CHOLESTEROL AS AN EVOLUTIONARY RESPONSE TO LIVING WITH OXYGEN

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Although often considered in a negative light, cholesterol is an essential molecule with unusually diverse functions. Cholesterol and related sterols (ergosterol in yeast, phytosterols in plants) is considered a hallmark of eukaryotes, and may even have triggered the evolution of multicellular organisms. Synthesis of cholesterol is an extremely oxygen-intensive process and requires sufficient terrestrial oxygen to proceed. In turn, several lines of evidence support the argument that cholesterol evolved at least in part as an adaptation to the hazards of oxygen. This evolutionary perspective usefully informs medical research on cholesterol to address health-related issues, as illustrated by examples drawn from three prominent human diseases: cataracts, heart disease, and cancer.

KEY WORDS: Adaptation, molecular evolution.

Puzzling over why a particular feature may have evolved can be an amusing pastime and keeps many a biologist off the street. Cholesterol is a case in point. The presence of this multifaceted molecule and related sterols (ergosterol in yeast, phytosterols in plants) is a defining characteristic of eukaryotes.

Cholesterol is best known as something to avoid—people tend to know their blood cholesterol levels almost as well as their weight or shoe size. But has cholesterol deserved its bad reputation? Yes, it is implicated in a number of diseases, most notably heart disease. But the fact is that we need cholesterol, as highlighted by the evolution of a hundred or more proteins that deal with its acquisition, transport, and elimination (Kurzchalia and Ward 2003). Konrad Bloch, who shared a Nobel Prize for elucidating the cholesterol synthetic pathway, wrote of cholesterol representing evolutionary perfection, being superior in its membrane functions to sterol precursors (Bloch 1994).

Cholesterol is an essential membrane reinfacer, and is also a precursor for steroid hormones, vitamin D, and bile acids. Its key role in cell signaling, via lipid rafts and as a covalent attachment to the important developmental protein Hedgehog, is also becoming more widely recognized (Stevenson and Brown 2009). An amazing diversity of lipid species has evolved to confer the necessary intrinsic properties of biological membranes, such as flexibility and selective permeability. The exclusivity of cholesterol to the membranes of animal cells suggests that it has a specialized role. In model bilayers, cholesterol displays selective properties by creating liquid ordered domains enriched in cholesterol together with high transition temperature lipids. These domains or lipid rafts “float” in the liquid disordered phase of the rest of the membrane (Berkowitz 2009). Thus, sterols regulate membrane dynamics by maintaining the membrane in a state of microfluidity suitable for cell function on large temperature scales (Dufou尔 2008). Specifically, cholesterol generates organized heterogeneity, providing a useful means for membrane subcompartmentalization that is essential for eukaryotic existence (Lingwood and Simons 2010). Indeed, cholesterol may have removed a bottleneck in evolution, allowing the development of multicellular organisms due to cholesterol’s role in processes needed for communication between cells like endocytosis and exocytosis (Mouritsen and Zuckermann 2004). Understanding the evolution of sterol biosynthesis is important to evolutionary biology, biochemistry, and the earth sciences, because the only known route to its synthesis...
requires molecular oxygen (Pearson et al. 2003). Here, we extend this concept to encompass the extraordinary relationship between cholesterol and oxygen.

The Root of All Sterol?
The evolution of the cholesterol biosynthetic pathway in animals is highly derived, but its roots remain obscure. The evolution of oxygenic photosynthesis precipitated a sharp rise in atmospheric oxygen ~2.45 billion years ago which had a profound impact on life on earth (Rasmussen et al. 2008). Many organisms retreated to anoxic environments. Others evolved to use oxygen in a vast array of metabolic networks (including sterol synthesis), while developing protective measures to counteract its toxicity (Raymond and Segre 2006). The evidence for the appearance of sterols in the geological record is a subject of rigorous debate. Molecular fossils of sterols (steranes) have been reported in shales that are 2.7 billion years old (Brocks et al. 1999), that is, predating the Great Oxidation Event (~2.45–2.3 billion years ago). However, more recent work argues that all of the earliest biomarker evidence is compromised by contamination, and that there is no molecular fossil evidence for eukaryotes prior to the Great Oxidation Event (Rasmussen et al. 2008). The last common ancestor that was able to synthesize sterols is not known.

However, the need for oxygen in sterol synthesis suggests that this ancestor lived in a fully oxygenated environment (Desmond and Gribaldo 2009). Furthermore, this last common ancestor was probably an ancient eukaryote, considering that there are only a few sporadic instances of bacteria with a proven ability to synthesize sterols (although not cholesterol), and that this likely occurred through horizontal gene transfer from such an organism (Pearson et al. 2003; Summons et al. 2006). As more genomes become available, further phylogenomic profiling may yield new clues about the roots of cholesterol synthesis (Desmond and Gribaldo 2009).

Cholesterol and Oxygen Caught in an Evolutionary Embrace
Recently, we provided a fresh perspective on why natural selection may have crafted cholesterol. We gathered evidence that sterols like cholesterol may have in fact been a protective response to the increasing levels of molecular oxygen in earth’s atmosphere (Galea and Brown 2009). The evolution of aerobic metabolism had advantages by increasing energy generation, but this came at a price. As we all know from the proliferation of antioxidant-containing creams, pills, and potions, oxygen breathing has its associated dangers.
The relationship between cholesterol and oxygen is tangled. Cholesterol synthesis is an extremely oxygen-intensive process, with 11 molecules of oxygen being needed to make one molecule of cholesterol (Summons et al. 2006). Therefore, cholesterol could only evolve as atmospheric oxygen levels rose. In fact, there is evidence that the chronology of the pathway ultimately leading to cholesterol synthesis coincides with the rise in oxygen (Bloch 1994). There are many instances across eukaryotic life whereby cholesterol synthesis is tied to oxygen levels (reviewed in Galea and Brown 2009). Indeed, it has been noted that the lung surfactant of the earliest air breathers, lungfish, is particularly rich in cholesterol (Orgeig et al. 2003) (Fig. 1).

There is also the issue of feedback control, a guiding principle in biology. This is where one substance controls the levels of another, metabolically related substance, allowing organisms to conserve resources, adapt to changing environments, and prevent the accumulation of toxic products. Lessons from the study of heart disease attest to the toxicity of excess cholesterol; no doubt explaining why we have evolved elaborate systems to keep our cellular cholesterol levels under tight control. In fact, cholesterol was one of the first examples of feedback control to be described (Schoenheimer and Breusch 1933), inhibiting its own production at multiple levels (Goldstein et al. 2006). Because oxygen is a necessary component in cholesterol production, the idea that cholesterol in turn may limit oxygen uptake by eukaryotic cells has a certain elegant symmetry to it.

Indeed, there are several studies suggesting that cholesterol limits oxygen diffusion across membranes (e.g., Khan et al. 2003; Mencaca et al. 2004; Widomska et al. 2007). This may appear to be a logical extension of cholesterol’s general barrier function, but although cholesterol in membranes certainly is important for excluding polar molecules, it should be remembered that oxygen is not polar. Actually, oxygen readily permeates lipids, such as oils. So, the fact that cholesterol impedes oxygen transport across membranes is not a given. This phenomenon is observed in model membranes and a variety of cell types. For instance, oxygen diffusion across mammalian cell membranes is considerably influenced by the cholesterol content of the plasma membrane (Khan et al. 2003).

Another example of the intricate link between sterols and oxygen is the idea that sterols are oxygen sensors. This concept was first discovered in yeast (Hughes et al. 2005) but also translates to mammalian cells (DeBose-Boyd 2008). It is not too much of a stretch to think of the surveillance role of sterols in sensing oxygen, to becoming an active defense against this potential hazard.

Cholesterol has even been considered to act as an antioxidant (Smith 1991). In this context, it is noteworthy that one of the first responses by yeast on exposure to hydrogen peroxide is to induce sterol synthesis; disruption of which renders the yeast cell more susceptible to this oxidant (Folmer et al. 2008). Another curious link between oxidant stress and cholesterol is that one of the enzymes that catalyses a concluding step in cholesterol synthesis (3β-hydroxysterol-Δ24-reductase, also called Seladin-1), directly scavenges hydrogen peroxide (Lu et al. 2008). Furthermore, there is considerable evidence that membrane cholesterol can limit cellular damage to reactive oxygen species like hydrogen peroxide. The mechanism is believed to involve sealing the membrane to limit the oxidant’s access to the cell’s interior, or even by directly intercepting the oxidant. Of course, this apparent antioxidant role of cholesterol may have been largely superseded by the development of a vast armory of more sophisticated enzyme-based antioxidant defenses.

Medical Implications

We have focused on the complex interplay between cholesterol and oxygen. This evolutionary perspective usefully informs medical research on cholesterol to address health-related issues, as illustrated by three examples.

CATARACTS

Ultraviolet radiation, oxygen, and perturbed cholesterol metabolism in the lens can converge to precipitate cataracts. For example, an increase in oxygen can cause cataracts, following hyperbaric oxygen treatment or surgical removal of the clear gel that fills the middle of the eye (Palmquist et al. 1984; Holekamp et al. 2005). The fiber cells that comprise the lens are especially rich in cholesterol. These cells are packed into a honeycomb-like array—a regular arrangement that confers ideal optical properties to allow efficient transmission of light, and is partially maintained by the high cholesterol content of the plasma membrane. The fiber cell’s high cholesterol content may also limit oxygen entry (Widomska et al. 2007) which could damage intracellular lens proteins contributing to cataract formation. Consequently, disturbances in cholesterol synthesis, either caused by genetic lesions or pharmacologically, tend to be associated with increased cataractogenesis. For instance, the first available cholesterol-lowering drug, triparanol (which incidentally targets 3β-hydroxysterol-Δ24-reductase), was taken off the markets due to increased incidence of cataracts in patients (Steinberg 2006). Fortunately, the current cholesterol-lowering drugs of choice, the statins, are not associated with increased risk of cataracts (Tan et al. 2007). Statins, unlike triparanol, may avoid cataractogenesis by targeting an earlier step of the pathway, and thereby resulting in the accumulation of a water-soluble rather than lipid-soluble intermediate, and also perhaps by tending to selectively target the liver rather than peripheral tissues including the eye.

Considering this interplay between cholesterol and oxygen in cataract formation, we should be mindful of the cataractogenic
potential of new drugs being developed which affect cholesterol metabolism.

HEART DISEASE
Elevated levels of blood cholesterol are a long-established and notorious risk marker for heart disease. Current thinking is that cholesterol in an inflammatory setting is deposited via modified lipoproteins in macrophages in the artery wall, leading to the development of early atherosclerotic lesions. Over time, these lesions develop further and may eventually rupture, precipitating a clinical event such as a heart attack (Libby et al. 2009).

In addition to this paradigm, the role of cholesterol in impeding oxygen diffusion across membranes may also have relevance to the etiology and treatment of heart disease. For example, the cholesterol content of red blood cell membranes affects the extent of oxygenation of hemoglobin—patients with high blood cholesterol levels have less oxygenation, which can be reversed with cholesterol-lowering drugs (Menchaca et al. 2004). Therefore, another benefit of cholesterol-lowering therapies is to improve tissue oxygenation and hence to reduce the damage caused by ischemia, as experienced during chest pain. It is also possible that reducing the cholesterol content of mitochondria in the cardiomyocyte will improve their aerobic capacity. In turn, this should improve heart function and ameliorate the compensatory enlargement of the heart (ventricular hypertrophy) that contributes to heart failure.

CANCER
Although cholesterol is best known as a risk factor for heart disease, emerging evidence is now providing a number of intriguing links between cholesterol and cancer (Brown 2007). A particularly promising case can be made for involvement of cholesterol in the development and progression of prostate cancer. First, cholesterol is the starting material that is converted into male sex hormones, and hormones like testosterone are critical drivers in prostate cancer (Chen et al. 2009). Second, some (but not all) studies have linked increased blood cholesterol levels with increased risk of prostate cancer (e.g., Mondul et al. 2010). Furthermore, there is an increasing number of studies finding an association between the use of the statin class of cholesterol-lowering drugs and reduced risk of aggressive prostate cancer (reviewed in Hamilton and Freedland 2008).

As in the case of heart disease, cholesterol and oxygen may also have implications for cancer research, again involving the mitochondrion. This powerhouse of the cell has the lowest cholesterol content of any eukaryotic organelle (van Meer et al. 2008), which may relate to its prokaryotic (i.e., cholesterol-free) origin. However, higher cholesterol contents have been observed in certain disease states, such as cancer (Garcia-Ruiz et al. 2009). Cholesterol, mitochondria, and cancer cross paths in the Warburg Effect, which after 80 years, is enjoying a strong revival. Otto Warburg was an early biochemist who, following in the footsteps of Louis Pasteur, studied respiration in tumors. He found that unlike normal cells, cancer cells shun mitochondrial oxidative phosphorylation in favor of glycolysis (Warburg 1930). This altered mode of energy generation apparently gives cancer cells a survival advantage. Increased mitochondrial cholesterol levels, possibly by inhibiting oxygen entry into the mitochondria, reduces oxidative phosphorylation and promotes the Warburg Effect (Campbell and Chan 2008). Therefore, reducing mitochondrial cholesterol may provide a novel chemotherapeutic strategy: compromising cancer cell survival by increasing oxygen entry and altering energy production pathways.

Conclusions
We do not propose that cholesterol’s defensive role against oxygen is its most important function today in eukaryotes—just as the human arm is more than a swimming aid when viewed telescopically from its evolutionary forebear, the fish fin. Nor can we exclude the possibility that cholesterol’s apparent protection against oxygen is a fortuitous side effect of natural selection. However, our evolutionary perspective on cholesterol’s special relationship with oxygen has important medical implications as exemplified by our illustrations drawn from three prominent human diseases: cataracts, heart disease, and cancer.

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LITERATURE CITED


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