Coenzyme Q10 Increases Cholesterol Efflux and Inhibits Atherosclerosis Through MicroRNAs

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Atherosclerosis is a progressive inflammatory disease of the artery wall and the underlying basis for cardiovascular disease (CVD), which accounts for ≈32% of all deaths in the United States and is the leading cause of mortality in the world. Atherosclerosis is classically defined by the accumulation of lipid and cholesterol deposits within the subendothelial space in the artery wall which leads to chronic inflammation and proinflammatory, cholesterol-laden macrophages which differentiate into resident foam cells in the lesion, ultimately forming an acellular necrotic core. To date, the statin drug class has been overwhelmingly successful at reducing circulating total cholesterol levels, namely low-density lipoproteins cholesterol, the number one risk factor for CVD. Although pharmacological intervention with statins has dramatically reduced the number of cardiovascular events, many patients do not tolerate statins and a substantial disease burden remains even in patients who are on statins. Therefore, a great need remains to identify new drug targets and novel approaches to prevent and treat atherosclerosis and CVD. One strategy that has been extensively studied, but remains central to atherosclerosis reduction, is identifying new ways to increase the removal of excess cholesterol from peripheral cells and lesions through the reverse cholesterol transport (RCT) pathway. Briefly, the RCT pathway involves the transport of cholesterol from peripheral tissues (ie, foam cells within atherosclerotic lesions) to the liver by high-density lipoproteins (HDL), where cholesterol is excreted from the body as bile. This pathway is mediated by a number of lipid and cholesterol transporters, including the widely studied cholesterol efflux proteins, ATP-binding cassette transporters A1 and G1 (ABCA1 and ABCG1). Although this pathway has been studied for 35 years, regenerating total cholesterol levels, namely low-density lipoproteins cholesterol, the number one risk factor for CVD. Although pharmacological intervention with statins has dramatically reduced the number of cardiovascular events, many patients do not tolerate statins and a substantial disease burden remains even in patients who are on statins. Therefore, a great need remains to identify new drug targets and novel approaches to prevent and treat atherosclerosis and CVD. One strategy that has been extensively studied, but remains central to atherosclerosis reduction, is identifying new ways to increase the removal of excess cholesterol from peripheral cells and lesions through the reverse cholesterol transport (RCT) pathway. Briefly, the RCT pathway involves the transport of cholesterol from peripheral tissues (ie, foam cells within atherosclerotic lesions) to the liver by high-density lipoproteins (HDL), where cholesterol is excreted from the body as bile. This pathway is mediated by a number of lipid and cholesterol transporters, including the widely studied cholesterol efflux proteins, ATP-binding cassette transporters A1 and G1 (ABCA1 and ABCG1). Although this pathway has been studied for 35 years, a new class of drugs has emerged as a viable strategy to control RCT and inhibit atherosclerosis. Nucleic acid-based drugs represent a novel approach to manipulate cholesterol transport and inflammation associated with CVD. Currently, only one drug, Mipomersen (antisense RNA against apolipoprotein B), has gained Food and Drug Administration approval; however, many other small RNA, antisense, or microRNA (miRNA)-based drugs are in clinical phase trials and are the next frontier in controlling metabolic diseases.

Since their discovery in mammals nearly 15 years ago, miRNAs (19–22 nucleotides in length) have rapidly emerged as powerful regulators of metabolism and promising drug targets for numerous metabolic diseases. miRNAs are one class of small noncoding RNAs that bind to partially complementary sequences in target mRNA 3′-untranslated regions and promote posttranscriptional repression. Although gain- and loss-of-function studies have revealed a tremendous amount about miRNA regulation and function, relatively little attention has been given to understanding dietary, nutritional, and environmental regulation of mammalian miRNAs in metabolic disease. Based on evidence from a few early studies, miRNAs likely serve as critical intermediary nodes in diet or environment-driven metabolic networks, namely in the pathogenesis of metabolic disease. Dietary botanicals and the genes and miRNAs they regulate have gained considerable interest in the past 4 years. Epigallocatechin gallate, found in green tea, was one of, if not, the first dietary polyphenols reported to alter miRNAs in mammalian cells (human hepatoma HepG2 cells). Most interestingly, Wang et al demonstrated in a beautiful 2012 study that the breakdown of anthocyanins into protocatechuic acid by gut microbiota repressed macrophage miR-10b expression. miR-10b was found to directly target both ABCA1 and ABCG1 in macrophages; therefore, protocatechuic acid was found to increase macrophage cholesterol efflux and inhibit atherosclerosis progression. In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Wang et al report another fascinating study linking diet, cholesterol efflux, and atherosclerosis through miRNA function. Here, Wang et al have continued their pioneering work linking a vitamin-like supplement, Coenzyme Q10 (ubiquinone, ubiquinol, CoQ10), with enhanced RCT and reduced atherosclerosis through a novel miR-378 regulatory module. CoQ10 is a fat-soluble antioxidant vitamin found in most cells and membranes and is highly enriched in the inner membrane of mitochondria where it produces energy (ATP) as a component in the electron transport chain. CoQ10 is also a widely popular dietary supplement with sales exceeding $500 million dollars in the United States in 2011 (Nutrition Business Journal). Cholesterol biosynthesis, the target of statins, and CoQ10 share biosynthetic intermediate steps; therefore, statins dramatically reduce CoQ10 biosynthesis and may contribute to complications of statin use. As such, CoQ10 supplementation is often used in adjunct with statins. Several clinical trials have indicated that CoQ10 supplementation is beneficial and has protective cardiovascular effects in humans. Specifically, CoQ10 has been found to improve endothelial dysfunction associated with atherosclerosis, likely through regulating endothelial nitric oxide levels. Previous studies in apolipoprotein E-deficient (Apoe−/−) mice have demonstrated
its effectiveness in promoting atherosclerotic regression.\textsuperscript{15} Nevertheless, CoQ10 failed to protect \textit{ApoE}\textsuperscript{−/−} from cigarette smoke-induced atherosclerosis.\textsuperscript{16} Dietary supplementation with CoQ10 and other antioxidants has a long history with atherosclerosis, CVD, and statins; however, Wang et al\textsuperscript{10} have discovered a novel critical network with potentially multiple drug targets that may be manipulated to increase cholesterol efflux and reduce atherosclerosis.

Wang et al\textsuperscript{10} first observed profound inhibitory effects of CoQ10 on in vitro foam-cell formation using both mouse (\textit{J774.A1}) and human (\textit{THP-1}) macrophage-like cell lines. This observation was found to be linked to dose-dependent increases in cholesterol efflux to HDL but not lipid-poor apolipoprotein A-I. Cells, namely macrophages, efflux cholesterol to spherical native HDL through ABCG1 and to lipid-poor discoidal apolipoprotein A-I through ABCA1. Based on the lack of CoQ10-induced efflux to lipid-poor apolipoprotein A-I, the cholesterol efflux response was linked to ABCG1. The authors then demonstrated that CoQ10 treatment increased ABCG1 expression. Most importantly, the CoQ10 effect on \( \text{ABCG1} \) stability was found to be mediated by ABCG1’s 3′-untranslated regions, as CoQ10 was found to reduce gene (luciferase) reporter activity in reporters containing the \( \text{ABCG1} \) 3′-untranslated regions, thus suggesting a miRNA posttranscriptional regulatory mechanism. The authors used a high-throughput miRNA microarray strategy to identify miR-378 as one of several miRNAs whose expression was significantly reduced in macrophages by CoQ10 treatment. Although putative miR-378-\( \text{ABCG1} \) target sites were not uniformly conserved among mammals, conserved miR-378 target sites were found in the 3′-untranslated regions of primates and rodents. Using classic in vitro cholesterol efflux assays, the authors confirmed that miR-378 was negatively correlated with ABCG1 expression and macrophage cholesterol efflux to HDL. Notably, exogenous transfection of miR-378 abolished the observed increase in cholesterol efflux associated with CoQ10, whereas silencing of miR-378 was not additive to CoQ10 treatment. These convincing data support a model by which CoQ10 inhibits miR-378 suppression of ABCG1 expression, thus increasing macrophage cholesterol efflux to HDL. To determine how CoQ10 inhibits miR-378 expression, the authors scanned miR-378’s promoter region for potential regulatory elements, and a putative activator protein-1 (AP-1) response element was identified in the proximal promoters of both mouse and human miR-378. These functional AP-1 response elements were validated in both mouse and human constructs using mutagenesis and demonstrated that miR-378 sensitivity to CoQ10 supplementation was mediated through c-Jun, a key heterodimeric partner in the AP-1 transcriptional complex. CoQ10 was found to powerfully inhibit c-Jun expression in macrophages. Most interestingly, CoQ10 induction of macrophage cholesterol efflux through miR-378 was also demonstrated in vivo using \textit{ApoE}\textsuperscript{−/−} mice fed CoQ10 (600 mg/kg body weight, daily oral gavage) for 2 weeks. Strikingly, both aortic lysates and primary macrophages were found to have decreased expression of both c-Jun and miR-378, as well as markedly increased macrophage ABCG1 expression and cholesterol efflux to HDL. Moreover, the authors demonstrated that CoQ10 supplementation (1) increased RCT through the liver and feces, (2) decreased foam cell formation, and (3) stimulated lesion regression in \textit{ApoE}\textsuperscript{−/−} mice, as determined by Oil-Red-O staining of lesions (lesion area). Collectively, results from this study provide ample evidence to support an AP-1/miR-378/ABCG1 regulatory axis that links CoQ10 to macrophage cholesterol efflux to HDL and atheroprotection. As such, the authors suggest that these powerful findings provide mechanistic support for CoQ10 supplementation in the management of atherosclerosis and CVD in humans.

The miR-378/ABCG1 axis arrives in the time of heightened interest in miRNA control of cholesterol metabolism, namely cholesterol efflux. Recently, multiple miRNA-regulation networks have been identified to regulate the RCT pathway. The most extensively studied cholesterol-regulating miRNA, miR-33, is cotranscribed with its host gene, sterol regulatory element-binding protein, during periods of low intracellular cholesterol states or in response to statins, and has been shown by several independent laboratories to limit sterol flux at multiple levels within the RCT pathway.\textsuperscript{17} Therefore, silencing miR-33 has been highly regarded as a attractive target for promoting RCT and enhancing atherosclerosis regression. Nevertheless, the effect of sustained miR-33 silencing on atherosclerosis progression in mice has proved complex and controversial.\textsuperscript{18,19} Other miRNA targets have also emerged, including miR-144, which was found by 2 independent laboratories to suppress cholesterol efflux to HDL by targeting ABCA1. miR-144 was found to be activated by nuclear receptors for oxysterols, liver-X-receptor,\textsuperscript{20} and bile acids, Farnesoid-X-receptor,\textsuperscript{21} which provide clear links between cholesterol accumulation and oxidation in the liver and ABCA1-mediated cholesterol efflux. In addition to miR-33 and miR-144, many other miRNAs have been reported to target ABCA1, compared with few for ABCG1. Moreover, there is a general lack of understanding for how cholesterol efflux-regulating miRNAs are influenced by diet, supplements, or environments. For example, fisetin, a dietary antioxidant flavonoid present in many fruits, including grapes and strawberries, was recently found to block high-fat diet–induced miR-378 expression in the liver.\textsuperscript{22} Through miR-378, fisetin was found to regulate hepatic lipid metabolism and protect against high-fat diet–induced hepatosteatosis.\textsuperscript{22} If CoQ10 suppresses miR-378 levels in other cell types or tissues, namely the liver, then CoQ10 may be a potential therapeutic strategy to increase cholesterol efflux in peripheral macrophages and prevent concomitant hepatic lipid accumulation through increased hepatic nuclear respiratory factor-1 and increased fatty acid oxidation.\textsuperscript{23} Activating liver-X-receptor to drive ABCA1 and ABCG1 has been explored as a potential method to increase cholesterol efflux and inhibit atherosclerosis\textsuperscript{23,24}; however, liver-X-receptor activation was found to cause hepatosteatosis and hypertriglyceridemia in animals.\textsuperscript{25} Therefore, manipulating cholesterol efflux through the CoQ10/AP-1/miR-378/ABCG1 axis may overcome barriers that have plagued previous strategies. In summary, the work presented by Wang et al\textsuperscript{10} in this issue is a significant advancement for miRNAs in metabolic disease, cholesterol homeostasis, and atherosclerosis. This well-crafted and designed study should
not only re-energize investigation into the benefits of CoQ10 and the underlying molecular mechanisms but also serve as an impetus to gain a greater understanding of miRNA axes that link diet and nutrients to CVD.

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References

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