Apolipoprotein A1 Regulates Coenzyme Q10 Absorption, Mitochondrial Function, and Infarct Size in a Mouse Model of Myocardial Infarction1–3

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Abstract

HDL and apolipoprotein A1 (apoA1) concentrations inversely correlate with risk of death from ischemic heart disease; however, the role of apoA1 in the myocardial response to ischemia has not been well defined. To test whether apoA1, the primary HDL apolipoprotein, has an acute anti-inflammatory role in ischemic heart disease, we induced myocardial infarction via direct left anterior descending coronary artery ligation in apoA1 null (apoA1−/−) and apoA1 heterozygous (apoA1+/−) mice. We observed that apoA1−/− and apoA1+/− mice had a 52% and 125% increase in infarct size as a percentage of area at risk, respectively, compared with wild-type (WT) C57BL/6 mice. Mitochondrial oxidation contributes to tissue damage in ischemia–reperfusion injury. A substantial defect was present at baseline in the electron transport chain of cardiac myocytes from apoA1−/− mice localized to the coenzyme Q (CoQ) pool with impaired electron transfer (67% decrease) from complex II to complex III. Administration of coenzyme Q10 (CoQ10) to apoA1 null mice normalized the cardiac mitochondrial CoQ pool and reduced infarct size to that observed in WT mice. CoQ10 administration did not significantly alter infarct size in WT mice. These data identify CoQ pool content leading to impaired mitochondrial function as major contributors to infarct size in the setting of low HDL/ apoA1. These data suggest a previously unappreciated mechanism for myocardial stunning, cardiac dysfunction, and muscle pain associated with low HDL and low apoA1 concentrations that can be corrected by CoQ10 supplementation and suggest populations of patients that may benefit particularly from CoQ10 supplementation. J. Nutr. 144: 1030–1036, 2014.

Introduction

Plasma concentrations of HDL and its major protein, apoA1, are both inversely associated with cardiovascular disease morbidity and mortality (1,2). apoA1 is a single polypeptide of ~28,000 kDa primarily synthesized in the liver and small intestine (3,4). Human individuals with apoA1 deficiency (5) and apoA1-deficient mice (6) fail to form normal HDL particles, cannot effectively transport cholesterol from tissues back to liver and, as a result, are predisposed to premature coronary artery disease and death (7,8). More recently, novel therapeutic approaches to treat coronary artery disease, such as the administration of apoA1 or its analogs to alter the development of atherosclerosis, were investigated in animal models and in humans (9,10).

Recent studies (11–14) show that apoA1 possesses anti-inflammatory and antioxidant properties, in addition to its role in reverse cholesterol transport. Given the role of inflammation (15–17), redox state, and mitochondrial electron transport (18–20) in determining cardiac function during postmyocardial infarction, we sought to determine whether apoA1 has acute anti-inflammatory properties that modulate myocardial infarct size. In fact, we found that apoA1 deficiency sharply increased infarct size. The pathology was traced to a functional defect in mitochondrial electron transport that arose from a deficiency in the coenzyme Q (CoQ)10 pool of apoA1 null (apoA1−/−) mice. This defect was reversed by coenzyme

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3 Supplemental Tables 1 and 2 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
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10 Abbreviations used: apoA1−/−, apoA1 heterozygous; apoA1+/−, apoA1 null; ck-1, CDC-like kinase 1; CoQ, coenzyme Q; CoQ10, coenzyme Q10; KCl, potassium chloride; LAD, left anterior descending coronary artery; MOPS, 3-(/N-morpholino)propanesulfonic acid; ROS, reactive oxygen species; TMPO, N/N,N,N-tetramethyl p-phenylenediamine; TTC, 2,3,5-triphenyltetrazolium chloride; WT, wild-type.
Mitochondrial techniques. Colorimetric HDL assay kit (KA1656). Minced, and placed in Chappell-Perry (CP1) buffer [100 mmol/L potassium HDL assay] hearts (apoA1

Terminal deoxynucleotidyl transferase–mediated biotinylated dUTP nick end labeling assay. Heart sections were stained with the In Situ Cell Death Detection kit (Roche Applied Science) per the instructions of the manufacturer and stained with 4',6-diamidino-2-phenylindole. Terminal deoxynucleotidyl transferase–mediated biotinylated dUTP nick end labeling–positive cells were counted at 40× magnification in 5 randomly selected areas within the infarct zone and expressed as positive cells per square millimeter and then compared between WT and apoA1−/− mice. At least 10 sections were analyzed throughout the entire longitudinal axis of the hearts (n = 5 hearts per group).

HDL assay. HDL was quantified in duplicate in serum using the Abnova colorimetric HDL assay kit (KA1656).

3.0 mg/dL (n = 4), and 60.3 ± 3.0 mg/dL (n = 5) in apoA1−/−, apoA1+/−, and WT mice, respectively, and are consistent with previous reports (6,31).

Loss of apoA1 and HDL affected outcomes after chronic ligation of the proximal LAD in which ~80–90% of apoA1−/−, but not WT, mice died within the first 24 h (data not shown). Using an ischemia (30 min)–reperfusion (3 h) model, we observed no differences in the area at risk after LAD ligation between WT, apoA1−/−, and apoA1+/− mice after LAD ligation (50 ± 11%, 50 ± 3%, and 52 ± 4%, respectively). Infarct size as a percentage of the area at risk was significantly (P < 0.05)
Mitochondrial oxidative phosphorylation is compromised by loss of apoA. Mitochondrial oxidative metabolism was measured using glutamate, succinate, duroquinol, and TMPD-ascorbate as substrates for complexes I through IV, respectively. Oxygen consumption under ADP-stimulated (state 3) and ADP-limited (state 4) conditions are shown in Supplemental Table 1. State 3 respiration, state 4 respiration, respiratory control ratio, and the ADP-to-oxygen ratio were similar in WT and apoA1−/− mice when glutamate was used as the substrate (Supplemental Table 1). However, there was a decrease in both state 3 and uncoupled respiration in the apoA1−/− mice using a complex II substrate (succinate plus rotenone). The decreased coupling of respiration observed in apoA1−/− mice indicated by the decrease in the respiratory control ratio was mainly due to a decrease in state 3 respiration rather than an increase in state 4 respiration. Dinitrophenol-uncoupled respiration was decreased in apoA1−/− mice, localizing the respiratory defect to the electron transport chain.

Substrates that donate electrons to specific electron transport complexes were used under conditions of maximal ADP-stimulated respiration to further localize the sites of defects within the electron transport chain. Maximal ADP-stimulated respiration, measured using succinate as substrate for complex II, was decreased in apoA1−/− mice (Supplemental Table 1). These data uncover a defect in the pathway from complex II → CoQ pool → complex III → cytochrome c → cytochrome oxidase → oxygen. To assess the function of the electron transport distal to complex II, duroquinol (electron donor to complex III) and TMPD-ascorbate (electron donor to cytochrome oxidase via cytochrome c) were used. Maximal ADP-stimulated respiration was not affected in the apoA1−/− mice when duroquinol or TMPD-ascorbate were substrates (Supplemental Table 1). Maximal ADP-stimulated respiration was also similar in WT and apoA1−/− mice with a complex I substrate (which, like complex II, donates electrons to complex III), confirming the integrity of the electron transport chain other than at complex II.

Electron transport is compromised by inadequate CoQ. Nicotinamide adenine dinucleotide cytochrome c reductase, a measure of the activity of the electron transport segment I → CoQ → III, was similar in apoA1−/− and WT mice (Fig. 2). The activity of succinate cytochrome c reductase (antimycin A sensitive) was markedly decreased in apoA1−/− mice, localizing a defect to either complex II, CoQ, or complex III of the electron transport chain (Fig. 2; Supplemental Table 2). However, the activity of cardic myocyte cell death suggests that the majority of the increased death in the absence of apoA1 is secondary to increased necrosis.
complex III was not changed compared with WT mice, and the activity of complex II itself was surprisingly normal (Fig. 2A). Thus, the defect in electron transport in mitochondria of apoA1−/− mice must be localized to the CoQ pool that transfers electrons from complex II to complex III. The activity of citrate synthase, a mitochondrial matrix enzyme, was similar in complex I to complex III. The activity of citrate synthase, a mitochondrial matrix enzyme, was similar in apoA1−/− and WT mice (Fig. 2B), indicating that mitochondrial integrity was not compromised in apoA1−/− mice.

Cardiac CoQ10 concentrations are reduced in apoA1−/− mice. The foregoing analyses suggest that a defect exists in the mitochondria of apoA1−/− mice consistent with an inability of the CoQ pool to adequately support complex II function. We determined whether the cardiac pool of CoQ10 was indeed sensitive to circulating HDL and apoA1 concentrations and found that the lack of apoA1 reduced the cardiac CoQ10 pool by almost 75% (Fig. 3A). This was a selective reduction because Western blotting for cytochrome oxidase showed that mitochondrial mass was not diminished in the apoA1−/− mice (Fig. 3B). We sought to determine whether the cardiac pool of CoQ10 could be manipulated by administration of exogenous CoQ10 (22). CoQ10 concentrations did not increase in hearts of WT mice, but this supplementation did raise cardiac CoQ10 concentrations in the apoA1−/− mice to WT levels (Fig. 3A).

CoQ10 supplementation reduced infarct size in apoA1−/− mice. The ready manipulation of the cardiac CoQ10 pool is a potential therapy to reverse the adverse effects of apoA1/HDL deficiency on infarct size. Indeed, administration of CoQ10 to apoA1−/− mice completely corrected the defect in apoA1−/− mice because CoQ10 supplementation fully reduced infarct size as a percentage area at risk to that of WT mice (Fig. 4). We found that CoQ10 injected into WT mice were no different than WT mice that received vehicle (P = 0.15), suggesting that the CoQ10 content of WT mitochondria is sufficient.

Discussion

For >3 decades, population studies demonstrated an inverse correlation between plasma apoA1 and HDL concentration and coronary artery disease (32,33). Many of these same clinical populations’ low HDL concentrations are associated with increased mortality, which to date is presumed to be secondary to an increased risk of myocardial infarction in which decreased HDL concentrations place a greater burden of atherosclerotic coronary artery disease at presentation (34,35). Here, we showed that in fact reducing HDL concentrations, even just by half, acutely alters infarct size. Processes occurring during the ischemia or reperfusion are affected by the previous history of circulating HDL concentrations, and we trace this to a sharply reduced cardiac CoQ10 pool that is insufficient to support electron transport from complex II to complex III. This deficit can more than double infarct size.

Our initial studies indicated that anterior wall myocardial infarction led to substantial cardiac dysfunction to such a degree that mice could not successfully be weaned from the ventilator in the apoA1−/− mice. These initial findings suggested that there was a significantly greater tissue death and perhaps increased strain on surviving myocardium in the absence of apoA1. This increased death led us to assess the effects of apoA1 in an acute ischemia–reperfusion model with 30 min ischemia and 3 h reperfusion. Our data indicate that necrotic, but not apoptotic, cell death was enhanced in apoA1-deficient mice in this model.
Current literature suggests that the increased death in apoA1⁻/⁻ mice from reperfusion injury should be due to the loss of free-radical scavenging by apoA1 itself (14). However, our data demonstrate that a defect that persists after mitochondrial isolation is present in the hearts of apoA1⁻/⁻ mice. This defect is the relevant change responsive to circulating apoA1 concentrations because correction of this defect by CoQ10 supplementation normalized infarct size. CoQ10 is a facile antioxidant, but neither reduction of circulating apoA1 nor cardiac mitochondrial CoQ10 content enhanced cardiac ROS production detectable by hydroethidine staining.

Given that there was CoQ10 in the diet of the mice, the fact that i.p. supplementation restored CoQ10 concentrations in the myocardium in the absence of an increase in apoA1 or HDL suggests that apoA1 could have an as-yet undefined role in CoQ10 absorption or trafficking from the gastrointestinal tract. Abnormal gastrointestinal absorption was postulated in patients with heart failure due to intestinal edema, and the increasing doses of CoQ10 supplementation were shown to increase plasma concentrations of CoQ10 and be associated with increases in cardiac function (36). Furthermore, emulsification of CoQ10 was shown to enhance the oral bioavailability of CoQ10 (37). The potential role for apoA1 in the bioavailability of oral CoQ10 remains to be determined.

Cardiac mitochondria of apoA1⁻/⁻ mice exhibit a defect in succinate-driven respiration that, notably, exists before the onset of ischemic injury and may predispose mice with low HDL concentrations to heightened responses to other insults. This defect in oxidative phosphorylation was limited to the pathway from complex II → CoQ → complex III even while the electron transport chain from complex I → CoQ → complex III remained intact. Differential interaction of complex I and complex II with the CoQ pool was first described (38) in CDC-like kinase 1 (clk-1) mutants of Caenorhabditis elegans defective in the last step of CoQ synthesis (39). These mutants display defective electron transport (38), although in this organism, the decrease in respiration was with glutamate, a complex I donor, and not succinate (40). clk-1 Mutants have an increased lifespan (41), suggesting that defective coupling of the CoQ pool to complex I is protective. This outcome is in line with the protective effect of blocking electron flow from complex II into complex III during ischemia (19). In contrast, the current observation suggests that a blockade of electron flow from complex II into complex III exacerbates ischemic damage. Ischemia by itself allows cytochrome c to escape from mitochondrial isolation is present in the hearts of apoA1⁻/⁻ mice. This finding suggests novel mechanisms for the recent findings, such as improved functional status associated with higher HDL concentrations (50) or a worse outcome in patients with non-ischemic heart failure associated with low HDL concentrations (51).

In summary, our findings demonstrate an important link between circulating apoA1 concentrations and cardiac mitochondrial function. Low apoA1/HDL decreases the CoQ10 pool, which in turn decreases electron transfer from complex II to complex III. This deficiency clearly has relevant implications in the setting of acute myocardial infarction and defines novel mechanisms for the recent findings, such as improved functional status associated with higher HDL concentrations (50) or a worse outcome in patients with non-ischemic heart failure associated with low HDL concentrations (51).

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