Published in final edited form as:
Arterioscler Thromb Vasc Biol. 2013 February ; 33(2): . doi:10.1161/ATVBAHA.112.300921.

Apolipoprotein A-II: Still second fiddle in HDL metabolism?

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Keywords

Atherosclerosis; HDL; apolipoprotein A-II; cholesterol

Apolipoprotein A-II (apo A-II) is the second most abundant protein on HDL, accounting for approximately 20% of total HDL protein[1]. Like apo A-I, the most abundant HDL protein, it contains a tandem array of amphipathic helices. It is considered an exchangeable apolipoprotein, because it can dissociate from lipoproteins, although it has greater lipid affinity than apo A-I[1]. Despite the relative abundance of apo A-II, its role in HDL metabolism has long been a mystery, particularly whether it is an anti or a pro-atherogenic protein. Data from *in vitro* HDL fuctional studies[2, 3], mouse transgenic models[4, 5], and human epidemiologic studies[6, 7] have all been conflicting concerning its pathophysiologic role. A patient with apo A-II deficiency has been described[8], but this patient was largely unaffected in terms of her lipoprotein profile and appeared to be free of cardiovascular disease (CVD), suggesting that the role of apo A-II may be redundant with the other exchangeable apolipoproteins, such as apo A-I.

In this issue of *ATVB*, Jianglin Fan and colleagues investigate the expression of human apo A-II in transgenic rabbits on lipoprotein metabolism and atherosclerosis.

Although rabbits have many advantages over mice as animal models for atherosclerosis, such as the expression of CETP, there have only been a few previous reports of transgenic rabbits being used to study atherosclerosis[9, 10]. One important difference between rabbits and humans in lipoprotein metabolism, which facilitated this study, is that rabbits do not normally express apo A-II. Using the human apo A-II promoter, Jianglin Fan and colleagues were thus able to produce transgenic rabbits that produce levels of human apo A-II comparable to what is found in human serum. The expression of apo A-II in rabbits resulted in numerous but relatively subtle effects on the lipoprotein profile. On a normal chow diet, the transgenic rabbits had slightly higher levels of total cholesterol and lower levels of HDL-C, but interestingly HDL-C did not decrease when placed on a high fat diet unlike in normal rabbits, in which it decreased to a level lower than in transgenic rabbits. Apo A-II was found in all HDL fractions but was enriched in smaller size fractions of HDL and was associated with a decrease in the apo A-I content of HDL. Interestingly, on a high fat diet much of the HDL in the transgenic rabbits was found by electrophoresis to be in the pre-beta HDL fraction.

The function of HDL from apo A-II transgenic rabbits was compared to HDL from non-transgenic sibling controls by studying cholesterol efflux, anti-inflammation and anti-oxidation and it appeared to be superior by all 3 measures. Cholesterol efflux from

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macrophages to isolated HDL^2 and HDL^3 was slightly better for transgenic rabbits versus controls when normalized to the protein content of HDL. Phospholipids appeared to be enriched on HDL from transgenic rabbits, which could possibly account for this finding. Increased pre-beta HDL in the transgenic rabbits would also be expected to increase cholesterol efflux, particularly by the ABCA1 transporter, although this HDL subfraction was not directly in this study. Similarly, isolated HDL from transgenic rabbits was superior to HDL from control rabbits in suppressing cytokine mRNA levels in LPS-induced macrophages, although again the difference was relatively modest.

There was, however, a striking decrease in CRP levels and neutrophil and monocyte counts in the blood of transgenic rabbits on the high fat diet compared to control rabbits, which suggests that apo A-II has potent anti-inflammatory effects. The oxidizability of beta-VLDL was also decreased in transgenic versus control rabbits, which may be due to the fact that apo A-II was also found on all the apoB-containing lipoproteins.

Given the beneficial *in vitro* effects of apo A-II expression on HDL function, it was perhaps not surprising that the transgenic rabbits had significantly less aortic atherosclerosis, as well as decreased atherosclerosis in coronary vessels. The atherosclerotic lesions in the transgenic rabbits also had less macrophage infiltration and smooth muscle proliferation. Immunohistochemically, apo A-II was detected in the atherosclerotic lesions of the transgenic rabbits, but one can not infer whether this is simply just a consequence of increased endothelial permeability and subsequent deposition of apo A-II in plaques or whether apo A-II is exerting a beneficial anti-atherogenic effect in plaques.

So where do we go from here in regard to future studies on apo A-II? For the two areas of translational research that directly impact on patient care, diagnostics and therapeutics, there are several possible implications from the findings from this paper, but there is still much to do. Given that there have already been several large epidemiologic studies that have examined the utility of apo A-II as a diagnostic biomarker but have not consistently shown a strong association with CVD[6, 7], it is unlikely that clinical laboratory tests based on total plasma levels of apo A-II will add much over our current CVD biomarkers. Recent studies showing that the proteome of HDL is much more complex than initially thought and that apo A-I and apo A-II represent the “skeleton” of HDL on which numerous other proteins can attach[11], it is still possible that a particular subfraction of HDL, containing apo A-II and other specific proteins or lipids, may be of diagnostic value, but this has yet to be shown.

Apo A-I, which has been much more investigated, and for which there is better evidence that raising it may be beneficial for preventing CVD, there are still not effective drugs, although there is at least one that is being investigated in late clinical trials[12]. A more likely fruitful area of apo A-II research, would be investigating the use of the full length protein or peptide mimics of apo A-II for acute intravenous HDL therapy, as has been done for apo A-I in the rapid stabilization of patients with acute coronary syndrome[13].

In fact, the term apo A-I mimetic peptides is somewhat of a misnomer, because many of the currently studied peptides for intravenous HDL therapy are not necessarily based on the primary amino acid sequence of apo A-I but simply contain an amphipathic helix like the other exchangeable apolipoprotiens[14, 15]. This study confirmed previous findings[16] that apo A-II is just as effective as apo A-I in promoting cholesterol efflux by the ABCA1 transporter, suggesting that it may be a good agent for promoting reverse cholesterol transport. Recently, it was also shown that apo A-II was much better than apo A-I in suppressing inflammation in a concanavalin A-induced hepatitis mouse model[17], which may perhaps help explain the observed marked anti-inflammatory effects seen in this study on CRP levels and on the white count in apo A-II transgenic rabbits.

Although there is undoubtedly a lot of redundancy in the function of the different exchangeable type apolipoproteins, because of their close protein homology and genetic relatedness[1], each apolipoprotein may have evolved to also have a unique function. Future studies of the separate structural domains of apo A-II may thus lead to a better understanding of the specific role of apo A-II in lipoprotein metabolism and may also reveal new strategies for the treatment of CVD. Someday our view of apo A-II as only a supporting player on HDL may perhaps change, and apo A-II could even supplant apo A-I as “first chair” of HDL metabolism.

Acknowledgments

The author is supported by intramural research funds from the National Heart, Lung and Blood Institute of the NIH.

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