Quality Control of HDL: Nutrition and Not Numbers May Determine HDL Functionality

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Abstract: The strong inverse relationship between high density lipoproteins-cholesterol (HDL-C) levels and cardiovascular disease (CVD) has aroused a strong interest in the research of lifestyle and pharmacological agents capable of elevating plasma HDL levels. HDL is essential in reverse cholesterol transport (RCT), thus its anti-atherogenic function. However, torcetrapib, a compound that increases plasma HDL-C levels, was unexpectedly associated with an increased cardiovascular mortality. The findings led to consider that HDL functionality and quality might be more relevant to CVD than the total circulating HDL quantity itself. Adherence to the Mediterranean diet is known to be associated with increased HDL-C and decreased risk of CVD. However, the mechanism by which this happens has been yet poorly investigated and the effect of nutrition on HDL functionality and quality needs further attention.

Keywords: HDL functionality, HDL quality, nutrition, cardiovascular disease, reverse cholesterol transport (RCT), ATP-binding cassette transporters.

HIGH DENSITY LIPOPROTEIN AND CARDIOVASCULAR DISEASE: A BRIEF EXCURSUS

Extensive epidemiological data have shown that low circulating concentrations of high density lipoprotein (HDL) are associated with increased risk of cardiovascular disease (CVD). HDL is considered atheroprotective because of its role in reverse cholesterol transport (RCT), which consists of the transport of excess cholesterol from peripheral tissues to the liver or intestine for excretion into the bile and lumen respectively, and its final disposal through the faeces. Thus, increasing plasma HDL levels has become a substantial therapeutic target for preventing CVD. Nevertheless, pharmacological increases in HDL have not always been correlated with a decrease in CVD risk. For example, Torcetrapib was shown to efficiently increase HDL levels, but phase III trials were interrupted because of an increase in cardiovascular mortality [1]. Khera *et al*. have recently shown an inverse relationship between cholesterol efflux capacity and carotid intima-media thickness, both before and after adjusting for HDL-cholesterol (HDL-C) [2]. These surprising results lead to consider that it might not be the *quantity* of HDL that should be raised, but, rather, the *quality* of the HDL particles that should be increased for more efficient cholesterol efflux and atheroprotection. In mice lacking endothelial lipase or hepatic lipase despite an increase in HDL-C, macrophage RCT was not increased [3]. Indeed, HDL plasma levels are not predictive of the degree of RCT because HDL constitutes a heterogeneous group of particles differing in density, size, lipid composition, and apolipoprotein content. These different HDL particles should have different efficiencies of cholesterol efflux. Castro and Fielding found that a significant part of cell-derived cholesterol is transferred specifically to a pre-beta-migrating lipoprotein A1 (pre-β-HDL population) [4]. Statins [5] and niacin [6] increase the large, apoA1 containing HDL subpopulations, but fibrates tend to selectively increase smaller HDL subpopulations [7]. Thus, determining the efficiency of cholesterol efflux of the different populations of HDL is essential in determining HDL functionality.

HDL AND REVERSE CHOLESTEROL TRANSPORT

HDL is formed by the lipidation of apoA1, mediated by several ATP-binding cassette transporters (ABCs). A key protein in RCT is the ATP-binding cassette transporter A1 (ABCA1). Defects in this protein cause Tangier's disease, characterized by very low levels of HDL, accumulation of macrophage foam cells, and increased atherosclerosis [8]. Mice over-expressing ABCA1 exhibit higher HDL levels and a decreased risk of atherosclerosis relative to wild type mice [9], indicating the indispensable role of ABCA1 in HDL biogenesis. ABCA1 binds to apoA1 and effluxes cholesterol and phospholipids to apoA1; then, this partially lipidated apoA1 particle is further lipidated by ABCG1 and/or ABCG4. The HDL thus formed is then matured in the plasma by the incorporation of apoproteins (apoAII, apoAIV, ApoCs, and apoE) and other HDL-associated proteins. The resulting particle is

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further remodeled by other proteins, including PLTP, CETP, LCAT, SR-BI, and several lipases. The role of these proteins in HDL functionality - especially concerning the ability of HDL to efflux cholesterol - deserves further investigation.

It remains unclear which component(s) of the HDL particle specifically determine its overall quality. It has been shown that trypsinized HDL is not as efficient in facilitating cholesterol efflux, because hydrophobicity of the C-terminal amphipathic α-helix plays a critical role in determining apoA1 functionality [10]. Shao *et al*. described that oxidation by myeloperoxidase-generated chlorinating intermediates of apoA1 in the arterial wall which could contribute to atherogenesis by impairing cholesterol efflux from macrophages [11]. These results suggest that apoA1 structure and oxidative state affect cholesterol efflux. HDL prevents low density lipoprotein (LDL) oxidation, thus conferring anti-inflammatory, antioxidant, and anti-atherogenic properties with potential positive consequences on atherogenesis. Taken together, these results suggest that oxidation of HDL is both atheroprotective because of the protection of LDL from oxidation and atherogenic because of reduced cholesterol efflux to oxidized apoA1. The effects of other HDL-components (phospholipids, fatty acids, triglycerides, proteins) on the efficiency of cholesterol efflux need yet to be determined.

Functional HDL particles will not only uptake cholesterol from peripheral tissues, but also efflux cholesterol to liver and intestinal cells in order to redirect this cholesterol for excretion. HDL functionality has mainly been assessed in terms of its ability to uptake cholesterol from macrophages. Accumulation of cholesterol in macrophages leads to the formation of foam cells and atherosclerotic plaque; thus, the interest in decreasing cholesterol levels in macrophages before they become foam cells. However, when determining HDL functionality one should not only consider cholesterol efflux to HDL but also cholesterol uptake by SR-BI for secretion into the gut. If the final step of RCT is not efficient, then excess cholesterol will not be disposed of properly. Indeed, mice lacking SR-BI were found to have increased plasma cholesterol associated with large HDL particles [12], but biliary cholesterol secretion was reduced [13]. On the other hand, hepatic over expression of SR-BI in mice resulted in decreased plasma HDL [14, 15], increased RCT from macrophages to feces [16], and increased biliary cholesterol secretion [15]. In mice prone to atherosclerosis, hepatic over expression of SR-BI reduced the risk of atherosclerosis [17], indicating a protective role of SR-BI in RCT. These results suggest that removal of cholesterol by SR-BI is essential in RCT and that this removal might allow further efflux of peripheral cholesterol to the HDL which previously lost its cholesterol via SR-BI.

POTENTIAL ROLE OF DIET ON HDL QUANTITY AND QUALITY

The role of nutrition on HDL quality and functionality is currently poorly understood. Certain nutrients are known to increase HDL-C levels, but the question is whether they can alter HDL functionality. Indeed, whereas one nutrient could increase the *concentration* of one HDL subpopulation which is, however, not efficient in RCT, another nutrient could also increase HDL *functionality*. Thus, total HDL-C levels could be increased by both diets, but only the latter would reduce the risk of CVD. For this reason, it is imperative to asses which HDL populations are affected by nutritional interventions and whether these changes are beneficial in terms of RCT. For example, the ingestion of olive and palm oils is thought to be atheroprotective because - in humans - it decreases LDL and increases HDL levels. However, the expression of ABCA1 is decreased *in vitro* by oleic acid [18, 19], the main fatty acid of olive oil, suggesting that HDL should decrease after olive oil consumption. Then, why does olive oil increase HDL? Are the other components of olive oil modulating ABCA1, ABCG1 and ABCG4 expression or it is that a more functional HDL particle is formed in the presence of olive oil? Berrougui *et al*. have shown that HDL preincubated with phenolic extracts of virgin argan oil increased cholesterol efflux from macrophages (THP-1) [20], suggesting that polyphenolic extracts of olive oil might also be playing a role in the increase of HDL-C. Indeed, Covas *et al*. reported that the phenolic content of olive oil provided benefits for plasma lipid levels and oxidative damage [21].

Fish oil consumption is cardioprotective, largely because of the presence, in fish, of long-chain omega-3 fatty acids, namely docosahexaenoic (DHA, 22:6n-3) and eicosapentaenoic (EPA, 20:5n-3) acids [22]. DHA consumption increases HDL but the mechanisms by which this occurs are not known [23]. Indeed, DHA incorporates into phospholipids, but is also found in free form (as a fatty acid bound to albumin) in plasma. It remains to be determined which of these forms, i.e. phospholipid or free fatty acid, could be affecting the HDL rise and whether or not they affect HDL functionality.

Finally, moderate consumption of alcohol increases HDL and, thus, is considered cardio-protective. The mechanisms by which alcohol increases HDL are not clear, but Berrougui *et al*. showed that resveratrol (which is contained in minute amounts in red wine) induces the efflux of cholesterol to apoA1 [24]. Nevertheless, the effects of alcohol on HDL functionality need in-depth investigation.

Mechanistically, ABCA1 is known to not only efflux cholesterol and phospholipids but also β-sitosterol (plant sterol) [25], α-tocopherol (vitamin E) [26] and retinol (vitamin A) [27] and probably other dietary lipids that have not yet been determined. The role of these lipids in HDL functionality deserves further investigation as differences in phospholipid, sterol, triglyceride, and vitamin composition of HDL could affect its size, charge, or oxidative state, and, in turn, cholesterol efflux. Indeed, oxidation of apoA1 was shown to severely impair cholesterol efflux by the ABCA1 pathway [28], hence the interest of anti-oxidant supplementation for the prevention of CVD. Notably, the effect of antioxidants such as vitamin E and β-carotene have shown inconclusive results as to their protection in CVD [29, 30]. However, the haptoglobin (Hp) protein is associated with HDL and, in humans, there exist two genetic variants: Hp1 and Hp2. Vitamin E supplementation appeared to provide substantial cardiovascular benefit to Hp 2-2 diabetic [31] individuals, but it also appeared to promote CVD in Hp 2-1 diabetic individuals [32]. The protein product of the Hp2 allele is an inferior antioxidant compared to the Hp1 allele product, which could explain these results since vitamin E is an antioxidant transported in plasma associated to lipoproteins. Farbstein *et al*., showed that cholesterol efflux to HDL was increased after vitamin E supplementation in the Hp2-2 genotype and decreased in the Hp 2-1 genotype [33]. These results suggest a role for nutrition in HDL functionality with a genotype-dependent basis.

CONCLUSIONS

The notion of HDL functionality rather than (or in addition to) HDL concentration is gaining momentum. Most epidemiological studies on the beneficial effects of diet on CVD have focused on measuring increases in HDL-C, decreases in LDL-C, decreases in triglycerides, plasma antioxidant capacity, other variables, and surrogate markers. Yet, little attention has been given to the functionality of HDL and its efficiency in RCT. Even though it is known that some food items such as olive oil, palm oil, walnuts, polyphenols, alcohol, and DHA increase HDL-C, the mechanisms by which they do so remain unclear and HDL functionality is still not well defined. Thus, more future attention should be given to the capacity of the HDL particle to efflux cholesterol from macrophages and to transfer cholesterol from the HDL particle to the liver and/or intestine for the disposal of excess cholesterol.

REFERENCES

[1] Barter PJ, Caulfield M, Eriksson M, Grundy SM, Kastelein JJ, Komajda M et al. Effects of torcetrapib in patients at high risk for coronary events. N Engl J Med 2007; 357: 2109-22. http://dx.doi.org/10.1056/NEJMoa0706628

[2] Khera AV, Cuchel M, de la Llera-Moya M, Rodrigues A, Burke MF, Jafri K et al. Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis. N Engl J Med 2011; 364: 127-35. http://dx.doi.org/10.1056/NEJMoa1001689

[3] Brown RJ, Lagor WR, Sankaranaravanan S, Yasuda T, Quertermous T, Rothblat GH et al. Impact of combined deficiency of hepatic lipase and endothelial lipase on the metabolism of both high-density lipoproteins and apolipoprotein B-containing lipoproteins. Circ Res 2010; 107: 357-64. http://dx.doi.org/10.1161/CIRCRESAHA.110.219188

[4] Castro GR, Fielding CJ. Early incorporation of cell-derived cholesterol into pre-beta-migrating high-density lipoprotein. Biochemistry 1988; 27: 25-9. http://dx.doi.org/10.1021/bi00401a005

[5] Asztalos BF, Le Maulf F, Dallal GE, Stein E, Jones PH, Horvath KV et al. Comparison of the effects of high doses of rosuvastatin versus atorvastatin on the subpopulations of high-density lipoproteins. Am J Cardiol 2007; 99: 681-5. http://dx.doi.org/10.1016/j.amjcard.2006.09.117

[6] Morgan JM, Capuzzi DM, Baksh RI, Intenzo C, Carey CM, Reese D et al. Effects of extended-release niacin on lipoprotein subclass distribution. Am J Cardiol 2003; 91: 1432-6. http://dx.doi.org/10.1016/S0002-9149(03)00394-1

[7] Asztalos BF, Collins D, Horvath KV, Bloomfield HE, Robins SJ, Schaefer EJ. Relation of gemfibrozil treatment and high-density lipoprotein subpopulation profile with cardiovascular events in the Veterans Affairs High-Density Lipoprotein Intervention Trial. Metabolism 2008; 57: 77-83. http://dx.doi.org/10.1016/j.metabol.2007.08.009

[8] Kolovou GD, Mikhailidis DP, Anagnostopoulou KK, Daskalopoulou SS, Cokkinos DV. Tangier disease four decades of research: a reflection of the importance of HDL. Curr Med Chem 2006; 13: 771-82. http://dx.doi.org/10.2174/092986706776055580

[9] Joyce CW, Amar MJ, Lambert G, Vaisman BL, Paigen B, Najib-Fruchart J, et al. The ATP binding cassette transporter A1 (ABCA1) modulates the development of aortic atherosclerosis in C57BL/6 and apoE-knockout mice. Proc Natl Acad Sci U S A 2002; 99: 407-12. http://dx.doi.org/10.1073/pnas.012587699

[10] Lyssenko NN, Hata M, Dhanasekaran P, Nickel M, Nguyen D, Chetty PS et al. Influence of C-terminal alpha-helix hydrophobicity and aromatic amino acid content on apolipoprotein A-I functionality. Biochim Biophys Acta 2011; in press.

[11] Shao B, Tang C, Heinecke JW, Oram JF. Oxidation of apolipoprotein A-I by myeloperoxidase impairs the initial interactions with ABCA1 required for signaling and cholesterol export. J Lipid Res 2010; 51: 1849-58. http://dx.doi.org/10.1194/jlr.M004085

[12] Rigotti A, Trigatti BL, Penman M, Rayburn H, Herz J, Krieger M. A targeted mutation in the murine gene encoding the high density lipoprotein (HDL) receptor scavenger receptor class B type I reveals its key role in HDL metabolism. Proc Natl Acad Sci U S A 1997; 94: 12610-5. http://dx.doi.org/10.1073/pnas.94.23.12610

[13] Mardones P, Quinones V, Amigo L, Moreno M, Miquel JF, Schwarz M et al. Hepatic cholesterol and bile acid metabolism and intestinal cholesterol absorption in scavenger receptor class B type I-deficient mice. J Lipid Res 2001; 42: 170-80.

[14] Wang N, Arai T, Ji Y, Rinninger F, Tall AR. Liver-specific overexpression of scavenger receptor BI decreases levels of very low density lipoprotein ApoB, low density lipoprotein ApoB, and high density lipoprotein in transgenic mice. J Biol Chem 1998; 273: 32920-6. http://dx.doi.org/10.1074/jbc.273.49.32920

[15] Kozarsky KF, Donahee MH, Rigotti A, Iqbal SN, Edelman ER, Krieger M. Overexpression of the HDL receptor SR-BI alters plasma HDL and bile cholesterol levels. Nature 1997; 387: 414-7. http://dx.doi.org/10.1038/387414a0

[16] Zhang Y, Da Silva JR, Reilly M, Billheimer JT, Rothblat GH, Rader DJ. Hepatic expression of scavenger receptor class B type I (SR-BI) is a positive regulator of macrophage reverse cholesterol transport in vivo. J Clin Invest 2005; 115: 2870-4. http://dx.doi.org/10.1172/JCI25327

[17] Arai T, Wang N, Bezouevski M, Welch C, Tall AR. Decreased atherosclerosis in heterozygous low density lipoprotein receptor-deficient mice expressing the scavenger receptor BI transgene. J Biol Chem 1999; 274: 2366-71. http://dx.doi.org/10.1074/jbc.274.4.2366

[18] Wang Y, Oram JF. Unsaturated fatty acids inhibit cholesterol efflux from macrophages by increasing degradation of ATP-binding cassette transporter A1. J Biol Chem 2002; 277: 5692-7. http://dx.doi.org/10.1074/jbc.M109977200

[19] Murthy S, Born E, Mathur SN, Field FJ. Liver-X-receptor-mediated increase in ATP-binding cassette transporter A1 expression is attenuated by fatty acids in CaCo-2 cells: effect on cholesterol efflux to high-density lipoprotein. Biochem J 2004; 377: 545-52.

[20] Berrougui H, Cloutier M, Isabelle M, Khalil A. Phenolic-extract from argan oil (Argania spinosa L.) inhibits human low-density lipoprotein (LDL) oxidation and enhances cholesterol efflux from human THP-1 macrophages. Atherosclerosis 2006; 184: 389-96. http://dx.doi.org/10.1016/j.atherosclerosis.2005.05.018

[21] Covas MI, Nyyssonen K, Poulsen HE, Kaikkonen J, Zunft HJ, Kiesewetter H et al. The effect of polyphenols in olive oil on heart disease risk factors: a randomized trial. Ann Intern Med 2006; 145: 333-41.

[22] Holub BJ. Docosahexaenoic acid (DHA) and cardiovascular disease risk factors. Prostaglandins Leukot Essent Fatty Acids 2009; 81:199-204. http://dx.doi.org/10.1016/j.plefa.2009.05.016

[23] Wei MY, Jacobson TA. Effects of Eicosapentaenoic Acid Versus Docosahexaenoic Acid on Serum Lipids: A Systematic Review and Meta-Analysis. Curr Atheroscler Rep 2011; 13: 474-83. http://dx.doi.org/10.1007/s11883-011-0210-3

[24] Berrougui H, Grenier G, Loued S, Drouin G, Khalil A. A new insight into resveratrol as an atheroprotective compound: inhibition of lipid peroxidation and enhancement of cholesterol efflux. Atherosclerosis 2009; 207: 420-7. http://dx.doi.org/10.1016/j.atherosclerosis.2009.05.017

[25] Field FJ, Born E, Mathur SN. LXR/RXR ligand activation enhances basolateral efflux of beta-sitosterol in CaCo-2 cells. J Lipid Res 2004; 45: 905-13. http://dx.doi.org/10.1194/jlr.M300473-JLR200

[26] Oram JF, Vaughan AM, Stocker R. ATP-binding cassette transporter A1 mediates cellular secretion of alpha-tocopherol. J Biol Chem 2001; 276: 39898-902. http://dx.doi.org/10.1074/jbc.M106984200

[27] During A, Harrison EH. Mechanisms of provitamin A (carotenoid) and vitamin A (retinol) transport into and out of intestinal Caco-2 cells. J Lipid Res 2007; 48: 2283-94. http://dx.doi.org/10.1194/jlr.M700263-JLR200

[28] Bergt C, Pennathur S, Fu X, Byun J, O'Brien K, McDonald TO et al. The myeloperoxidase product hypochlorous acid oxidizes HDL in the human artery wall and impairs ABCA1-dependent cholesterol transport. Proc Natl Acad Sci U S A 2004; 101: 13032-7. http://dx.doi.org/10.1073/pnas.0405292101

[29] Bleys J, Miller ER, 3rd, Pastor-Barriuso R, Appel LJ, Guallar E. Vitamin-mineral supplementation and the progression of atherosclerosis: a meta-analysis of randomized controlled trials. Am J Clin Nutr 2006; 84: 880-7; quiz 954-5.

[30] Nunez-Cordoba JM, Martinez-Gonzalez MA. Antioxidant vitamins and cardiovascular disease. Curr Top Med Chem 2011; 11: 1861-9. http://dx.doi.org/10.2174/156802611796235143

[31] Blum S, Vardi M, Brown JB, Russell A, Milman U, Shapira C et al. Vitamin E reduces cardiovascular disease in individuals with diabetes mellitus and the haptoglobin 2-2 genotype. Pharmacogenomics 2010; 11: 675-84. http://dx.doi.org/10.2217/pgs.10.17

[32] Blum S, Vardi M, Levy NS, Miller-Lotan R, Levy AP. The effect of vitamin E supplementation on cardiovascular risk in diabetic individuals with different haptoglobin phenotypes. Atherosclerosis 2010; 211: 25-7. http://dx.doi.org/10.1016/j.atherosclerosis.2010.02.018

[33] Farbstein D, Blum S, Pollak M, Asaf R, Viener HL, Lache O et al. Vitamin E therapy results in a reduction in HDL function in individuals with diabetes and the haptoglobin 2-1 genotype. Atherosclerosis 2011; 219: 240-4. http://dx.doi.org/10.1016/j.atherosclerosis.2011.06.005

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