SciMedCentral

JSM Atherosclerosis

Review Article

Increased Binding of Apolipoproteins A-I and E4 to Triglyceride-Rich Lipoproteins is linked to Induction of Hypertriglyceridemia

Irina N. Gorshkova* and David Atkinson

Department of Physiology and Biophysics, Boston University School of Medicine, USA

***Corresponding author**

Irina N. Gorshkova, Department of Physiology and Biophysics, Boston University School of Medicine, 700 Albany Street, Boston, Massachusetts 02118, USA, Tel: 1-617-638-4207; Email: igorshko@bu.edu

Submitted: 21 January 2017

Accepted: 12 February 2017

Published: 13 February 2017

Keywords

- Hypertriglyceridemia
- Triglyceride-rich lipoproteins
- Apolipoproteins A-I and apoE4
- Biophysical properties

Abstract

Hypertriglyceridemia (HTG) is an independent factor of atherosclerotic cardiovascular disease and a hallmark of many metabolic disorders. However, the molecular etiology of HTG is still largely unknown. In mice, severe HTG may be induced by expression of specific mutants of apolipoprotein (apo) A-I or wild type (WT) apoE4. Expression of a certain apoE4 mutant results in mild HTG, while expression of another apoE4 mutant or WT apoA-I results in normal plasma triglyceride (TG) levels. Biophysical studies of the apoA-I and apoE4 forms associated with HTG help better understand the molecular mechanisms of induction of HTG by these proteins. The studies show that the apoA-I and apoE4 forms that induce HTG have a destabilized and more loosely folded conformation in solution than their counterparts not associated with HTG. Disruption of the protein salt bridge networks by the mutations is likely responsible for the observed structural changes. Each apoA-I and apoE4 form that induced HTG show enhanced binding to model TG-rich particles. HTG appeared to positively correlate with the apolipoprotein ability to bind to TG-rich particles. This observation implies that in vivo, the conformational changes in the apolipoproteins that induce HTG facilitate their binding to plasma TG-rich lipoproteins. We discuss metabolic pathways leading to the development of HTG that may result from enhanced binding of the apolipoproteins to TG-rich lipoproteins in circulation. While various factors may be involved in the development of HTG in humans, it is possible that structural alterations that increase affinity of apolipoproteins to TG-rich lipoproteins may contribute to some cases of this disorder.

ABBREVIATIONS

HTG: Hypertriglyceridemia; Apo: Apolipoprotein; WT: Wild Type; TG: Triglyceride; VLDL: Very Low Density Lipoprotein

INTRODUCTION

Hypertriglyceridemia (HTG) is a hallmark of many disorders, including metabolic syndrome, diabetes, obesity, and atherosclerosis [1-3]. A growing number of studies indicate that triglyceride (TG) levels are strong independent predictors of atherosclerotic cardiovascular disease risk [1,4,5]. Recent genetic analyses have linked genes that modulate TG metabolism with cardiovascular disease [6,7]. TG-lowering therapy in patients with HTG has been shown to reduce cardiovascular disease events [6,8]. Yet, the molecular etiology of HTG remains largely unknown. Animal studies showed that several mutations in apolipoproteins (apo) A-I and E may dramatically affect plasma TG in mice expressing the mutant proteins [9-14]. Here, we review biophysical aspects of the mutant apoA-I and apoE forms associated with HTG, in order to look into the molecular mechanisms of induction of HTG by the proteins. This information may help to unravel the mechanisms contributing to some cases of HTG in humans.

ApoA-I, a 243 amino acid protein, is a major protein constituent of plasma HDL. Plasma concentrations of apoA-I and most apoA-I mutations usually affect plasma levels of cholesterol and HDL, but do not correlate in general with plasma concentrations of TG or TG-rich lipoproteins [15,16]. However, three engineered mutant forms of apoA-I, apoA-I[E110A/E111A], apoA-I[Δ(61-78)], and apoA-I[D89A, E91A, E92A], cause severe HTG when expressed in apoA$^{-/-}$ mice [9-11]. It was also reported that a naturally occurring human apoA-I mutation, apoA-I[K107del], was associated with elevated plasma TG in men [17,18], although a more recent population study [19] found an increase in TG levels in carriers of this mutation being not statistically significant. Most recently, another natural human mutation associated with HTG, apoA-I $_{Nashua}$, was identified [20].

ApoE is one of the major protein components of TG-rich lipoproteins, chylomicrons and very low density lipoproteins (VLDL). ApoE mediates the hepatic uptake of TG-rich lipoprotein remnants via the LDL receptor and thereby helps to control plasma lipid levels [21]. However, elevated plasma concentrations of apoE in humans were shown to correlate positively with plasma TG levels [12]. In apoE$^{-/-}$ mice, severe HTG can be induced by overexpression of apoE2, apoE3, or apoE4 [12,13]. Similar levels of expression of an apoE4 mutant, apoE4 [W276A, L279A, V280A, V283A], induces only mild HTG, while another apoE4 mutation, apoE4 [L261A, W264A, F265A, L268A, V269A], has been shown to prevent the induction of HTG in apoE$^{-/-}$ mice [14].

The altered function of the apoA-I and apoE4 forms that cause HTG may be dictated by altered biophysical properties of these apolipoproteins. To investigate the properties of the apoA-I and apoE4 forms associated with HTG and compare them to those of the counterpart proteins that are not associated with HTG, the conformation and stability of the purified recombinant apolipoproteins were studied using circular dichroism and fluorescent techniques [9,11,22-24]. It was found that the apoA-I mutations that cause HTG in mice, apoA-I[E110A/E111A], apoA-I[Δ(61-78)], and apoA-I[D89A, E91A, E92A], as well as the natural mutation apoA-I[K107del], lead to destabilization of lipid-free apoA-I and a more loosely folded tertiary conformation with greater expose of hydrophobic surfaces [9,11,22,23]. Similarly, WT apoE4 that may cause severe HTG in mice has a less stable and more loosely folded conformation with greater exposure of hydrophobic surfaces in solution, as compared to the apoE4[W276A, L279A, V280A, V283A] associated with mild HTG and particularly, as compared to the apoE4[L261A, W264A, F265A, L268A, V269A] that prevented the induction of HTG [24]. The destabilized, loosely folded conformation with greatly exposed hydrophobic surfaces may facilitate binding of apoA-I and apoE4 to lipids and large TG-rich lipoproteins [21,24-27] and thereby affect metabolism of the lipoproteins. In-vitro experiments were conducted to test this notion for the apoA-I and apoE4 forms associated with HTG.

Lipid affinity of the apolipoproteins was assessed by assays that monitored the kinetics of solubilization of DMPC multilamellar vesicles by the proteins. ApoA-I and apoE4 forms associated with HTG solubilized DMPC vesicles faster than their counterparts not associated with HTG [9,11,24]. The ability of the apolipoproteins to bind to TG-rich particles was evaluated using synthetic triolein-egg phosphatidylcholine emulsion particles that resembled plasma VLDL in respect of the average particle size and the triacylglyceride to phosphatidylcholine ratio [28]. The binding assays conducted at various molar ratios of apoA-I to TG-rich particles demonstrated enhanced binding to the particles of the apoA-I forms associated with HTG [22,23]. Notably, the trend in binding to TG-rich particles of the apoA-I forms {WT apoA-I < apoA-I[Δ(61-78)] < apoA-I[E110A/E111A] < apoA-I[D89A, E91A, E92A]} correlates positively with the trend in plasma TG concentrations in mice expressing the corresponding apoA-I form. In contrast, an apoA-I mutant apoA-I[Δ(89-99)] that causes hypercholesterolemia with normal plasma TG levels when expressed in apoA$^{-/-}$ mice [10], did not show enhanced binding to model TG-rich particles [22]. The apoA-I[K107del] mutant associated with HTG in men [17] also showed enhanced binding to model TG-rich particles [23]. The binding studies for apoE4 showed that similar to apoA-I, the forms that induce HTG, WT apoE4 and apoE4[W276A, L279A, V280A, V283A], have enhanced binding to model TG-rich particles as compared to apoE4[L261A, W264A, F265A, L268A, V269A] that does not induce HTG [24]. Similar to apoA-I, the trend in binding to TG-rich particles of the apoE4 forms {apoE4[L261A, W264A, F265A, L268A, V269A] < apoE4 [W276A, L279A, V280A, V283A] < WT apoE4} correlates positively with the trend in plasma TG concentrations in mice expressing the corresponding apoE4 form.

These findings imply that *in vivo*, the apoA-I and apoE4 forms associated with HTG have increased affinity to TG-rich lipoproteins in circulation, which may have implications for catabolism of these lipoproteins. First, the enhanced binding of the apolipoproteins to TG-rich lipoproteins may result in a higher protein content and reduced fluidity of the surface monolayer of the lipoproteins [29,30]. These surface changes may inhibit the interaction of lipoprotein lipase with the particles and ensuing hydrolysis of TG. Reduced fluidity of the surface monolayer may account for the reported reduced level of hydrolysis of TG-rich lipoproteins isolated from plasma of mice expressing the apoA-I mutants associated with HTG, when in-vitro lipolysis was promoted using purified lipoprotein lipase [9-11]. A higher content of apoA-I or apoE on the surface of TG-rich lipoproteins may also result in an altered apolipoprotein conformation and function, including a compromised interaction of apoE with the LDL receptor and reduced clearance of remnant lipoproteins from the circulation [21,31]. One of the possibilities is that under conditions of the more "dense" protein packing on the surface of TG-rich particles, the N-terminal domain of apoE is displaced from the lipid surface and adopts an inactive four-helix bundle conformation [31,32]. Enhanced binding of apoA-I or apoE4 to TG-rich lipoproteins may also lead to displacement from the lipoproteins of apoC-II that is required for the activation of lipoprotein lipase [12] and thereby, result in elevated plasma TG levels. Displacement of apoC-II from TG-rich lipoproteins may account for lower apoC-II content in TG-rich lipoprotein fractions isolated from plasma of mice expressing apoA-I[Δ(61-78)], apoA-I[E110A/E111A], orapoA-I[D89A, E91A, E92A] [9-11]. Thus, structural mutations in apoA-I and apoE that facilitate binding of the proteins to TG-rich lipoproteins in circulation may trigger various metabolic changes leading to the development of HTG.

Mapping the apoA-I mutations associated with HTG in mice, apoA-I[E110A/E111A], apoA-I[Δ(61-78)], and apoA-I [D89A, E91A, E92A], on the 2.2Å crystal structure of the truncated apoA-I[33] shows that all three mutations disrupt salt bridge networks that stabilize the unique protein structure. Lysine 107, which is absent in the natural occurring apoA-I mutant [17-19], is the key residue in two critical intermolecular salt bridges stabilizing apoA-I [23]. Disruption of the salt bridges likely leads to a greater exposure of the protein hydrophobic surfaces. While various factors may be involved in the development of HTG in humans, it is possible that structural mutations in apoA-I and apoE (and perhaps some other apolipoproteins) that disrupt intermolecular salt bridges and increase protein affinity to TG-rich lipoproteins may contribute to some cases of this disorder.

ACKNOWLEDGEMENTS

This work was funded by NIH grant R01HL116518.

REFERENCES

1. Miller M, Stone NJ, Ballantyne C, Bittner V, Criqui MH, Ginsberg HN. Triglycerides and Cardiovascular Disease. A Scientific Statement from the American Heart Association. Circulation. 2011; 123: 2292-2333.

2. Eckel RH. The complex metabolic mechanisms relating obesity to hypertriglyceridemia. Arterioscler Thromb Vasc Biol. 2011; 31: 1946-1948.

3. Hassing HC, Surendran RP, Mooij HL, Stroes ES, Nieuwdorp M, Dallinga-Thie GM. Pathophysiology of hypertriglyceridemia. Biochim Biophys Acta. 2012; 1821: 826-832.

4. Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease,

and death in men and women. JAMA. 2007; 298: 299-308.

5. Kannel WB, Vasan RS. Triglycerides as vascular risk factors: new epidemiologic insights. Curr Opin Cardiol. 2009; 24: 345-350.

6. Agrawal N, Freitas Corradi P, Gumaste N, Goldberg IJ. Triglyceride treatment in the age of cholesterol reduction. Prog Cardiovasc Dis. 2016; 59: 107-118.

7. Johansen CT, Hegele RA. The complex genetic basis of plasma triglycerides. Curr Atheroscler Rep. 2012; 14: 227-234.

8. Brinton EA. Management of Hypertriglyceridemia for Prevention of Atherosclerotic Cardiovascular Disease. Endocrinol Metab Clin North Am. 2016; 45: 185-204.

9. Chroni A, Kan H-Y, Kypreos KE, Gorshkova IN, Shkodrani A, Zannis VI. Substitutions of Glu110 and Glu111 in the middle helix 4 of human apoA-I by alanine affect the structure and in vitro functions of apoA-I and induce severe hypertriglyceridemia in apoA-I-deficient mice. Biochemistry. 2004; 43: 10442-10457.

10. Chroni A, Kan H-Y, Shkodrani A, Liu T, Zannis VI. Deletions of helices 2 and 3 of human apoA-I are associated with severe dyslipidemia following adenovirus-mediated gene transfer in apoA-I-deficient mice. Biochemistry. 2005; 44: 4108-4117.

11. Kateifides AK, Gorshkova IN, Duka A, Chroni A, Kardassis D, Zannis VI. Alteration of negatively charged residues in the 89 to 99 domain of apoA-I affects lipid homeostasis and maturation of HDL. J Lipid Res. 2011; 52: 1363-1372.

12. Huang Y, Liu XQ, Rall SC Jr, Taylor JM, von Eckardstein A, Assmann G, et al. Overexpression and accumulation of apolipoprotein E as a cause of hypertriglyceridemia. J Biol Chem. 1998; 273: 26388-26393.

13. Kypreos KE, van Dijk KW, van Der Zee A, Havekes LM, Zannis VI. Domains of apolipoprotein E contributing to triglyceride and cholesterol homeostasis in vivo. Carboxyl-terminal region 203-299 promotes hepatic very low density lipoprotein-triglyceride secretion. J Biol Chem. 2001; 276: 19778-19786.

14. Kypreos KE, van Dijk KW, Havekes LM, Zannis VI. Generation of a recombinant apolipoprotein E variant with improved biological functions: hydrophobic residues (Leu-261, Trp-264, Phe-265, Leu-268, Val-269) of apoE can account for the apoE-induced hypertriglyceridemia. J Biol Chem. 2005; 280: 6276-6284.

15. Pownall HJ, Ehnholm C. The unique role of apolipoprotein A-I in HDL remodeling and metabolism. Curr Opin Lipidol. 2006; 17: 209-213.

16. Rhee EJ, Byrne CD, Sung KC. The HDL cholesterol/apolipoprotein A-I ratio: an indicator of cardiovascular disease. Curr Opin Endocrinol Diabetes Obes. 2017 24: 148-153..

17. von Eckardstein A, Funke H, Walter M, Altland K, Benninghoven A, Assmann G. Structural analysis of human apolipoprotein A-I variants. Amino acid substitutions are nonrandomly distributed throughout the apolipoprotein A-I primary structure. J Biol Chem. 1990; 265: 8610-8617.

18. Nofer JR, von Eckardstein A, Wiebusch H, Weng W, Funke H, Schulte H, et al. Screening for naturally occurring apolipoprotein A-I variants: apo A-I(delta K107) is associated with low HDL-cholesterol levels in men but not in women. Hum Genet. 1995; 96: 177-182.

19. Haase CL, Frikke-Schmidt R, Nordestgaard BG, Tybjærg-Hansen A. Population-based resequencing of APOA1 in 10,330 individuals: spectrum of genetic variation, phenotype, and comparison with extreme phenotype approach. PLoS Genet. 2012; 8: e1003063.

20. Lee EY, Klementowicz PT, Hegele RA, Asztalos BF, Schaefer EJ. HDL deficiency due to a new insertion mutation (ApoA-I_{Nashua}) and review of the literature. J Clin Lipidol. 2013; 7: 169-173.

21. Phillips MC. Apolipoprotein E isoforms and lipoprotein metabolism. IUBMB Life. 2014; 66: 616-623.

22. Gorshkova IN, Atkinson D. Enhanced binding of apolipoprotein A-I variants associated with hypertriglyceridemia to triglyceride-rich particles. Biochemistry. 2011; 50: 2040-2047.

23. Gorshkova IN, Mei X, Atkinson D. Binding of human apoA-I[107del] variant to TG-rich particles: implications for mechanisms underlying hypertriglyceridemia. J Lipid Res. 2014; 55: 1876-1885.

24. Gorshkova IN, Kypreos KE, Gantz DL, Zannis VI, Atkinson D. Biophysical properties of apolipoprotein E4 variants: implications in molecular mechanisms of correction of hypertriglyceridemia. Biochemistry. 2008; 47: 12644-12654.

25. Gorshkova IN, Liu T, Kan HY, Chroni A, Zannis VI, Atkinson D. Structure and stability of apolipoprotein A-I in solution and in discoidal high-density lipoprotein probed by double charge ablation and deletion mutation. Biochemistry. 2006; 45: 1242-1254.

26. Tanaka M, Dhanasekaran P, Nguyen D, Nickel M, Takechi Y, Lund-Katz S, et al. Influence of N-terminal helix bundle stability on the lipid-binding properties of human apolipoprotein A-I. Biochim Biophys Acta. 2011; 1811: 25-30.

27. Nguyen D, Dhanasekaran P, Phillips MC, Lund-Katz S. Molecular mechanism of apolipoprotein E binding to lipoprotein particles. Biochemistry. 2009; 48: 3025-3032.

28. Miller KW, Small DM. Triolein-cholesteryl oleate-cholesterol-lecithin emulsions: structural models of triglyceride-rich lipoproteins. Biochemistry. 1983; 22: 443-451.

29. Gorshkova IN, Menschikowski M, Jaross W. Alterations in the physicochemical characteristics of low and high density lipoproteins after lipolysis with phospholipase A2. A spin-label study. Biochim Biophys Acta 1996; 1300: 103-113.

30. Tetali SD, Budamagunta MS, Simion C, den Hartigh LJ, Kálai T, Hideg K, et al. VLDL lipolysis products increase VLDL fluidity and convert apolipoprotein E4 into a more expanded conformation. J Lipid Res. 2010; 510: 1273-1283.

31. Narayanaswami V, Ryan RO. Molecular basis of exchangeable apolipoprotein function. Biochem Biophys Acta. 2000; 1483: 15-36.

32. Satio H, Dhanasekaran P, Baldwin F, Weisgraber KH, Lund-Katz S, Phillips MC. Lipid binding-induced conformational change in human apolipoprotein E. Evidence for two lipid-bound states on spherical particles. J Biol Chem. 2002; 276: 40949-40954.

33. Mei X, Atkinson D. Crystal structure of C-terminal truncated apolipoprotein A-I reveals the assembly of high density lipoprotein (HDL) by dimerization. J Biol Chem. 2011; 286: 38570-38582.

Cite this article

Gorshkova IN, Atkinson D (2017) Increased Binding of Apolipoproteins A-I and E4 to Triglyceride-Rich Lipoproteins is linked to Induction of Hypertriglyceridemia. JSM Atheroscler 2(2): 1026.