Research article

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Apolipoprotein A2 reduces the levels of circulating triglyceride-rich lipoproteins, an effect blocked by apolipoprotein E

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ABSTRACT

Apolipoprotein A2 (APOA2), the second in quantity apolipoprotein of high density lipoprotein (HDL) is synthesized by the liver and much less by the intestine. Studies in humans, failed to establish a clear role for APOA2 in coronary heart disease and overall human physiology. Even though we know that APOA2 plays a key role in the biogenesis and functionality of HDL particles and can interact physically with other apolipoproteins such as apolipoprotein E (APOE), forming dimers, our knowledge on its role in triglyceride-rich lipoprotein (TRL) metabolism remains limited. Here, we investigated how functional interactions between APOA2 and APOE may affect plasma lipoprotein metabolism in the absence of apolipoprotein A1 (APOA1). For this purpose, APOA1 deficient and APOA1xAPOE double deficient mice were fed high fat diet for two weeks and were subsequently infected with either an adenovirus expressing the human APOA2 (AdAPOA2) or a control adenovirus AdGFP. Five days post-infection blood was collected, and plasma and lipoprotein fractions were isolated. After confirmation of human APOA2 expression *in vivo* by western blot we measured plasma and lipoprotein total cholesterol and triglyceride levels. APOA2 expression increased total cholesterol and triglyceride levels in APOA1 deficient mice. To the contrary, when APOA2 was expressed in APOA1xAPOE double deficient mice, which lack functional APOE a significant reduction in both plasma cholesterol and triglyceride levels associated with a notable reduction in TRL was observed. Overall, our data support that a significant functional interaction between APOA2 and APOE impacts plasma TRL metabolism.

KEY WORDS: *Apolipoprotein A2, apolipoprotein E, triglyceride-rich lipoproteins*

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INTRODUCTION

Apolipoprotein A2 (APOA2) is the second, most abuandant apolipoprotein of high density lipoprotein (HDL) particles after apolipoprotein A1 (APOA1). APOA2 consists of 77 amino acids and is synthesized mainly by the liver

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and to a lesser extent by the intestine[1]. It has been suggested that APOA2 impacts the synthesis and functionality of HDL particles[2].

In transgenic mice, APOA2 overexpression is associated with abnormal lipoprotein composition, elevated HDL-cholesterol (HDL-C) levels, and susceptibility to atherosclerosis[3,4]. Clinical data from animal models and humans show that elevated APOA2 levels affect the size and distribution of HDL particle subpopulations[5,6] as well as their APOA1 content. Similarly, Mice expressing human APOA2 have smaller size APOA2-containing HDL particles and significantly reduced levels of APOA1-containing HDL particles[6–8]. Furthermore, the HDL particles of these mice show reduced levels of esterified cholesterol in relation to total cholesterol, due to reduced activity of lecithin–cholesterol acyltransferase (LCAT)[7,8]. Mice overexpressing endogenous APOA2 show increased levels of triglycerides in HDL particles[7,8]. In addition, transgenic mice expressing human APOA2 contain high levels of APOA2 in their very low density lipoprotein (VLDL) particles and reduced activity of lipoprotein lipase (LpL) and hepatic lipase (HL) in plasma[5]. Some atherosclerotic lesions develop in these mice, even when they are fed a standard low-fat diet[9]. These lesions develop further when these mice are fed an atheromatous diet[3,10,11]. In contrast, human studies have so far failed to identify a precise role of APOA2 in coronary heart disease (CHD). In some studies, APOA2 appears to promote the development of atherosclerosis[3,12]. However, in a study of 126 subjects with different degrees of atherosclerosis (calcified and non-calcified atherosclerotic plaques), APOA2 appeared to be positively correlated with reverse cholesterol transport and negatively correlated with non-calcified plaque burden[13]. Studies in mice in which the *Apoa2* gene was deleted, revealed increased levels of other apolipoproteins in HDL particles. APOA2 deficiency in mice was associated with 50% reduced HDL-C levels, due to increased catabolism of HDL particles[14], indicating that the simultaneous expression of APOA1 and APOA2 is necessary to maintain plasma HDL levels[14]. Furthermore, the interactions of APOA2 with APOA1 but also with other lipoproteins involved in the HDL metabolic pathway, affect the distribution of HDL particle subpopulations, as well as their functionality.

Despite that 40 years have elapsed since *Apoa2* gene was sequenced and the structure of APOA2 protein was identified[15], its functional role in human physiology and disease development have not yet been elucidated.

In a recent study, we showed that overexpression of human APOA2 in full-genome expressing mice (C57BL/6) resulted in the generation of APOA2-containing HDL particles with distinctly different apolipoprotein composition and geometry from control HDL particles, with features which were associated with increased functionality[16]. It is possible that these changes in HDL functionality are not due to a direct effect of APOA2, but rather arise from the functional interaction of APOA2 with other apolipoproteins, such as apolipoprotein E (APOE). *In vitro* studies have shown that APOA2 forms dimers with APOE and that this interaction was proposed to affect the ability of APOE to bind to lipoprotein particles[17]. However, to this date there is limited knowledge on the effect of APOA2-APOE interaction on TRL metabolism.

To fill this void, we investigated how functional interactions between APOA2 and APOE may affect plasma lipoprotein metabolism. To this end we used APOA1 deficient mice (*apoa1*$^{-/-}$) and mice with a combined deficiency in APOA1 and APOE (*apoa1*$^{-/-}$×*apoe*$^{-/-}$), fed a lipid-rich diet for 2 weeks and subsequently infected them with a recombinant adenovirus expressing either human APOA2 (AdAPOA2) or a control adenovirus expressing the green fluorescent protein (AdGFP).

Our results show that in *apoa1*$^{-/-}$ mice hepatic production of APOA2 and subsequent generation of APOA2-containg HDL, is associated with an increase in both plasma cholesterol and triglycerides due to increased deposition of TRL particles in blood. In contrast, in *apoa1*$^{-/-}$×*apoe*$^{-/-}$ mice that do not express APOE, a significant decrease in TRLs particles was observed and was associated with a measurable decrease in plasma cholesterol and triglyceride levels.

MATERIALS AND METHODS

Animals

APOA1 deficient (*apoa1*$^{-/-}$) mice and mice deficient in both APOA1 and APOE (*apoa1*$^{-/-}$×*apoe*$^{-/-}$) on the C57BL/6 genetic background were purchased from Jackson Labs (Bar Harbor, Maine, USA). Mice aged 16-20 weeks were individually caged under a 12-hour light/dark cycle and had unrestricted access to food and water. Their age was approximately 16-20 weeks. For a period of 2 weeks mice were fed a standard Western-type diet (WTD, Mucedola SRL, Milano, Italy, 4.5 kcal/g) composed of 17.3% protein, 48.5% carbohydrate, 21.2% fat and 0.2% cholesterol (0.15% added, 0.05% from fat source). Immediately after the 2 weeks mice were infected with either an adenovirus expressing human APOA2 or a control adenovirus. Sample size was determined based on the desired power of statistical analysis, using an online statistical tool (http://www.stat.ubc.ca/~rollin/stats/ssize/n2.html). All animal experiments were conducted according to the EU guidelines of the Protocol for the Protection and Welfare of Animals. The work was authorized by the Laboratory Animal Centre com-

mittee of The University of Patras Medical School and the Veterinary Authority of the Prefecture of Western Greece.

Development and purification of recombinant adenovirus expressing human APOA2

The development and purification of the recombinant adenovirus expressing human APOA2 (AdAPOA2) was performed as described previously[16]. Briefly, both AdAPOA2 and AdGFP (control adenovirus expressing the green fluorescent protein) were expanded in HEK293 cells and then purified by double CsCl ultracentrifugation, followed by titration. The adenovirus title was approximately 5×10^{13} pfu/L.

Expression of human APOA2 in mice

Mice were infected with 2x109 pfu of AdGFP-APOA2, by tail vein injection, after WTD feeding. To assess potential non-specific effects of viral infection in subsequent analyses, an additional mouse group will also be infected with 2x109 pfu of the control AdGFP virus. Five days post-infection and four hours after fasting, mice were euthanized, and blood and tissue samples were collected for further analyses. To assess the expression of APOA2 in the infected mice plasma and lipoprotein fractions were analyzed by western blot as described previously[16]

Lipid levels of plasma and lipoproteins

Total cholesterol and triglycerides levels were measured spectrophotometrically in plasma samples and lipoprotein fractions, using the DiaSys Cholesterol FS kit (ref# 11300, Diagnostic Systems, GmbH, Holzheim, Germany) and the DiaSys Triglycerides FS kit (ref# 15710, Diagnostic Systems, GmbH, Holzheim, Germany), respectively, according to manufacturers' instructions and as described previously[16].

Plasma density gradient ultracentrifugation and isolation of lipoprotein fractions

Pooled plasma (0.4 ml) from each mouse group was fractionated by KBr density gradient ultracentrifugation, over a 4 ml KBr (Sigma-Aldrich, St. Louis, MO, USA) gradient (1.23 g/ml over 1.21 g/ml over 1.063 g/ml over 1.019 g/ml over saline), as described previously[16].

Assessment of hepatic VLDL-triglyceride secretion

Mice were infected with 2x109 pfu of AdGFP-APOA2, by tail vein injection, after WTD feeding. To assess potential non-specific effects of viral infection in subsequent analyses, an additional mouse group was also infected with 2x109 pfu of the control AdGFP virus. On the 4th day post-infection mice were fasted for 16h and subsequently injected with Tyloxapol (Triton-WR1339) at a dose of 500 mg/kg body weight using a 15% solution (w/v) in 0.9% NaCl. Blood samples were isolated at 5, 10, 20, 30, 40, 50, and 60 min after injection with Triton-WR 1339. As a control, blood samples were isolated 1 min immediately after the injection with the detergent. Triglyceride levels were assessed spectrophotometrically in plasma samples using the DiaSys Triglycerides FS kit (ref# 15710, Diagnostic Systems, GmbH, Holzheim, Germany). The rate of VLDL-triglyceride secretion (expressed in mg/dl/min) was calculated from the slope of the linear regression for each individual mouse. Then, slopes were grouped together and reported as means ± SEM in the form of a bar graph.

Statistical analyses

All data sets were tested for normality using the Kolmogorov-Smirnov and the Shapiro-Wilk tests. Data are reported as Mean ± SEM. Parametric ($p>0.1$) or non-parametric tests ($p<0.1$) were performed using the GraphPad Prism 6 software.

RESULTS

Confirmation of human APOA2 expression *in vivo*

To confirm human APOA2 expression *in vivo*, mice were infected with 2×10^9 pfu AdGFP or AdAPOA2 by tail-vein injection. Five days post-infection plasma samples were collected and analyzed by western blot for APOA2 protein, confirming the efficient expression of APOA2 *in vivo* (Figure 1). In *apoa1*$^{-/-}$ mice, infection with the AdAPOA2 resulted in the presence of significant amounts of APOA2 on both HDL and LDL, but not VLDL, particles. No lipid free APOA2 was detected in the plasma of these mice. In *apoa1*$^{-/-}\times$*apoe*$^{-/-}$mice APOA2 was present mainly in HDL particles and to a lower extent in LDL particles with a significant amount of APOA2 present in its lipid-free form.

Effects of human APOA2 expression on plasma cholesterol and triglyceride levels

Mice were infected with $2x10^9$ pfu of AdAPOA2 or AdGFP to assess potential non-specific effects of viral infection in subsequent analyses. Analysis of plasma lipid levels five days post-infection showed that the expression of human APOA2 resulted in a significant increase of plasma total cholesterol and triglyceride levels of *apoa1*$^{-/-}$ mice, compared to the same mice

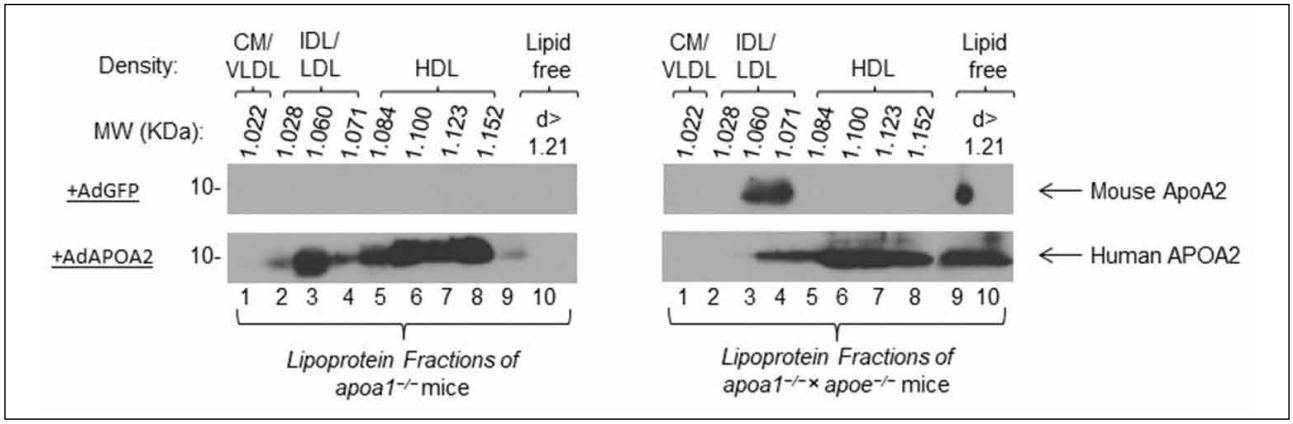

FIGURE 1. Western Blot analysis of plasma lipoprotein fractions isolated from *apoa1*$^{-/-}$ and *apoa1*$^{-/-}$× *apoe*$^{-/-}$ mice, infected with either AdAPOA2 or AdGFP.

infected with the control AdGFP adenovirus (Figure 2A, 2B). This increase was associated with an increase in chylomicrons/VLDL, IDL, LDL and HDL cholesterol and an increase in chylomicrons/VLDL and IDL triglycerides (Figure 3A, 3B). On the other hand, the expression of human APOA2 in *apoa1*$^{-/-}$×*apoe*$^{-/-}$ mice five days post-infection, resulted in a significant decrease of both cholesterol and triglyceride levels (Figure 2A, 2B). This decrease was also evident in the analysis of lipoprotein fractions (Figure 3C, 3D). Specifically, both cholesterol and triglyceride levels were significantly lower in chylomicrons/VLDL, IDL and LDL fractions of *apoa1*$^{-/-}$×*apoe*$^{-/-}$ mice infected with AdAPOA2 compared with control group (Figure3C, 3D). Moreover, our data show that APOA2 expression in the absence of functional APOE, results in elevated triglycerides levels in the HDL fractions (Figure 3C, 3D).

Effects on hepatic VLDL-triglyceride secretion

The lower VLDL- triglyceride content detected in the *apoa1*$^{-/-}$×*apoe*$^{-/-}$ mice infected with AdAPOA2 could be a result of reduced VLDL- triglyceride secretion rate. Therefore, we performed a VLDL- triglyceride secretion assay in both *apoa1*$^{-/-}$ and *apoa1*$^{-/-}$×*apoe*$^{-/-}$ mice following infection with AdGFP or AdAPOA2. Our results show that APOA2 expression led to a significant reduction of the hepatic VLDL- triglyceride secretion rate *apoa1*$^{-/-}$×*apoe*$^{-/-}$ mice infected with AdAPOA2 compared to those infected with the control AdGFP adenovirus (1.396 ±0.13 mg/dL/min for AdAPOA2 group vs 3.314 ± 0,83 mg/dL/min for AdGFP group, p<0.05) (Figure 4B, 4C). Interestingly, in *apoa1*$^{-/-}$ mice which express APOE, infection with AdAPOA2 did not alter the rate of hepatic VLDL- triglyceride secretion (7.460 ± 0.59 mg/dL/min for AdAPOA2 group vs 7.779 ± 0.06 mg/dL/min for AdGFP group, p>0.05) (Fig. 4A, C).

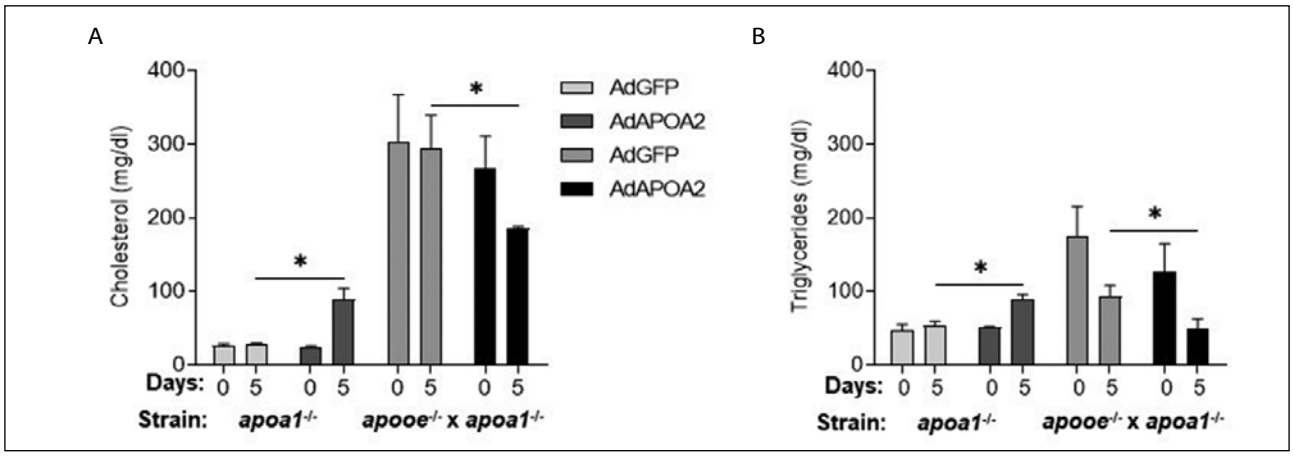

FIGURE 2. Plasma total cholesterol (A) and triglyceride (B) levels of *apoa1*$^{-/-}$ and *apoa1*$^{-/-}$× *apoe*$^{-/-}$ mice before (day 0) and after (day 5) the administration of AdAPOA2 or AdGFP. Data are presented as Mean ± SEM and were analyzed using T-test. *, p value<0.05. (n=5)

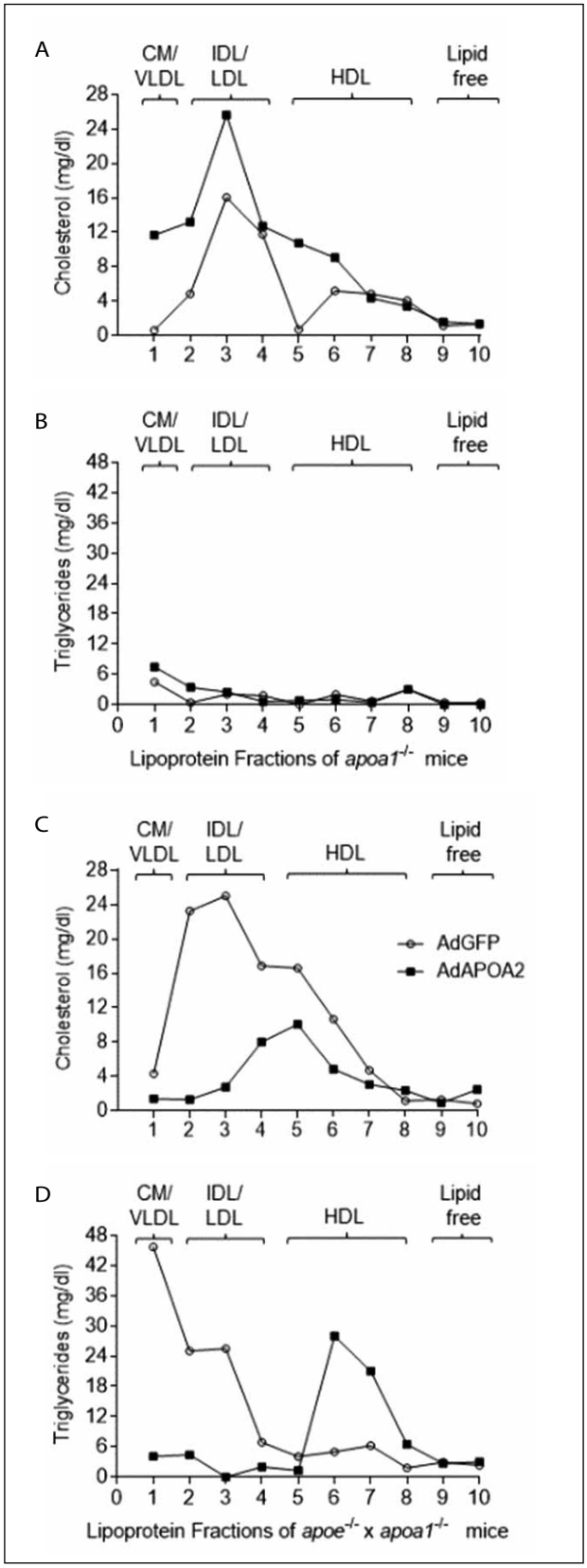

FIGURE 3. Lipoprotein total cholesterol (A) and triglyceride levels (B) of *apoa1*$^{-/-}$ mice and lipoprotein total cholesterol (C) and triglyceride levels (D) of *apoa1*$^{-/-}$× *apoe*$^{-/-}$ mice five days post-infection with AdAPOA2 or AdGFP. (n=5).

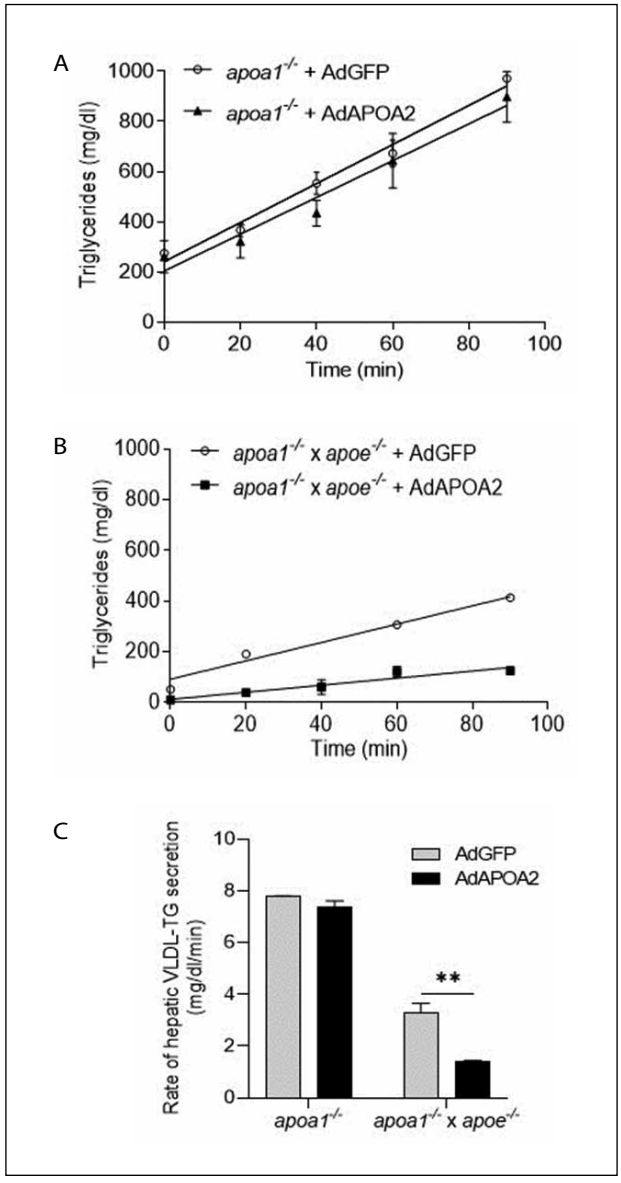

FIGURE 4. Rate of hepatic VLDL triglyceride secretion in apoa1−/− (A) and apoa1−/x apoe−/− (B) mice five days post-infection with AdAPOA2 or AdGFP. The bar graph (C) represents the mean ± SEM of the individual secretion rates. (n=6)

DISCUSSION

Forty years have passed since the human APOA2 nucleotide sequence was identified[15]. Yet, our knowledge of the specific origin of APOA2-containing lipoproteins and their role in human physiology remain unclear. *In vitro* studies have shown that APOA2 forms dimers with APOE[17]. It is possible that this kind of interaction affects the ability of APOE to bind to HDL particles or interact with its receptors including the low density lipoprotein receptor (LDLR). Led by previous observations from our laboratory, such as the effects of APOC3 in triglycerides levels[26], here we investigated the effect of APOA2 on TRL metabolism

in the presence and absence of functional APOE, in mice lacking classical APOA1-containing HDL particles.

Western blot analysis of of plasma lipoprotein fractions isolated from *apoa1*$^{-/-}$ and *apoa1*$^{-/-}$× *apoe*$^{-/-}$ mice five days post-infection with either AdAPOA2 or AdGF, confirmed the *in vivo* expression of human APOA2. Specifically, human APOA2 was present in the IDL, LDL and HDL fractions of both *apoa1*$^{-/-}$ and *apoa1*$^{-/-}$× *apoe*$^{-/-}$ mice. Our results from plasma and lipoprotein fractions lipid analysis, show that in *apoa1*$^{-/-}$ mice expression of human APOA2 results in a significant increase in both plasma cholesterol and triglycerides due to increased deposition of all lipoprotein fractions. In contrast, in *apoa1*$^{-/-}$×*apoe*$^{-/-}$ mice that do not express APOE, a significant decrease in plasma cholesterol and triglycerides was observed, with the most significant decrease was identified in the triglyceride levels of TRLs.

Our data thus far suggest that in the absence of APOE, APOA2 suppresses hepatic VLDL-triglycerides secretion, an effect that disappears when APOE is expressed (Figure 4). However, other mechanisms could also mediate the observed phenotypes and need to be further investigated. For example, it is possible that the positive effect of APOA2 on plasma TRLs in the absence of APOE is also associated with reduced post-prandial intestinal lipid absorption. Another possibility is that the formation of APOE-APOA2 heterodimers that has been previously reported in the literature[17], may lead to a potent inhibition of LpL, an effect that disappears in the absence of APOE. Another possibility is that APOA2 may also promote the direct clearance of TRLs via a receptor mediated process which is inhibited in the presence of functional APOE.

Overall, our data support that a significant functional interaction between APOA2 and APOE impacts plasma TRL metabolism.

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Conflict of interest

The authors declare no conflict of interests/financial disclosure statement.

ΠΕΡΙΛΗΨΗ

Η απολιποπρωτεΐνη Α2 μειώνει τα επίπεδα των πλούσιων σε τριγλυκερίδια λιποπρωτεϊνών της κυκλοφορίας, ένα φαινόμενο που αναστέλλεται από την απολιποπρωτεΐνη Ε

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Η απολιποπρωτεΐνη Α2 (APOA2), η δεύτερη σε ποσότητα απολιποπρωτεΐνη της λιποπρωτεΐνης υψηλής πυκνότητας (HDL) συντίθεται από το ήπαρ και πολύ λιγότερο από το έντερο. Μελέτες σε ανθρώπους, απέτυχαν να καθορίσουν έναν σαφή ρόλο για την APOA2 στη στεφανιαία νόσο και τη συνολική ανθρώπινη φυσιολογία. Παρόλο που γνωρίζουμε ότι η APOA2 παίζει βασικό ρόλο στη βιογένεση και τη λειτουργικότητα των HDL σωματιδίων και μπορεί να αλληλεπιδράσει φυσικά με άλλες απολιποπρωτεΐνες όπως η απολιποπρωτεΐνη Ε (APOE), σχηματίζοντας διμερή, η γνώση μας για το ρόλο της στον μεταβολισμό των πλούσιων σε τριγλυκερίδια λιποπρωτεϊνών (TRL) παραμένει περιορισμένη. Εδώ, διερευνήσαμε πώς οι λειτουργικές αλληλεπιδράσεις μεταξύ APOA2 και APOE μπορεί να επηρεάσουν τον μεταβολισμό των λιποπρωτεϊνών του πλάσματος απουσία της απολιποπρωτεΐνης Α1 (APOA1). Για το σκοπό αυτό, ποντίκια με έλλειψη στην APOA1 ή διπλή έλλειψη στις APOA1 και APOE τράφηκαν με δίαιτα υψηλής περιεκτικότητας σε λιπαρά για δύο εβδομάδες και στη συνέχεια μολύνθηκαν είτε με έναν αδενοϊό που εκφράζει την ανθρώπινη APOA2 (AdAPOA2), είτε με έναν αδενοϊό ελέγχου (AdGFP). Πέντε ημέρες μετά τη μόλυνση συλλέχθηκε αίμα και απομονώθηκαν πλάσμα και λιποπρωτεΐνες. Μετά την επιβεβαίωση της έκφρασης της ανθρώπινης APOA2 *in vivo* με western blot, μετρήσαμε τα επίπεδα ολικής χοληστερόλης και τριγλυκεριδίων στο πλάσμα και στις λιποπρωτεΐνες. Η έκφραση της APOA2 αύξησε τα επίπεδα ολικής χοληστερόλης και τριγλυκεριδίων σε ποντικούς με έλλειψη στην APOA1. Αντίθετα, όταν η APOA2 εκφράστηκε σε ποντίκια με διπλή έλλειψη στις APOA1 και APOE, τα οποία στερούνται λειτουργικής

APOE, παρατηρήθηκε σημαντική μείωση τόσο της χοληστερόλης όσο και των τριγλυκεριδίων στο πλάσμα, που σχετίστηκε με αξιοσημείωτη μείωση στις TRL. Συνολικά, τα δεδομένα μας υποστηρίζουν ότι μια σημαντική λειτουργική αλληλεπίδραση μεταξύ της APOA2 και της APOE επηρεάζει το μεταβολισμό των TRL στο πλάσμα.

ΛΕΞΕΙΣ ΚΛΕΙΔΙΑ: *Απολιποπρωτεΐνη Α2, απολιποπρωτεΐνη Ε, πλούσιες σε τριγλυκερίδια λιποπρωτεΐνες*

REFERENCES

1. Hussain MM, Zannis VI. Intracellular modification of human apolipoprotein AII (apoAII) and sites of apoAII mRNA synthesis: comparison of apoAII with apoCII and apoCIII isoproteins. Biochemistry. 1990 Jan;29(1):209-17.
2. Pownall HJ, Gillard BK, Gotto AM. Setting the course for apoAII: A port in sight? Clin Lipidol. 2013 Oct;8(5):551-60.
3. Warden CH, Hedrick CC, Qiao JH, Castellani LW, Lusis AJ. Atherosclerosis in transgenic mice overexpressing apolipoprotein A-II. Science (1979). 1993 Jul;261(5120):469-72.
4. Kalopissis AD, Pastier D, Chambaz J. Apolipoprotein A-II: beyond genetic associations with lipid disorders and insulin resistance. Curr Opin Lipidol. 2003 Apr;14(2):165-72.
5. Lusis AJ. Genetic factors affecting blood lipoproteins: the candidate gene approach. J Lipid Res. 1988 Apr;29(4):397-429.
6. Schultz JR, Gong EL, McCall MR, Nichols AV, Clift SM, Rubin EM. Expression of human apolipoprotein A-II and its effect on high density lipoproteins in transgenic mice. J Biol Chem. 1992 Oct;267(30):21630–6.
7. Marzal-Casacuberta A, Blanco-Vaca F, Ishida BY, Julve-Gil J, Shen J, Calvet-Márquez S, et al. Functional lecithin: Cholesterol acyltransferase deficiency and high density lipoprotein deficiency in transgenic mice overexpressing human apolipoprotein A-II. J Biol Chem. 1996 Mar;271(12):6720–8.
8. Boisfer E, Lambert G, Atger V, Tran NQ, Pastier D, Benetollo C, et al. Overexpression of Human Apolipoprotein A-II in Mice Induces Hypertriglyceridemia Due to Defective Very Low Density Lipoprotein Hydrolysis. J Biol Chem. [Internet]. 1999 Apr;274(17):11564–72. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0021925819734644
9. Schönfeld P, Wojtczak L. Fatty acids as modulators of the cellular production of reactive oxygen species. Free Radic Biol Med. 2008 Aug;45(3):231–41.
10. Schultz JR, Verstuyft JG, Gong EL, Nichols AV, Rubin EM. Protein composition determines the anti-atherogenic properties of HDL in transgenic mice. Nature. 1993 Oct;365(6448):762–4.
11. Escolà-Gil JC, Marzal-Casacuberta À, Julve-Gil J, Ishida BY, Ordóñez-Llanos J, Chan L, et al. Human apolipoprotein A-II is a pro-atherogenic molecule when it is expressed in transgenic mice at a level similar to that in humans: evidence of a potentially relevant species-specific interaction with diet. J Lipid Res. 1998 Feb;39(2):457–62.
12. van 't Hooft FM, Ruotolo G, Boquist S, de Faire U, Eggertsen G, Hamsten A. Human Evidence That the Apolipoprotein A-II Gene Is Implicated in Visceral Fat Accumulation and Metabolism of Triglyceride-Rich Lipoproteins. Circulation. 2001 Sep;104(11):1223–8.
13. Gordon SM, Chung JH, Playford MP, Dey AK, Sviridov D, Seifuddin F, et al. High density lipoprotein proteome is associated with cardiovascular risk factors and atherosclerosis burden as evaluated by coronary CT angiography. Atherosclerosis. 2018 Nov;278:278–85.
14. Weng W, Breslow JL. Dramatically decreased high density lipoprotein cholesterol, increased remnant clearance, and insulin hypersensitivity in apolipoprotein A-II knockout mice suggest a complex role for apolipoprotein A-II in atherosclerosis susceptibility. Proc Natl Acad Sci U S A. 1996 Dec 10;93(25):14788–94.
15. Lackner KJ, Law SW, Brewer HB. The human apolipoprotein A-II gene: complete nucleic acid sequence and genomic organization. Nucleic Acids Res. 1985;13(12):4597–608.
16. Zvintzou E, Xepapadaki E, Kalogeropoulou C, Filou S, Kypreos KE. Pleiotropic effects of apolipoprotein A-II on high-density lipoprotein functionality, adipose tissue metabolic activity and plasma glucose homeostasis. J Biomed Res. 2020 Jul;34(1):14-26.
17. Borghini I, James RW, Blatter MC, Pometta D. Distribution of apolipoprotein E between free and A-II complexed forms in very-low- and high-density lipoproteins: functional implications. Biochim Biophys Acta. 1991 May;1083(2):139-46.
18. Plump AS, Smith JD, Hayek T, Aalto-Setälä K, Walsh A, Verstuyft JG, et al. Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. Cell. 1992 Oct;71(2):343–53.
19. Schaefer EJ, Gregg RE, Ghiselli G, Forte TM, Ordovas JM, Zech LA, et al. Familial apolipoprotein E deficiency. J Clin Invest. 1986 Nov;78(5):1206–19.
20. Reddick RL, Zhang SH, Maeda N. Atherosclerosis in mice lacking apo E. Evaluation of lesional development and progression. Arterioscler Thromb. 1994 Jan;14(1):141–7.
21. Huang Y, Zhu Y, Langer C, Raabe M, Wu S, Wiesenhütter B, et al. Effects of genotype and diet on cholesterol efflux into plasma and lipoproteins of normal, apolipoprotein A-I-, and apolipoprotein E-deficient mice. Arterioscler Thromb Vasc Biol. 1997 Oct;17(10):2010–9.
22. Huang Y, von Eckardstein A, Wu S, Maeda N, Assmann G. A plasma lipoprotein containing only apolipoprotein Eand with gamma mobility on electrophoresis releases cholesterol fromcells. Proc Natl Acad Sci U S A. 1994 Mar;91(5):1834–8.
23. Kypreos KE, Zannis VI. Pathway of biogenesis of apolipoprotein E-containing HDL in vivo with the participation of ABCA1 and LCAT. Biochem J. 2007 Apr;403(2):359–67.

24. Linton MF, Atkinson JB, Fazio S. Prevention of atherosclerosis in apolipoprotein E-deficient mice by bone marrow transplantation. Science (1979). 1995 Feb;267(5200):1034–7.
25. Shimano H, Ohsuga J, Shimada M, Namba Y, Gotoda T, Harada K, et al. Inhibition of diet-induced atheroma formation in transgenic mice expressing apolipoprotein E in the arterial wall. J Clin Invest. 1995 Feb;95(2):469–76.
26. Zvintzou E, Lhomme M, Chasapi S, Filou S, Theodoropoulos V, Xapapadaki E, et al. Pleiotropic effects of apolipoprotein C3 on HDL functionality and adipose tissue metabolic activity. J Lipid Res. 2017 Sep;58(9):1869–83.