Lipoproteins and Atherosclerotic Cardiovascular Diseases

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Abstract: Metabolisms of triglycerides and cholesterol from both the intestine and the liver are important in humans. Generally, there are three lipoprotein pathways involving chylomicrons, chylomicron remnants, VLDL, LDL, and HDL. Started in arterial endothelium, accumulation of LDL and proteoglycans will form oxidized LDL and lead to arterial macrophages. Then, macrophage foam cells will enhance plaque progression. If the plaque is unstable, it is very likely to develop plaque rupture and form thrombus. To better understand the mechanism and find medications, it is important to choose adequate animal models based on lipid metabolism and characteristics of atherosclerosis. The combination of statistical models in recent studies helps to examine the effects of two independent factors on one dependent factor.

1 INTRODUCTION

Nowadays, people consume more and more food containing high fat and cholesterol in developed countries and the likelihood of getting atherosclerosis increases. To attenuate or avoid the symptoms of atherosclerotic cardiovascular diseases, it is necessary to understand the principles behind these diseases. In addition, the combination of statistical models to biological research may differentiate the influence of two independent factors or two dependent factors, which provide futural experiments with possibilities to include more factors in one research.

2 LIPOPROTEINS, RECEPTORS, ENZYMES, AND LIPOPROTEIN PATHWAYS

Lipoproteins are complexes with hydrophobic core, which is formed by triglyceride and cholesteryl esters, and hydrophilic phospholipids, free cholesterol, and apolipoproteins. There are seven classes of lipoproteins: chylomicrons, chylomicron remnants, very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), low density lipoproteins (LDL), high density lipoproteins (HDL), and lipoprotein (a) (Lp (a)).

Chylomicrons are triglyceride-rich particles produced by the intestine. The major structural protein of chylomicrons is apolipoprotein B-48 (Apo B-48) that cannot be recognized by LDL receptors. Chylomicron remnants are smaller particles after removing triglycerides from chylomicrons by peripheral tissues. These smaller particles are high in cholesterol and more pro-atherogenic. VLDL are triglyceride-rich particles produces by the liver, and they are smaller than chylomicrons. Compared with chylomicrons, the major structural protein is Apo B-100 which is a ligand for LDL receptors. IDL, or VLDL remnants, is a smaller particle after removing triglycerides by muscle and adipose tissue. Similar to chylomicron remnants, IDL is enriched in cholesterol and pro-atherogenic. LDL is derived from VLDL and IDL, and it is more enriched in cholesterol. Smaller LDL is more pro-atherogenic than larger LDL because it has a decreased affinity with LDL receptors, which leads to a longer retention time, and they bind more tightly to proteoglycans and are more likely to be oxidated, which increases the consumption by macrophages. (Feingold 2021) HDL is anti-atherogenic because it acts in reverse cholesterol transport. The major structural protein of HDL is Apo A-I. Apo A-I helps interaction between HDL and ATP-binding cassette transport A1

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(ABCA1) and ABCG1 that transport cholesterol from cells to HDL in reverse cholesterol transport. (Feingold, 2021) Lp (a) is an LDL particle with apolipoprotein (a) and it is pro-atherogenic. Another important apolipoprotein is Apo C that is found in chylomicrons, VLDL, and HDL. Apo C-II is a co-factor of lipoprotein lipase (LPL) and hydrolyzes triglycerides, which means that it is anti-atherogenic. However, Apo C-III is an inhibitor of LPL and inhibits triglycerides interact with their receptors.

In addition to ABCA1 and ABCG1, several receptors and transporters are important in lipid metabolism. LDL receptors determine the LDL level in the plasma: the high level of LDL receptors is corresponding to a low LDL level, while the low level of LDL receptors is corresponding to a high LDL level. (Feingold 2021) With the delivery of cholesterol to cells, the level of HMG CoA reductase decreases, and the level of acyl-CoA cholesterol acyl transferase (ACAT) increases. As a result, the level of LDL receptors, which are controlled by HMG CoA reductase, decreases. When the cellular cholesterol level is low, the transcription factor SREBP will be activated, and LDL receptors will be stimulated to express. In contrast, when the cellular cholesterol level is high, the SREBP will be inactive, and the expression of LDL receptors is low. (Feingold 2021) Similar to ABCA1, AND ABCG1, class B scavenger receptor B1 (SR-B1) facilitates selective uptake of cholesterol esters from HDL particles.

There are several enzymes involved in lipoprotein metabolism. Lipoprotein lipase (LPL) hydrolyzes triglycerides in chylomicrons and VLDL and forms chylomicron remnants and IDL. Apo C-II and Apo A-V are cofactors of LPL, while Apo C-III and Apo A-II are inhibitors. Lecithin cholesterol acyltransferase (LCAT) catalyzes the synthesis of cholesterol esters and transfers free cholesterol from the surface of the HDL particle to the core and forms cholesterol esters. (Feingold 2021) This process reduces the concentration of cholesterol on the HDL particles and allows the consumption of more cholesterol from cells. Cholesteryl ester transfer protein (CETP) helps transfer cholesterol esters from HDL to VLDL, chylomicrons, and LDL, and triglycerides from VLDL and chylomicrons back to HDL. (Feingold 2021)

There are three major lipoprotein pathways: exogenous lipoprotein pathway, endogenous lipoprotein pathway, and reverse cholesterol transport. Exogenous lipoprotein pathway starts in the intestine: dietary lipids are incorporated into chylomicrons. Triglycerides in chylomicrons are hydrolyzed into muscle and adipose tissue into free fatty acids by LPL, and chylomicrons form chylomicron remnants. (Feingold 2021) Endogenous lipoprotein pathway starts in the liver with VLDL. Triglycerides in VLDL are hydrolyzed into free fatty acids, and VLDL forms IDL. IDL can be further metabolized into LDL and can be consumed in tissues via LDL receptors. (Feingold 2021) Reverse cholesterol transport begins with nascent HDL in the liver and intestine. Cells donate cholesterol and phospholipids via ABCA1 to nascent HDL and form mature HDL. Additional cholesterol can be transferred to mature HDL via ABCG1, SR-B1, or passive diffusion. (Feingold 2021) Facilitated by CETP, HDL transports cholesterol back to the liver via SR-B1 or indirectly to VLDL or LDL.

3 ATHEROSCLEROTIC CARDIOVASCULAR DISEASE

Accumulation of LDL, which is one of the major culprits of atherosclerotic cardiovascular disease, is due to interaction between positive-charged amino acyl residues in Apo B-100 with negative-charged sulfate and carboxylic acid groups of proteoglycans in the artery wall. Both changes in the core or on the surface of LDL may enhance atherosclerosis. For example, enrichment of cholesterol in LDL or Apo E, Apo C-III, and serum amyloid A can increase the binding affinity of LDL and arterial wall proteoglycans. (Borén 2020) In humans, there is an inclination to develop atherosclerosis at branches and bifurcations with laminar blood flow and low or fluctuating shear stress.

There are four subfractions of LDL: large LDL-I, LDL-II with intermediate size and density, small LDL-III, and very small LDL-IV. People with medium plasma triglyceride levels will release VLDL1 and VLDL2 that is further metabolized into LDL-II; people with low plasma triglyceride levels mainly release smaller VLDL and form predominantly LDL-I, along with some LDL-II; people with high plasma triglyceride level release dense LDL-IV due to high level of VLDL, and they generally lack lipolysis due to inhibition of overproduced Apo C-III on LPL. (Borén 2020) Small dense LDL is more pro-atherogenic than larger LDL because it enters the artery faster and has a longer retention time due to impaired binding affinity to LDL receptors. In addition, small dense LDL is enriched in Apo C-III and glycated Apo B, and unsaturated cholesteryl esters are more susceptible to hydroperoxide. (Borén 2020)

With a longer retention time, LDL particles are more likely to form oxidized LDL and trigger the entrance of monocytes into the artery. Monocytes differentiate in the artery and become macrophages and intensify oxidized LDL that can be consumed by scavenger receptors like clusters of differentiation-36 (CD36) and form foam cells. Modified LDL triggers a series of innate and adaptive immune responses and leads to inflammation. Defective efferocytosis, which results in non-resolving inflammation, is due to signals like CD47 in the artery and will lead to the accumulation of cell debris. (Borén 2020) Apoptotic cell will stimulate secondary necrosis that results in unstable plaque, plaque rupture, and later thrombus formation. Both plaque rupture and plaque erosion may lead to thrombus formation. With lipid cores or thin fibrous cap tissue between the lipid core and blood that reaches the luminal surface, the blood can enter and core material may leak out. (Borén 2020) This process forms plaque rupture that always accompanying by protruding cholesterol crystals. In contrast, lesions without lipid cores or thick fibrous cap will not lead to plaque rupture but instead plaque erosion, where the plaque is intact but endothelial cells are deficient.[1] Recent researches show that a spotty pattern of calcium deposits is prone to be more dangerous. An elevated LDL-cholesterol level is one of the risk factors of calcification. (Borén 2020) In contrast, HDL-mediated efflux of cholesterol inhibits calcification. (Borén 2020) While the formation of atherosclerosis is attributed to the accumulation of oxidated LDL, the relation between lowering aggressive LDL and lesion area remains unclear. Similarly, while HDL features anti-inflammatory and anti-oxidative functions, its role in attenuating lesion areas is indistinct but probable.

4 CHOICE OF ANIMAL MODELS

Since it is impossible to track the lengthy development of atherosclerosis in arteries of humans, it is necessary to observe that in animal models which are representative of humans. The murine model is ideal because of its small size and its relative homogeneity to humans. For example, both the mechanism of triglyceride-rich lipoprotein inducing atherosclerosis and Apo A-I lowering atherosclerosis can be applied to humans. (Daugherty 2017) However, while a murine transports cholesterol primarily in HDL, humans utilize LDL. This difference in lipoprotein profile protects a murine from atherosclerosis because there is no binding site of Lp-PLA2 to LDL. Besides, there is a lower probability to form oxidated LDL and trigger atherosclerosis, but a higher probability to form stable plaques. Furthermore, the much higher level of LDL receptors in the liver in a murine than in humans leads to lower LDL levels in a murine and a lower probability to develop atherosclerosis. There is a lower level of plasma Apo B on LDL in a murine than in humans. The chylomicron is from the intestine, and the VLDL is from the liver in humans. However, Apo B-48 exists in the VLDL, and Apo B-100 exists in chylomicrons in a murine. Fed with a high-fat high cholesterol diet, humans are prone to develop increased plasma cholesterol and triglycerides, while a murine may develop increased plasma cholesterol but lowered triglycerides. In addition, the murine accumulates lesions primarily in the aortic root, arch, and other side branches instead of coronary arteries in humans. Recent studies mainly utilize either Apo E knock-out mice or LDL receptor knock-out mice. Although Apo E knock-out mice carry more VLDL, LDL receptor knock-out mice have higher LDL particles that are more atherogenic. (Getz, & Reardon 2016) However, Apo E has some athero-protective functions other than lower plasma lipids like anti-inflammation and anti-oxidation to lower atherosclerosis, which generalizes how Apo B-containing lipoproteins influence atherosclerosis more difficult. (Getz, & Reardon 2016) While it is convenient to study characteristics of atherosclerosis in a murine, there are limitations when applying the same mechanism to humans.

Pigs are more relevant to humans than murine. Like humans, pigs transport cholesterol primarily in LDL, and pigs also have a binding site of Lp-PLA2 to LDL. Moreover, similar to humans, the LDL receptors level is low in pigs, and a high-fat high cholesterol diet can stimulate the increase in both plasma cholesterol and triglycerides. All these properties determine the susceptibility of atherosclerosis and unstable characteristics of plaques. Pigs are inclined to develop lesions at branches with low shear stress and laminar blood flow including coronary, and it is more convenient to observe changes in arteries because of the huge size of pigs. (Daugherty 2017) However, since there is no CETP in pigs, the mechanism of atherosclerosis in pigs is different from that of humans. In addition, the huge size of pigs will increase the cost of feeding and increase the difficulty of experiments.

Recently, scientists find great similarities between hamsters and humans. Hamsters utilize LDL to transport cholesterol, and they have CETP, which means that hamsters share a similar mechanism of atherosclerosis development. Besides, most Lp-

PLA2 in hamsters binds to LDL instead of HDL, and this increases the susceptibility of atherosclerosis and forms unstable plaques. All these similarities with humans, along with the small size of hamsters, ensure that hamsters are one of the most appropriate animal models when there is a need to how atherosclerosis may develop in humans. However, information about the place of atherosclerosis is still limited, and more experiments about atherosclerosis in hamsters are needed.

5 LCAT DEFICIENT HAMSTERS AND STATISTIC MODELS

In recent scientific studies, it is common to combine statistical models to analyze research results. In research conducted by Guo, et al., it is noted that aged male and female hamsters that are deficient in LCAT develop atherosclerosis with higher plasma oxidative lipids but not total cholesterols. (Guo, Liu, Xu, Ma, Huang, Gao, Wang, Liu, & Xian 2020) In the study, Guo, et al. employ a two-way ANOVA test and linear regression.

In linear regression, there are three important values: total variability, remaining residue, and the explained viability due to regression. Total variability, or SS_{TOT}, can be written as Y – $\bar{Y}$, which stands for the difference between the exact value Y and the mean $\bar{Y}$. Remaining residue, or SS_{RES}, can be written as Y – $\hat{Y}$, and it stands for the difference between the exact value Y and the value on the model $\hat{Y}$. The explained viability due to regression can be written as $\hat{Y} - \bar{Y}$, and it stands for the difference between the value on the model $\hat{Y}$ and the mean $\bar{Y}$. Based on these three values, the coefficient of determination R square in linear regression is defined as:

$$R^2 = 1 - \frac{SS_{RES}}{SS_{TOT}} = 1 - \frac{\sum_i (Y_i - \hat{Y}_i)^2}{\sum_i (Y_i - \bar{Y})^2} \quad (1)$$

When R^2 is closer to 1, the linear model is more accurate. In the study of Guo, et al., the linear model fits well to samples. In the aorta of male hamsters $R = 0.804$, and in female hamsters $R = 0.862$ (Figure 1, 2). In the aortic root of male hamsters, $R = 0.602$. Based on these data, it is reliable to conclude that as the plasma malondialdehyde (MDA) level increases, the lesion volume, or the severity of atherosclerosis in the aorta of LCAT deficient hamsters increases.

In the ANOVA test, a statistic F test uses F value to compare two variances. First, the formula of regression sum of squares (SSR) is:

$$SSR = n \sum_j (x_j - \overline{x_{overall}})^2 \quad (2)$$

Where n is the sample size of group j; x_j is the mean of group j; and $\overline{x_{overall}}$ is the mean of all samples.

Then, the formula of the error sum of squares (SSE) is:

$$SSE = \sum_j \sum_i (x_i - \bar{x_j})^2 \quad (3)$$

Where x_i is the i[th] term in group j; and $\bar{x_j}$ is the mean of group j.

The total sum of squares (SST) is defined as:

$$SST = SSR + SSE. \quad (4)$$

Finally, based on formulas (2), (3), and (4), the F value in F test is:

$$F = \frac{MS\ treatment}{MS\ error} = \frac{{}^{SST}/_{df\ treatment}}{{}^{SSE}/_{df\ error}} = \frac{{}^{SST}/_{k-1}}{{}^{SSE}/_{n-1}} \quad (5)$$

Where k is the number of groups; and n is the total sample size.

Based on the calculated F value and the table of critical values of the F distribution, it is convenient to find out the P-value that stands for the probability of coincident results. If the calculated F value is larger than the critical F value, then it is statistically significant to reject the null hypothesis that the variance between the means of two samples has no significant difference. In the study of Guo et al., P-values for aorta in male hamsters and aortic root in female hamsters are less than 0.01, while the P-value for aortic root in male hamsters is less than 0.05 (Figure 1, 2). In addition, a two-way ANOVA test is applied to consider the influence of both gender and age on lesion area in the study of Guo et al. Different from the one-way ANOVA test, a two-way ANOVA test considers two conditions: the influence of gender only on the lesion area and the influence of age only on the lesion area. Since all P-values for lesion areas in the aorta and aortic root are less than 0.01 in male hamsters and 0.001 in female hamsters, it is statistically significant to conclude that LCAT deficient hamsters tend to develop higher plasma MDA levels and larger lesion areas than wild-type hamsters (Figure 3, 4).

6 CONCLUSIONS

Recent studies develop some generalized ideas about the characteristics of lipoproteins and their pathways in transporting cholesterol. Defects in lipoproteins, lipoprotein receptors, transporters, or enzymes will

lead to retention of LDL in the artery and the formation of atherosclerosis. However, there are still confusions about the functions of some apolipoproteins like Apo C-III and Apo E in the formations of atherosclerosis. Furthermore, since current animal models of mice are not appliable to humans in some cases, futural research about how these defects express on other animal models like hamsters may be insightful. When conducting research, the employment of statistical models helps to differentiate the influence of various independent factors that will reduce potential errors when there are several sets of variables.

7 SUPPLEMENT

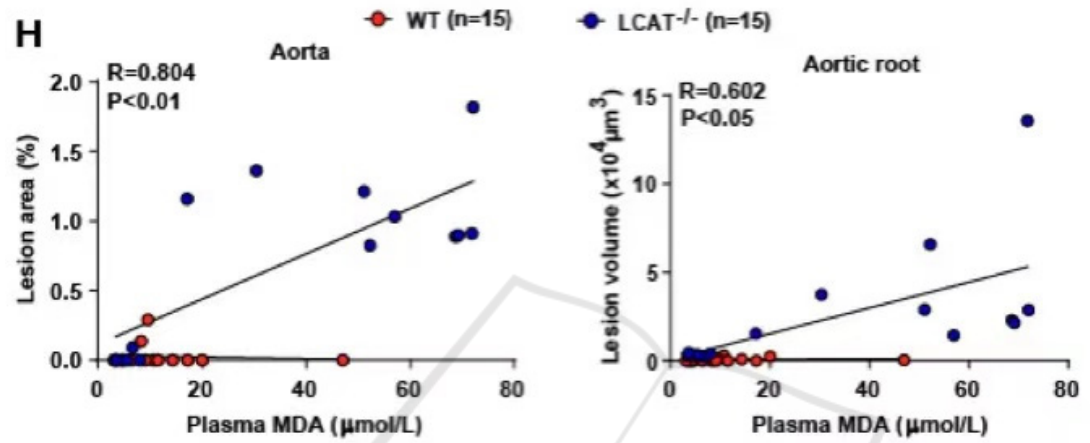

Figure 1: The correlation of plasma MDA level and lesion area in aorta and aortic root in wild-type and LCAT deficient male hamsters (adapted from Guo, et al. 2020).

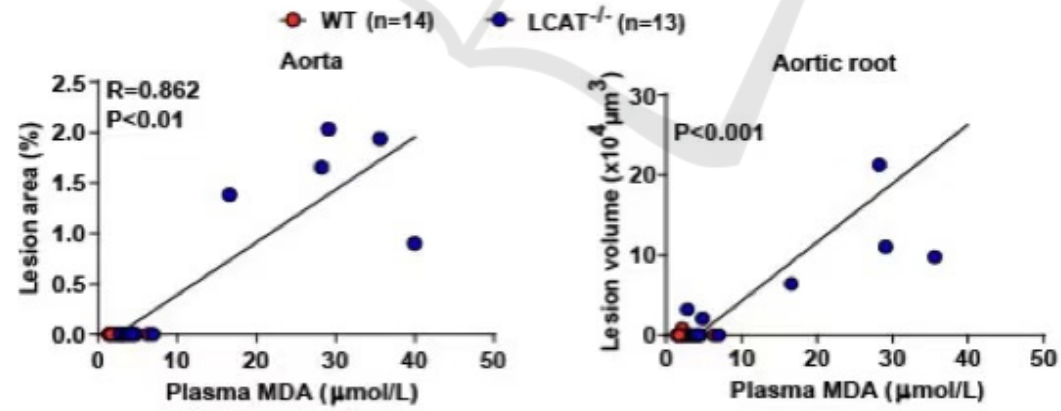

Figure 2: The correlation of plasma MDA level and lesion area in aorta and aortic root in wild-type and LCAT deficient female hamsters (adapted from Guo, et al. 2020).

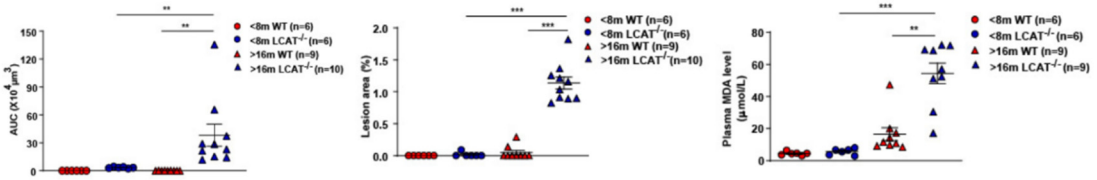

Figure 3: Quantification of atherosclerosis lesion area in aortic roots in male hamsters; Quantification of atherosclerosis plaque area in the aorta in male hamsters; Plasma MDA levels in male hamsters. Scale bar: 1mm. P<0.01 by Two Way ANOVA/Bonferronis post-test (adapted from Guo, et al. 2020).

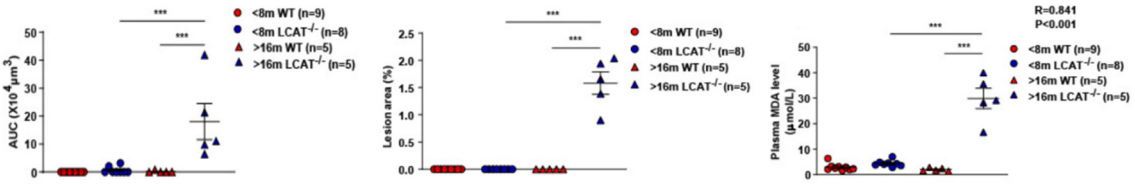

Figure 4: Quantification of atherosclerosis lesion area in aortic roots in female hamsters; Quantification of atherosclerosis plaque area in the aorta in female hamsters; Plasma MDA levels in female hamsters. Scale bar: 1mm. P<0.001 by Two Way ANOVA/Bonferronis post-test (adapted from Guo, et al. 2020).

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