Published in final edited form as:
Arterioscler Thromb Vasc Biol. 2010 January ; 30(1): 20–23. doi:10.1161/ATVBAHA.109.196329.

Chylomicronemia Elicits Atherosclerosis in Mice

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Abstract

Objective—The risk of atherosclerosis in the setting of chylomicronemia has been a topic of debate. In this study, we examined susceptibility to atherosclerosis in *Gpihbp1*-deficient mice (*Gpihbp1*$^{-/-}$), which manifest severe chylomicronemia as a result of defective lipolysis.

Methods and Results—*Gpihbp1*$^{-/-}$ mice on a chow diet have plasma triglyceride and cholesterol levels of 2812 ± 209 and 319 ± 27 mg/dl, respectively. Even though nearly all of the lipids were contained in large lipoproteins (50–135 nm), the mice developed progressive aortic atherosclerosis. In other experiments, we found that both *Gpihbp1*-deficient "apo-B48–only" mice and *Gpihbp1*-deficient "apo-B100–only" mice manifest severe chylomicronemia. Thus, GPIHBP1 is required for the processing of both apo-B48– and apo-B100–containing lipoproteins.

Conclusions—Chylomicronemia causes atherosclerosis in mice. Also, we found that GPIHBP1 is required for the lipolytic processing of both apo-B48– and apo-B100–containing lipoproteins.

Keywords

lipoprotein lipase; chylomicronemia; lipolysis; GPIHBP1

Introduction

Human and mouse studies have proven that high levels of cholesterol-rich remnants cause atherosclerosis, but the relevance of triglyceride-rich lipoproteins (TRLs) to atherogenesis remains controversial.[1] In part, this controversy stems from the fact that elevated levels of TRLs and remnants often coexist.

Humans with chylomicronemia have severe hypertriglyceridemia but low levels of cholesterol-rich remnants. Chylomicronemia patients are often assumed to be protected from atherosclerosis because experiments in rabbits had shown that very large lipoproteins cannot enter the arterial wall and were not particularly atherogenic.[2] However, this assumption was recently challenged by the finding of atherosclerosis in four chylomicronemia patients with lipoprotein lipase (LPL) deficiency.[3]

Mice lacking GPIHBP1 (*Gpihbp1*$^{-/-}$) have severe chylomicronemia, even on a low-fat chow diet, as a result of defective lipolysis.[4] Very recently, chylomicronemia has been observed in

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Disclosures None.

a young man with a homozygous missense mutation in GPIHBP1.[5] Thus far, no one has examined whether *Gpihbp1*$^{-/-}$ mice have an increased susceptibility to atherosclerotic lesions. In this study, we examined that issue.

We also tackled a second issue; we addressed whether GPIHBP1 is required for the lipolytic processing of both apo-B48–containing lipoproteins and apo-B100–containing lipoproteins.

Materials and Methods

Gpihbp1$^{-/-}$ mice (>90% C57BL/6)[4] were housed in a barrier facility and fed a 4.5%-fat chow diet. We also bred "apo-B48–only" and "apo-B100–only" *Gpihbp1*$^{-/-}$ mice (*Gpihbp1*$^{-/-}$ *Apob*$^{48/48}$, *Gpihbp1*$^{-/-}$ *Apob*$^{100/100}$).[6] All experiments were approved by the Animal Research Committee.

Plasma lipid levels were measured with enzymatic kits, and their distribution within lipoproteins was assessed by Fast Protein Liquid Chromatography (FPLC).[6] Lipoprotein diameters were measured by laser light scattering.[6] Western blots were performed as described.[6]

Sections of the aortic root were stained with Oil Red O and lesions were quantified as described.[6] Macrophages were stained with a monoclonal antibody against CD68 (Serotec; 1:100).

Results

Gpihbp1$^{-/-}$ mice had lipemic plasma (Figure 1A) and nearly all of the plasma lipids were contained in large lipoproteins (as judged by FPLC) (Figure 1B). Also, plasma apo-B48 levels in *Gpihbp1*$^{-/-}$ mice were increased (Figures 1C–D). *Gpihbp1*$^{-/-}$ mice had triglyceride and cholesterol levels of 2812 ± 209 and 319 ± 27 mg/dl, respectively (n = 42) (*vs.* 35 ± 5 and 90 ± 6 mg/dl in littermate *Gpihbp1*$^{+/+}$ mice, n = 24; $p < 0.0001$ for both) (Figure 1E).

The fact that amphibians and birds have neither apo-B48 nor GPIHBP1 led Beigneux *et al.*[4] to speculate that GPIHBP1 might have arisen in mammals as a new protein specifically dedicated to the lipolytic processing of apo-B48–lipoproteins. They further hypothesized that GPIHBP1 might be unimportant for the processing of apo-B100–containing lipoproteins and that mice lacking *both* apo-B48 and GPIHBP1 might be normolipidemic. While this hypothesis initially seemed attractive, it was incorrect. *Gpihbp1*$^{-/-}$ *Apob*$^{+/+}$ mice and littermate *Gpihbp1*$^{-/-}$ *Apob*$^{48/48}$ mice had very similar plasma lipid levels (Figure 1F). Similarly, *Gpihbp1*$^{-/-}$ *Apob*$^{+/+}$ mice and littermate *Gpihbp1*$^{-/-}$ *Apob*$^{100/100}$ mice had very similar plasma lipid levels (Figure 1G).

Most of the $d < 1.006$ g/ml lipoproteins in *Gpihbp1*$^{-/-}$ mice were large (50–135 nm), overlapping little with the sizes of lipoproteins in *Gpihbp1*$^{+/+}$ mice (Figure 1H). Lipoprotein sizes were similar in *Gpihbp1*$^{-/-}$ *Apob*$^{+/+}$ mice and littermate *Gpihbp1*$^{-/-}$ *Apob*$^{48/48}$ mice (Figure 1I). Also, lipoprotein sizes were similar in *Gpihbp1*$^{-/-}$ *Apob*$^{+/+}$ mice and littermate *Gpihbp1*$^{-/-}$ *Apob*$^{100/100}$ mice (Figure 1J).

Chow-fed *Gpihbp1*$^{-/-}$ mice developed lipid-rich atherosclerotic lesions in the aortic root and coronary arteries (Figures 2A–B). The lesions also stained strongly for macrophages (Figure 2C). The lesions were small at 11–12 months (median 500 μm^2/section) but were much larger at 16–22 months (median 3000 μm^2/section) (Figure 2D). Lesions were evident in both males and females [3947; 3172–6071 (median; 33rd–66th percentile) and 1680; 1000–4688 μm^2/section, respectively, p = 0.74].

Discussion

GPIHBP1-deficient mice develop severe chylomicronemia as a result of defective lipolysis of triglyceride-rich lipoproteins.[4] In this study, we address two timely and important issues. First, we show that mice with chylomicronemia develop spontaneous atherosclerosis, despite the fact that most of the lipids in these mice are found in large lipoproteins—lipoproteins that are often considered to be nonatherogenic.[2] Thus, chylomicronemia leads to atherosclerotic lesions, even in mice fed a low-fat chow diet. These findings in mice add plausibility to the concept[3] that chylomicronemia in humans could lead to increased susceptibility to atherosclerosis. Second, we show that the severe chylomicronemia in *Gpihbp1*$^{-/-}$ mice is not due to a selective defect in the processing of apo-B48–containing lipoproteins. Beigneux *et al.*[4] had hypothesized that GPIHBP1 might be a mammalian protein specifically dedicated to the processing of apo-B48–containing lipoproteins and further speculated that the processing of apo-B100–containing lipoproteins might not depend on GPIHBP1. This speculation is incorrect. The plasma lipid levels and lipoprotein sizes in *Gpihbp1*$^{-/-}$ *Apob*$^{+/+}$ mice are very similar to those in littermate *Gpihbp1*$^{-/-}$ *Apob*$^{100/100}$ mice (or littermate *Gpihbp1*$^{-/-}$ *Apob*$^{48/48}$ mice). Thus, GPIHBP1 is required for the processing of both apo-B48–containing lipoproteins and apo-B100–containing lipoproteins.

Finding atherosclerosis in *Gpihbp1*$^{-/-}$ mice is consistent with a recent report of aortic lesions in *Lpl*$^{-/-}$ mice that had been rescued with an injection of an LPL adenovirus.[7, 8] The severity of the hypertriglyceridemia in *Gpihbp1*$^{-/-}$ mice and LPL adenovirus–rescued *Lpl*$^{-/-}$ mice is quite similar; however, *Gpihbp1*$^{-/-}$ mice appear to be the most reasonable choice for future research. *Gpihbp1*$^{-/-}$ mice can be bred in limitless numbers, while the production of adenovirus-rescued *Lpl*$^{-/-}$ mice is more challenging. Also, systemic infections with an adenovirus could cloud the interpretation of mouse atherosclerosis studies.

The lesions in *Gpihbp1*$^{-/-}$ mice are small and require longer to develop than those of *Apoe*$^{-/-}$ or *Ldlr*$^{-/-}$ mice.[6] Nevertheless, these mice will be useful to the research community. With *Gpihbp1*$^{-/-}$ mice, it should be possible to determine if the atherogenicity of TRLs depends on their cholesterol content. Also, this model opens the door to defining the impact of different dietary fatty acids (including dietary oxidized fatty acids) on the atherogenicity of TRLs.

Acknowledgments

Sources of Funding HL094732-01 (APB); T32HL69766 (MMW); Texas AgriLife 8738 (RLW); HL090553 and HL087228 (SGY).

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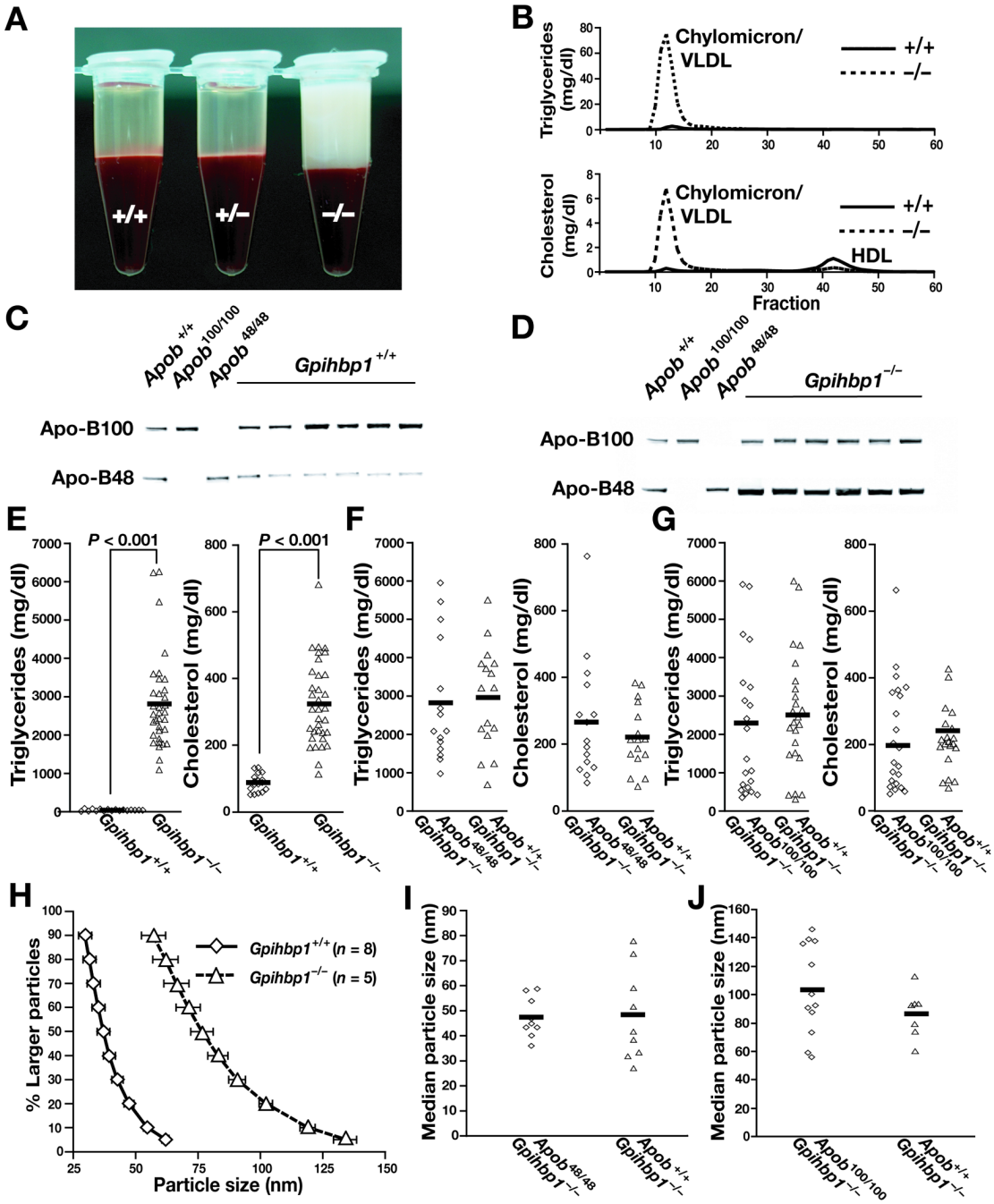

Figure 1.

Lipids and lipoproteins in *Gpihbp1*$^{-/-}$ mice. A, Lipemic plasma in 15-month-old chow-fed *Gpihbp1*$^{-/-}$ mice. B, Distribution of lipids in FPLC-fractionated plasma. C–D, Western blots of *Gpihbp1*$^{+/+}$ (C) and *Gpihbp1*$^{-/-}$ (D) plasma with an apo-B–specific antibody. *Apob*$^{100/100}$ and *Apob*$^{48/48}$ mice were used as controls. E–G, Plasma triglyceride and cholesterol levels in *Gpihbp1*$^{-/-}$, *Gpihbp1*$^{-/-}$ *Apob*$^{48/48}$, and *Gpihbp1*$^{-/-}$ *Apob*$^{100/100}$ mice on a chow diet. H, Distribution of $d < 1.006$ g/ml lipoprotein diameters in *Gpihbp1*$^{-/-}$ mice on a chow diet (deciles plus the 95% point). The sizes of the $d < 1.006$ g/ml lipoproteins in *Gpihbp1*$^{-/-}$ mice were similar to those observed previously in mouse lymph chylomicrons during active lipid absorption.[9] We did not compare the sizes of lipoproteins in chow-fed *Gpihbp1*$^{-/-}$ mice and

diabetic rabbits (such as those studied by Nordestgaard *et al.*[2]). Because the diabetic rabbits examined by Nordestgaard *et al.*[2] were on a high-fat diet, it is possible that their lipoproteins were larger than those in chow-fed *Gpihbp1*$^{-/-}$ mice. (I) Median diameters of $d < 1.006$ g/ml lipoproteins in *Gpihbp1*$^{-/-}$ *Apob*$^{+/+}$ and littermate *Gpihbp1*$^{-/-}$ *Apob*$^{48/48}$ mice. (J) Median diameters of $d < 1.006$ g/ml lipoproteins in *Gpihbp1*$^{-/-}$ *Apob*$^{+/+}$ mice and littermate *Gpihbp1*$^{-/-}$ *Apob*$^{100/100}$ mice. In the two different experiments shown in panels I and J, the median diameter of lipoproteins in *Gpihbp1*$^{-/-}$ *Apob*$^{+/+}$ mice were different. We do not fully understand the lipoprotein size differences in the two different experiments. The experiments were performed independently, many months apart, on different sets of mice. Both experiments were performed on mice consuming *ad libitum* diets (not fasting mice), and the mice examined in panel I were three months younger than the mice examined in panel J. In any case, the important point (for the experiments in both panel I and panel J) is that lipoprotein sizes in littermates were not affected by *Apob* genotype.

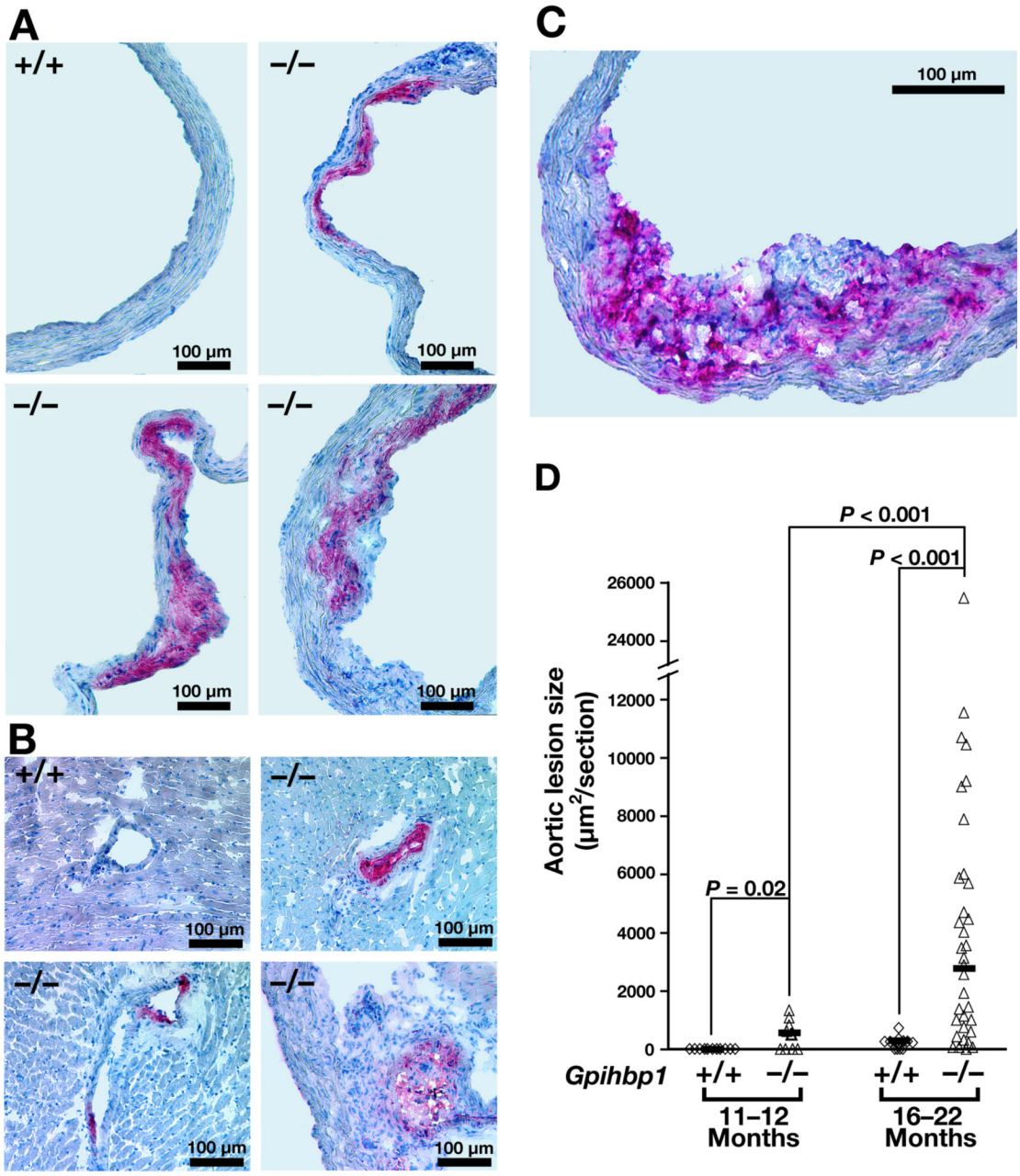

Figure 2.

Atherosclerosis in chow-fed *Gpihbp1*$^{-/-}$ mice. A, Oil red O–stained atherosclerotic lesions in the aortic root of three 22-month-old *Gpihbp1*$^{-/-}$ mice. B, Oil red O–stained coronary atherosclerotic lesions in three 18-month-old *Gpihbp1*$^{-/-}$ mice. C, Staining of macrophages in an atherosclerotic lesion with an antibody against CD68. D, Atherosclerotic lesion sizes (mean μm^2/section) at 11–12 months (*Gpihbp1*$^{+/+}$, $n = 12$; *Gpihbp1*$^{-/-}$, $n = 12$) and 16–22 months (*Gpihbp1*$^{+/+}$, $n = 20$; *Gpihbp1*$^{-/-}$, $n = 18$). Horizontal bars represent medians; differences tested with the Mann-Whitney test.