Lipolytic Modification of LDL by Phospholipase A₂ Induces Particle Aggregation in the Absence and Fusion in the Presence of Heparin

Jukka K. Hakala, Katriina Öörni, Mika Ala-Korpela, Petri T. Kovanen

Abstract—One of the first events in atherogenesis is modification of low density lipoprotein (LDL) particles in the arterial wall with ensuing formation of aggregated and fused lipid droplets. The accumulating particles are relatively depleted in phosphatidylcholine (PC). Recently, secretory phospholipase A₂ (PLA₂), an enzyme capable of hydrolyzing LDL PC into fatty acid and lysoPC molecules, has been found in atherosclerotic arteries. There is also evidence that both LDL and PLA₂ bind to the glycosaminoglycan (GAG) chains of extracellular proteoglycans in the arterial wall. Here we studied the effect of heparin GAG on the lipolytic modification of LDL by PLA₂. Untreated LDL, heparin-treated LDL, and heparin-bound LDL were lipolyzed with bee venom PLA₂. In the presence of albumin, lipolysis resulted in aggregation in all 3 preparations of the LDL particles. Lipolysis of untreated LDL did not result in aggregation if albumin was absent from the reaction medium, and the lipolytic products accumulated in the particles rendering them negatively charged. However, heparin-treated and heparin-bound lipolyzed LDL particles aggregated even in the absence of albumin. Importantly, in the presence of albumin, some of the heparin-treated and heparin-bound lipolyzed LDL particles fused, the proportion of fused particles being substantially greater when LDL was bound to heparin during lipolysis. In summary, lipolysis of LDL PC by PLA₂ under physiological conditions, which allow transfer of the lipolytic degradation products to albumin, leads to fusion of LDL particles in the presence, but not in the absence, of heparin. Thus, it is possible that within the GAG meshwork of the arterial intima, PLA₂-induced modification of LDL is one source of the lipid droplets during atherogenesis. (Arterioscler Thromb Vasc Biol. 1999;19:1276-1283.)

Key Words: aggregation ■ fusion ■ heparin ■ LDL ■ phospholipase A₂

In experimental atherosclerosis a fraction of circulating low density lipoprotein (LDL) enters the intima; some of the particles fuse and form larger particles, which are visible in electron microscopy as aggregated lipid droplets.¹⁻³ There is also evidence that the first morphologic sign of intimal lipid accumulation in man is appearance of extracellular lipid droplets in the subendothelial proteoglycan-rich layer.⁴⁻⁵ The accumulating extracellular lipid in atherosclerotic lesions has been suggested to originate either from dead foam cells or directly from LDL. Two important observations favor the latter pathway. First, the fatty acyl pattern of cholesteryl esters of the extracellular droplets resembles that of plasma lipoproteins rather than that of the cytoplasmic cholesteryl ester droplets of foam cells,⁶⁻⁷ and second, the diameter of most extracellular lipid droplets (range 30 to 400 nm) is smaller than that of intracellular lipid droplets (range 400 to 6000 nm).⁸

According to current understanding, LDL particles do not aggregate or fuse until they have been modified.⁹ In vitro experiments have shown that LDL particles will aggregate, fuse, or both aggregate and fuse if modified by proteolytic enzymes,¹⁰⁻¹¹ or by lipolytic enzymes such as sphingomyelinase¹⁰,¹¹ or phospholipase C.¹⁴,¹⁵

When the LDL particles become aggregated, the surface protein or lipid environments of the attached individual particles will become connected. Particle aggregation, therefore, brings the surface monolayers of different lipoprotein particles into contact, but does not unite them, and thus does not change the size of the individual particles. If the modifications of the particles are prominent enough to lead to interpenetration of the surfaces, energetic stabilization can drive subsequent fusion of the attached particles. It is important to note that particle fusion leading to enlarged individual particles is an irreversible phenomenon, in contrast to aggregation, which in principle, is a reversible reaction.

The apoB100-containing particles and small lipid droplets isolated from the arterial intima are relatively enriched in sphingomyelin and lysophosphatidylcholine (lysoPC), but have a relatively low content of phosphatidylcholine (PC),¹⁶⁻²⁰ suggesting that the particles are modified by an enzyme of the phospholipase A type. In fact, a type II secretory nonpancreatic phospholipase A₂ (PLA₂) capable of lipolysing LDL²¹ has recently been shown to be located...
extracellularly in the arterial intima.22–24 However, our recent findings showed that LDL particles, when lipolyzed in vitro by PLAr in the presence of albumin, aggregate, but do not fuse.25

The enzymatic attack on LDL particles, leading to the modification of LDL, takes place in the proteoglycan-rich areas of the arterial intima. In vitro experiments suggest that proteoglycans and glycosaminoglycans (GAGs) present in this layer create a special environment that favors LDL modification, and thus, affect proteolysis26,27 and oxidation28 of LDL. Moreover, arterial proteoglycans have been shown to induce irreversible changes in the structure of LDL particles,26 including changes in the conformation of apoB100 and in the organization of LDL lipids.26,29,30 We therefore modeled the effect of GAGs on the PLAr-induced aggregation of LDL particles by including in the incubation system heparin that interacts with LDL particles in a similar way to the GAGs in the arterial intima. The results showed that, in combination with heparin, treatment of LDL with PLAr induces fusion of the lipolyzed particles. Therefore, we suggest that in such areas of the arterial intima, in which LDL particles can interact with GAGs, lipolysis of LDL by PLAr leads to the formation of aggregated and fused particles characteristic for atherosclerotic lesions.

Methods

Materials

Essentially fatty acid free BSA, phospholipase A2 (from bee venom), and all lipids (lysoPC, PC, and sphingomyelin) were from Sigma. t-Butoxy carbonyl-L-[^3H] methionine N-hydroxysuccinimidyl ester (the ‘^3H-labeling reagent) was from Amersham. Superose 6 and 12 HR 10/30, HiTrap heparin (1 mL) and PD-10 columns were from Bio-Rad. human LDL (d=1.019 to 1.050 g/mL) was isolated from plasma of healthy volunteers by sequential ultracentrifugation in the presence of 3 mMol/L EDTA.31,32 ‘H-LDL was prepared by labeling the protein component of LDL by the Bolton-Hunter procedure33 with a ‘^3H-labeling reagent, as described previously.34 For each experiment, labeled LDL was diluted with unlabeled LDL to give the specific radioactivities indicated in the figure legends. The amount of LDL is expressed in terms of its protein concentration, which was determined by the method of Lowry et al.,35 with BSA as a standard.

Lipolysis of Untreated LDL With PLAr

Lipolysis of Heparin-Treated LDL With PLAr

Lipolysis of Heparin-Bound LDL With PLAr

Isolation of Aggregated/Fused and Monomeric LDL Particles by Gel Filtration

Electron Microscopy

For electron microscopy, aggregated/fused and monomeric particles of various H-LDL preparations were separated by gel filtration chromatography and the peak fractions were negatively stained. Samples (3 μL) were dried on carbon-coated grids, after which 3 μL of 1% potassium phosphotungstate, pH 7.4, was also dried on the grids.36 The negatively stained samples were viewed and photographed in a JEOL 1200EX electron microscope at the Institute for Biotechnology, Department of Electron Microscopy, University of Helsinki, Helsinki, Finland. For the determination of the size distribution of the LDL particles, the diameters of 200 randomly selected lipoprotein particles were measured from the electron micrographs.

Cellulose Acetate Electrophoresis

The net charge of the various LDL preparations was analyzed with cellulose acetate plates. These were equilibrated for 15 minutes with the Electra HR® Buffer and then heated the plate at 150°C for 20 minutes. The spots were visualized with Ponceau-S.

Analysis of the Phospholipid Composition

Lipid extracts37 of various LDL preparations were analyzed by thin layer chromatography (TLC) using chloroform/methanol/concentrated acetic acid/H2O (50:30:8:3.5, vol/vol/vol/vol). Individual lipid classes were visualized by dipping the TLC plate into CuSO4 (3%)/H3PO4 (8%) and then heating the plate at 150°C for 20 minutes. The spots were scanned with an automatic plate scanner (CAMAG TLC Scanner No. 3).

Analysis of ApoB100 Integrity

After lipolysis of the LDL samples with PLAr, 10 μL samples corresponding to 10 μg of LDL were run in Bio-Rad 4% to 20%
and aliquots of the reaction mixtures corresponding to 100 μg LDL were run in buffer B through a Superose 6 HR 10/30 column. The flow rate was 0.5 mL/min, and 0.5-mL fractions were collected. The radioactivities of the fractions were measured (A). Samples (3 μL) of the fractions with the highest radioactivity in peaks I and II were negatively stained and studied with an electron microscope (insets in B to D). The diameters of 200 randomly selected particles were measured from the micrographs of each LDL peak: B, control LDL; C, lipolyzed particles in peak I; D, lipolyzed particles in peak II. The bar under the inset in B represents 100 nm.

**Results**

To study the effect of lipolytic modification of LDL on aggregation/fusion of the particles, 1 mg/mL of 3H-LDL was incubated at 37°C with or without 50 ng/mL PLA2 in the presence of 2% BSA for 24 hours. The PLA2 activity was then inhibited by addition of EDTA, and aliquots of the reaction mixtures corresponding to 100 μg of LDL were run in buffer B through a Superose 6 HR 10/30 column. The flow rate was 0.5 mL/min, and 0.5-mL fractions were collected. The radioactivities of the fractions were measured (A). Samples (3 μL) of the fractions with the highest radioactivity in peaks I and II were negatively stained and studied with an electron microscope (insets in B to D). The diameters of 200 randomly selected particles were measured from the micrographs of each LDL peak: B, control LDL; C, lipolyzed particles in peak I; D, lipolyzed particles in peak II. The bar under the inset in B represents 100 nm.

To analyze the morphology of the PLA2-treated LDL in greater detail, the fractions with the highest radioactivity in peaks I and II were examined and photographed with an electron microscope after negative staining of the particles. The size distribution of 200 randomly selected LDL particles was determined from the electron micrographs (Figure 1B to 1D). The individual lipolyzed LDL particles, eluting both in peak I and in peak II, were slightly smaller than the control LDL, showing that some (peak I) of the lipolyzed LDL particles had formed aggregates. The mean diameters (±SD) of the LDL particles in peak I and peak II were 18 nm (±3 nm, median 17 nm) and 19 nm (±3 nm, median 18 nm), respectively, whereas the mean diameter of the control LDL was 22 nm (±2 nm, median 23 nm). In summary, lipolysis of LDL with PLA2, in the presence of physiological concentration of albumin, decreases the size of individual LDL particles and induces aggregation, but not fusion, of these smaller-sized particles.

Next, the effect of GAGs on the lipolytic modification of LDL was studied by comparing the effect of PLA2 on LDL that had been released from the heparin-Sepharose column by treatment with high salt concentration (heparin-treated LDL), and on LDL that was bound to heparin-Sepharose beads (heparin-bound) as described in the Methods. Heparin-treated and heparin-bound 3H-LDL were incubated in the presence of 2% BSA with or without PLA2 for 24 hours. It was found that PLA2 had hydrolyzed all the PC molecules of heparin-treated LDL, showing that some (peak I) of the lipolyzed LDL particles had formed aggregates. The mean diameters (±SD) of the LDL particles in peak I and peak II were 18 nm (±3 nm, median 17 nm) and 19 nm (±3 nm, median 18 nm), respectively, whereas the mean diameter of the control LDL was 22 nm (±2 nm, median 23 nm). In summary, lipolysis of LDL with PLA2, in the presence of physiological concentration of albumin, decreases the size of individual LDL particles and induces aggregation, but not fusion, of these smaller-sized particles.

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and heparin-bound $^3$H-LDL. Aliquots of the reaction mixtures were analyzed on a gel filtration column. It appeared that 45% of the lipolyzed heparin-treated $^3$H-LDL (Figure 2A) eluted in the void volume of the column (peak I). The size distribution of the negatively stained particles in the peak fractions (Figure 2B to 2D) revealed that the mean diameters of the control LDL (21 nm $\pm$ 2 nm, median 21 nm) and lipolyzed heparin-treated LDL particles eluting in peak I (21 nm $\pm$ 4 nm, median 21 nm) were the same. A minor portion (~3%) of the individual lipolyzed particles in peak I were larger than the biggest particles in the control LDL (28 nm) and some particles (~10%) were smaller than the smallest particles in the control LDL (17 nm). However, the majority of the LDL particles eluting in the void volume of the column (peak I) had diameters within the size range of native LDL, and therefore they must have formed aggregates consisting of individual LDL particles. The lipolyzed LDL particles eluting in peak II were smaller than control LDL, with a mean diameter of 18 nm ($\pm$ 3 nm, median 18 nm). Of the lipolyzed heparin-bound LDL particles eluting in peak I, ~50% were larger than the biggest particles in the control LDL (26 nm), the mean diameters of these individual fused particles being 31 nm. The rest of the particles that eluted in the void volume of the column were regarded as aggregates because their mean diameter was 23 nm. In summary, interaction of LDL with heparin seems to be a prerequisite for the PLA$_2$-induced fusion of the LDL particles.

Next, we studied the effect of heparin both on the reaction kinetics of LDL lipolysis and on the aggregation/fusion of lipolyzed LDL. Untreated $^3$H-LDL, heparin-treated $^3$H-LDL, and heparin-bound $^3$H-LDL were incubated with PLA$_2$; in the presence of 2% BSA. At the time points indicated, lipolysis was stopped by addition of EDTA, and the fatty acids produced during lipolysis were measured. As shown in Figure 3A, neither heparin pretreatment nor the presence of heparin during lipolysis markedly altered the reaction kinetics. Analysis of the composition of the phospholipids in the lipolyzed LDL samples showed that, after lipolysis for 24 hours, all the PC molecules in each LDL preparation were completely hydrolyzed to lysoPC. The lipolyzed particles of untreated LDL, which were aggregated, contained less lysoPC than the corresponding monomeric particles. In contrast, nearly all of the lysoPC formed remained bound to both monomeric and aggregated/fused particles of the lipolyzed heparin-treated and heparin-bound LDL (data not shown). Furthermore, 90% of the fatty acids that had been released from the particles in

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**Figure 2.** Gel filtration chromatography and the size distribution of PLA$_2$ lipolyzed heparin-treated and heparin-bound LDL. Heparin-treated $^3$H-LDL, 100 $\mu$g, and 200 $\mu$g of heparin-bound $^3$H-LDL (1 mg/mL, 2000 dpm/$\mu$g LDL) were incubated at 37°C for 24 hours with (lipolyzed LDL) or without (control LDL) PLA$_2$ as described in the Methods. Lipolysis was stopped by addition of EDTA, and aliquots of the reaction mixtures were run in buffer B through a Superose 6 HR 10/30 column. The radioactivities of the fractions were measured (heparin-treated, A; heparin-bound, E) and the peak fractions were examined in an electron microscope. B, control LDL for the heparin-treated LDL; C, lipolyzed heparin-treated LDL in peak I; D, lipolyzed heparin-treated LDL in peak II; F, control LDL for the heparin-bound LDL; G, lipolyzed heparin-bound LDL in peak I; H, lipolyzed heparin-bound LDL in peak II. The bar under the inset in B represents 100 nm.
addition of EDTA, and the anodal migration of the samples time points during the incubation, lipolysis was stopped by PLA₂, we performed an experiment in which ³H-LDL was incubated with PLA₂ (50 ng/mL) in buffer A containing 2% BSA. After incubation at 37°C for the indicated times, lipolysis was stopped by addition of EDTA. Twenty-microliter samples of the supernatants were taken for analysis of the fatty acids generated during lipolysis (A). The degree of aggregation/fusion of the ³H-LDL (B) was determined by running samples of the reaction mixtures in buffer B through a Superose 6 HR 10/30 column and calculating the ratio of the radioactivity in the void volume peak to the total eluted radioactivity. The degree of aggregation/fusion is expressed as a percentage: (radioactivity in void volume/total eluted radioactivity)×100.

To evaluate the effect of the degree of lipolysis on the aggregation/fusion of the lipolyzed particles, aliquots of the various LDL samples were analyzed with gel filtration chromatography (Figure 3B). It was found that the degree of aggregation/fusion reached a maximum of 50% to 60% rapidly during LDL lipolysis. Surprisingly, further lipolysis slightly decreased the degree of aggregation/fusion. In an additional experiment with identical design, we inhibited the enzyme activity with EDTA after lipolysis for 60 minutes, and continued the incubation at 37°C for an additional 2 hours. The degree of aggregation/fusion then remained maximal, showing that the decrease depended on the ongoing PC lipolysis of the LDL particles (data not shown). When the above experiments were repeated with LDL from various donors, the degree of aggregation/fusion was found to vary 16% to 60%. The degree of lipolysis, however, did not depend on the donor of LDL.

If albumin is not added to the incubation medium, the lipolytic products of PLA₂ activity, fatty acids and lysoPCs, accumulate in the LDL particles.³⁹ To analyze the effect of accumulating lipolytic products on the modification of LDL by PLA₂, we performed an experiment in which ³H-LDL was incubated with PLA₂ in the absence of albumin. At various time points during the incubation, lipolysis was stopped by addition of EDTA, and the anodal migration of the samples all 3 LDL preparations could be recovered from the albumin molecules. Analysis by SDS-PAGE and determination of the amount of trichloroacetic acid-soluble apoB100 ³H-radioactivity revealed that in all the samples apoB100 remained intact (data not shown).

The accumulation of lipolytic products and REM of lipolyzed LDL. One hundred micrograms of untreated ³H-LDL (1 mg/mL, 1000 dpm/µg LDL) was incubated with PLA₂ (50 ng/mL) and without BSA at 37°C for the times indicated. Incubation was stopped with EDTA and the fatty acids generated during lipolysis were measured. A duplicate of the 4-hour lipolyzed LDL was incubated for an additional 15 minutes with BSA, after which LDL and BSA were separated on a Superose 12 HR 10/30 column, and the amount of fatty acids in the LDL was measured. Samples of the reaction mixtures were taken for analysis of REM of the lipolyzed LDL in cellulose acetate electrophoresis.

Finally, the effect of the reaction products accumulating in the lipolyzed LDL on aggregation/fusion of the particles was analyzed. For this purpose, untreated ³H-LDL, heparin-treated ³H-LDL, and heparin-bound ³H-LDL were incubated with or without PLA₂ in the absence of BSA. After incubation for 24 hours, lipolysis was stopped with EDTA and the amounts of fatty acids were measured. During this period, all the PC molecules of the lipolyzed LDL had been hydrolyzed into fatty acid and lysoPC. The degree of aggregation/fusion of the lipolyzed samples was determined by gel filtration chromatography. After lipolysis of untreated (Figure 6A) or heparin-treated LDL (Figure 6B), aggregation was only minimal, whereas the particles of the lipolyzed heparin-bound LDL, which remained bound to heparin after lipolysis, were able to aggregate (Figure 6C). In contrast, the LDL particles that had become detached during lipolysis did not aggregate unless they were treated with albumin (data not shown).
Analysis of the morphology of the lipolyzed heparin-bound LDL particles showed that the mean diameter of the particles, whether in peak I or peak II, was not changed (data not shown). Thus, even in the absence of albumin, ie, when the lipolytic products accumulated in the particles, heparin-bound lipolyzed LDL aggregated, but did not fuse.

**Discussion**

This study shows that lipolysis of LDL phospholipids with PLA₂ in the presence of albumin induces particle aggregation. Albumin has been shown to sequester fatty acids and lysoPC, the lipolytic products of PLA₂ activity, from the LDL particles. In the absence of albumin, these lipolytic products accumulated on LDL particles and increased their negative charge, thereby preventing particle aggregation. However, if LDL was bound to heparin during lipolysis, the particles aggregated even in the absence of albumin. Interestingly, the effect of heparin on the PLA₂-induced changes in LDL morphology was prominent also in the presence of a physiological concentration of albumin: among the aggregates of heparin-treated and, particularly, heparin-bound LDL, fused particles appeared.

Interactions between native LDL particles do not result in their aggregation or fusion. However, if the surface structure of the particles is modified, these interactions may promote aggregation, which may then lead to subsequent particle fusion. Lipolysis of LDL by PLA₂, particularly if albumin is present in the reaction mixture and the products of PC lipolysis, fatty acid and lysoPC molecules, can leave the LDL particles, has been shown to alter the structure of the particles: both conformational changes in the apoB100 component at the surface and a reorganization of lipids have been reported. The present results show that PLA₂ lipolysis of untreated LDL leads to aggregation of some of the lipolyzed particles in the presence of albumin, but does not trigger particle fusion. This finding indicates that even though PLA₂ lipolysis is able to affect the outcome of LDL particle interactions, the resultant structural changes are not sufficient to promote fusion of the particles. In fact, we found, consistent with previous reports, that in electron microscopy the lipolyzed LDL particles were slightly smaller than the native particles. The decrease in particle size and the accompanying reorganization of the lipids result in particles in which the proportion of core lipids at the surface monolayer is increased. There is evidence that this kind of lipoprotein lipid reorganization leads to a surface lipid environment that is more rigid and has a decreased mobility in comparison to native particles. Therefore, whereas the surface hydrophobicity of such modified LDL particles may have increased their tendency to aggregate, it seems that the enhanced structural rigidity of the particles stabilizes the aggregates and precludes particle fusion.

In contrast to lipolysis of untreated LDL, lipolysis of heparin-bound LDL, and even heparin-treated LDL, induced fusion among the aggregated particles. The interactions of LDL particles with proteoglycans and GAGs have also been reported to cause changes in both the apoB100 component and the lipid pool of the particles. The interactions of LDL particles with GAGs have been shown to induce such conformational changes in apoB100 that increase the exposure of arginine- and lysine-containing segments and, in contrast to the PLA₂ effect on the lipid pool of LDL, decrease the organization of the core and surface regions of the particles. These structural changes induced by GAGs do not alone lead to aggregation or fusion of LDL, but they have...
been shown to accelerate both proteolytic\textsuperscript{27,28} and oxidative modifications\textsuperscript{28} of the particles. The current results also revealed that if the structure of the LDL particles was altered by heparin-induced changes, the PLA\textsubscript{2} lipolysis produced fused particles within the aggregates of LDL. The relative importance of the structural changes of LDL apoB100 and the reorganization of LDL lipids on particle aggregation is still unclear. It seems likely, however, that the destabilization of the particles induced by heparin\textsuperscript{26,30,45} is an important event to outweigh the rigidifying effect of PLA\textsubscript{2} on LDL, and thereby to trigger fusion of the lipidized particles.

The first visible change during atherogenesis is the focal accumulation of lipid droplets in the extracellular space of the arterial intima.\textsuperscript{4,5} There is substantial evidence supporting the idea that the droplets are derived directly from modified LDL that lipolysis of untreated LDL, in the presence of albumin, had interacted with heparin or was bound to heparin, PLA\textsubscript{2} lipolysis produced also fused particles. The mean diameter of the fused heparin-bound LDL particles was 31 nm, the largest particles being 44 nm. These fused particles were within the lower size range of the extracellular lipid droplets (30 to 400 nm)\textsuperscript{8} and the size distribution of lipolyzed heparin-bound LDL closely resembled the size distribution of LDL isolated from human atherosclerotic lesions.\textsuperscript{47}

Recently, secretory PLA\textsubscript{2} has been found in atherosclerotic human arteries\textsuperscript{22,23} and, very recently, it has been shown to be located in atherosclerotic lesions, especially in places where apoB100 containing lipoproteins and lipid droplets are trapped.\textsuperscript{24} Because there is evidence that hydrolysis of LDL phospholipids with PLA\textsubscript{2}-like activity is one of the earliest modifications of LDL in the arterial intima,\textsuperscript{16,17,20} the early PLA\textsubscript{2} activity may have a proatherogenic role in vivo. In fact, it has been shown that pretreatment of LDL with PLA\textsubscript{2} in vitro increases its susceptibility to lipoproteinase-mediated oxidation\textsuperscript{48} and SMase-induced lipolysis\textsuperscript{49} and promotes aggregation and fusion of SMase-treated LDL.\textsuperscript{25} It has also been shown by Öörni et al, that lipolysis of LDL by PLA\textsubscript{2} increases the binding strength of aggregated lipidized LDL to extracellular proteoglycans\textsuperscript{25} and can thereby increase retention of LDL in the arterial intima, and thus increase the probability for further modifications. In addition, hydrolysis of LDL PCs by PLA\textsubscript{2} can simply promote atherogenesis because of the production of proinflammatory molecules, lysoPCs, and fatty acids.

In conclusion, colocalization of LDL and PLA\textsubscript{2} in the GAG meshwork of the arterial intima may promote several atherogenic processes, including fusion of LDL particles. The generation of enlarged LDL-derived lipid droplets and the concomitant lipid accumulation induced by PKA\textsubscript{2} could therefore be critical events during atherogenesis.

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References


Absence and Fusion in the Presence of Heparin
Induces Particle Aggregation in the
Lipolytic Modification of LDL by Phospholipase A2

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