Hypertension exacerbates the effect of hypercholesterolemia on the myocardial microvasculature

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Abstract

Objective: Hypercholesterolemia (HC) and hypertension (HT) are both major risk factors for the development and progression of atherosclerotic heart disease, and their co-existence has been associated with an increased incidence of cardiac events in clinical studies. HC and HT are individually associated with abnormal myocardial vascular function, but whether HT exacerbates the HC-induced myocardial vascular dysfunction remains unclear. Methods: We studied in pigs the effect of renovascular HT superimposed on diet-induced HC (HC+HT) on myocardial perfusion and microvascular permeability in vivo (using electron-beam computed tomography) in response to cardiac challenge (i.v. adenosine and dobutamine). The involvement of systemic and myocardial tissue oxidative stress in vitro was assessed by oxidizability of LDL, levels of endogenous antioxidants, and tissue activities of radical–scavenger systems. Results: While in normal animals myocardial perfusion increased in response to i.v. adenosine (+36±13%, \(P<0.05\)), in HC and HT alone the increase was blunted. In HC+HT myocardial perfusion response was further attenuated and significantly lower than normal, and myocardial vascular resistance failed to decrease (+7.6±8.8 vs. −21.0±5.8%, \(P=0.02\) versus normal). HC+HT also showed blunted response to dobutamine, and augmented increases in microvascular permeability in vivo. These functional abnormalities were associated with increased systemic and myocardial tissue oxidative stress compared to HC or HT alone, and a synergistic decrease in endogenous antioxidant defenses in myocardial tissue. Furthermore, chronic antioxidant vitamin supplementation in combined HC and HT improved myocardial vascular responses. Conclusion: HT amplifies the HC-induced myocardial microvascular dysfunction in vivo and increased oxidative stress in vitro. These alterations may potentially play a role in the increased incidence of cardiac events observed when HC and HT co-exist.

Keywords: Atherosclerosis; Hypertension; Coronary circulation; Microcirculation; Regional blood flow

1. Introduction

Hypercholesterolemia (HC) is a major risk factor for coronary atherosclerosis, and has been associated with impairment in coronary vascular function and myocardial perfusion [1–3], preceding development of overt atherosclerosis and potentially leading to cardiac events [4]. HC often co-exists with other cardiovascular risk factors such as hypertension (HT), and their combination is associated with a yet greater increase in the incidence of cardiac events [5], but the underlying mechanisms of this phenomenon remain unresolved [6]. HT alone also impairs vascular function and myocardial perfusion [7], especially after development of left ventricular hypertrophy (LVH) [8]. However, the role of early HT (prior to the development of LVH) remains unclear. Furthermore, whether superimposition...
Impairment in myocardial vascular function may manifest in an array of functions controlled by the vascular wall, such as inflammation, angiogenesis, and barrier function. Indeed, alterations in microvascular permeability (MVP) [9] may be a measure of coronary endothelial dysfunction, and indicate loss of coronary vascular integrity [10]. One of the mechanisms that may be activated in both HC [1,11] and HT [12,13] and hinder myocardial vascular function is a shift in scavenging activity and redox status [14]. Increased vascular oxidative stress is characterized by a decrease in endogenous tissue or plasma antioxidants and bioavailability of nitric oxide, which may alter MVP [15]. Concurrent modification of low-density lipoprotein (LDL) to its oxidized form (ox-LDL) and its increased uptake by endothelial and intimal cells may also impair vascular function [16]. Thus, these mechanisms may conceivably play a role in the interaction between HC and HT to impair myocardial vascular function.

Elucidation of the deleterious mechanisms by which HC and HT interact and their functional significance could advance our understanding of the pathophysiology of coronary atherosclerosis and its early clinical manifestations. However, few available methods are capable of accurately and non-invasively measuring myocardial perfusion and vascular integrity in vivo. Electron-beam computerized tomography (EBCT) provides a unique tool to reliably [17] and reproducibly [18,19] study in vivo myocardial perfusion and MVP [14,20,21] non-invasively.

Therefore, the present study was designed to test the hypothesis that HT might exacerbate the impairment of myocardial perfusion and MVP response to increased cardiac demand observed in HC, and, furthermore, that these abnormalities are accompanied by greater alterations in redox status and scavenging activity.

2. Methods

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Domestic, pre-menstrual female pigs (55–65 kg) were studied after a 12-week either normal (n=9) or atherogenic (HC, n=9) diet of 2% cholesterol and 15% lard (TD-93296, Harlan-Teklad, Madison, WI). In additional pigs, unilateral renal artery stenosis was induced at baseline, followed by gradual development of renovascular hypertension 7–10 days later, as previously described [22–24]. A 12-week normal (HT, n=6) or HC (HC+HT, n=7) diet was then initiated at baseline.

EBCT in vivo studies were then performed to assess myocardial perfusion, MVP, and LV muscle mass. Myocardial functional studies were performed under resting conditions and repeated after cardiac challenge with i.v. adenosine and dobutamine, substances routinely used for cardiac stress testing. Subsequent in vitro studies included plasma lipids (Roche, Nutley, NJ) and renin activity (PRA, New England Nuclear, Boston, MA), and redox status (LDL oxidizability, systemic and tissue endogenous antioxidant levels, and tissue radical scavenger enzymes activities).

2.1. In vivo studies

2.1.1. EBCT scans

Animals were anesthetized with ketamine and xylazine, intubated, and ventilated. Anesthesia was maintained with constant infusion of ketamine (17.5 mg/kg per h) and xylazine (2.3 mg/kg per h). Catheters were placed fluoroscopically in the aorta, for measurement of mean arterial pressure (MAP), and right atrium (for contrast media injections) [14]. Animals were then positioned in the EBCT (C-150, Imatron, San Francisco, CA), and blood samples collected. Two mid-LV levels were then identified, and a baseline myocardial functional (perfusion and MVP) study performed. Forty consecutive end-diastolic scans were obtained [20] during 40 s (at one to three heart beat intervals) after a 2-s injection of iopamidol-370 (Squibb, Princeton, NJ, 0.3 cc/kg) into the right atrium [14]. This was followed 15 min later by a myocardial muscle mass study, for which eight end-diastolic tomographic scans (from LV apex to base) were acquired simultaneously [17] during infusion of iopamidol (4 ml/s over 7 s). Myocardial functional studies were then repeated at 20–30-min intervals towards the end of a 10-min i.v. infusion of either adenosine (400 µg/kg per min) or dobutamine (15 µg/kg per min, to a target heart rate of 150 bpm) [21]. Studies were performed after hemodynamic stabilization, since infusion of i.v. adenosine produced a transient decrease in blood pressure, while infusion of i.v. dobutamine was associated with a transient increase in blood pressure, both of which returned to pre-infusion levels within 5–10 min. After a 5-day recovery from in vivo studies, animals were killed by 100 mg/kg i.v. pentobarbital sodium (Fort Dodge Laboratories, IA). Coronary and myocardial tissue was flash-frozen and preserved at −80 °C or in formalin.

2.1.2. EBCT data analysis

For measurement of myocardial vascular function, regions-of-interest were traced in the anterior cardiac wall and LV chamber (Fig. 1, top) [20]. For transmural distribution, the myocardial region-of-interest was further subdivided into equidistant sub-epicardium and sub-endocardium. Time-density curves were then generated and the intra-vascular and extra-vascular transit of contrast media [14,20] was modeled (Fig. 1, bottom). The area and first moment of each curve were then calculated.

Myocardial perfusion (ml/min per g) was calculated [14,20,21] as: \( 60 \times (BV/MTT)/(1.05 \times (1-BV)) \), where BV is intramyocardial blood volume, MTT is the first pass time.
moment (index of mean transit time), and 1.05 g/cc is the specific density of the myocardium. Myocardial blood flow was subsequently calculated as perfusion×LV muscle mass. Perfusion of the sub-endocardial and sub-epicardial regions was similarly obtained, and their ratio (endocardial/epicardial) calculated.

MVP (arbitrary units; AU) was calculated as [14,21,25]: 60×1.05×[slope of extravascular curve×MTT]/Area under input curve/BV, where slope is the maximal slope of the ascending arm of the extravascular curve. BV was used as a surrogate for vascular surface area [26].

For LV muscle mass, the epicardial and endocardial LV surfaces were traced at end-diastole, and the product of myocardial muscle area, density, and slice thickness calculated [17]. Then, myocardial vascular resistance (MVR) was then calculated as: 80×MAP/myocardial blood flow.

2.2. In vitro studies

2.2.1. Oxidative status

LDL oxidizability was assessed as previously detailed [14,24,27]. LDL rapidly isolated from plasma was incubated with 1 μM copper sulphate. Malondialdehyde (MDA) content was assayed by the thiobarbituric acid assay, and relative electrophoretic mobility (REM) on agarose gel (0.8%) was assessed. Lag-time was measured spectrophotometrically, and vitamin E (α-tocopherol) and C (ascorbate) concentrations in plasma and tissue were determined by HPLC.

Tissue scavenging activities of catalase, glutathione peroxidase, and the copper-zinc (CuZn)-superoxide dismutase (SOD) and manganese (Mn)-SOD were determined spectrophotometrically in the myocardium and coronary tissue, and normalised for protein content [14,23].

2.3. Histomorphometry

H&E-stained coronary artery histological cross-sections obtained from the four different groups were used for morphometric analysis using a computer image analysis program. The intima and media layers were defined by the borders of the internal and external elastic lamina, respectively. Areas for each region were traced and calculated in
square millimeters. To correct for vessel size, areas were normalized for lumen area.

2.4. Antioxidant supplementation

To further explore the role of oxidative stress in co-existent HC and HT, additional animals with combined HC and HT received chronic daily antioxidant supplementation (vitamin E 100 IU/kg and vitamin C 1000 mg, n=6) [14,27]. A combination of vitamin E and vitamin C provides synergistic blockade of the endogenous oxidative stress system [28,29], and we have also previously shown that this regimen was highly effective for this purpose [14,27]. After 12 weeks of diet and intervention, animals were instrumented and in vivo studies performed using EBCT to measure myocardial perfusion and microvascular permeability) at baseline and in response to i.v. adenosine and i.v. dobutamine, as described above.

2.5. Statistical analysis

Data are mean±S.E.M. Comparisons among the groups were performed using ANOVA, followed by Bonferroni corrected t-test, and within groups using paired Student’s t-test. Regressions were calculated by least squares. Statistical significance was accepted for \(P<0.05\). Synergism was tested using linear regression with an interaction term (JMP statistic program 4.0).

3. Results

Cholesterol levels were significantly and similarly increased in HC and HC+HT pigs (Table 1). Baseline MAP was similarly and significantly increased in HT and HC+HT (Table 1), but heart rate (\(P=0.58\)), LV muscle mass (\(P=0.8\)) and PRA (\(P=0.8\)) were similar among all groups. There were no significant differences among the normal, HC, HT, and HC+HT groups in MAP response to either i.v. adenosine or dobutamine. Similarly, there was no difference in heart rate response to i.v. adenosine. As expected by protocol design, the heart rate in response to i.v. dobutamine was significantly higher compared to baseline, and that change was similar among the groups.

The media to lumen ratio in animals with either HC or HT alone was similar to normal (normal, 1.02±0.11; HC, 0.90±0.12; HT, 1.04±0.11, respectively, \(P=\text{NS}\)). However, in HC+HT the medial/lumen ratio was significantly higher compared to normal, HC, or HT animals (1.37±0.14, \(P<0.03\) compared to all other groups). There was no difference in the intima to lumen ratio among the four groups (normal, 0.99±0.0023; HC, 0.86±0.012; HT, 0.108±0.023; and HC+HT, 0.115±0.025. \(P=0.33\), \(P=0.40\), and \(P=0.315\) compared to normal, respectively).

3.1. In vivo studies

3.1.1. Myocardial perfusion

Basal anterior wall perfusion was similar in the normal, HC, HT, and HC+HT groups (1.02±0.1, 0.78±0.1, 1.01±0.2, and 1.15±0.3 ml/min per g, respectively, \(P=0.4\)). Adenosine induced a significant increase in myocardial perfusion in normal animals (\(P=0.02\), Figs. 1 and 2, top right panel), which was accompanied by a significant decrease in MVR (−21.0±5.8%, \(P<0.05\)). The myocardial perfusion response to adenosine was attenuated in HC, HT and HC+HT animals (Figs. 1 and 2, top right panel). Furthermore, in response to i.v. adenosine HC+HT animals had a decrease in myocardial perfusion (−14.0±3.9% compared to baseline, \(P<0.05\), and only in the HC+HT group the degree of attenuation in myocardial perfusion response was significantly different from the response observed in normal animals (\(P<0.05\), Figs. 1 and 2, top right panel), as was the blunted MVR response (+7.6±8.8%, \(P=0.02\) versus normal). Dobutamine significantly increased myocardial perfusion and decreased MVR in all the groups (all \(P<0.05\)), although in HT myocardial perfusion response tended to be attenuated (Fig. 2, bottom right, \(P=0.07\) versus normal). Moreover, only in HC+HT myocardial perfusion response to dobutamine was again significantly blunted compared to normal (\(P=0.05\), Figs. 1 and 2, bottom right panel) and

Table 1

<table>
<thead>
<tr>
<th>Lipids</th>
<th>Normal</th>
<th>HC</th>
<th>HT</th>
<th>HC+HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>1.68±0.13</td>
<td>9.86±1.75*</td>
<td>1.73±0.15</td>
<td>9.68±0.75*</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>0.55±0.08</td>
<td>7.63±1.53*</td>
<td>0.95±0.18</td>
<td>8.52±0.60*</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1.10±0.08</td>
<td>2.37±0.54*</td>
<td>0.78±0.13</td>
<td>2.25±0.44*</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>0.70±0.13</td>
<td>0.89±0.09</td>
<td>0.72±0.10</td>
<td>0.79±0.14</td>
</tr>
<tr>
<td>Plasma renin activity (ng/ml per h)</td>
<td>0.41±0.2</td>
<td>0.56±0.0</td>
<td>0.32±0.1</td>
<td>0.30±0.2</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>97±4</td>
<td>99±2</td>
<td>124±6*</td>
<td>121=9*</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>81±4</td>
<td>72±5</td>
<td>82±4</td>
<td>77±9</td>
</tr>
<tr>
<td>LV muscle mass (g)</td>
<td>124±9</td>
<td>124±5</td>
<td>123±2</td>
<td>130±6</td>
</tr>
</tbody>
</table>

\(\text{*P}<0.05\) versus normal and HT; \(\text{†P}<0.05\) versus normal and HC.

LDL, low-density lipoprotein; HDL, high-density lipoprotein; LV, left ventricle.
MVR response tended to be attenuated as well (−25.1±7.2 vs. −53.5±17.6%, \(P=0.09\)), suggesting greater impairment than in HC or HT alone.

Trans-mural perfusion ratio (endocardial/epicardial) appeared to be lower in HT and HC+HT compared to normal and HC, but using ANOVA this has not reached statistical significance either under basal conditions (1.07±0.09 and 0.94±0.08 vs. 1.25±0.19 and 1.32±0.12, respectively, \(P=0.2\)) or during both challenges.

3.1.2. Myocardial MVP

Basal MVP was similar in the four groups (1.55±0.2, 1.35±0.2, 1.54±0.2, and 1.01±0.1 AU, respectively, \(P=0.3\)). In response to adenosine, MVP remained unchanged in normal and HT pigs (Fig. 2, top left panel). However, in HC adenosine infusion induced a significant increase in MVP (\(P=0.005\)), similar to that observed in HC+HT (\(P=0.01\), Fig. 2, top left panel). Dobutamine significantly increased MVP in all groups (\(P<0.03\)). However, the degree of this increase was significantly greater in HC+HT compared to the other groups (\(P=0.04\), Fig. 2, bottom left panel).

3.2. In vitro studies

3.2.1. Redox status

In HC pigs, but not in HT, plasma and tissue levels of the endogenous antioxidant vitamins E and C were significantly decreased compared to normal, associated with a significant increase in LDL propensity for oxidation (increased LDL-REM and -MDA, and shortened LDL-lag time), and decreased myocardial levels of all the intracellular radical scavenger enzymes (Table 2). In HT pigs glutathione peroxidase and CuZn-SOD were also significantly decreased (Table 2). Nonetheless, in HC+HT the decrease in plasma levels of vitamins E and C and LDL oxidation were significantly greater compared to either HC or HT alone (Table 2), as were myocardial levels of the scavenger radical enzymes, and those of catalase and CuZn-SOD achieved statistical significance (Table 2). Furthermore, the deleterious decrease in tissue levels of...
3.3. Antioxidant supplementation

Animals with HC and HT that received chronic antioxidant vitamin supplementation had similar levels of baseline MAP (120.3 ± 3.3 mmHg) and cholesterol levels (total, 9.22 ± 1.19 and LDL, 6.82 ± 0.96 mmol/l) compared to untreated HC+HT (all P = NS), as well as comparable systemic hemodynamic responses to both adenosine and dobutamine (data not shown). Baseline myocardial perfusion and coronary flow (perfusion, 1.01 ± 0.07 ml/min per g tissue; coronary blood flow, 1.00 ± 0.13 AU) was similar to HC+HT animals (P = 0.31 and 0.34, respectively). In response to i.v. adenosine, there was an improvement in the responses of both the myocardial perfusion (to 1.06 ± 0.03 ml/min tissue, P = 0.04 compared to HC and HT, P = 0.063 compared to normal, Fig. 3, top left panel) and MVR (−12.11 ± 6.7% compared to baseline, Fig. 3, bottom left panel). In addition, a significant improvement was found in the MVP responses in these animals (to 1.33 ± 0.12 AU, P = 0.06 compared to baseline, and P = 0.018 compared to HC+HT). Significant, albeit less pronounced improvement was also found in response to i.v. dobutamine (Fig. 3, right panel).

4. Discussion

This study demonstrates, for the first time, that superimposition of a short-term (12 weeks) HT on HC is associated with marked impairment in myocardial perfusion and coronary flow responses to increased cardiac demand. The functional impairments were associated and correlated with coexisting measures of increased systemic LDL oxidizability and decreased levels of endogenous antioxidants, and were significantly improved upon chronic antioxidant supplementation. The cardiovascular alterations were significantly augmented compared to either risk factor alone, and may play a role in the ‘cross-talk’ between them to increase the incidence of cardiac events.

HC and HT are major risk factors for coronary atherosclerosis [30], and clinically their coexistence is associated with increased incidence of cardiac events, even in the absence of significant atherosclerotic coronary artery disease [4]. Each can impair coronary vascular function [3], lead to myocardial perfusion defects [2], and increase the incidence of cardiac events [4]. However, whether superimposition of early HT on HC exacerbates myocardial microvascular abnormalities remained unclear. We have previously shown in the pig model that relatively short exposure to cardiovascular risk factors was not accompanied by development of advanced vascular lesions [14,31]. The present study corroborates our previous findings and further demonstrates that while short-term mild HT alone did not have a significant structural impact on the coronary arterial wall, in combined HC and similar HT there was arterial wall remodeling and increased media thickness. The presence of such changes only in the HC+HT group likely represent an acceleration of the remodeling process when the two risk factors co-exist, and suggests the existence of an interaction among the different pathological mechanisms triggered by these risk factors.
These findings were associated with a more pronounced impairment in myocardial perfusion and MVR responses to challenge, further underscoring a cross-talk between these two cardiovascular risk factors that may potentially contribute to increased incidence of cardiac events. Homogeneity of this impairment throughout the cardiac wall was suggested by the unaltered transmural distribution of perfusion. The slight differences in myocardial perfusion response to i.v. adenosine and dobutamine in HC+HT might reflect different degrees of cardiac stress achieved and different mechanisms of action of these cardiac challenges. Intravenous adenosine has a greater flow-mediated endothelium-dependent component [32] that may initially be more impaired in HC, while dobutamine has a more direct impact on vascular smooth muscle cells [33], which may be more impaired in HT. Nevertheless, amplification of vascular functional impairments elicited by early but concurrent HC and HT was probably responsible for the markedly blunted myocardial perfusion responses detected in HC+HT.

The barrier function of the vascular wall may be regarded as a parameter of vascular integrity and endothelial function. HC can transiently dissociate adjoining endothelial cells [34] and dynamically change endothelial permeability [35], as previously shown by our group [14,21]. The effect of HT on MVP has not been fully explored, and our study indicates that early HT may not have a major effect on this parameter, and the MVP response to adenosine in HC+HT was therefore similar to that in HC pigs. Interestingly, the substantial challenge imposed by dobutamine revealed significantly impaired MVP response in the HC+HT group. Indeed, the observed MVP increase might be related to the degree of concurrent myocardial ischemia [36]. The alterations in MVP response in vivo may reflect or compel abnormal delivery and deposition of blood-borne substances in the myocardium or vascular wall.

One of the mechanisms underlying vascular dysfunction may be alteration in systemic and/or tissue oxidative status that both HC and HT individually elicit. HC is characterized by increased propensity for LDL oxidation and decreased activity of endogenous antioxidant and radical scavenger systems [37]. Increased oxidative stress can decrease the bioavailability of nitric oxide and tilt the balance between vasodilators and vasoconstrictors in HC [15,38] and HT [13]. This study shows that in pigs with a...
relatively short exposure, HC constitutes a greater impetus than HT for increased plasma and tissue oxidation. Nevertheless, HT superimposed on HC accentuated the alterations of redox status and scavenging activity, potentially contributing to the abnormal myocardial vascular function. Furthermore, increased LDL oxidizability might have also provoked myocardial vascular dysfunction, since ox-LDL directly impairs vascular reactivity [39]. Our study supports this notion by showing that in HC+HT all the measures of plasma LDL oxidizability were increased compared not only to normal animals, but also to each condition alone. A concerted and more pronounced action of increased oxidative stress and inflammatory changes [24,40] likely contributed to the arterial wall changes observed in combined HC+HT. Indeed, although the differences among the groups in oxidative stress indices may not appear to be substantial, they likely conferred significant impairment in vascular function, as the redox balance is very tightly controlled [41], and small deviations may bear significant functional consequences. The association between increased oxidative stress and myocardial vascular dysfunction is also supported by the significant correlation observed between measures of the two, e.g., levels of vitamin E and LDL-MDA (an index of lipid peroxidation) and myocardial perfusion and MVP responses to challenge. Lastly, the involvement of oxidative stress in combined HC and HT is further underscored by the significant improvement in myocardial vascular function observed during chronic antioxidant supplementation. Nevertheless, the lack of complete normalization in myocardial perfusion responses in HC+HT may signify the activation of additional mechanisms other than oxidative stress, or irreversible changes.

Measurements of myocardial perfusion were obtained in this study using an EBCT technique, which has been previously validated using both radioactive microspheres [17,42] and intracoronary Doppler [18,19]. Although EBCT might underestimate very high myocardial flow rates (>2.5 ml/min per g), this was unlikely to interfere with our results, since in a physiological range of myocardial perfusion, like those observed in the current study, EBCT measurements agree well with reference standards. The modest response to adenosine may be due to species variability in the response to administered dose, as our [14,21] and other [43] previous studies showed that in the pig model i.v. administration of adenosine at doses that elicit greater myocardial perfusion responses was associated with substantial systemic hemodynamic alterations. Nonetheless, our measurements disclosed significant differences in myocardial perfusion responses among experimental groups with different cardiovascular risk factors. Furthermore, the EBCT technique enabled concomitant examination of transmural distribution and detection of changes in MVP, and allowed subsequent ex vivo utilization of tissue obtained from the same animals to support functional significance of in vitro observations. Studies were performed in juvenile pre-menstrual pigs that have a mature cardiovascular system [44,45] but minimal influence of sex hormones. Nevertheless, developmental changes throughout the course of the study, or effects of maturation on their degree of response to HC or HT, cannot be excluded.

In summary, this study demonstrates that a 12-week combination of HC and HT accentuates impairment in myocardial perfusion and microvascular permeability responses to cardiac challenge. This functional impairment was associated and correlated with marked alterations in systemic and myocardial oxidative status, LDL oxidizability, and scavenging activity, albeit without establishing a causal relationship. Such interaction may partially account for increased incidence of cardiac events observed in concurrent HC+HT, and longer exposure may also accelerate development of coronary atherosclerotic lesions. Further elucidation of underlying molecular mechanisms involved in this interaction may provide important insight into the pathophysiological framework involving early vascular dysfunction, atherogenesis, and coronary heart disease.

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