[Original article]

Angiotensin II increases coronary fibrosis, cardiac hypertrophy and the incidence of myocardial infarctions in ApoE^{-/-}Fbn1^{C1039G+/-} mice

Lynn ROTH, PharmD, PhD; Dorien M. SCHRIJVERS, PhD; Wim MARTINET, PhD;
Guido R.Y. DE MEYER, PharmD, PhD

Laboratory of Physiopharmacology, University of Antwerp, Antwerp, Belgium.

Objective Apolipoprotein E deficient mice (ApoE^{-/-}) with a heterozygous mutation in the fibrillin-1 gene (Fbn1^{C1039G+/-}) develop increased arterial stiffness, accelerated plaque progression, spontaneous plaque ruptures, myocardial infarction (MI) and sudden death. In the present study we used angiotensin II (Ang II) infusion to assess the responsiveness of the ApoE^{-/-}Fbn1^{C1039G+/-} mouse model to risk factors of atherosclerosis, such as high blood pressure and inflammation.

Methods and results Osmotic minipumps filled with phosphate buffered saline or Ang II (1,000 ng/kg/day) were implanted in ApoE^{-/-}Fbn1^{C1039G+/-} mice at week 14 of Western diet (WD). After 4 weeks of therapy, we were not able to detect an increase in blood pressure, but we observed severe aneurysms in both the thoracic and abdominal aorta of Ang II treated mice. Plaque size and composition in the proximal ascending aorta was not altered. Nevertheless, coronary fibrosis, cardiac hypertrophy and a 6-fold increase in the occurrence of MI in Ang II-treated mice was observed, which did not result in a higher mortality.

Conclusion The study showed that Ang II treatment in ApoE^{-/-}Fbn1^{C1039G+/-} mice increased cardiac hypertrophy, coronary fibrosis and the incidence of MI, without affecting blood pressure, plaque vulnerability and survival.

Keywords Atherosclerosis – cardiac remodelling – plaque vulnerability – blood pressure – mouse model – mortality.

INTRODUCTION

Atherosclerosis is a progressive inflammatory disease of the large and medium-sized arteries, characterized by the formation of plaques in the vessel wall. During the development of the disease, the stability of the atherosclerotic plaque plays a major role. Features of plaque instability include a large necrotic core, a high infiltration of inflammatory macrophages and a thin fibrous cap, composed of few smooth muscle cells (SMCs) and collagen fibres. When a

plaque develops such an unstable phenotype, it may easily rupture, followed by thrombosis and clinical complications such as myocardial infarction and stroke¹⁻³.

Angiotensin II (Ang II), an important substrate in the renin-angiotensin-aldosterone system (RAAS), can influence atherogenesis via several mechanisms. Firstly, Ang II causes vasoconstriction and the release of aldosterone, leading to an increase in blood pressure which is a well-known risk factor for atherosclerosis^{4,5}. Secondly, Ang II is responsible for a range of blood pressure independent effects, such as an upregulation of adhesion molecules on endothelial cells, activation of macrophages and an increased expression of matrix metalloproteinases (MMPs) and inflammatory cytokines⁴⁻⁶. Eventually, these effects will result in the formation of rupture-prone atherosclerotic plaques with an increased risk of cardiovascular events⁷.

In the present study, we used apolipoprotein E deficient mice (ApoE^{-/-}) with a heterozygous mutation in

Address for correspondence:

Lynn Roth, University of Antwerp,
Universiteitsplein 1, B-2610 Antwerp,
Belgium. E-mail: lynn.roth@uantwerpen.be

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the fibrillin-1 gene ($Fbn1^{C1039G/+}$). These mice develop increased arterial stiffness due to fragmentation of the elastin fibres⁸. Recently, we reported that this mouse model shows accelerated plaque progression, spontaneous plaque ruptures, myocardial infarction and sudden death^{8,9}. The goal of this study was to evaluate whether the $ApoE^{-/-}Fbn1^{C1039G/+}$ mouse model is responsive to risk factors of atherosclerosis, such as high blood pressure and inflammation. Therefore, we administered Ang II for a period of 4 weeks and examined plaque stability, the presence of myocardial infarctions (MI) and sudden death.

METHODS

Mice

Female $ApoE^{-/-}Fbn1^{C1039G/+}$ mice (C57BL/6J background) were fed a Western diet (WD; TD88137, Harlan Teklad) starting at an age of 6 weeks. The animals were housed in a temperature-controlled room with a 12-hour light/dark cycle and had free access to water and food. Cases of sudden death were documented. At the end of the experiment (18 weeks WD) body weight was determined and plasma samples were obtained from the retro-orbital plexus of anaesthetized mice (sodium pentobarbital 75 mg/kg, i.p.). Subsequently, the mice were sacrificed with sodium pentobarbital (250 mg/kg, i.p.). Analysis of total plasma cholesterol was performed via a commercially available kit (Randox, Crumlin, UK). The animal procedures were performed in accordance with the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes and all experiments were approved by the ethics committee of the University of Antwerp.

Angiotensin II infusion

At 14 weeks WD, $ApoE^{-/-}Fbn1^{C1039G/+}$ mice were anaesthetized with sevoflurane (8% for induction and 4.5% for maintenance, SevoFlo[®], Penlon vaporizer) and subcutaneously implanted with osmotic minipumps (Alzet, model 1004) filled with either Ang II (1,000 ng/kg/day, $n = 12$, Sigma) or phosphate buffered saline (control, $n = 11$). The minipumps allowed continuous delivery of Ang II over a period of 28 days.

Blood pressure measurements

Peripheral blood pressure was measured at week 14 (baseline), 16 and 18 of WD in conscious mice via a tail cuff. Mice were placed in Plexiglas restrainers in a heating chamber (37°C) and kept in the dark. Ten minutes

of acclimatization was allowed before measurements were initiated. At the distal end of the tail, a pulse sensor and occluding cuff controlled by a programmed Electro-Sphygmomanometer (Narco Bio-systems, Austin, TX) were placed. Voltage output from the sensor and cuff were recorded and analyzed by a PowerLab signal transduction unit and associated Chart software (ADInstruments, Colorado Springs, CO). To be accustomed to the procedure, mice were trained for four weeks before the baseline measurement. At least eight separate pressure measurements were averaged from each animal on each recording day.

Histology

After sudden death or sacrifice of $ApoE^{-/-}Fbn1^{C1039G/+}$ mice, the proximal ascending aorta and the heart were collected. After determining heart weight, tissues were fixed in 4% formaldehyde (pH 7.4) for 24 hours, dehydrated overnight in 60% isopropanol and embedded in paraffin. Serial cross sections (5 μ m) of the proximal ascending aorta and heart were prepared for histological analysis. Atherosclerotic plaque size, the length of the internal elastic lamina (IEL), stenosis and necrotic core (acellular areas with a threshold of 3,000 μ m²) of the proximal ascending aorta were analyzed on haematoxylin-eosin (H-E) stained sections. Immunohistochemical staining with a primary antibody against Mac-3 (553322, Pharmingen, San Diego, CA) was used to determine the percentage of macrophages in the plaques. Collagen content was measured in Sirius red stained sections. Fibrous cap thickness was determined as the median value of 10 measurements per atherosclerotic plaque on α -SMC actin (Sigma, St Louis, MO) stained sections. The occurrence of myocardial infarctions, coronary plaques and perivascular fibrosis, measured as the perivascular collagen area divided by the luminal area (PVCA/LA) of 10 coronary arteries (microcirculation) per mouse, was analyzed on Masson's trichrome stained sections (cut from the middle of the heart to the apex). If plaques were present in the coronary arteries (microcirculation), plaque size and percentage stenosis were measured on Masson's trichrome stained sections. All images were acquired with Universal Grab 6.1 software using an Olympus BX40 microscope and were quantified with Image J software.

Statistical analysis

All data are expressed as mean $\pm$ SEM. Statistical analyses were performed using SPSS software (version 20, SPSS Inc., Chicago, IL). Statistical tests are specified in the figure legends. Differences were considered significant at $P < 0.05$.

RESULTS

Blood pressure

Baseline measurements before onset of Ang II treatment, did not show differences in systolic blood pressure (Sys BP), diastolic blood pressure (Dia BP) and pulse pressure (PP) between both groups (table 1). Treatment with Ang II did not influence Sys BP, Dia BP and PP over a period of 4 weeks (table 1).

Total cholesterol, plaque size and composition

Total plasma cholesterol in Ang II treated mice (384 ± 55 mg/dl, $n=8$) was not different from controls (452 ± 67 mg/dl, $n=8$).

Angiotensin II did not affect atherosclerotic plaque size, vascular remodelling measured as the length of the IEL and stenosis in the proximal ascending aorta of ApoE^{-/-}Fbn1^{C1039G+/-} mice (table 2). The size of the necrotic core was also not different between both groups (table 2). Furthermore, the composition of the plaques was equal between Ang II treated mice and controls, since no differences were detected for the percentage

of macrophages, fibrous cap thickness and collagen content (table 2).

Aneurysms in the thoracic aorta

Treatment with Ang II resulted in severe aneurysm formation in the thoracic aorta of ApoE^{-/-}Fbn1^{C1039G+/-} mice, which impaired atherosclerotic plaque evaluation in this region (figure 1).

In the Ang II treated group, 50% was diagnosed with an aneurysm. In contrast, no aneurysms were seen in control mice (Pearson's chi-squared test; $P=0.006$).

Coronary plaques, perivascular fibrosis and heart weight

The number of mice showing plaque in the coronary arteries was slightly, but not significantly increased after Ang II infusion (table 3). Coronary plaque size and the degree of stenosis was not different between both treatment groups (table 3 and figure 2A).

Perivascular fibrosis of the coronary arteries was measured by dividing the perivascular collagen area by the luminal area of the blood vessel. This fibrosis was significantly more pronounced in Ang II treated mice ($25 \pm 4\%$ versus $15 \pm 2\%$ in control mice; figure 2A and B).

As a result of Ang II infusion, heart weight (divided by body weight) of ApoE^{-/-}Fbn1^{C1039G+/-} mice was significantly higher than in control conditions (control: $0.8 \pm 0.1\%$ versus Ang II: $1.7 \pm 0.4\%$; figure 2C).

Table 1 Blood pressure

Weeks treatment	0	2	4
Systolic BP (mm Hg)			
Control	134 ± 6	132 ± 4	131 ± 5
Angiotensin II	132 ± 5	119 ± 2	144 ± 11
Diastolic BP (mm Hg)			
Control	113 ± 6	109 ± 4	104 ± 2
Angiotensin II	108 ± 4	97 ± 3	126 ± 11
Pulse pressure (mm Hg)			
Control	21 ± 2	23 ± 3	22 ± 2
Angiotensin II	24 ± 3	22 ± 3	18 ± 1

Data are shown as mean $\pm$ SEM, BP: blood pressure, Repeated measures ANOVA; $P > 0.05$.

Table 2 Atherosclerotic plaque size, stenosis and composition

	Control	Angiotensin II
Plaque size ($10^3 \mu\text{m}^2$)	629 ± 88	616 ± 76
Internal elastic lamina (μm)	$3,789 \pm 212$	$3,802 \pm 128$
Stenosis (%)	46 ± 4	52 ± 5
Necrotic core (%)	10 ± 2	13 ± 3
Macrophages (%)	3 ± 1	3 ± 1
Fibrous cap thickness (μm)	13 ± 2	14 ± 1
Collagen (%)	33 ± 3	33 ± 4

Data from proximal ascending aorta, mean $\pm$ SEM, control $n=9$, Ang II $n=8-9$, Independent samples t test; $P > 0.05$.

Control

Ang II

Fig. 1 Aneurysms in the thoracic aorta.

In control ApoE^{-/-}Fbn1^{C1039G+/-} mice no aneurysms were observed. Ang II treatment resulted in severe aneurysm formation in 50% of mice.

Table 3 Coronary plaque parameters and occurrence of myocardial infarctions

	Control	Angiotensin II
Coronary plaque (%) ^a	27 (3/11)	42 (5/12)
Coronary plaque size (μm ²)	3,368 ± 1,693	2,384 ± 757
Coronary stenosis (%)	67 ± 4	58 ± 17
Myocardial infarctions (%) ^b	9 (1/11)	58 (7/12)*

Data are shown as mean ± SEM, control n = 3-11, Ang II n = 5-12, Independent samples t test; *P* > 0.05.
^a Percentage of mice showing coronary plaques, Pearson's chi-squared test; *P* > 0.05.
^b Percentage of mice showing myocardial infarctions, Pearson's chi-squared test; **P* < 0.05.

Myocardial infarctions and survival

The occurrence of MI was significantly higher in Ang II treated mice as compared to controls (table 3). In the Ang II group 67% of mice remained alive until the end of the experiment, which was not different from control mice where survival was 73%.

DISCUSSION

It is known that Ang II can promote the development of atherosclerotic plaques by increasing systemic blood pressure⁴. However, in the present study no elevation in blood pressure was observed. Similar studies in mice

Fig. 2 Coronary perivascular fibrosis and heart weight. (A) Trichrome Masson staining of the coronary arteries showed the presence of fibrosis and atherosclerotic plaques, with no difference in plaque size and the degree of stenosis between control and Ang II treated mice. Fibrosis was more pronounced after Ang II treatment and the example shows a coronary artery in close proximity to an infarcted area (P=plaque, L=lumen, scale bar=50 μm). (B) The percentage of perivascular fibrosis (perivascular collagen area divided by luminal area = PVCA/LA) was significantly increased in Ang II treated mice as compared to controls (control n = 10, Ang II n = 11, Independent samples t test; **P* < 0.05). (C) Heart weight divided by body weight (bw) was higher in mice that received Ang II treatment (control n = 7, Ang II n = 9, Independent samples t test; **P* < 0.05).

were also not able to detect relevant differences in blood pressure between control and Ang II treated mice^{10,11}. This might be caused by tolerance to Ang II or a compensatory cardiovascular reflex¹⁰. Moreover, other research suggests that the observed effects of Ang II on plaque development and inflammation are independent of high blood pressure¹², underlining the importance of the non-haemodynamic effects of Ang II.

Size and composition of plaques in the proximal ascending aorta was not altered in Ang II treated mice. This is in contrast with previous studies that report accelerated plaque development and enhanced inflammation in ApoE^{-/-} mice where Ang II treatment was started before the onset of atherosclerosis^{10,12,13}. At the time point we chose to initiate the study (14 weeks WD), advanced plaques were already present in the proximal ascending aorta. These plaques might have been too evolved to be influenced by a relatively short Ang II treatment, which can explain the absence of significant results. Plaques in the thoracic aorta develop at a later stage and are therefore a good alternative to investigate. However, in the present study plaque analysis in the thoracic aorta was not possible due to severe aneurysm formation. Other studies have shown that Ang II is able to induce abdominal aortic aneurysms^{10,11,14-16}. We observed aneurysms in both the thoracic and abdominal aorta of mice, indicating that aneurysm formation in ApoE^{-/-}Fbn1^{C1039G+/-} mice is more severe than in ApoE^{-/-} mice. It is described that Ang II can cause elastin fragmentation, resulting in arterial stiffness and aneurysm formation^{15,16}. Combined with the fibrillin-1 gene mutation in our mouse model, which is also responsible for fragmentation of elastic fibres^{8,9}, it is conceivable that the process of vascular stiffening was enhanced. Eventually this led to the formation of large aneurysms.

Importantly, Ang II treatment resulted in a 6-fold increase in the number of mice with MI. However, this was not accompanied by an increased presence of plaques in the coronary arteries. Also plaque size and the degree of stenosis were not influenced by Ang II treatment. It has been described that even in areas with no coronary obstructive lesions, myocardial necrosis was observed in patients with a highly stimulated RAAS⁷. This can be explained by coronary vasoconstriction⁷, impairment of blood flow and subsequent myocardial ischaemia⁵. In the clinical situation, this type of cardiac damage is described as myocardial infarction with no obstructive coronary atherosclerosis (MINOCA)¹⁷. The lowering of coronary blood flow can result in increased perivascular fibrosis¹⁸, which was indeed observed in the present study. Furthermore, coronary perivascular fibrosis can be caused by post-myocardial ventricular remodelling¹⁹. Thus, we presume that the cardiac fibrosis is reparative and the result of cardiomyocyte loss.

However, we cannot exclude that the fibrotic response is partly mediated by direct effects of Ang II, since it can enhance fibrosis via the production of transforming growth factor- β ²⁰⁻²².

In this study, a significant increase in heart weight over body weight in the Ang II treated mice was observed, indicative of cardiac hypertrophy. This observation can be explained by the higher number of MI present, resulting in progressive left ventricular remodelling²¹. Moreover, Ang II can stimulate the release of noradrenaline and therefore promotes myocardial cell growth and hypertrophy^{7,21,22}.

Although we observed a significant increase in the occurrence of MI after Ang II infusion, no effect on survival of the ApoE^{-/-}Fbn1^{C1039G+/-} mice could be detected. Since previous research with this mouse model was performed over longer periods of time, we presume that the time frame of the current study was too narrow to observe an effect on survival^{9,23,24}.

A limitation of the present study is the lack of echocardiographic data, which could have provided additional information on left ventricular function and hypertrophy, regional contractility abnormalities, aneurysm formation and aortic/mitral valve regurgitation.

In conclusion, Ang II treatment in ApoE^{-/-}Fbn1^{C1039G+/-} mice did not affect blood pressure, atherosclerotic plaque size and composition. However, the occurrence of MI, coronary perivascular fibrosis and cardiac hypertrophy was significantly increased. Ang II was also responsible for the formation of large thoracic aneurysms, which might be of interest in future aortic aneurysm research. However, the present study showed that Ang II treatment for a period of 4 weeks is not the ideal trigger to accelerate and aggravate atherosclerosis in ApoE^{-/-}Fbn1^{C1039G+/-} mice. In contrast, we previously reported that chronic mental stress, another clinically relevant risk factor, did increase plaque vulnerability and mortality²⁵, which proves the validity of the ApoE^{-/-}Fbn1^{C1039G+/-} mouse as a model for plaque rupture and supports the use of this model to evaluate potential plaque stabilising therapies.

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CONFLICT OF INTEREST: none.

REFERENCES

- Lusis AJ. Atherosclerosis. *Nature* 2000; **407**: 233-241.
- Libby P. Mechanisms of acute coronary syndromes and their implications for therapy. *N Engl J Med* 2013; **368**: 2004-13.
- Yla-Herttuala S, Bentzon JF, Daemen M, Falk E, Garcia-Garcia HM, Herrmann J, Hoefer I, Jauhainen S, Jukema JW, Krams R, Kwak BR, Marx N, Naruszewicz M, Newby A, Pasterkamp G, Serruys PW, Waltenberger J, Weber C, Tokgozoglu L. Stabilization of atherosclerotic plaques: An update. *Eur Heart J* 2013; **34**: 3251-8.
- Sata M, Fukuda D. Crucial role of renin-angiotensin system in the pathogenesis of atherosclerosis. *J Med Invest* 2010; **57**: 12-25.
- da Silva AR, Fraga-Silva RA, Stergiopoulos N, Montecucco F, Mach F. Update on the role of angiotensin in the pathophysiology of coronary atherothrombosis. *Eur J Clin Invest* 2015; **45**: 274-87.
- Benigni A, Cassis P, Remuzzi G. Angiotensin II revisited: New roles in inflammation, immunology and aging. *EMBO Mol Med* 2010; **2**: 247-57.
- Gavras I, Gavras H. Angiotensin II as a cardiovascular risk factor. *J Hum Hypertens* 2002; **16 Suppl 2**: S2-6.
- Van Herck JL, De Meyer GRY, Martinet W, Van Hove CE, Foubert K, Theunis MH, Apers S, Bult H, Vrints CJ, Herman AG. Impaired fibrillin-1 function promotes features of plaque instability in apolipoprotein E-deficient mice. *Circulation* 2009; **120**: 2478-87.
- Van der Donckt C, Van Herck JL, Schrijvers DM, Vanhoutte G, Verhoye M, Blockx I, Van Der Linden A, Bauters D, Lijnen HR, Sluimer JC, Roth L, Van Hove CE, Franssen P, Knaapen MW, Hervent AS, De Keulenaer GW, Bult H, Martinet W, Herman AG, De Meyer GR. Elastin fragmentation in atherosclerotic mice leads to intraplaque neovascularization, plaque rupture, myocardial infarction, stroke, and sudden death. *Eur Heart J* 2015; **36**: 1049-58.
- Daugherty A, Manning MW, Cassis LA. Angiotensin II promotes atherosclerotic lesions and aneurysms in apolipoprotein E-deficient mice. *J Clin Invest* 2000; **105**: 1605-12.
- Johansson ME, Wickman A, Fitzgerald SM, Gan LM, Bergström G. Angiotensin II, type 2 receptor is not involved in the angiotensin II-mediated pro-atherogenic process in apoe(-/-) mice. *J Hypertens* 2005; **23**: 1541-9.
- Weiss D, Kools JJ, Taylor WR. Angiotensin II-induced hypertension accelerates the development of atherosclerosis in apoe-deficient mice. *Circulation* 2001; **103**: 448-54.
- da Cunha V, Martin-McNulty B, Vincelette J, Choy DF, Li WW, Schroeder M, Mahmoudi M, Halks-Miller M, Wilson DW, Vergona R, Sullivan ME, Wang YX. Angiotensin II induces histomorphologic features of unstable plaque in a murine model of accelerated atherosclerosis. *J Vasc Surg* 2006; **44**: 364-71.
- Wang YX. Cardiovascular functional phenotypes and pharmacological responses in apolipoprotein E deficient mice. *Neurobiol Aging* 2005; **26**: 309-16.
- Saraff K, Babamusta F, Cassis LA, Daugherty A. Aortic dissection precedes formation of aneurysms and atherosclerosis in angiotensin II-infused, apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol* 2003; **23**: 1621-6.
- Tham DM, Martin-McNulty B, Wang YX, De Cunha V, Wilson DW, Athanassios CN, Powers AF, Sullivan ME, Rutledge JC. Angiotensin II injures the arterial wall causing increased aortic stiffening in apolipoprotein E-deficient mice. *Am J Physiol Regul Integr Comp Physiol* 2002; **283**: R1442-R1449.
- Niccoli G, Scalone G, Crea F. Acute myocardial infarction with no obstructive coronary atherosclerosis: mechanisms and management. *Eur Heart J* 2015; **36**: 475-81.
- Dai ZH, Fukumoto Y, Shimokawa H. Coronary perivascular fibrosis is associated with impairment of coronary blood flow in patients with non-ischemic heart failure. *J Cardiol* 2012; **60**: 416-21.
- Kalkman EA, van Suylen RJ, van Dijk JP, Saxena PR, Schoemaker RG. Chronic aspirin treatment affects collagen deposition in non-infarcted myocardium during remodeling after coronary artery ligation in the rat. *J Mol Cell Cardiol* 1995; **27**: 2483-94.
- Zaman A, Fujii S, Goto D, Furumoto T, Mishima T, Nakai Y, Dong J, Imagawa S, Sobel BE, Kitabatake A. Salutary effects of attenuation of angiotensin II on coronary perivascular fibrosis associated with insulin resistance and obesity. *J Mol Cell Cardiol* 2004; **37**: 525-35.
- Gajarsa JJ, Kloner RA. Left ventricular remodeling in the post-infarction heart: a review of cellular, molecular mechanisms, and therapeutic modalities. *Heart Fail Rev* 2011; **16**: 13-21.
- Bader M. Role of the local renin-angiotensin system in cardiac damage: a minireview focussing on transgenic animal models. *J Mol Cell Cardiol* 2002; **34**: 1455-62.
- Roth L, Van Dam D, Van der Donckt C, Schrijvers DM, Lemmens K, Van Brussel I, De Deyn PP, Martinet W, De Meyer GR. Impaired gait pattern as a sensitive tool to assess hypoxic brain damage in a novel mouse model of atherosclerotic plaque rupture. *Physiol Behav* 2015; **139**: 397-402.
- Van der Donckt C, Roth L, Vanhoutte G, Blockx I, Bink DI, Ritz K, Pintelon I, Timmermans JP, Bauters D, Martinet W, Daemen MJ, Verhoye M, De Meyer GR. Fibrillin-1 impairment enhances blood-brain barrier permeability and xanthoma formation in brains of apolipoprotein E-deficient mice. *Neuroscience* 2015; **295**: 11-22.
- Roth L, Rombouts M, Schrijvers DM, Lemmens K, De Keulenaer GW, Martinet W, De Meyer GR. Chronic intermittent mental stress promotes atherosclerotic plaque vulnerability, myocardial infarction and sudden death in mice. *Atherosclerosis* 2015; **242**: 288-94.