Animal models for plaque rupture: a biomechanical assessment

Kim Van der Heiden1*; Ayla Hoogendoorn1*; Mat J. Daemen2; Frank J. H. Gijsen1
1Department of Biomedical Engineering, Thoraxcenter, Erasmus Medical Center, Rotterdam, The Netherlands; 2Department of Pathology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Summary
Rupture of atherosclerotic plaques is the main cause of acute cardiovascular events. Animal models of plaque rupture are rare but essential for testing new imaging modalities to enable diagnosis of the patient at risk. Moreover, they enable the design of new treatment strategies to prevent plaque rupture. Several animal models for the study of atherosclerosis are available. Plaque rupture in these models only occurs following severe surgical or pharmaceutical intervention. In the process of plaque rupture, composition, biology and mechanics each play a role, but the latter has been disregarded in many animal studies. The biomechanical environment for atherosclerotic plaques is comprised of two parts, the pressure-induced stress distribution, mainly - but not exclusively - influenced by plaque composition, and the strength distribution throughout the plaque, largely determined by the inflammatory state. This environment differs considerably between humans and most animals, resulting in suboptimal conditions for plaque rupture. In this review we describe the role of the biomechanical environment in plaque rupture and assess this environment in animal models that present with plaque rupture.

Keywords
Animal models, atherosclerosis, biomechanics, devices

Introduction
Atherosclerosis-induced clinical events like coronary heart disease, ischaemic stroke and peripheral vascular disease are a result of arterial stenosis and/or thrombosis. The latter is caused by atherosclerotic plaque rupture (A), endotheial plaque erosion (B) or protruding calcified nodules (C) (1–3). In man, only a subset of plaques with distinct morphological features is prone to rupture. These plaques were termed “vulnerable” in 1989 by Dr. James E. Muller (4). Please note that the term vulnerable plaque is valid for all three plaque morphologies (A-C) that can cause arterial thrombosis (5). In the past decades, the disease process has changed, not only by means of our medical interventions, e.g. use of statins, but also by changes in risk factors, e.g. increase in diabetic patients, increase in obesity and decrease in smoking (6). Plaque rupture is still considered the main cause of cardiovascular events, but plaque erosion appears to be on the rise (6). As animal models for plaque erosion and calcified nodules are currently lacking, we will focus on plaque rupture and will therefore only take into account the vulnerable plaque models that show events of rupture.

The burden of atherosclerotic disease in humans is traditionally estimated from the percentage of stenosis detected by coronary angiography or carotid computed tomography, magnetic resonance imaging or intravascular ultrasound. However, these techniques fail to detect non-stenotic – possibly vulnerable – plaques as the degree of stenosis does not correlate to plaque vulnerability (7). Patients without stenosis could still present with vulnerable atherosclerosis and consequently be at risk of cardiovascular events. In the 1990s, work by the groups of Dr. Virmani (8), Dr. Davies (9) and Dr. Falk (10) demonstrated that plaque composition correlates to plaque vulnerability, which eventually resulted in the publication of a consensus paper on vulnerable plaque terminology (11).

However, finding the vulnerable plaque in humans with current imaging techniques appears not to be the ‘holy grail’ to predict rupture-related future events. Different large clinical trials like the PROSPECT study (12) showed a very low future event rate in patients diagnosed with plaques characterised as vulnerable. One could state that not all vulnerable plaques rupture, or take it even further and state that most vulnerable plaques will never rupture. Moreover, even plaques characterised as stable did show some events of plaque rupture. This apparent contradiction in terminology can be explained in many cases by looking at the different biomechanical factors influencing plaque rupture. Both progression and regression of plaques is influenced by the whole organism environment which includes aspects like blood composition, blood pressure, but also genetics. All these processes can be described by three factors: the plaque composition, the underlying biological processes and the mechanics. All these factors have an
Biomechanical assessment of plaque rupture animal models

During the past decade, a lot of research effort was put into development of new imaging modalities to detect specific plaque components in order to identify the patient at risk. Moreover, defining the biological and biomechanical mechanisms responsible for plaque destabilisation and rupture would enable the development of new treatment strategies. Disease progression was studied in several longitudinal clinical studies, but these studies were limited by the fact that patients were already symptomatic upon study inclusion. Prospective cohort studies, like the Rotterdam ERGO study (13), provide insight into carotid plaque development and progression since the subjects are asymptomatic upon inclusion. However, imaging modalities to delineate plaque composition like nuclear or intravascular imaging, cannot be tested on these subjects. Therefore, we are restricted to post mortem or ex vivo imaging of diseased human arteries, accepting the major limitation of a cross sectional study and fully advanced disease state. To study the natural history of a plaque, test experimental imaging modalities and treatments, and gain mechanistic insight into plaque destabilisation, we have to resort to animal models.

The use of the term ‘vulnerable plaque’ in humans (11) is, however, different from what is termed a vulnerable plaque in most animal models. While vulnerability in humans is related to a high risk of rupture, in animals, the term vulnerability is usually used only to refer to plaques with morphological characteristics of a vulnerable plaque, without actually resulting in plaque rupture. Moreover, animal models for vulnerable plaque pose a problem in the fact that all three factors, i.e. the plaque composition, the biological processes and the mechanics, are different compared to the human setting. To promote plaque rupture in an animal model, all these parameters can potentially be altered to optimise the biomechanical conditions. In this review we will further discuss plaque composition in the context of biomechanics and rupture for both humans and appropriate animal models.

The vulnerable plaque classification

The American Heart Association (AHA) classification, which was slightly adapted by Virmani et al., describes the histological composition of the different stages of human atherosclerotic plaques (14). In short, the natural accumulation of smooth muscle cells (SMCs) in the intima, i.e. adaptive intimal thickening, is regarded as the first step in lesion formation. When foam cells are observed in these lesions, the lesions are called xanthoma or fatty streaks. The term pathological intimal thickening is used when, besides SMCs and foam cells, extracellular lipids are present in the form of a lipid pool. Upon the introduction of inflammatory cells and necrosis, lesions have developed into fibroatheroma (FA; type IV lesion) which have a very low risk of rupture. These are highly cellular lesions with few lipids, a thick cap with abundant SMCs and collagen, little inflammation, and little necrosis/apoptosis. FAs can present with a thick fibrous cap or a thin cap (<65 µm in coronary plaques) overlying the necrotic core/lipid pool. The latter are termed thin cap fibroatheroma (TCFA) and are seen as the precursor lesions of plaque rupture.

It is important to realise that a classification based on composition/histology is a snapshot in time. During the natural history of atherosclerosis, plaques undergo compositional changes which are influenced by many different biological processes like inflammation and oxidative stress (15). These biological processes can in turn be influenced by the composition of the plaque itself but also by changes in mechanically-induced signalling. Due to this complex interaction, plaques constantly change in composition and size and can change into a different plaque type in a relative short period of time (16). Stable plaques can become more vulnerable over time, but vulnerable plaques can also stabilise (17).

During atherosclerosis development, plaques are exposed to changing mechanical conditions. These include not only the stresses inside the plaque but also the strength of the plaque itself. The fate (will the plaque rupture, progress or regress) of any given plaque at any given time point is determined by the mechanical forces acting upon the plaque. This means that every plaque has
the potential to rupture, and could therefore be called ‘vulnerable’. The likelihood of rupture however depends both on the composition as well as on the mechanical stimulus (Figure 1).

**Plaque rupture: a mechanical process**

Failure of a structure is a mechanical event in which the structure loses its integrity due to the forces it is exposed to. Failure, or rupture, occurs when the forces exceed a certain threshold value. From an engineering perspective, forces in a structure are often described using ‘stress’, while the threshold value is referred to as ‘strength’. Irrespective of the rupture mechanism, the underlying paradigm is identical: cap rupture will occur if local stress exceeds local strength.

To understand and predict cap rupture, we therefore need to know the cap stress distribution and the strength of the cap. Almost all studies in human plaques focus on cap stress. This quantity is usually determined using a numerical technique called finite element analyses (FEA). This tool is a commonly used technique in various engineering disciplines and is applied to predict the mechanical behaviour of complex structures under various loading conditions. Applications of FEA range from optimising airplane design to predicting mechanical heart valve failure. FEA requires three main input parameters, all of them relevant for the cap stress computations. First, the mechanical loading conditions are required. The main mechanical load for the cap is the intraluminal blood pressure. The second input parameter is the plaque geometry, including size and shape of the individual plaque components. Finally, the material properties of these components need to be described by a material model, which relates the forces to the resulting deformation of a material. Cap strength is much less intensively studied, and is mostly investigated in an experimental setting (18).

**Plaque rupture in human plaques**

Many FEA studies investigated the impact of various geometrical risk factors on peak cap stress. In idealised geometries, cap thickness and necrotic core size were identified most influential (19, 20). The often reported threshold for cap thickness of a vulnerable plaque is 65 µm. This threshold value was derived from morphometric analyses in 41 ruptured caps (8), in which 95% had a thickness smaller than 64 µm. The mean cap thickness was 23 ± 19 µm, indicative of the large variation in that small sample. This is further corroborated by the study of Akyildiz et al. (21) in which they studied 77 intact human coronary plaques. The median cap thickness in that population was 190 µm, with 25% of the plaques having a cap thickness lower than 65 µm. In conclusion, there are caps thicker than 65 µm that did rupture, and there are many intact caps thinner than 65 µm. Thin caps and large necrotic cores lead to high peak cap stresses. In realistic geometries (21), this picture changes: cap thickness is still the most relevant parameter, but necrotic core size is less important, and lumen curvature and radius emerge as independent predictors of peak stress. From these studies, it can be concluded that geometrical features of type IV plaques indeed are associated with elevated peak cap stress. However, there is also considerable variation, and not all type IV plaques will harbour caps with high peak cap stress, while some other – histologically stable – plaque types will have high peak cap stress.

The stresses in the plaque are also influenced by the mechanical properties of the plaque components. The properties of the plaque components can be described using material models. Most of the material models used in numerical studies are relatively simple (22, 23) and characterise the property of a plaque component with a single value, the stiffness. The influence of the stiffness of the plaque components was investigated in several numerical studies. Especially the stiffness of the intima was shown to be important for peak cap stress (22): stiffer intima properties will generally induce higher stress.

Cap strength is much less investigated. The most influential study used a combination of experimentally observed rupture locations and determined the peak stress at the rupture location. A threshold value was derived from that study (24), and this threshold value is used in almost all studies investigating rupture risk. A single threshold value is unlikely to be sufficient to determine rupture risk, since it was shown that cap strength shows quite some variation and is influenced by macrophages (18), collagen content (25), and possibly microcalcifications (26). The fact that plaque rupture occurs in human plaques reflects their biomechanical environment. This environment comprises of two parts, on the one hand pressure-induced stress distribution throughout the plaque and on the other hand the strength distribution throughout the plaque. Apparently the conditions for human plaques are such that cap stress locally exceeds cap strength, leading to cap rupture. Although many uncertainties need to be dealt with, certain general statements can be made for the human situation. Histological plaque classification makes sense from a biomechanical perspective: plaques with a large lipid core, thin fibrous cap and complex shapes lead to high peak cap stresses (27). The presence of macrophages and reduction of collagen content decrease cap strength. Together with elevated blood pressure, these plaques are at high risk of rupture. However, the true risk of rupture depends on the whole organism environment described by the interplay between plaque composition, biology and the mechanical environment (Figure 1), rendering a histologically-deemed stable plaque still at risk of rupture in a mechanically harmful environment. Conditions that can be mimicked –or exaggerated- in a lab setting, but how are these relevant biomechanical conditions for rupture in animal models?

**The mouse as a model for plaque rupture**

The mouse has so far been the most often used model for vulnerable plaque due to its low costs, small size, fast plaque development and the many options available to induce genetic modifications. There are, however, some pronounced differences between mice and men regarding metabolism and physiology. Mice do not...
develop plaques naturally, but, in transgenics, plaque growth can be induced by different stimuli. Plaques in humans are more commonly located in the coronary arteries, carotid bifurcations, abdominal aorta, iliofemoral arteries, and carotid bifurcations, while atherosclerotic mice present plaques in the aorta, carotid bifurcation and brachiocephalic artery (28, 29). Furthermore, mice have a different LDL/HDL balance compared to humans (30). Regarding plaque composition, murine plaques mainly consist of inflammatory components while these form only a very small part of the total plaque volume in humans (31). Intraplaque haemorrhage (IPH), one of the driving factors of plaque destabilisation in humans (32), is rarely reported in mice, just as the presence of luminal thrombi after an event. The latter could, however, be the result of a very fast clearance of the thrombus due to differences in the fibrinolytic system between mice and men (28, 29).

Plaque rupture in mice: general biomechanical considerations

The prevalence of plaque rupture in mice was debated extensively (29, 33–38). Although some vulnerable plaque features can be copied in genetically and surgically manipulated mouse models, plaque rupture, according to the human definition, is rare. Apparently the biomechanical environment for murine atherosclerotic plaques significantly differs from the human situation. The most striking difference between human and murine plaques is their size. Size does influence the mechanical environment significantly, and this is easily demonstrated using the well-known law of Laplace, which predicts the circumferential stress \( \sigma \) in a tube with wall thickness \( h \) and radius \( r \) loaded by a pressure \( p \). It reads:

\[
\sigma = \left( \frac{r}{h} \right) p
\]

This law is often used to predict average stress in a healthy arterial wall, and it demonstrates that the average stress in the wall of an artery with a radius of 2 mm and a wall thickness of 0.2 mm is identical to the average stress in an artery with a radius of 0.1 mm and a wall thickness of 0.01 mm. It also predicts that if the radius of the artery decreases and the wall thickness remains constant, the average stress in the wall would decrease accordingly. Translating this scaling law to atherosclerotic plaques, this implies that a 65 \( \mu \)m thick murine cap has much lower stresses than a human cap of similar thickness (39).

A second notable difference between human and murine plaques lies in their morphology. A comprehensive study (40) investigated plaque stresses in three different murine models, and concluded that morphology of plaques in the mouse models differed considerably from the human situation, leading to a different stress distribution inside the plaque. The murine plaques were often focally adhered to a relatively intact vessel wall. The mechanical load exerted by the intraluminal pressure is mainly carried by the media and adventitia, leading to elevated stresses in these structures. The soft lipid rich plaques deform along with the media and adventitia, leading to lower stresses, especially in the cap of the murine plaque.

The third difference between the biomechanical environment in human and murine plaques might be found in the differences in the material properties. Although experimental data are scarce (41, 42), they seem to indicate that murine plaque components are generally softer, leading to lower plaque stresses (19, 43).

No experimental data on cap strength in mice is available, although it has to be mentioned that calcifications, thought to reduce cap strength in human plaques (44) are not reported in murine plaque caps. Compared to the biomechanical studies on human plaques, the biomechanical environment in murine plaques is less well characterised and many issues need to be resolved. Not only plaque properties, but also murine cap strength should be investigated in detail. Keeping these uncertainties in mind, the smaller size, the morphological features and the softer plaque components all indicate that cap stresses in murine plaques are lower than in human plaques. The biomechanical environment of most murine plaques seems to be such that plaque rupture will not occur without –drastic– interventions.

To induce plaque rupture in mice, biological and/or mechanical stimuli that alter the biomechanical environment are required. The most applied stimuli include blood pressure elevation, changing local blood flow, increasing inflammation or a combination of these. Below we will describe these models and discuss the potential consequences of the applied stimuli in a biomechanical context.

Biomechanically suitable mouse models for plaque rupture

Some models do show clear signs of plaque rupture, as seen in humans, including luminal thrombi and erythrocytes in the cap cleft but they often use a combination of complex stimuli, which have an individual but also a combined effect on plaque biology and mechanics. Combination of cast (a perivascular device) placement around the carotid artery and p53 adenoviral overexpression to induce apoptosis spontaneously resulted in ‘cap breaks’ with extrusion of the plaque contents in three of 14 animals. Additional short-term administration of phenylephrine moderately increased the blood pressure, but only resulted in luminal thrombosis and cap breakage in two different mice (45).

The main long term effect of p53 on the biomechanical environment presumably acts through its effect on SMC apoptosis. The SMC is the main source of collagen production in atherosclerotic plaques. Increased SMC apoptosis can therefore be expected to decrease the total amount of collagen in the plaque, having two important effects on cap biomechanics. First, the overall stiffness of the plaque will decrease, generally leading to a minor decrease in cap stress (19). Second, a decrease in collagen in the cap will decrease cap strength, and this effect can be expected to outweigh the first effect. The biomechanical environment is also changed due to administration of phenylephrine. This will increase the systolic blood pressure with approximately 15%, which will induce a similar relative increase in cap stress. This explains that the effect of such a minor pressure elevation through phenylephrine administration on the frequency of cap breakage is limited as well.
Of note, the effect of cast placement on the stresses in the plaque was never investigated. Manipulation of arteries through cast placement or ligations will inherently lead to changes in the biomechanical environment. Especially near the edges of cast and partial ligations, the 3D biomechanical stress environment will change drastically. The stiff structures surrounding the wall will prevent the artery to deform, leading to high strains, and therefore high stresses near the edges of the structure. If the cast or ligation induces a severe restriction, differences in luminal pressure between the upstream and downstream side will change the mechanical environment with higher stresses in the upstream region. Furthermore, the development of scar tissue potentially restricts the deformation of the arterial wall immediately upstream and downstream. In conclusion, the effect of cast placement or ligation is very complex, but is potentially important for plaque rupture. Especially near the edges of the cast, stress concentrations occur that are unlikely to be present in human plaques, potentially triggering unnatural failure mechanisms.

A different biological approach was used by Gough et al. (41) who induced molecular weakening of the cap by transfection of ApoE<sup>−/−</sup> mice with haematopoietic stem cells overexpressing matrix metalloproteinase (MMP) 9 (46). Proven signs of rupture in the form of cap breaks in the shoulder region were observed in almost half of the mice. The main biomechanical effect of overexpression of MMP9 is on cap strength: since it was demonstrated that collagen content is related to cap strength, degradation of this component reduces cap strength and therefore enhances the risk of cap rupture. The overexpression of MMP9 potentially has another important biomechanical effect: if the collagen in the media and adventitia is degraded, the overall stiffness of these two layers will also decrease. The overall change in deformation could lead to increased deformation in the cap, inducing elevated peak cap stress.

Another study combined a cast and a short-term lipopolysaccharide (LPS) administration together with phenylephrine treatment and cold (47). In around 30% of the mice they found cast disruption with luminal thrombus and cap breakage with core extrusion. Administration of LPS increases the inflammatory state in general. It was shown that LPS leads to an increase in Th17 cells, which through IL17 induces SMC apoptosis. This potentially decreases collagen content in the plaque, with previously discussed consequences. Phenylephrine will maximise the metabolic state of the heart, leading to increased heart rates and blood pressure. Exposing the animals to cold will increase microvascular resistance, potentially leading to a significantly larger increase in blood pressure, and therefore cap stress, compared to phenylephrine alone. However, no experimental data are available to confirm this.

Some models have used less complex methods to induce plaque rupture. Mice with a partial ligation of the carotid artery and renal artery to induce hypertension (48), formed plaques containing high numbers of macrophages, high MMP activity and there were signs of collagen breakdown and decreased actin content. More importantly, half of the mice showed rupture with luminal thrombi and IPH. The induced hypertension leads to a chronic increase in blood pressure of approximately 40%. This pressure increase exceeds the effect of phenylephrine, leading to a larger increase in peak cap stress. However, this relatively mild increase in the mechanical loading of the plaque alone cannot explain the high prevalence of plaque rupture in these mice. Chronic hypertension might influence biomechanical environment in several other ways. First, the observed increased plaque size leads to a redistribution of the mechanical load, inducing lower stresses in the cap of the plaque. On the other hand, one can argue that, although the plaque is larger, increased blood pressure leads to increased strain inside the plaque, inducing IPH (49). This is potentially related to the increased inflammatory state of the plaque, which in turn could lead to the reported increased MMP levels in the plaques of these mice. This might induce local weakening of the cap, overruling the overall decrease in cap stress due to increased plaque size.

Clear events of plaque rupture and possible rupture-related events like buried caps (50) were observed in a new mouse model developed by Chen et al. (51). Besides a large necrotic core and thin cap, the lesions proximal to the tandem ligation presented with high numbers of buried caps, ruptured fibrous caps and luminal thrombosis. The prevalence of rupture for the most proximal lesion most probably springs from a combined effect. First, plaque composition in the upstream lesion favours high cap stresses most. Second, the luminal pressures that the plaques are exposed to are highest for the proximal lesion. Especially the two, highly stenotic, lesions can induce a significant pressure drop leading to large differences in the mechanical loading conditions for the lesions.

Another recent model targeted vessel wall stiffness by putting an ApoE<sup>−/−</sup>/Fibrillin-1<sup>−/−</sup> double knockout mice on a high-fat diet. These mice present with reduced levels of elastin in their vessel walls and consequently stiffer vessels (52). On a high fat diet these mice developed large plaques with multiple features of vulnerability around the aortic valves, brachiocephalic artery and aorta. The plaques contained low levels of collagen and SMCs, large necrotic cores, macrophage infiltration, numerous buried caps and over half of the mice showed events of acute plaque rupture (52, 53). Uniquely, a high number of mice showed ruptured coronary plaques with thrombi and events of myocardial and cerebral infarction leading to sudden death (53). The biomechanical effect of knocking out Fibrillin-1<sup>−/−</sup> most likely is determined by its effect on elastin. The loss of elastin leads to a larger vessel area, even if affected by atherosclerosis. Assuming plaque morphology and blood pressure are unchanged, this would result in exposure of the cap to increased strain levels, and therefore higher stress.

Very recently, a new mouse model was introduced with an inducible adeno-virus mediated gain-of-function mutation in the PCSK9 gene (54). Induction of this mutation in ApoE<sup>−/−</sup> mice resulted in hypercholesterolaemia and early plaque growth double as extensive as compared to control ApoE<sup>−/−</sup> mice. Although no further long term studies have been published so far on advanced atherosclerotic plaque development in this model, these results are promising.

**Rabbits as a model for plaque rupture**

Another animal often used for vulnerable plaque and plaque rupture research, is the rabbit. The larger size might make rabbits into
Pigs as a model for plaque rupture

Since size and composition matter considerably, larger animal models should be considered for investigating plaque rupture. Primate studies have been performed in the early years but lost focus after growing ethical concern. With regard to its size, anatomy and physiology, the pig now is the number one choice to mimic the human atherosclerotic disease process. From a biological point of view, pigs more closely resemble human metabolism with a comparable LDL/HDL balance due to their omnivorous nature (65). Moreover, the plaques are predominantly lipid rich, and the cap covering the core does not contain stiff collagen fibres. This implies that the mechanical stresses inside the plaque will be low, and from that perspective, it is not surprising that cap rupture in rabbits was not observed.

Rabbits with inheritable hyperlipidaemia (WHHL rabbits) large, stable plaques develop in the aorta and coronary arteries (reviewed by Fan et al. [63]). However, a mutant colony of the WHHL, termed the myocardial infarction prone WHHL rabbit (WHHLMI), presents with vulnerable-type coronary plaques displaying a large lipid core, thin fibrous cap, accumulation of macrophages and even intraplaque haemorrhage at an age of 20 months (reviewed by Shiomi and Fan [64]). Plaque rupture was, however, never observed and the observed myocardial infarctions were attributed to stenotic plaques or vasospasm. Despite the fact that the mechanical environment appears more optimal in rabbits compared to mice due to the larger size of the plaques, rupture only occurs after severe stimuli which potentially induce different failure modes in the plaques. The main underlying cause seems to lie in the plaque composition. Although very frequently labelled as ‘vulnerable’, subtle differences between human and rabbit plaques render them much less rupture prone. Heterogeneity within the plaque, an important source of stress peaks, is generally absent. Moreover, the plaques are predominantly lipid rich, and the cap covering the core does not contain stiff collagen fibres. This implies that the mechanical stresses inside the plaque will be low, and from that perspective, it is not surprising that cap rupture in rabbits was not observed.

In rabbits, atherosclerosis can be induced in the aorta by a high fat diet often in combination with induced endothelial dysfunction or injury (55–59). It then takes several months before plaques develop, which are small in size, mainly contain lipids and are lacking many of the compositional vulnerable plaque features seen in humans. Rupture can, however, be induced by a severe, non-physiological and biomechanical relevant stimulus like injection of Russell’s viper venom (acute coagulation) plus histamine which directly affects coagulation, endothelial cell attachment and vasoconstriction (60, 61) or injection of liquid nitrogen which leads to acute endothelial cell damage (62).

In rabbits with inheritable hyperlipidaemia (WHHL rabbits) large, stable plaques develop in the aorta and coronary arteries (reviewed by Fan et al. [63]). However, a mutant colony of the WHHL, termed the myocardial infarction prone WHHL rabbit (WHHLMI), presents with vulnerable-type coronary plaques displaying a large lipid core, thin fibrous cap, accumulation of macrophages and even intraplaque haemorrhage at an age of 20 months (reviewed by Shiomi and Fan [64]). Plaque rupture was, however, never observed and the observed myocardial infarctions were attributed to stenotic plaques or vasospasm. Despite the fact that the mechanical environment appears more optimal in rabbits compared to mice due to the larger size of the plaques, rupture only occurs after severe stimuli which potentially induce different failure modes in the plaques. The main underlying cause seems to lie in the plaque composition. Although very frequently labelled as ‘vulnerable’, subtle differences between human and rabbit plaques render them much less rupture prone. Heterogeneity within the plaque, an important source of stress peaks, is generally absent. Moreover, the plaques are predominantly lipid rich, and the cap covering the core does not contain stiff collagen fibres. This implies that the mechanical stresses inside the plaque will be low, and from that perspective, it is not surprising that cap rupture in rabbits was not observed.

In normocholesterolaemic pigs, a combination of interventions has been applied to accelerate the process of vulnerable plaque formation. The combination of high fat diet and diabetes was used in multiple studies (70–73) and resulted in large, advanced plaques in the coronary arteries, aorta and iliac arteries within half a year. Gerrity et al. (70) used pigs on high-fat diet and induced diabetes to study plaque formation. The observed plaques displayed acellular necrotic cores, fibrous cap, medial thinning, IPH and calcifications and were often almost totally occlusive but plaque rupture was not observed. Similar results were obtained in smaller pig studies but none of these showed events of plaque rupture either (71–73). From a biomechanical point of view, plaque rupture is expected to occur more frequently in the diabetic pig models since – on first sight – morphological plaque features and loading conditions resemble the human situation. Closer inspection of plaque composition reveals subtle, but biomechanically relevant differences between plaque composition in diabetic pigs and humans. First, the necrotic core is described as ‘relatively acellular’ (70). The accompanying histological data reveal plaques with extracellular lipid, but no discernible cholesterol crystals or cell debris. This seems to be indicative of an immature necrotic core. Compared to a true necrotic core, the immature necrotic core contains more extracellular components, and they will account for a higher stiffness. This will reduce deformation of the cap, and therefore lower cap stress values will be present in these models. Furthermore, the cap covering the region with extracellular lipids appears to be rather thick (74). Finally, the absence of a necrotic core will also reduce the inflammatory state of the cap, leading to increased cap strength. The combined effect of these observations, together with the limited number of vulnerable plaques included in most of the studies, might explain the lack of plaque ruptures observed in diabetic pig models.

Multiple studies (75–77) showed the effectiveness of partial carotid ligation in combination with a high-fat diet. Large plaques with vulnerable characteristics like a necrotic core, calcium, IPH and thin fibrous cap were seen proximal to the stenosis in the carotid arteries (75, 76). Strikingly, adding diabetes as an additional risk factor, did not result in more vulnerable plaques (78). The only
pig model that did show frequent signs of rupture in the form of cap rupture and luminal thrombus formation was developed by Jiang et al. (77). At 12 weeks after surgery these pigs displayed large, rupture prone plaques with neovascularisation and IPH which were characterised up to AHA stage VI. As an additional sign of rupture even distal emboli were found in the rete mirabile at the basis of the skull. The effect of ligation on the biomechanical environment in pigs will share features with the previously discussed effects in mouse models employing a cast or a ligation. In short, the ligation could prevent the artery to deform, leading to high strains and concomitant high stresses. The 70% stenosis created by ligation in the study by Jiang et al. (77) could affect the luminal pressure resulting in higher stresses in the upstream region, where most of the advanced plaques were seen. Note that the stress concentrations that occur are unlikely to be present in human plaques, potentially triggering unnatural failure mechanisms. The fact that rupture is observed in this ligation model but not in the other pig models, suggests a potential role for artery deformation and/or luminal pressure in plaque rupture in pigs. The use of relatively young animals, which still have elastic arteries that are capable of deformation, preventing a rise in luminal pressure, could therefore explain the lack of rupture in the other pig studies. Recently, a new pig model with a mutation in the PCSK9 gene was developed by the group of Falk et al. (79). The first study with this model (n=13) showed promising results, but the most advanced plaque were characterised as FA and did not show signs of a vulnerable plaque type. In a new study design, hypertension was added as an additional risk factor by performing suprarenal aortic banding. The observed FAs appear to develop faster, but final study results have not been published so far (79).

Concluding remarks

An optimal animal model to study plaque rupture is currently non-existent. Size, morphology and material properties of mice all result in lower cap stresses compared to human, thus prohibiting plaque rupture. Without drastic intervention, mouse and rabbit models do not demonstrate plaque rupture. Some of the intervention models that do show rupture have potential. Of these, manipulation of the biological processes with the possibility of vascular adaptation is preferred over models that create different cap failure modes by restricting vascular adaptation, e.g. ligation and cast placement. From a biomechanical perspective, plaque composition in rabbits differs too much from human plaques to be relevant for rupture studies. The pig model most resembles the human biomechanical environment and plaque morphology. The lack of rupture could be due to subtle differences in plaque composition, limited number of vulnerable plaques, or animal age, and requires more research. Induction of rupture in pigs should preferably focus on increasing stress, by reducing cap thickness, or reducing strength, by manipulating the inflammatory status. We conclude that despite differences between animal models of atherosclerosis and the human disease process, animal models are still useful and necessary to study separate mechanistic disease processes.

Conflicts of interest
None declared.

References
2. Van der Wal AC, et al. Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. Circulation 1994; 89: 36–44.