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DISSERTATION

Vessel function of the atherosclerotic low-density-lipoprotein-receptor-deficient apolipoprotein-B-100-only mouse

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1 Introduction

1.1 Atherosclerosis

Atherosclerosis is a major health problem. It is a chronic, slowly progressing, non-reversible disease affecting the blood vessel walls leading to a "hardening" or "stiffening" of the arteries. Atherosclerosis is the most important underlying cause of cardiovascular disease (CVD) [64]. Today, CVD rank as the most common cause of death in European men under 65 years and as the second most common cause of death in European women [32].

Major manifestations of atherosclerosis-related CVD are:

1. Coronary heart disease or coronary artery disease such as angina pectoris and myocardial infarction
2. Cerebrovascular disease such as transient ischemic attack and stroke
3. Peripheral vascular disease affecting blood vessels in legs, arms, stomach or kidneys

1.2 Literature overview

1.2.1 Pathological vessel alteration

Morphological substrate of atherosclerotic alteration is the formation of multiple atheromatous plaques within the vessel wall. Atheromatous plaques consist of three components [34][90]:

1. The atheroma, which is a nodular accumulation in the center of a plaque consisting of yellowish, soft material and macrophages
2. Cholesterol crystals
3. Calcification at the outer base of the plaque of older lesions

Within the process of plaque formation, the vessel wall loses elasticity through calcification, collagen, and vascular smooth muscle cell (VSMC) proliferation. For a period of time, the vessel compensates for changes in the wall and lumen through artery enlargement. After depletion of compensatory mechanisms, several outcomes can occur, such as [34][90]:

1. Obliteration of the lumen through sudden plaque rupture and consecutive stenosis and ischemia causing stroke or heart infarction.

2. Continuous narrowing of the vessel lumen leading to relative ischemia in stress situations, such as in angina pectoris.
3. Development of aneurysms through sagging of the stressed vessel wall, which can cause massive internal bleeding.

1.2.2 Disturbances of the lipid metabolism

High blood cholesterol is the most important risk factor for atherosclerosis. For its transportation and for the transportation of triglycerides within blood, lipoproteins are necessary. Major lipoprotein classes are: low density lipoprotein (LDL), high density lipoprotein (HDL), very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), and chylomicrons.

The protein part of lipoprotein complexes consists of apolipoproteins typical for each lipoprotein class. The apolipoproteins bind to the lipids, keep them soluble and act as a receptor ligand for cellular uptake. They are produced in the liver and gastro intestinal tract and are critical for the distribution of lipids from these organs to the periphery or vice versa. The risk of atherosclerotic processes rises with the concentration of cholesterol-rich LDLs in the plasma. LDLs, which are the smallest of the different lipoproteins, are far more atherogenic than larger particles such as VLDL or IDL [83]. The small size of LDLs makes it easy for them to leave the blood stream and enter the vessel wall. This is the first key step in the development of atherosclerosis (Figure 1-1).

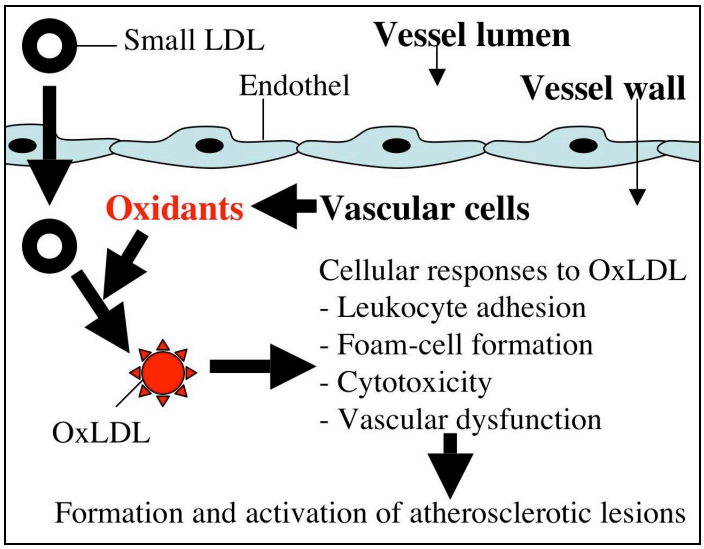

Figure 1-1: LDL and Atherosclerosis (modified from Diaz et al. [17])

The second key process in the development of atherosclerosis is the oxidation of LDL to oxidized LDL (oxLDL) within the vessel wall [59].

In the oxidation process, focus is set on specific proinflammatory oxidized phospholipids containing arachidonic acid in LDL particles. Enzymes such as the lipoxygenase and the myeloperoxidase cause the transformation of LDL into oxLDL thus generating proinflammatory

compounds. They are recognized by the innate immune system causing monocyte entry into the vessel wall [59]. In the vessel wall, the monocytes internalize the oxLDL. Since the oxLDL cannot be adequately metabolized by the monocytes, the monocytes start to degenerate and turn into foam cells. At the same time, the monocytes release potent proinflammatory cytokines causing cytotoxicity, vascular and endothelial dysfunction and atherosclerotic plaque formation.

1.2.3 Adhesion molecules

Blankenberg et al. wrote in their review article that adhesion molecules are "molecules expressed on the surface of a cell that mediate the adhesion of the cell to other cells or to the extracellular matrix" [6]. Several studies found that one of the early stages in the development of atherosclerosis and plaque instability is the overexpression of cellular adhesion molecules as seen in response to an inflammatory stimuli. The recruitment and subsequent interaction and migration of inflammatory cells through the vessel wall mediated by these adhesion molecules play a pivotal role in the pathogenesis of atherosclerosis [6][14][15][38].

1.2.4 Atherosclerotic vessel function

The endothelium:

Changes in the endothelial layer due to atherosclerotic processes are of special importance. The conglomerate of all vessel endothelial cells of the human body can be considered as an organ with a surface area of approximately $1000m^2$, consisting of approximately 10^{12} cells and weighing 0,1kg [40]. The endothelial cells have a very important function mediating the vessel tonus according to different pressure situations and substances arriving to the vessel via the blood stream. Therefore, pathological changes in the endothelium result in changes in circulation parameters and vice versa. The most common outcome following damage to the endothelial surface is thrombosis, causing dramatic effects such as heart infarction or stroke [34][90]. However, prior to the final damage to the endothelial surface, changes have taken place that can be explored both morphologically and functionally.

Vascular smooth muscle cells:

Due to atherosclerotic processes, VSMCs in the vessel media start to proliferate [90]. Although this should increase the contractile capacity of the vessel wall, the effect can be antagonized by plaque formation and calcification of the vessel wall, which will attenuate its elasticity.

Cellular mechanisms:

It has been established in different research models that the responses to vasoactive substances can be altered during the course of atherosclerosis [37][44][88]. Furthermore, high blood cholesterol levels and oxidized lipoproteins can change functional vessel characteristics even before the manifestation of atherosclerosis [12][13][22][24][28][30][46][57].

Several mechanisms are supposedly responsible for these changes:

1. Altered cell surface expression for receptors of vasoactive substances.
2. Alteration in the quantity of excreted vasoactive substances.
3. Changes in inner cell reactions, leading to altered levels of secondary messengers, such as nitric oxide (NO), inositoltriphosphate (IP_3), cyclic AMP (cAMP), cyclic GMP (cGMP), or other intracellular signaling mechanisms.
4. The inflammatory process of atherosclerosis induces the production of cytokines, cyclooxygenase (COX) products, reactive oxygen species (ROS), and other products that may interfere with the effect of vasoactive substances.
5. Instead of leading to an altered reaction for each individual cell, the proliferation of VSMCs due to atherosclerosis could change the contractile capacity simply through an increase in cell numbers. This effect could be counteracted by plaque formation and calcification of the vessel.

Potential interactions between these effects may not be ruled out.

1.3 Animal models in atherosclerosis research

In general, excessively high cholesterol intake will lead to high blood cholesterol levels, high LDL levels and the development of atherosclerosis. Yet, the severity of atherosclerotic alteration is modified by the genetic background.

Atherosclerosis has been described as a disease involving a genetic network rather than a single linear pathway. In a normal physiological state, this genetic network is sensitive to the known risk factors, which also include different organ systems and cell types.

In human research, all efforts to identify those genes responsible for the most common forms of atherosclerosis have had little success [29]. Furthermore, the understanding of atherosclerotic pathways and the possible interventions remain unclear [65]. For the common forms of atherosclerosis, genes will only have a modest influence on disease pathogenesis and are, in addition, often masked by environmental stimuli and the varying genetic backgrounds of subjects

in cohorts studied. Thus, different animal models have been established to address specific aspects of this disease and its morphological and functional alterations.

Experiments can be performed on intact animals, revealing mostly changes in the biological system. In vitro experiments can be done with isolated blood vessel in myographs or on cultured cells, thereby providing valuable information about local and cellular changes.

Mammals have a very conservative gene structure. For instance, humans and mice differ only in approximately 300 genes, therefore it can be hypothesized that the overall features and interactions on disease development should be similar [29]. Yet, the time for the development of atherosclerosis might play an important role in disease progress. In humans, the first atherosclerotic changes can already be seen during childhood, while manifest cardiac events might take place in the 6th decade of life or even later. For mouse models, the development of atherosclerosis will only take several months and they will mostly develop mild forms of the disease.

1.3.1 Mouse models

Naturally, rodents do not develop atherosclerosis. Their lipoprotein profile is different from humans. In mice, approximately 90 % of the plasma cholesterol circulates in the HDL fraction [66], which is known for its atheroprotective effect [5][9][10][21][50][59][63][72][82][83]. The rest of their plasma cholesterol is carried in the VLDL fraction and atherogenic LDL levels are very low.

One way of inducing atherosclerosis in mice is by feeding an excessively high fat diet. Another way is through genetic modification in order to introduce perturbations in their lipid metabolism. Both ways have to be viewed critically as an "artificial" atherosclerosis setup.

Different types of models include [19]:

1. Inbred strains of mice with specific susceptibility to developing (diet induced) atherosclerosis (for example C57BL/6 mice)
2. Transgenic models with genes derived from other species (for example human apolipoprotein B mice)
3. Gene-targeted models, so called "knock-out" models, which are unable to express a certain gene (for example LDL receptor (-/-) mice)

4. Mouse models with compound genetic manipulations that are unable to express several genes (for example LDL receptor (-/-) x apolipoprotein B (100/100) mice)

Animal models with high-fat diet-induced atherosclerotic lesions are often used for research. This was considered controversial [19], because it induced atherosclerosis too different from the pathogenesis in humans since the amount of fat has to be excessively high. It was discussed that the metabolism and transport via lipoproteins were not responsible for the atherosclerotic consequences but that the diet itself was so „unhealthy“ that it was already inflammatory. This problem was solved when the method of gene-targeted animal modification was established. It was now possible to create specific diet-independent genetic lipid metabolism perturbations for research.

1.3.2 Development of the mouse model used in this study, the LDL receptor (-/-) x ApoB (100/100) mouse, called "ApoB mouse"

LDLr knock-out mouse (LDLr (-/-))

The disruption of the LDL receptor (LDLr) was achieved through a gene-targeting vector of the replacement type in embryonic stem cells by Ishibashi, Herz et al. in 1993. These mice express a truncated form of the LDLr and are unable to bind LDL particles [39].

In humans, this defect causes familial hypercholesterolemia. Humans homozygous for this defect have a two- to threefold increase in blood cholesterol and usually succumb to a myocardial infarction during their second decade of life [8]. But mice have differences in lipid metabolism, which protect them from developing atherosclerosis even in the absence of the LDLr [39] (Figure 1-2).

ApoB100-only mouse (ApoB (100/100))

ApoB100-only mice were produced by Farese, Young et al. in 1996 by "hit and run" gene targeting in embryonic stem cells [25]. A "non stop" mutation was implemented into the ApoB48 editing codon, changing "A" for "T". Thus, these mice could only synthesize ApoB100 and no ApoB48 [25].

ApoB100 is necessary for the assembly of VLDLs in the liver and is virtually the only apolipoprotein of LDLs, which are derived from VLDLs [85]. ApoB100 is also present in the yolk sac during embryonic development. The other ApoB, ApoB48, is a truncated form of

ApoB100 (48% of the length of ApoB100), a result of mRNA editing by the enzyme ApoBec-1 [35]. This ApoB48 is produced in the intestine for the formation of chylomicrons.

In contrast to the human liver, the mouse liver has ApoB mRNA editing activity. Here, ApoB100 and ApoB48 are both synthesized and used for VLDL assembly [65]. In fact, 70 % of murine ApoB is comprised of ApoB48 [85]. Different from ApoB100, ApoB48 lacks the portion of the ApoB100 molecule that is necessary for binding to the LDLr [85]. Instead, ApoB48-containing lipoproteins lodge a large amount of ApoE, which enables them to bind to the LDLr, the LDL-receptor-related-protein (LRP) [85], and the VLDL receptor (VLDLr) [74]. The LRP and the VLDLr are not affected when there is a deficiency in LDLr. Thus, the mouse lipoprotein levels do not rise as high as in humans in the absence of LDLr and they develop very little atherosclerosis [62], unless fed a high fat diet [19].

LDLr (-/-) x ApoB100-only mouse ("ApoB mouse")

In 1998, Veniant, Young et al. bred LDLr-deficient mice with mice that synthesize exclusively ApoB100 by mixing the two models described above [83][85]. The official strain name is B6;129S-ApoBtm2SgyLdlrtm1Her (ApoB mouse).

This mouse model suffers from extremely high cholesterol levels in the LDL fraction, which cannot be cleared from the blood by the dysfunctional LDLr or the functional LRP.

The lipid metabolism of the ApoB mouse is pictured schematically in Figure 1-2.

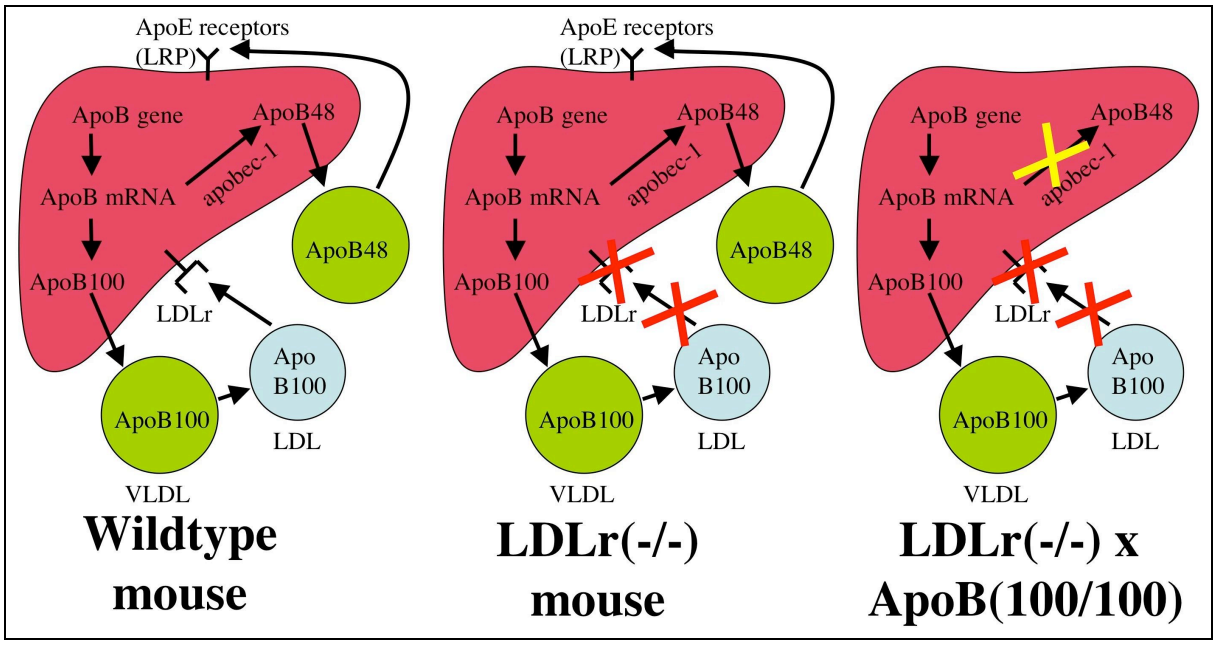

Figure 1-2: Lipid metabolism of different mice models (modified from Rader et al. [73])

The ApoB mouse develops atherosclerotic lesions throughout the arterial tree even on a chow diet [83][85]. Therefore, the criticism raised regarding mouse models with high-fat diet-induced atherosclerosis cannot be made with the ApoB mouse model [19].
Also, while most other mouse models have lipoprotein profiles dissimilar to humans [84], the ApoB mouse has high plasma levels of ApoB100 containing LDL. This is very similar to most humans with atherosclerosis [85].

1.4 Functional alterations due to atherosclerosis

1.4.1 Endothelial vasodilative function

Lacy et al. [48] wrote that three sorts of substances could lead to vessel relaxation:

1. Nitric oxide (NO)
2. Endothelium-derived hyperpolarizing factor (EDHF), which induces vasodilation and acts in a NO-independent, prostanoid-independent manner
3. Vasodilator prostanoids (such as prostacyclin (PGI_2)); prostanoids include all eicosanoids except leukotrienes

The role of nitric oxide (NO)
One of the most important substances produced by the endothelium is nitric oxide (NO). NO has only a very limited lifetime before it is degraded, yet its effects on the VSMCs in the media of the vessel are enormous, causing a fast and pronounced relaxation. Once this pathway is pathologically damaged, the vessel cannot react appropriately to high-pressure challenges resulting in an overall higher blood pressure.
NO is produced by NO-synthases (NOS) from the amino acid l-arginine. NO diffuses through the VSMC membrane. Here, it causes vessel relaxation by activating the guanylate cyclase resulting in an increase in cyclic GMP (cGMP) [44].
There are two constitutive isoforms of NOS. One is found mainly in endothelial cells (eNOS), the other in neuronal cells (nNOS). NO production of eNOS is activated by either the stimulation of cell surface receptors or by mechanical forces such as shear stress [23]. There is a third form of NOS, the cytokine inducible isoform (iNOS) found in different cell types including macrophages, hepatocytes and VSMC. In endothelial cells, eNOS and iNOS are crucial for the vasodilative vessel function [94]. It was described that iNOS is important in VSMC dilation and in inflammatory reactions [46] and iNOS is expressed in advanced atherosclerotic lesions [68]. Kim et al. found that in atherosclerotic rats iNOS expression was stronger and diffused into the

media as lesions progressed. They hypothesized a correlation between lesion gravity and iNOS expression [46]. Furthermore, iNOS protein expression and activity can be enhanced as a result of hypercholesterolemia [64].

The substance NO is also pivotal in blood vessel metabolism. Rabelo et al. wrote that NO not only influences the vessel tone via VSMC [64], but also inhibits various atherogenic processes such as:

1. VSMC proliferation and migration [64]
2. Platelet aggregation [64]
3. Oxidation of LDL particles [64]
4. Monocyte and platelet adhesion [64]
5. Production of inflammatory cytokines [64]
6. Leucocyte adhesion and migration into the vessel wall [9]

Attenuated production of endothelial NO plays a central role in the development and progression of atherosclerosis. This is referred to as "endothelial dysfunction", resulting in impaired vasodilation or increased VSMC contractions in response to vasoconstrictors [94].

Endothelial dysfunction

Endothelial dysfunction is a characteristic associated with atherosclerosis and is for that reason considered to be an early trait in atherogenesis [9].

Possible reasons for this dysfunction are:

1. Altered eNOS gene expression [94]
2. Degradation of NO (through oxidative stress by reactive oxygen species (ROS)) [23][64][94]
3. Reduction in concentration or activity of both iNOS and eNOS with subsequent impaired release of NO [56][58][64][94]
4. Impaired NO diffusion from the endothelium to VSMC followed by decreased sensitivity to its vasodilator action [58]
5. Functional abnormalities ("uncoupling") of eNOS due to deficiency of substrate or cofactors with production of ROS instead of NO [23]
6. Impairment of membrane receptors in the arterial wall that interact with agonists or physiological stimuli capable of generating NO [56]
7. Reduced concentrations or impaired utilization of l-arginine [56]

8. Impaired interaction of NO with guanylate cyclase and the consequent limitation of cGMP production [56]

The role of reactive oxygen species (ROS)

NO degradation by ROS is very important in atherosclerotic vessel function [64][94]. D'Uscio et al. discovered that the key mechanisms of endothelial dysfunction are increased production of $O_2\bullet^-$ and reduced eNOS activity in the aorta of atherosclerotic ApoE (-/-) mice [23]. Endothelial cells, VSMCs, and atheromatous plaques appeared to be the site of enhanced ROS production. It was shown that application of cell-permeable superoxide dismutase (SOD) mimetics enhanced endothelium dependent and endothelium independent relaxations [23]. Since indomethacin had no effect on endothelium-dependent relaxations in the aorta of their mouse model, it is unlikely that the COX pathway is responsible for ROS production [23].

eNOS itself can become atherogenic due to an uncoupling mechanism, which causes eNOS to produce ROS instead of NO. Furthermore, enzymes that produce superoxides, such as subcomponents of NADPH oxidase, are increased in atherosclerosis [94]. This ROS production not only inactivates NO but also again enforces eNOS uncoupling [94].

In combination with ROS, NO could cause lipid peroxidation or cellular damage. De Angelis et al. suggested that $O_2\bullet^-$ superoxide anions may directly inactivate NO, producing peroxynitrite ($ONOO^-$). This peroxynitrite can hydroxylate nitrate aromatic compounds and induce cellular injury [20]. This could have an effect on the pathogenesis in later disease stages. In line with this, Kim et al. described the effects of NO as a "double-edged knife." Both the profusion and the scarcity of NO causes disease [46].

Yet, it is improbable that enhanced ROS production fully explains the attenuated endothelium-dependent vasodilation in atherosclerotic blood vessels. In an ApoE (-/-) mice model, in vitro or in vivo application of SOD did not completely re-establish relaxations [92].

Even more, the role of low, strictly controlled $O_2\bullet^-$ levels is essential for normal vessel function. $O_2\bullet^-$ has an important regulatory function. It is involved in activating the hypertrophic responses of arteries and cardiomyocytes. In some vascular beds, $O_2\bullet^-$ may even contribute to endothelium-dependent vasodilation. The results suggest that the fine equilibrium between the positive and harmful effects of $O_2\bullet^-$ are clearly a basic feature of vessel control [20].

The role of blood lipids

There are different findings concerning the influence of lipids on endothelial dysfunction. Esenabhalu et al. [24] examined the vascular function of mice transgenic for vascular lipoprotein

lipase. In transgenic mice, this enzyme led to increases in aortic free fatty acids (FFAs), in acetylcholine (ACh) relaxation, and no change in the sodium nitroprusside (SNP, a NO donor) response. After application of SOD, the reduced ACh-induced relaxation in the transgenic group was normalized. The authors concluded that the reduced relaxation is due to increased NO scavenging by $O_2\bullet^-$. Although the lipoprotein levels are not increased, increased FFAs initiate vascular dysfunction through protein kinase C-mediated activation of endothelial NADPH oxidase [24]. In accordance with these findings, Jiang et al. found in experiments using the aortas of rabbits fed cholesterol for 6 months that the impairment of endothelium-dependent vasodilation is caused by attenuated bioavailability of NO due to increased inactivation of NO by ROS rather than a decrease in NO release [44]. Furthermore, artificial elevation of nonesterified FFA concentrations can reduce NO production [13]. These studies suggest an influence of systemic blood lipid levels on endothelial dysfunction.

Nevertheless, Crauwels et al. found ACh relaxations inversely correlated with plaque size. In adjacent atherosclerosis-free vessel segments of ApoE (-/-) mice, responses to ACh and all other agents were unaffected, although they had undergone long-term hypercholesterolemia. The authors concluded that endothelial dysfunction in ApoE (-/-) mice was not affected by hypercholesterolemia; however, it was closely associated with atherosclerotic plaque development [16]. Similar results were obtained in Watanabe heritable hyperlipidemic (WHHL) rabbits. The decrease in ACh-induced relaxation of their aortas correlated in a linear manner with the area covered with atherosclerotic lesions [75]. Johansson et al. found in in vitro experiments in ApoE (-/-) mice hypercholesterolemia unable to induce endothelial dysfunction. Atherosclerotic lesions seemed to be essential. Yet, they suggested that in vivo, endothelial dysfunction due to hypercholesterolemia was highly conceivable [45].

The effect of oxLDL on NO and prostacyclin (PGI_2) release is still unclear. oxLDL has been demonstrated to increase or decrease NO and PGI_2 production [43]. Nevertheless, it is widely accepted that oxLDL and hyperlipidemia impair endothelial-dependent vasodilation [43] and impairment of endothelial function occurs even before vascular structural changes begin to take place [23]. In humans, various studies showed effects of hypercholesterolemia on endothelial function [45]. oxLDL can suppress eNOS gene expression in endothelial cell cultures [94]. Furthermore, incubation with oxLDL inhibits endothelium-dependent vasodilation in isolated porcine coronary arteries [45].

1.4.2 Cyclooxygenase inhibition

In in vitro models, cyclooxygenase (COX) inhibitors such as indomethacin can be used to verify if COX products are involved in the impaired ACh response in endothelial dysfunction [27]. The findings in literature concerning the results of COX inhibition are not consistent. Most studies found that the ACh response was unchanged after incubation with indomethacin [23][27][48][64][78]. In a few models, the ACh response was increased after indomethacin incubation [17][20]. In some models, the ACh sensitivity also increased [17].

COX-inhibitors such as aspirin are commonly used for the prevention and treatment of CVD [70]. COX regulates the production of eicosanoids [51]. These eicosanoids adjust the physiologic processes in the arteries that are essential for the development of atherosclerosis and thrombosis. This includes platelet aggregation, regulation of blood vessel tone, and inflammatory responses within the vessel wall. Of the two existing COX subtypes, COX-1 is necessary for synthesis of platelet thromboxane A_2 (TxA_2), which is a potent vasoconstrictor and platelet agonist. COX-1 is constitutively present in most cells for maintenance functions. Both COX-1 and COX-2 contribute to prostacyclin synthesis, a vasodilator that inhibits platelet activation. COX-2 synthesis is quickly enhanced in situations characterized by inflammation. For instance, COX-2 is upregulated in activated macrophages, which play a pivotal role in the development of atherosclerosis. Thus, COX-1 and COX-2 might have important roles in atherosclerotic plaque formation. The inhibition of platelet COX activity [70] and TxA_2 production [51][70] has been associated with beneficial effects in the prevention of cardiovascular events. Furthermore, some COX-inhibitors are potent ROS-scavengers and are able to restrain intracellular oxidation [70]. Conversely, the COX product prostacyclin (PGI_2) is a powerful vasodilator and inhibits platelet aggregation and leukocyte adhesion. Thus, PGI_2 is believed to play an atheroprotective role [51].

Indomethacin potentiated ACh-induced relaxations (and increased the sensitivity) by enhancing ROS production in rat thoracic aortic rings of healthy male Wistar-Kyoto rats [20]. Simultaneously, the COX-inhibitors aspirin and ketoprofen did not have an effect on ACh-relaxations. Thus, it seems likely from this study indomethacin had a COX-independent effect. It was found that the increase in relaxations was not due to ACh-induced NO production. The authors concluded that indomethacin enhances ACh responses by causing an increase in ROS production (such as $O_2^{\bullet -}$) in the endothelium and in specific of peroxynitrite, which is produced

in the reaction of ROS and NO. They concluded that it remains unclear if the production of these substances in this circumstance can be considered atheroprotective or atherogenic [20].

1.4.3 Reaction to vasoconstrictive substances

Phenylephrine

Phenylephrine (Phe) responses were attenuated in different animal models of atherosclerosis [22][28][47][49]. Dowell et al. found that responses to agonists were inversely related to the extent of atheroma [22]. The Phe hyper-reactivity appeared to be overcome by disease progression as the structural changes started to occur in the vessels. As the atheroma progressively intruded into the media, causing disruption of the VSMCs and elastic laminae, the ability of the vessel to constrict was impaired. Migration of VSMCs from the media to the intima might have also contributed to the observed loss in contractile function. It has been observed that VSMCs, under certain conditions including atheroma, will alter their phenotype from the contractile status to secretory status, impairing overall contraction [22].

Furthermore, NO synthesis plays an important role in the Phe response. The Phe response is reported to increase after L-NAME application [2]. Thus, a possible explanation for the smaller Phe response might be the enhanced NO production [13][46][52].

These results are in contrast to reports that NO expression is actually impaired in vascular disease states [30], mostly through eNOS impairment [31]. Even hypercholesterolemia itself is known to be associated with a diminished eNOS activity [46]. In contrast, iNOS can be overexpressed [46][52]. For example, in atherosclerotic rabbits, cholesterol-rich diet impairs eNOS and enhances iNOS. At the same time, Phe reactions were diminished [46].

Other studies have found increased Phe responses in atherosclerotic vessels [24][37][44][61][73]. Vita et al. suggested that the endothelial dysfunction correlates with enhancement in sensitivity to the constrictor effects of catecholamines in humans [86]. Proposed mechanisms for the enhanced vasoconstrictor responses are augmented cholesterol content of VSMC membranes, impairment of the endothelium (NO release) and an increase in alpha-adrenergic receptors [12]; however, some studies found Phe responses unchanged in atherosclerotic vessels [12][22][33][77][87].

Angiotensin II

As a major vasoactive substance, angiotensin II (Ang II) is strongly associated with the development of hypertension and atherosclerosis [77]. Ang II is a multifunctional hormone.

Through a complex series of intracellular signaling it has various effects on the function of cardiovascular cells. These are induced by the binding of Ang II to AT_1 and AT_2 receptors. The effects of AT_1 receptor activation are vascular contraction, VSMC growth, inflammatory responses, intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) expression, and salt and water retention [80][81][93]. AT_2 receptor activation leads to apoptosis, vasodilation and natriuresis [80]. AT_2 receptors may play a role in lowering blood pressure [76].
These signaling pathways could have an essential role in structural and functional abnormalities in CVD such as cardiac hypertrophy, hypertension and atherosclerosis [80].

Yang and colleagues found a 5-fold increase in Ang II receptor expression in atherosclerotic rabbit aortas. The increase in Ang II receptors was due entirely to AT_1 receptor expression, as AT_2 expression was unaltered [93]. Even in vitro, the AT_1 receptor is upregulated in the presence of LDL (the effect was maximal after 12 hours incubation) [60]. AT_1 receptor density is increased in the medial lesion and angiotensin I-converting enzyme (ACE) activity rises in atherosclerotic vessels producing even more Ang II within the lesion area [69]. The source of this Ang II could be activated macrophages within atherosclerotic plaques [87]. The membrane-associated NADH-dependent oxidase, which is the predominant source of $O_2\bullet^-$ in vascular cells, is activated via the AT_1 receptor. Hypercholesterolemia is associated with an increase of NADH-dependent vascular $O_2\bullet^-$ production, inactivating NO [87]. Early stages of atherosclerosis, such as endothelial dysfunction, are characterized by a reduction in the bioavailability of vasodilators, especially NO. At the same time, there is an increase in the activity of vasoconstrictors, including Ang II and ROS. NO partially antagonizes the vasoconstrictive and atherogenic effects of Ang II. On the other hand, Ang II decreases NO bioavailability via the promotion of oxidative stress [67].

The changes reported on Ang II contraction in atherosclerotic vessels are not consistent. Some studies show increased vessel reactions, while others suggest unaffected or even diminished vessel responses [77][88]. The discrepant results may be due to the fact that Ang II receptor expression is not always responsible for the contractile results. In WHHL rabbits, the contractile response to Ang II was decreased despite an increased AT_1 receptor expression [77].

1.5 Derivation of the question

Atherosclerosis is a major health problem. Although an enormous amount of research has examined the pathogenesis of atherosclerosis and described its characteristics, a great deal remains unclear. Exploration in humans and animal models has very often been conducted on a morphological basis with pathohistological preparations of affected vessels and organs.
With our approach, we take into consideration the vessel physiology in terms of functional alterations of atherosclerotic vessels affecting blood circulation parameters. This will allow us to draw better conclusions about the development and characteristics of atherosclerosis.

The goal of this thesis was to explore the reaction of vessels in response to vasoactive compounds in the ApoB mouse model. The results concerning the altered vessel function from studies with different animal models are inconsistent. In contrast to most other animal models, the ApoB model has a lipoprotein profile similar to humans with atherosclerosis. Therefore, the functional alterations of the ApoB mouse vessels might allow us to draw more valid conclusions about the characteristics and mechanisms of this disease in humans.

The existing literature concerning atherosclerosis has prompted us to explore the following questions:

1. How has the contractile and vasodilative vessel physiology changed in the atherosclerotic ApoB mouse model?
2. Which pharmacological substances are the most useful substances to test these alterations?
3. Is there a correlation found in the ApoB model between serum parameters, vessel function, and morphological atherosclerotic vessel alterations?

The following pharmacological substances were used to find answers to these questions:

1.5.1 Dilative vessel function

To test the endothelial capacity to induce vasodilation, we chose **acetylcholine** (ACh). In most atherosclerosis models, the ACh-related vasodilation is impaired [16][17][23][26][44][64][75]. ACh is a potent agent to stimulate NO production in endothelial cells and thus to mediate vasorelaxation through NO stimulation of VSMCs. Due to atherosclerotic alterations, the endothelial capacity to produce NO could be decreased, leading to less NO outflow. Also, the

VSMC sensitivity to NO could be diminished or the increased production of reactive oxygen species (ROS) due to inflammation could inactivate NO.

To test the involvement of COX-products in the vessel reactivity, we used **indomethacin.** Indomethacin is a non-selective cyclooxygenase (COX) inhibitor. It is well established that the proinflammatory derivates of arachidonic acid, which are metabolized via the COX pathway, not only have an influence on thrombocyte aggregation but also on the VSMC tonus and on ACh mediated reactions [20][53].

Controversial results are reported on the influence of indomethacin on vasorelaxation in atherosclerotic situations. In some models, the endothelial function improved [17][20], while in others it remained unchanged [23][27][48][64][78], suggesting the involvement or non-involvement of COX products in these models.

NO-donors such as **DEA NONOate** induce endothelium-independent vessel relaxation [16], indicating the VSMC sensitivity to NO stimulation. Different results concerning the reaction to NO-donors in atherosclerotic vessels were described. The vessel responses were unchanged [17][26][36][44][45][64][75][79] or attenuated [16][23]. The attenuation is hypothesized to be due to reactive oxygen species (ROS) inactivating NO [92].

1.5.2 Contractile vessel function

Not only are the relaxation characteristics known to change during atherosclerotic alterations but also the contractile responses to vasoactive substances [37][44][88]. We decided to focus on two hormones that are especially important in the contractile regulation of the vessel tonus and circulation parameters: **adrenaline/noradrenaline** and **angiotensin II**.

A pharmacological substance mimicking the adrenaline/noradrenaline effect is **phenylephrine** (Phe), a selective alpha-1-receptor-agonist. Rather than using other catecholamines, such as norepinephrine, Phe has no effect on endothelial receptors that could lead to relaxation, such as the alpha-2- or beta-receptors. Therefore, Phe makes it possible to focus on the pathological contractile responses only [44][73]. Some studies found enhanced [24][37][44][61][73] others attenuated [22][28][47][49] or even unchanged Phe responses [12][22][33][77][87] in atherosclerotic setups.

The peptide hormone **angiotensin II** (Ang II) is the effector hormone of the renin-angiotensin-system (RAS). Ang II is one of the most important vasocontractile hormones [88]. Nevertheless, varying results have been reported on Ang II reactions in atherosclerotic vessels [77][88].
We chose to take the root of the aorta thoracica as research vessel, which consistently develops severe morphological atherosclerotic lesions as shown in preliminary studies in our lab. As a second vessel, we used a piece of the main branch of the arteria mesenterica superior (a. mes. sup.). Preliminary studies in our lab have shown that ApoB mice did not develop atherosclerosis in this vessel. The a. mes. sup. was chosen to explore functional alterations that might already exist without manifesting the morphological signs of atherosclerotic lesions due to hyperlipidemia.

We decided to not only compare ApoB mice with control mice but also young mice with old mice. Since atherosclerosis is a chronic, slowly progressing disease, this comparison might allow us to explore how vessel function changes as the disease progresses over time.
For background information and for correlation to our functional findings, we measured biochemical parameters such as the lipoprotein profile as well as total cholesterol and triglycerides. Furthermore, we measured the expression of two adhesion molecules found in the serum. The intercellular adhesion molecule 1 (ICAM-1) and the vascular cell adhesion molecule 1 (VCAM-1) play important roles in inflammation and atherosclerosis. They also exist in a soluble form within the blood serum (sICAM-1 and sVCAM-1). These adhesion molecules belong to the immunoglobulins and contain extracellular immunoglobulin domains responsible for the adhesion of inflammatory cells to the endothelium [6]. ICAM-1 and VCAM-1 are typically found within the atherosclerotic lesion [1][6], especially in the shoulder regions of atherosclerotic plaques but also in regions without plaque formation [38]. A lot of evidence has suggested that adhesion molecules play a crucial role especially in early stages of atherosclerosis and in plaque rupture. In studies, sVCAM-1 and sICAM-1 were also correlated to the severity of atherosclerosis. The function of the soluble forms, which result from shedding of ICAM-1 and proteolytic cleavage of VCAM-1, remains unclear [6].

To the best of our knowledge, no study has yet explored the functional changes resulting from atherosclerosis in this particular genetic background.

2 Materials and methods

2.1 Animals

The official name of the mice used in this study is B6;129S-Apobtm2SgyLdlrtm1Her mice ("ApoB mice"), bought at The Jackson Laboratory (Maine, USA). Theses mice have two mutations: one that results in the expression of only ApoB100 (instead of ApoB100 and ApoB48) and the other leading to a deficiency for the LDLr [41].

The corresponding control mice were B6129SF2/J mice ("B6 mice"), also bought at The Jackson Laboratory [42]. They are a hybrid cross between C57BL/6J and 129S1/SvImJ mice.
All mice were kept in cages under standard conditions (25°C, 12:12-h light-dark cycle) and given water and standard chow ad libitum. The local authority (Landesamt für Arbeitsschutz, Gesundheitsschutz und technische Sicherheit, Berlin, Germany) approved the experimental protocol, which complied with "APS Guiding Principles for Research Involving Animals and Human Beings."
For consistency, only male mice were taken. To compare the effect of age, a young and old group was chosen for each mouse strain. The so-called young ApoB and young B6 group were, on average, approximately 4 months old. This is the age required for mice to reach an age of sexual maturity and can be considered as „grown-ups“. At this early age, we estimated there would be only little atherosclerotic damage. The so-called old ApoB and old B6 group consisted of mice with an average age of 1 1/2 years, considering them as „old adults“.

2.2 Measuring of the vessel function

Measurement of vessel reactions was made with Dual Wire Myograph System Model 500 A (Danish Myo Technology, Aarhus N, Denmark) (Figure 2-1 and Figure 2-2). The vessels were mounted as ring preparations of 1-2 mm length by threading them over two parallel 40 μm tungsten wires and securing the wires with miniature screws to two jaws. One support was attached to a motor driven micrometer, which allowed the user to individually set the vessel stretch. The other support was attached to a force transducer for measurements of vasocontractile responses. Each of the two vessels was mounted in a 5.0 ml acid-resistant stainless steel chamber for separate independent testing. The vessels were kept in the heated vessel chamber in a physiological Krebs-Henseleit solution (KH solution) at 36.7°C, continuously gassed with

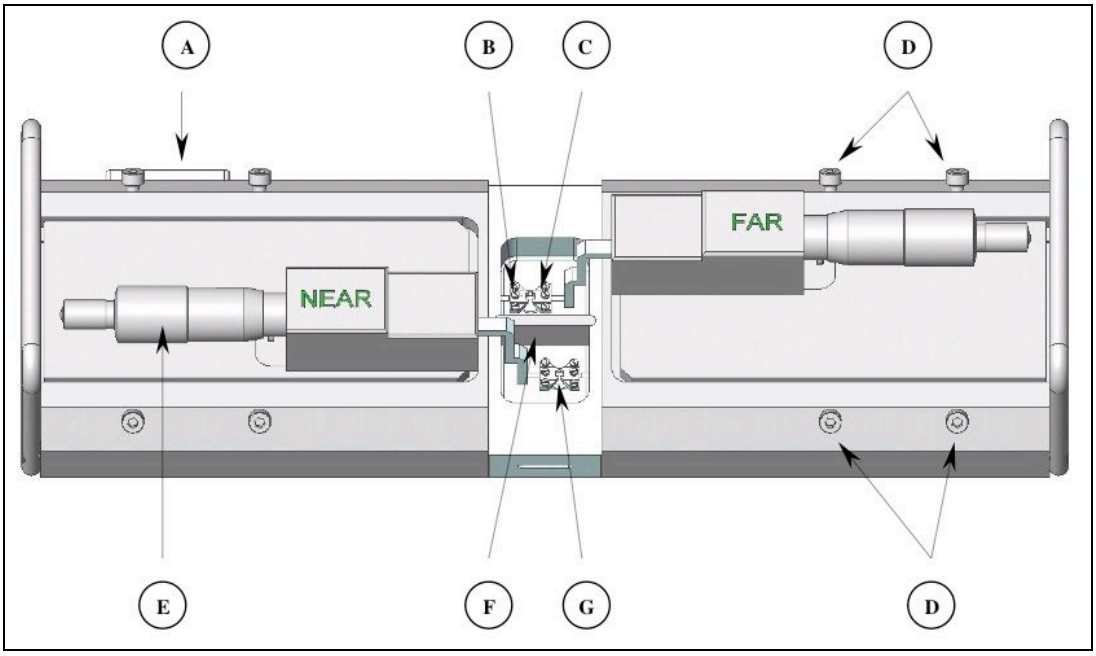

Figure 2-1: Dual wire myograph; A: Port for connection to Myo interface; B: Myograph jaw connected to force transducer; C: Myograph jaw connected to micropositioner; D: Allen screws for fine alignment of the myograph jaws; E: Micropositioner; F: Myograph chamber separator; Window at the bottom for myograph chamber imaging (source: Danish Myo Technology [4])

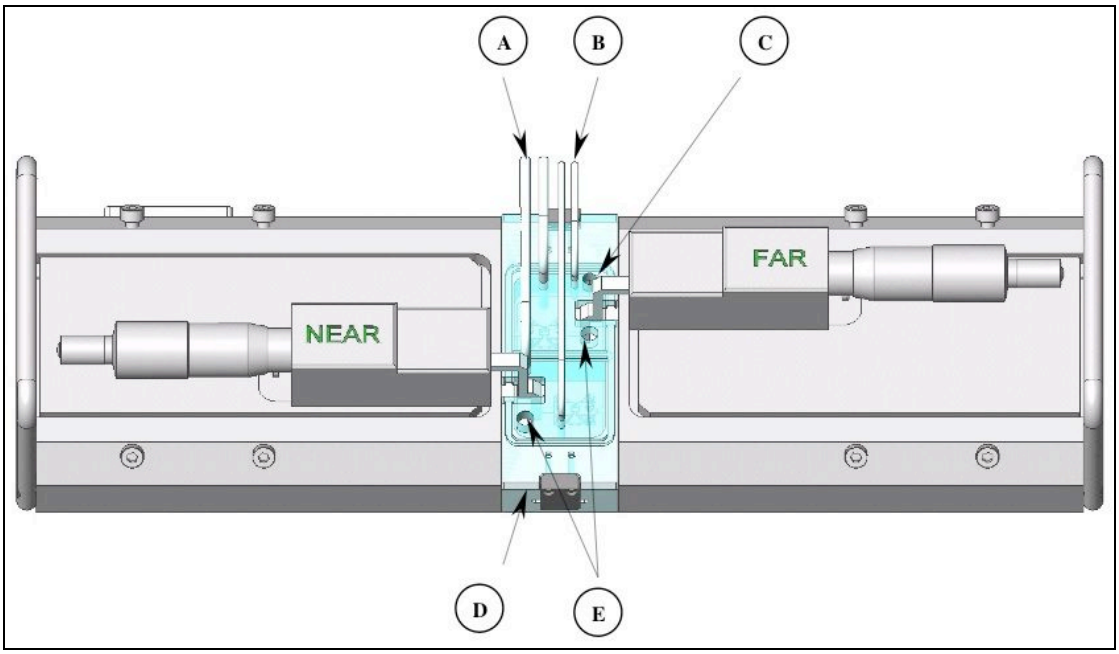

Figure 2-2: Dual wire myograph with chamber cover; A: Suction pipes for connection to vacuum pump; B: Pipes for carbogen supply; C: Access hole for temperature probe; D: Myograph chamber cover; E: Access hole for reagents and buffer (source: Danish Myo Technology [4])

carbogen (95 vol% O_2 for oxygen supply, 5 vol% CO_2 for buffering of the solution at pH 7.4). The chambers were covered by a lucent plastic lid with holes for application of substances and carbogen supply. Using the aforementioned set up, vessels can remain viable for at least 12 hours [3].

During the actual experiments, the stretch of the vessels was kept constant. Thus, the vessels could be examined under isometric conditions. Substances were pipetted directly into the chamber without removing the lid. The vessel tension was monitored and recorded for possible contractile or relaxing effects of the substance.

For recording of myograph data, the Linux program XMad was used. For evaluation of the data, the corresponding program XMana was used (both downloaded at http://www.motifdeveloper.com/non-comm.html).

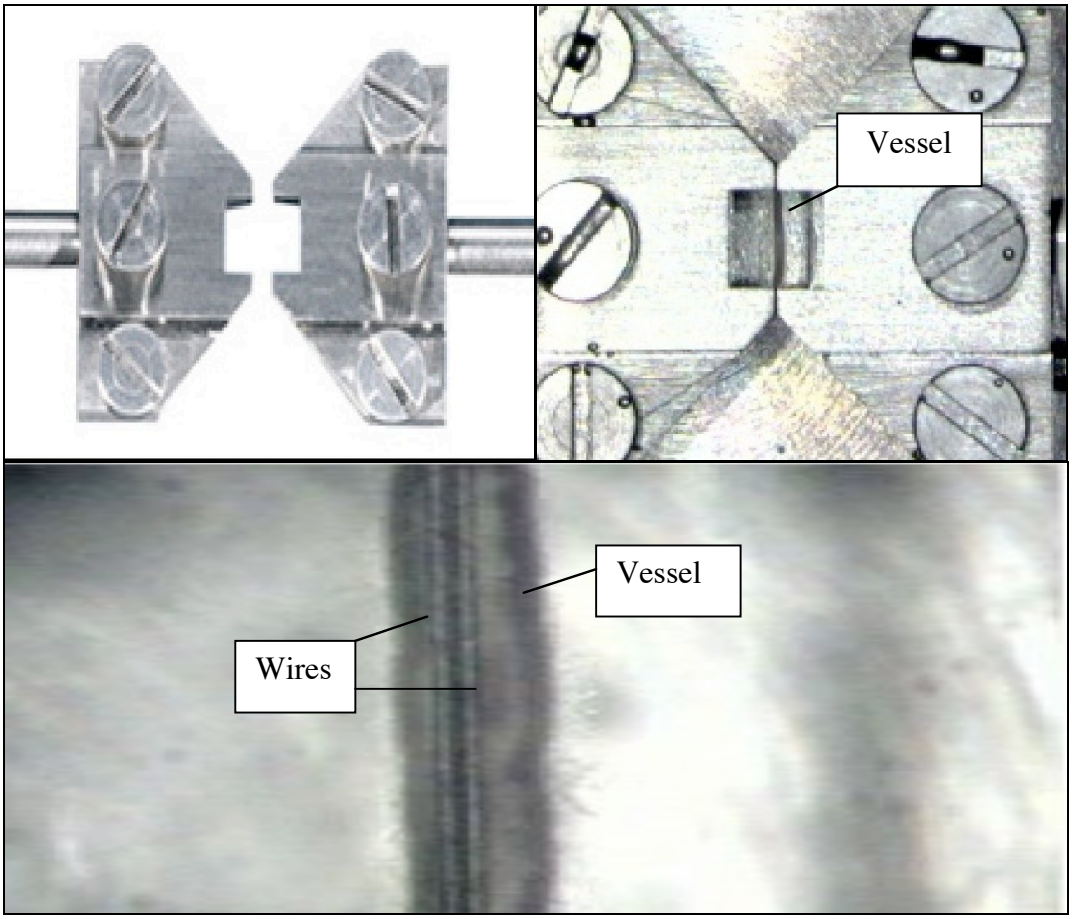

Figure 2-3: Upper left: Mounting jaws; upper right: Jaws with vessel mounted with wire only on left jaw (center of the picture); below: Vessel mounted on both wires, vessel unstretched (source: Danish Myo Technology [3])

2.3 Preparation of vessels

The mice were sacrificed in ether inebriation. Cellulose was soaked in liquid ether and placed in a glass cover and the mouse was put into the glass cover after weighing.

After opening the abdominal situs, the complete aorta thoracica, with the heart, was removed. The same procedure was done with the mesenterium after removal of the complete intestine.

Aorta thoracica and mesenterium were transferred into an ice-cooled petri dish containing physiological carbogenized KH solution. Attached fat and connective tissue of the vessels was

carefully removed. Then, a ring of 2 mm length was cut from the aortic root and the main branch of the a. mes. sup. The vessel rings were transferred into the dual wire myograph Model 500 A and each vessel was mounted in a separate chamber on two 40 μm tungsten wires (Figure 2-3). The rest of the vessels was used for pathohistological research.

2.4 Normalization of the vessels

After mounting, the vessels were equilibrated for 45 minutes in the two chambers of the myograph at 36.7°C and were constantly gassed with carbogen (95% O_2, 5% CO_2). Gassing with carbogen was constantly done until the end of all experiments of the protocol. The KH solution was changed every 15 minutes.

Force-stretch curves were then recorded. Different stretches of the vessel were set on the myograph interface and the corresponding force developed after high K^+ KH solution application was measured. After each application of the high K^+ KH solution, the chamber fluid was replaced with normal KH solution and a new stretch was set. The K^+ responses were measured and recorded for at least 5 different stretches with pauses of 5 minutes between each K^+ application. The stretch with the strongest K^+ response was calculated and the myograph was set to this optimal vessel stretch. The vessels were then again equilibrated at this stretch for 30 minutes, changing the KH solution every 10 minutes.

2.5 Protocols

The recording of a complete cumulative concentration response curve (CRC) with all doses took approximately 60 to 90 minutes for each substance. Doses were increased successively by a factor of 3.2 so that after two successive doses, the cumulative effective dose was increased by factor 10. The next higher dose was applied only after the vessel response had reached a plateau. At the end of each CRC, the chamber fluid was replaced with regular KH solution. Then the vessels were equilibrated for 40 minutes, renewing the KH solution every 10 minutes.

K^+ response as a reference for contractile responses:

The vessel response was recorded after application of the high K^+ KH solution. The first contractile peak of the K^+ contraction of the a. mes. sup. and the contractile response after 60 seconds of the aorta were evaluated, as the aorta never reached a plateau or peak. This measured K^+ response was used as a reference standard for the vasocontractile substances (i.e. Phe and Ang II) during experiments.

Afterwards, the chamber fluid was replaced with regular KH solution. Then, the vessels were equilibrated for 30 minutes, renewing the fluid every 10 minutes.

Recording of the cumulative Phe CRC:

The recording started with a Phe dose of 1.0E-10 (log M) and ended with a final dose of 1.0E-4 (log M). After applying the highest Phe dose and reaching the plateau, a 1.0E-4 (log M) ACh single bolus was given to verify the endothelial integrity by achieving a relaxation. Vessels that did not show a relaxation after ACh application were removed.

Recording of the cumulative Ang II CRC:

The starting dose for Ang II was 1.0E-12 (log M), and the end dose was 1.0E-05 (log M).
At the end of the recording, the solution was replaced with regular KH solution.

Recording of the cumulative ACh CRC:

The vessel was precontracted by applying the lowest Phe dose necessary for a force development of ≥ 50 % of the maximal Phe CRC response. This Phe dose was calculated from the Phe CRC recorded previously for this vessel (see above). If less than 50% of the maximal force was achieved with a given dose, then the next higher Phe dose was used until the force reached ≥ 50 % of the maximal Phe CRC response. This Phe end dose was also used for the subsequent CRCs (i.e. DEA NONOate CRC and ACh CRC after indomethacin incubation).
The starting dose for ACh was 1.0E-10 (log M) and the end dose 1.0E-5 (log M).

Recording of the cumulative DEA NONOate CRC:

For precontraction, the same Phe end dose as in the ACh CRC was applied. The DEA NONOate starting dose was 1.0E-12 (log M) and the end dose 1.0E-5 (log M).

Recording of the cumulative ACh CRC plus indomethacin incubation:

At the end of the recording of the DEA NONOate CRC, the KH solution was replaced with a KH solution containing 1.0E-06 (log M) indomethacin. Afterwards, equilibration was done for 40 minutes, renewing the KH solution containing 1.0E-06 (log M) indomethacin every 10 minutes.
For precontraction, the same Phe end dose as that for the ACh CRC was used. The ACh starting dose was 1.0E-10 (log M) and the end dose 1.0E-5 (log M).
At the end of the recording, the solution was replaced with KH solution without indomethacin. Afterwards, equilibration was done for 40 minutes, renewing the KH solution every 10 minutes.

Recording of the second K+ response to test the viability at the end of experiments:
The vessel response was recorded for 2 minutes after application of the high K^+ KH solution.

Annotations
All solutions were mixed freshly every day for every experiment and were kept on ice or at 4°C until use.
The curves for all solutions were loaded into the XMana interface for evaluation. Here, the maximum response of each vessel to each given dose was calculated. The data was transferred to STATISTICA and to GraphPad (see chapter 2.9: Statistical analysis).

2.6 Substances used during experiments

2.6.1 Solutions

Regular Krebs-Henseleit solution (KH solution)
Concentrations in mmol/l:
118.0 *Sodium chloride (NaCl)*[§]
4.7 *Potassium chloride (KCl)*[§]
25.0 *Sodium hydrogen carbonate ($NaHCO_3$)*[§]
0.45 *Magnesium sulphate 7-hydrate ($MgSO_4 x\ 7H_2O$)*[§]
1.03 *Potassium dihydrogen phosphate ($KH_2 x\ PO_4$)*[§]
5.5 *D(+)-glucose water free* (E. Merck, Darmstadt, Germany)
[§]NOTE: substances from Carl Roth GmbH Karlsruhe, Germany

High potassium (K^+) KH solution
Same chemicals as for regular KH solution with the following modifications:
Concentrations in mmol/l:
18.2 *Sodium chloride (NaCl)*
83.2 *Potassium chloride (KCl)*

Chemicals were mixed with distilled water. Prior to the experimental use, the solutions were heated to 37°C in a water bath and equilibrated with carbogen for several minutes.

2.6.2 Vasoactive substances for cumulative concentration response curves (CRC)

L-Phe hydrochloride (Sigma-Aldrich Chemie GmbH, Germany) was used as an alpha-1 adrenoceptor agonist in Phe CRC and for precontraction in ACh and DEA NONOate CRC.
ACh chloride (Sigma-Aldrich Chemie GmbH, Germany) was used in ACh CRC and for the single bolus application at the end of Phe CRC.
Ang II (H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-OH) (Bachem Biochemica GmbH, Germany) was used for Ang II CRC.
DEA NONOate (Acros Organics, Belgium) was used for DEA NONOate CRC as a NO donating substance.
Indomethacin (ICN Biomedicals Inc., France) was used in ACh CRC with indomethacin incubation.
Carbogen is a mixture of 95% O_2 (for oxygen supply) and 5% CO_2 (for solution buffering) used constantly during the vessel experiments.
Diethylether ≥99% was used for ether inebriation (Carl Roth GmbH Karlsruhe, Germany).

2.7 Morphometric analysis

Before starting our functional study, we performed brief pathohistological research in order to evaluate whether the chosen vessels of the ApoB and B6 mice were atherosclerotic or not. The aorta and the a. mes. sup. of the mice were dissected and placed in 10% buffered formalin (Carl Roth GmbH Karlsruhe, Germany). The formalin-fixed vessels were cut transversely into short segments (each approximately 5 mm long), embedded in paraffin and sectioned into 4-μm thick slices. The sections were then stained with Giemsa. The stenosis rate of the vessel lumen was quantified by using a digitizing morphometry image-analysis system (for digitalization of the pictures: Color View Video Camera; for evaluation of the digital images: AnalySIS; both Soft Imaging System GmbH, Münster, Germany) to determine whether vessels had atherosclerotic lesions. Three sections from different levels of the vessels for each mouse were used. The means of individual measurements were used for statistical analysis.

2.8 Serum parameters

Blood was drawn from the abdominal part of the vena cava inferior with a heparinized syringe. A proteinase inhibitor complex was added before centrifugating the blood for 10 minutes at 6000 rotations per minute at 4°C; the separated serum was pipetted into Eppendorf tubes and stored at –80°C until further analysis. Serum cholesterol and triglyceride levels were measured using

enzymatic colorimetric assays (Roche Diagnostics, Mannheim, Germany). The distribution of cholesterol among the different lipoproteins was determined by fast protein liquid chromatography (FPLC) gel filtration analysis of 50μl of pooled serum samples using a Superose 6 column (Pharmacia, Uppsala, Sweden). The serum was chromatographed at a constant flow rate of 0.5 ml/min using PBS as a buffer and lipoprotein fractions of 500 μl were collected. Total cholesterol and triglyceride levels within the individual fractions were measured enzymatically as described above. sICAM-1 and sVCAM-1 were measured using commercially available ELISA kits according to the manufacturer's instructions (R&D Systems, Minneapolis, USA).

2.9 Statistical analysis

2.9.1 ANOVA and Mann-Whitney U-test

Statistical evaluation was done with STATISTICA 1999 Edition Kernel-Version 5.5 A (Stat. Soft Inc., Tulsa, USA).

For experiments, the mouse groups consisted of 10 - 16 animals. Mean, standard deviation (SD), and standard error of mean (SEM) were calculated for all values. For all CRCs, an ANOVA for repeated measurements was calculated. If the p-value for the intergroup comparison was <0.05, the Mann-Whitney U-test (MWU-test) was also applied as a post-hoc test to test for significant intergroup differences for each applied dose.

The Mann-Whitney U-test was also used for intergroup comparison of K^+ response and serum parameters.

Mean and standard deviation were calculated for stenosis rate of vessel lumen.

2.9.2 Determination of EC_{50} and graphical presentation

The determination of the EC_{50} of the CRCs was done – if the CRC showed an appropriate sigmoidal shape - using GraphPad Prism Version 4.00 © 2003 (GraphPad Software Inc., USA).

The equation for the sigmoidal CRCs was:

$Y = Bottom + (Top – Bottom) / (1 + 10^((\log EC_{50} – X) * Hill Slope))$

X is the logarithm of concentration; Y is the response.

The Fisher-test was applied to test for significant intergroup differences in EC_{50} values. Furthermore, the graphical presentation of results was done using GraphPad. All data in graphs are presented as mean and standard error of the mean (SEM).

3 Results

The results are described separately for each vessel and solution. The CRCs are presented in graphs as means and standard error of mean (SEM). All CRCs are described threefold: The CRCs are described using an ANOVA whereas the single dose comparison within the CRCs was performed using a Mann-Whitney U-test. In addition, the EC_{50} for the CRCs is presented and tested for significance in intergroup comparison with the Fisher-test. Mouse type "ApoB" stands for the knockout mice strain B6;129S-Apobtm2SgyLdlrtm1Her. Mouse type "B6" stands for the control mice strain B6;129SF2/J. For general mice data see Table 3-1.

Table 3-1: General mice data

Mouse Type	Age (days) ± SD	Weight (g) ± SD
ApoB young (n=16)	117.4 (10.7)	28.7 (4.2)
ApoB old (n=15)	554.8 (84.6)	33.3 (3.5)
B6 young (n=10)	112.9 (11.5)	29.0 (2.7)
B6 old (n=12)	558.0 (61.4)	37.1 (5.2)

3.1 Serum parameters

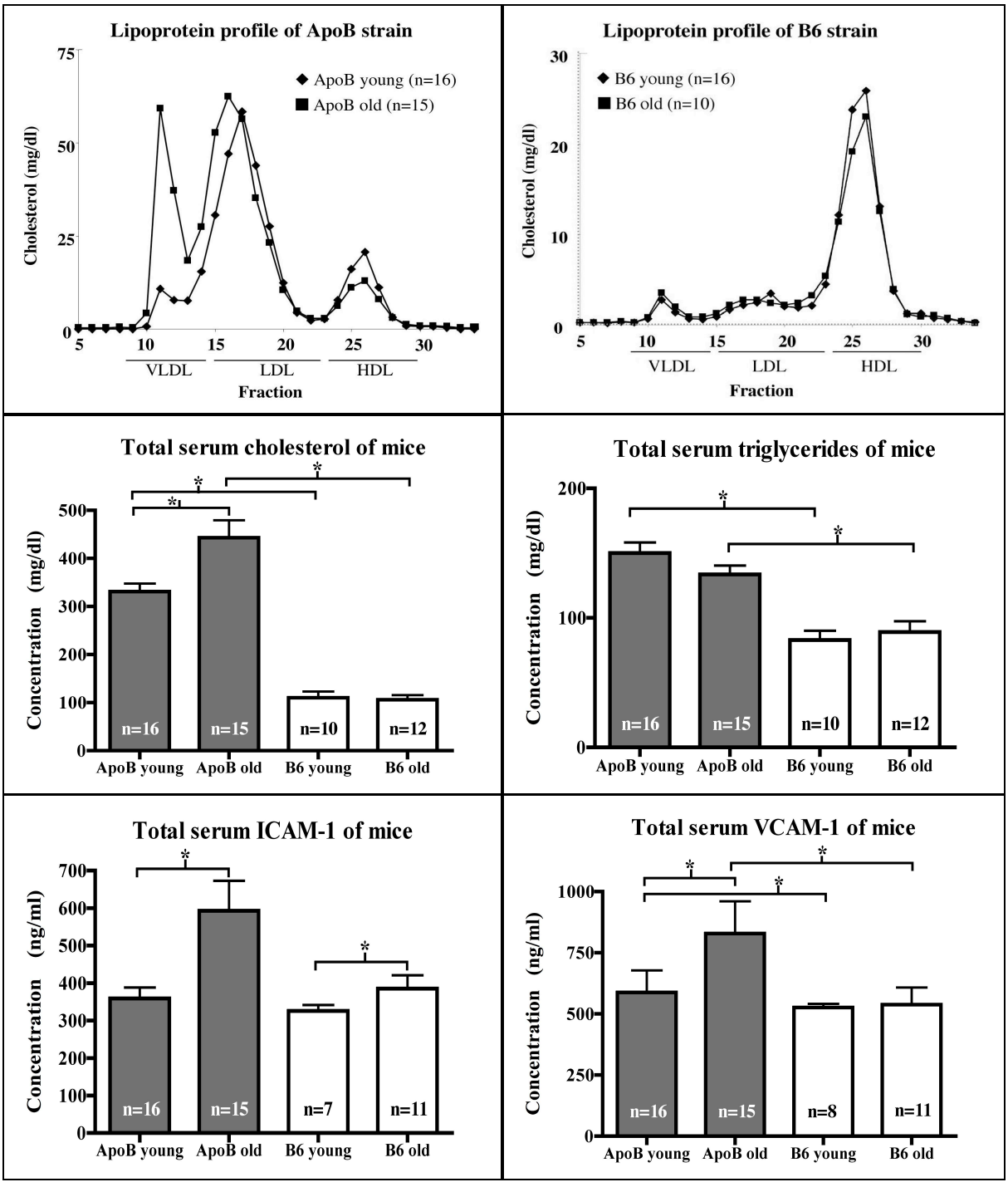

Figure 3-1: Serum parameters of mice groups; *, $p<0.05$ for intergroup comparison

The lipoprotein profile showed marked differences between the ApoB and the B6 strain. The B6 strain transported almost all of its cholesterol in the atheroprotective HDL fraction, whereas the ApoB mice had a low HDL level and a very high LDL level. In the B6 group, the lipoprotein profile was unchanged between the young and the old group. In the ApoB groups, the old ApoB

animals showed a marked increase in the VLDL fraction and a further decrease in the HDL fraction when compared to the young ApoB group (Figure 3-1). The ApoB strain showed a significantly increased triglyceride level compared to the B6 strain. There was no significant difference between the young and the old groups.

The ApoB strain also showed significantly increased cholesterol and vascular-cell adhesion molecule 1 (VCAM-1) levels compared to the wild-type B6 mice. In addition, the old ApoB animals showed an even further increase in total cholesterol and VCAM-1 in comparison with the young ApoB group.

The intercellular adhesion molecule 1 (ICAM-1) levels were not significantly different between the ApoB and the B6 strain; however, in both strains the old groups had significantly higher ICAM-1 levels compared to the young groups.

3.2 Overview on typical vessel responses

3.2.1 Cumulative Phe CRC as an example for vasocontractile substances

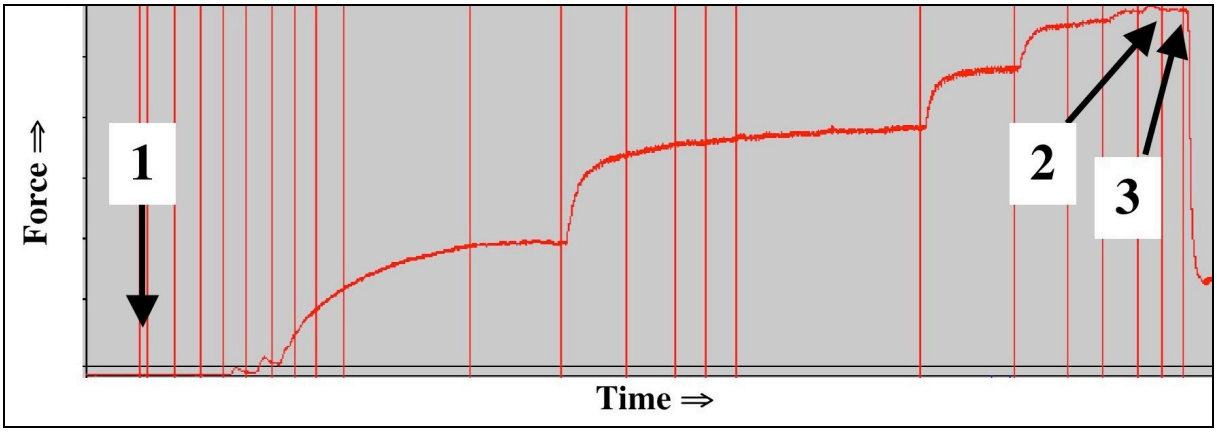

Figure 3-2: Aorta, cumulative Phe CRC plus ACh single bolus; B6 mouse, 109 days old; XMana interface; at tag number 1, the first Phe dose was applied; at tag number 2, the highest Phe dose was given; at tag number 3, the 10E-4 (log M) ACh bolus was applied, resulting in a deep relaxation

Increasing doses of phenylephrine were pipetted into the myograph chamber (starting with tag 1). After a contraction plateau was reached for a given dose, the next higher dose was applied. After the highest dose was applied (tag 2), a 10E-4 (log M) ACh bolus was given (tag 3). The resulting relaxation showed the endothelial integrity (Figure 3-2).

3.2.2 Cumulative ACh CRC as an example for vasodilative substances

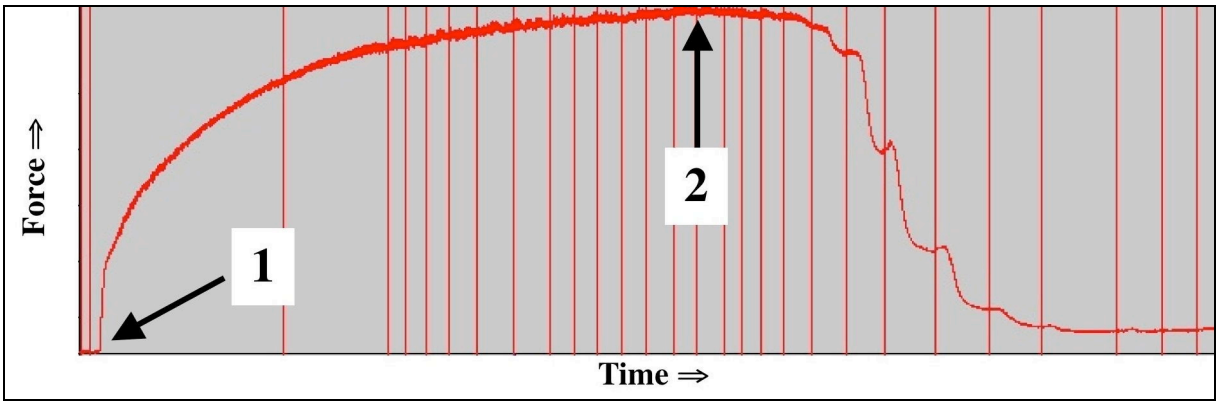

Figure 3-3: ACh CRC after submaximal Phe contraction; B6 mouse, 111 days; XMana interface; at tag number 1, Phe was applied; when a contraction plateau was established, increasing ACh doses were applied starting with the lowest dose at tag number 2

A submaximal dose of phenylephrine was pipetted into the myograph chamber (tag 1). After a contraction plateau was established (tag 2), increasing ACh doses were applied (tag 2); the next higher ACh dose was given after a relaxation plateau had been established (Figure 3-3).

3.3 Pathohistological analysis

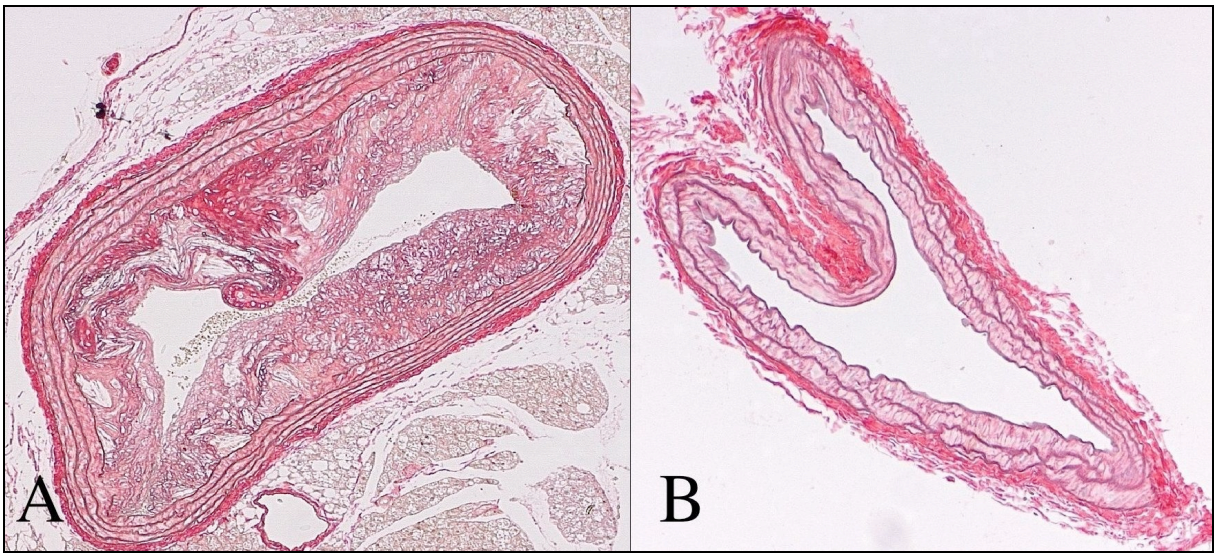

Figure 3-4: A: Aorta thoracica of ApoB male mice, 530 days; 125x; B: A. mes. sup. of ApoB male mice, 526 days; 250x; both Giemsa stain

Table 3-2: Aorta thoracica, stenosis rate of vessel lumen

Aorta thoracica	Stenosis of vessel lumen (%) ± SD
ApoB old (n=12)	57.63 (20.99)

We found that all ApoB old mice had developed severe atherosclerosis in the aorta while the arteria mesenterica superior was unaffected. More than half of the lumen of the aorta thoracica of the old ApoB animals was covered with atherosclerotic plaques (Figure 3-4 and Table 3-2). In contrast, young ApoB mice, as well as the B6 mouse strain, did not show light-microscopic signs of atherosclerosis (data not shown).

3.4 Functional data of the aorta

3.4.1 K^+ response

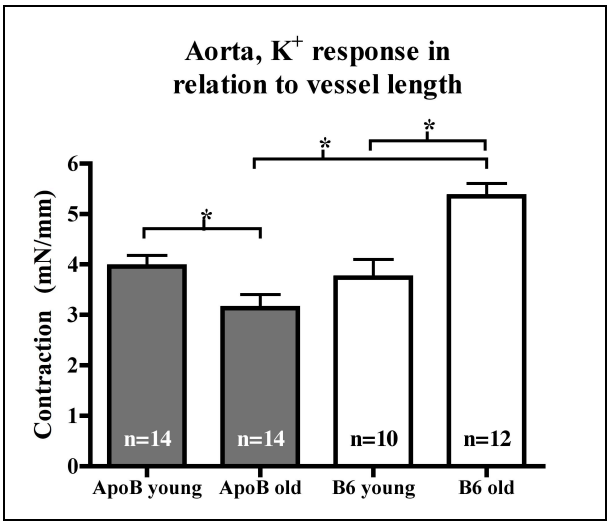

Figure 3-5: Aorta, K^+ response in relation to vessel length (mN/mm); *, $p<0.05$ for intergroup comparison

The MWU-test showed that the K^+ responses were significantly different between the various groups. Within the ApoB strain, the younger mice constricted more pronounced than the older mice, while within the B6 strain, more marked contractions were found in the old group. Additionally, the old B6 animals showed significantly larger contractions than the old ApoB mice (Figure 3-5).

3.4.2 Phenylephrine

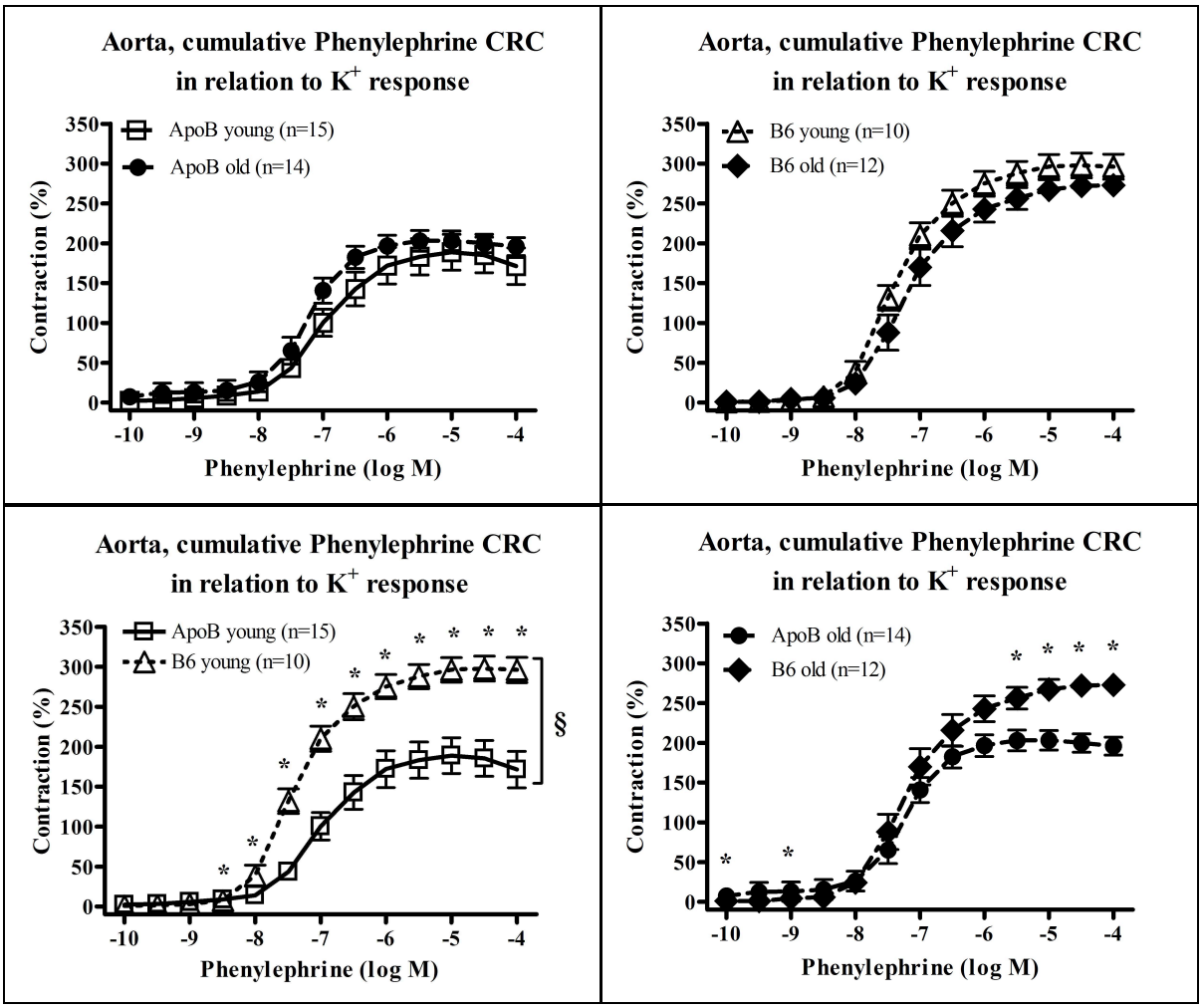

Figure 3-6: Aorta, Phe CRCs; *, $p<0.05$ between groups; §, $p<0.05$ for EC_{50} between groups

Table 3-3: Aorta, EC_{50} (log M) of Phe CRCs

Mouse type	EC_{50} (log M) ± SD
ApoB young	-7.03 (0.039)
ApoB old	-7.27 (0.02)
B6 young	-7.37 (0.04)
B6 old	-7.19 (0.04)

The young ApoB mice were less sensitive than young B6 animals (Figure 3-6 and Table 3-3). The ANOVA showed a significant difference in the curve between the young ApoB and the young B6 animals, with the young ApoB mice showing weaker responses than the B6 strain. The

same strain difference was observed in the older animals as well. However, at the low doses, the old B6 animals showed weaker reactions than the ApoB mice (Figure 3-6).

3.4.3 Angiotensin II

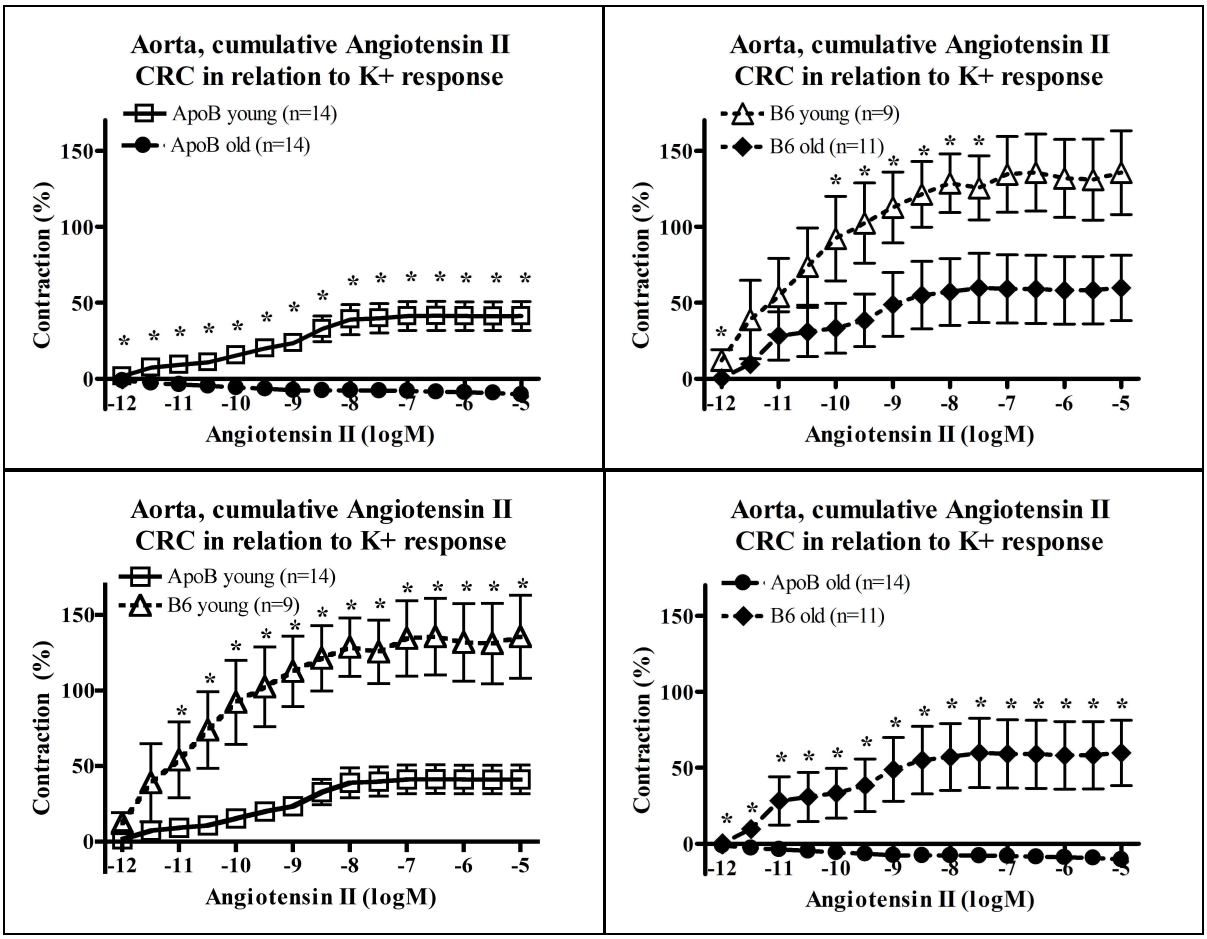

Figure 3-7: Aorta, Ang II CRCs; *, $p<0.05$ between groups; the EC_{50} was not measurable

The ANOVA showed that the curves were significantly different between all groups. In both comparisons with the young B6 mouse group, this group had the most marked contractions. The old ApoB mice showed the weakest contractions when compared to the other two groups (Figure 3-7).

We decided to perform some extra experiments to block the AT_2 receptor. This was accomplished with PD 123,319 di (trifluoroacetate) salt, a potent and selective non-peptide AT_2 Ang II receptor antagonist (Sigma-Aldrich Chemie GmbH, Germany). We incubated the vessel in a 1E-06 (log M) concentration before applying Ang II. No differences in vessel response were seen in the aorta and in the a. mes. sup. (data not shown).

3.4.4 Acetylcholine

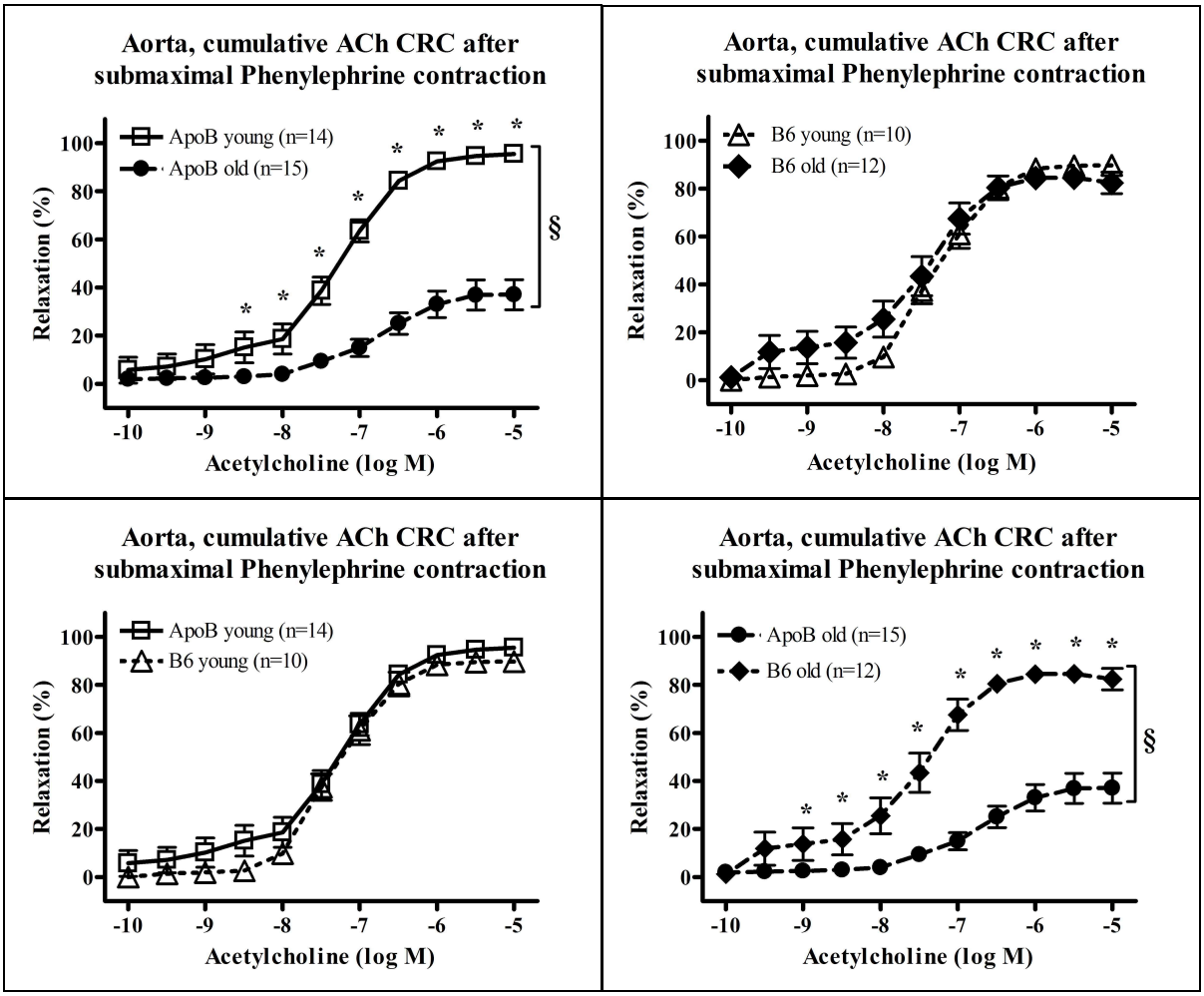

Figure 3-8: Aorta, ACh CRCs; *, $p<0.05$ between groups; §, $p<0.05$ for EC_{50} between groups

Table 3-4: Aorta, EC_{50} (log M) ACh CRCs

Mouse type	EC_{50} (log M) ± SD
ApoB young	-7.23 (0.03)
ApoB old	-6.75 (0.03)
B6 young	-7.31 (0.03)
B6 old	-7.47 (0.07)

The EC_{50} was significantly different between the young and old ApoB animals and between the old ApoB and B6 mice. The young ApoB animals were more sensitive than the older ApoB mice. The old B6 animals were more sensitive than the old ApoB animals (Figure 3-8 and Table 3-4). The ANOVA showed a significant difference between the curves of the young and old

ApoB animals, with the older animals showing less relaxation starting from a dose of 3.2E-9 (log M). The old ApoB mice also showed significantly weaker relaxations than the old B6 mice. Starting from dose 1.0E-9 (log M), the old B6 animals showed a deeper relaxation.

The ANOVA also showed a significant difference between the curves of the B6 mice. Yet, for this comparison, the post-hoc MWU-test did not show any significant differences (Figure 3-8).

3.4.5 DEA NONOate

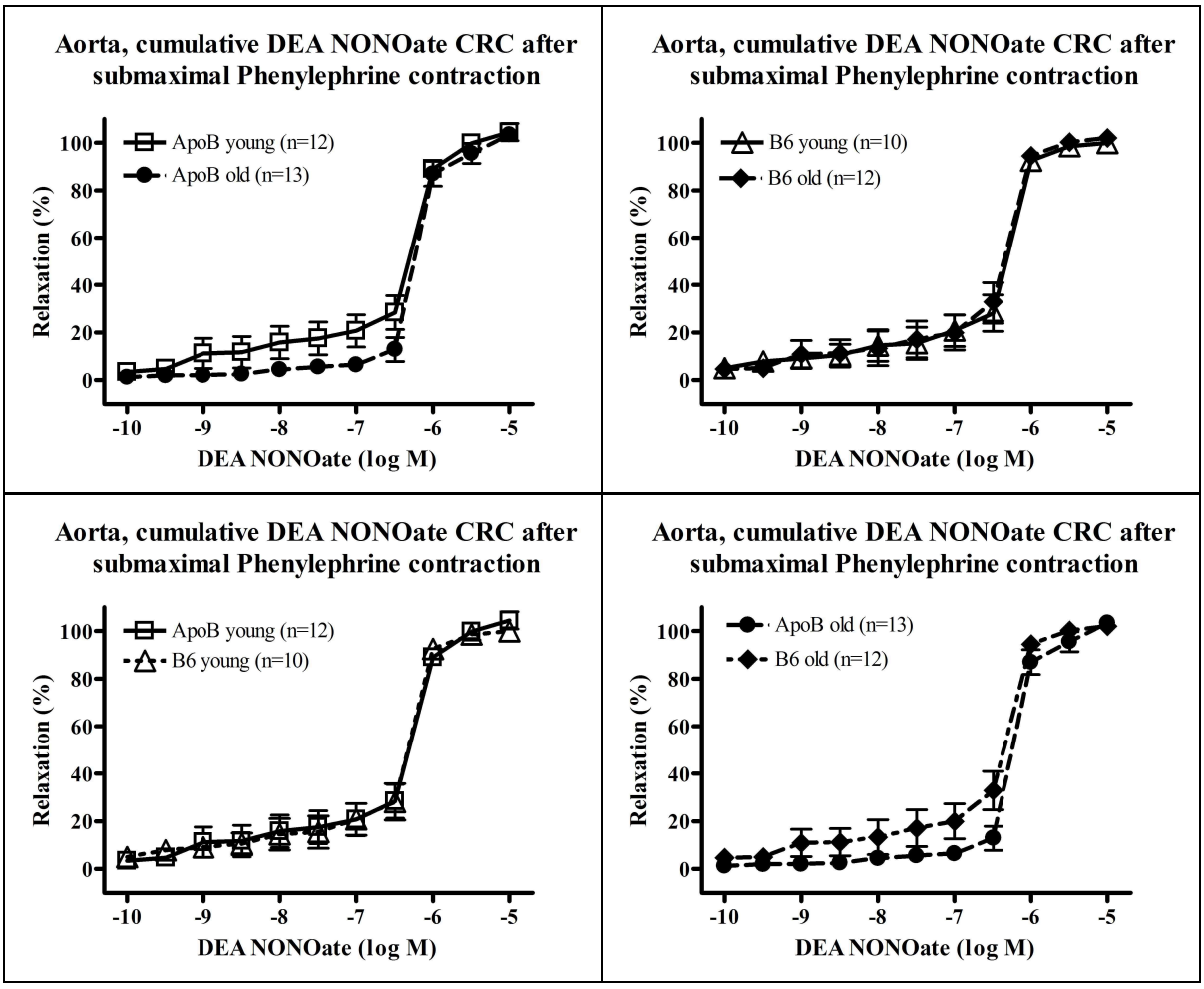

Figure 3-9: Aorta, DEA NONOate CRCs; ANOVA of intergroup comparison was not significant; there was no significant intergroup difference between the EC_{50} of any of the groups

Table 3-5: Aorta, EC_{50} (log M) of DEA NONOate CRCs

Mouse type	EC_{50} (log M) ± SD
ApoB young	-6.23 (0.01)
ApoB old	-6.23 (0.01)
B6 young	-6.31 (0.01)
B6 old	-6.33 (0.01)

There were no significant intergroup differences (Figure 3-9 and Table 3-5).

3.4.6 Acetylcholine after incubation with indomethacin

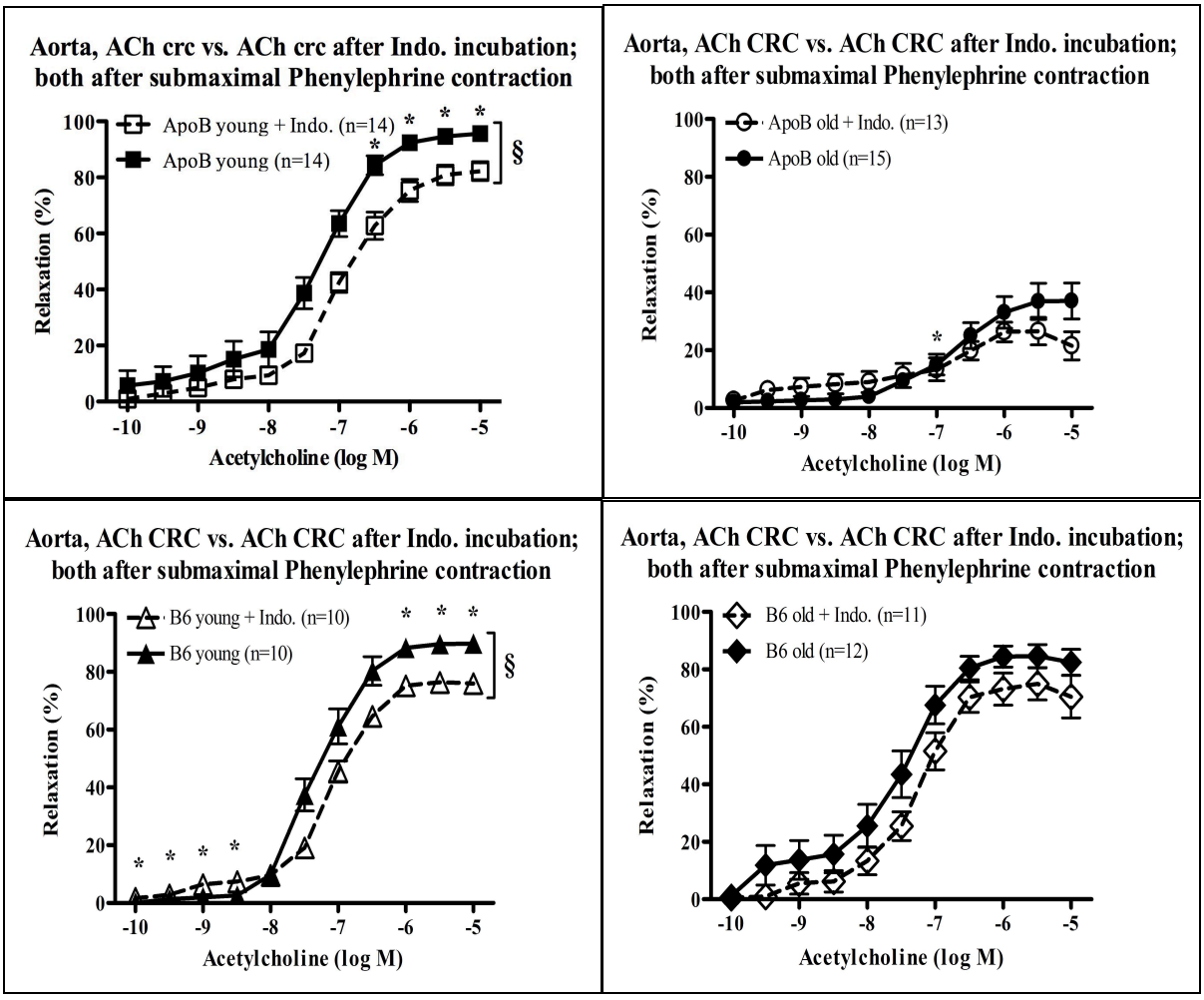

Figure 3-10: Aorta, ACh CRCs compared to ACh CRCs after indomethacin incubation; *, $p<0.05$ between groups; §, $p<0.05$ for EC_{50} between groups

Table 3-6: Aorta, EC_{50} (log M) of ACh CRCs after indomethacin incubation

Mouse type	EC_{50} (log M) ± SD
ApoB young	-6.95 (0.01)
ApoB old	-6.96 (0.01)
B6 young	-7.07 (0.01)
B6 old	-7.27 (0.01)

For the intragroup comparisons within the young ApoB mice and the young B6 mice, incubation with indomethacin led to less ACh sensitivity (Figure 3-10 and Table 3-4 and Table 3-6). The ANOVA showed a significant intragroup difference in the young B6 mice group. After incubation with indomethacin, the relaxation to smaller doses of ACh was deeper and the relaxation to higher doses of ACh was weaker. The young ApoB mice group showed weaker relaxations to high ACh doses after incomethacin incubation. Also, there was a significant difference in the old ApoB group, but only for the 1.0E-7 (log M) dose. Here, after indomethacin incubation, the relaxation was weaker (Figure 3-10).

3.5 Functional data of the arteria mesenterica superior

3.5.1 K^+ response

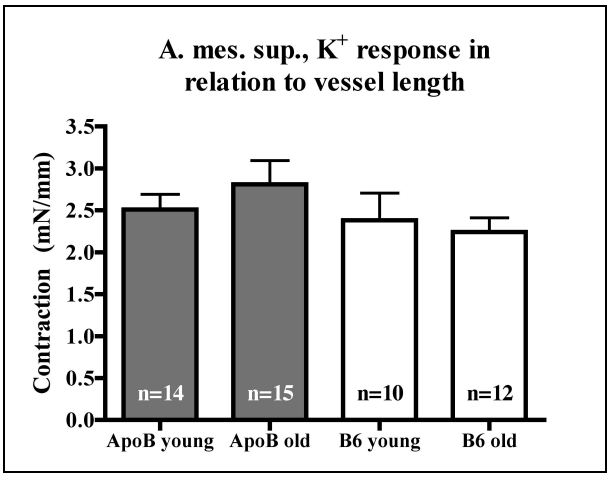

Figure 3-11: A. mes. sup, K^+ response in relation to vessel length (mN/mm); the MWU-test did not show any significant intergroup difference

There were no significant intergroup differences (Figure 3-11).

3.5.2 Phenylephrine

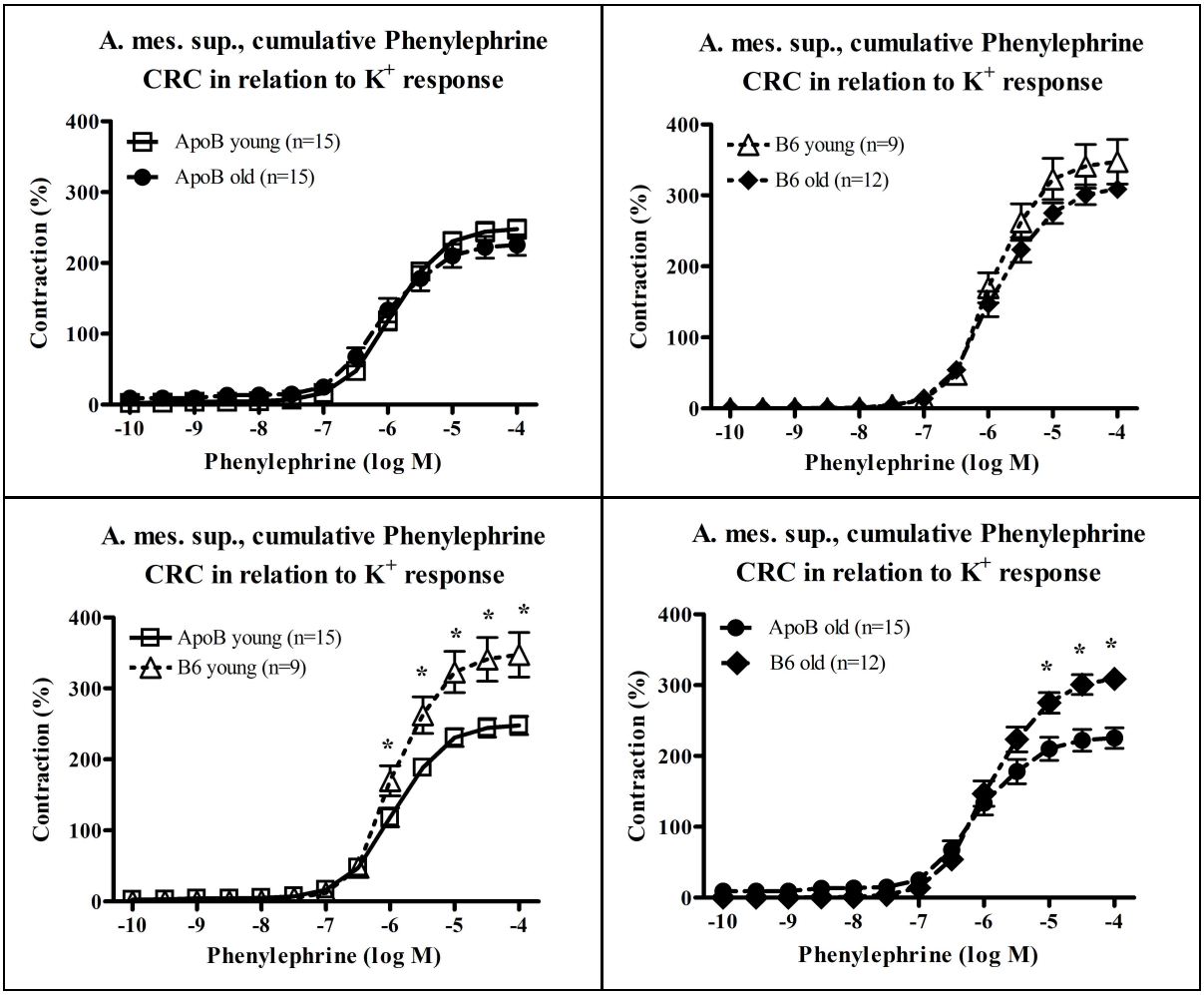

Figure 3-12: A. mes. sup., Phe CRCs; *, $p<0.05$ between groups; the EC_{50} was not significantly different for any of the groups

Table 3-7: A. mes. sup., EC_{50} (log M) of Phe CRCs

Mouse type	EC_{50} (log M) ± SD
ApoB young	-5.94 (0.00)
ApoB old	-6.08 (0.01)
B6 young	-5.94 (0.01)
B6 old	-5.92 (0.01)

There were no significant differences between the EC_{50} of the groups (Figure 3-12 and Table 3-7). Comparing the young ApoB animals to the young B6 animals and also the old ApoB animals to the old B6 animals, the ANOVA showed a significant difference between the curves.

In both cases, the B6 strain showed a more pronounced response at the higher doses than the ApoB strain (Figure 3-12).

3.5.3 Angiotensin II

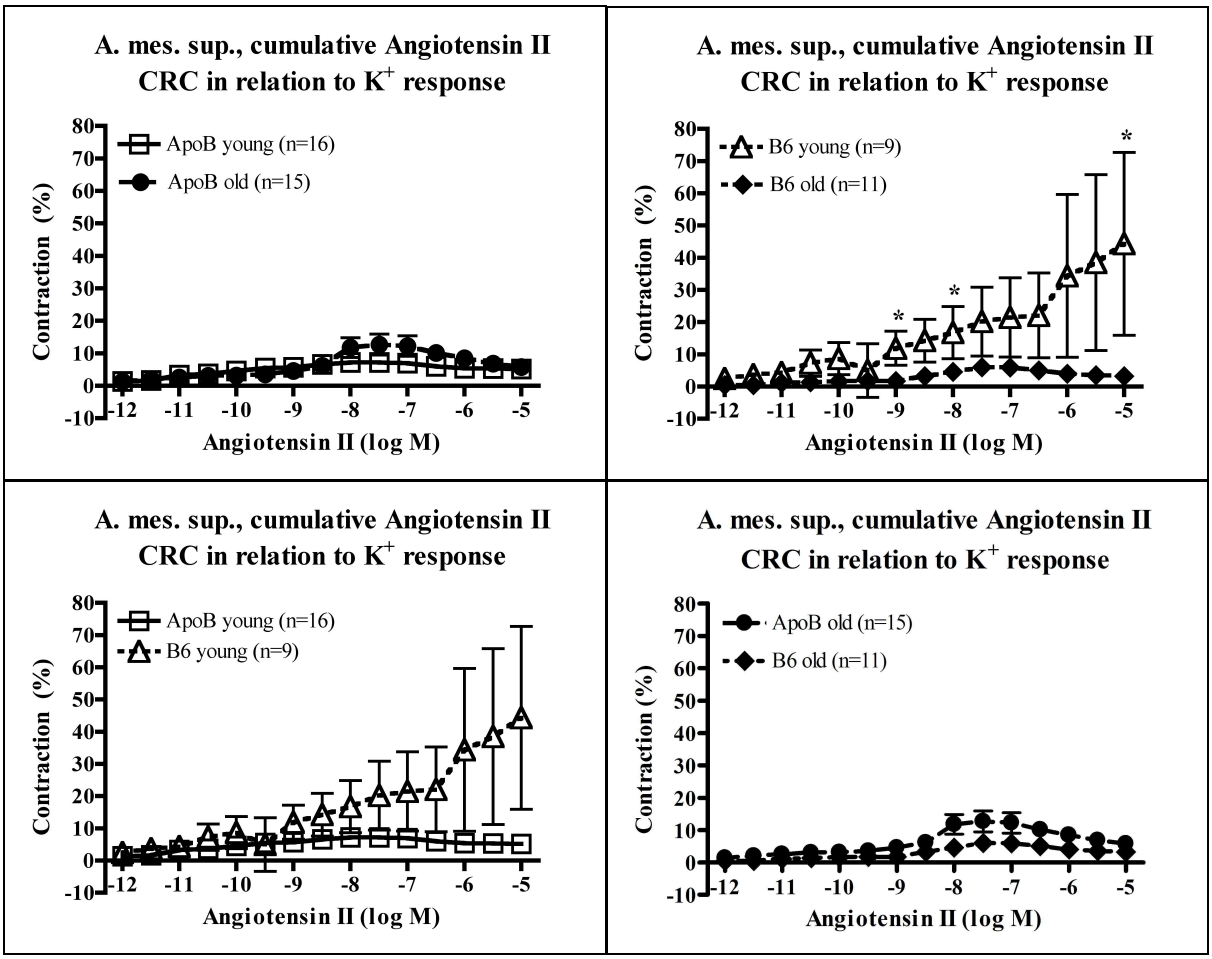

Figure 3-13: A. mes. sup., Ang II CRCs; *, $p<0.05$ between groups; the EC_{50} was not measurable

The ANOVA showed a significant difference between the young and old B6 mice, where the younger animals reacted more markedly than the older animals (Figure 3-13).

3.5.4 Acetylcholine

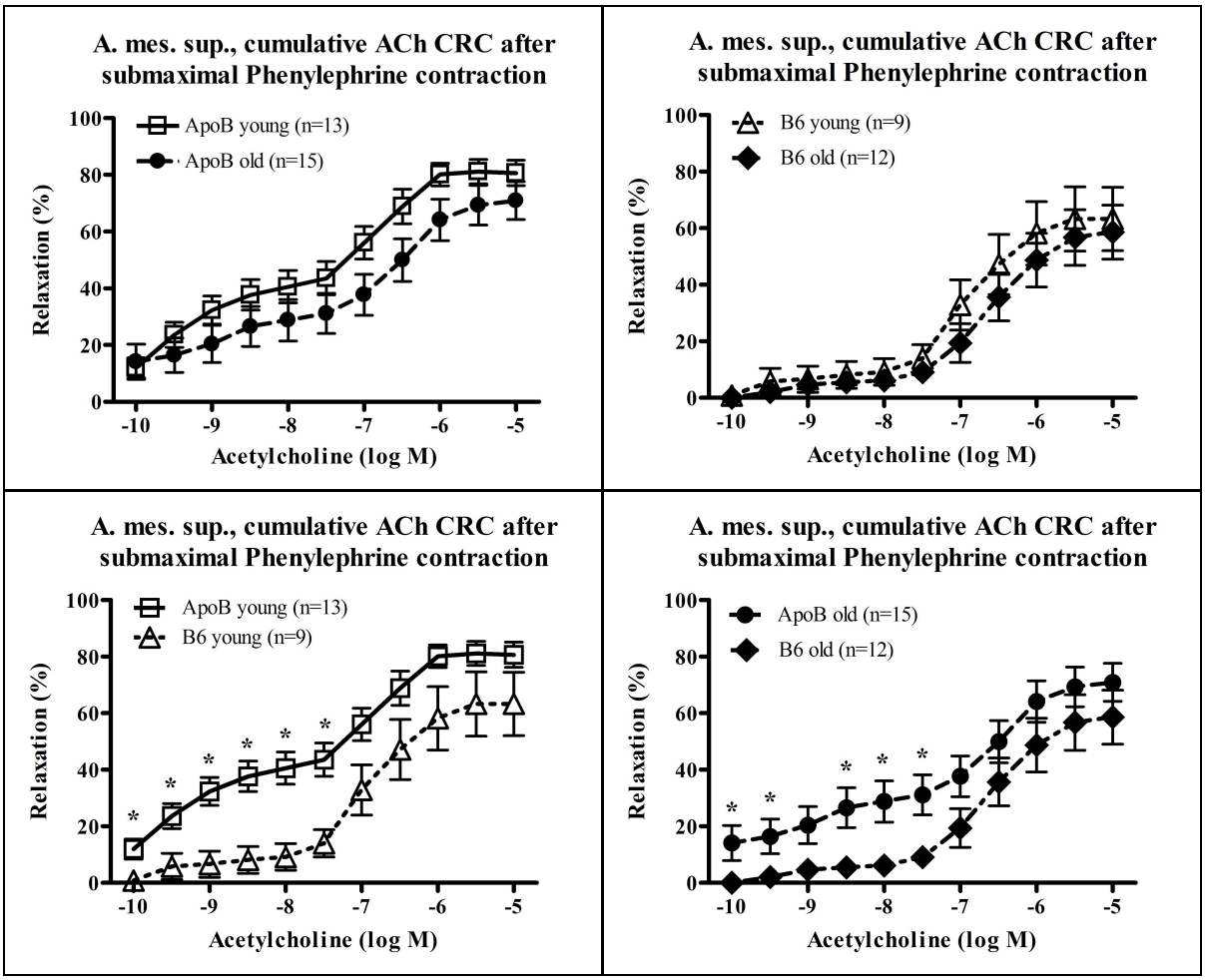

Figure 3-14: A. mes. sup., ACh CRCs; *, $p<0.05$ between groups; the EC_{50} was not significantly different for any of the groups

Table 3-8: A. mes. sup., EC_{50} (log M) of ACh CRCs

Mouse type	EC_{50} (log M) ± SD
ApoB young	-7.50 (0.14)
ApoB old	-6.60 (0.06)
B6 young	-6.90 (0.02)
B6 old	-6.61 (0.01)

There were no significant differences between the EC_{50} of the groups (Figure 3-14 and Table 3-8). The ANOVA showed a significant difference between the curves of the young ApoB and the young B6 mouse groups and also between the old ApoB and the old B6 mouse groups. The

young ApoB mouse group relaxed more deeply than the young B6 group at both the low and medium doses, whereas the reaction to the highest doses was similar. Comparing the old ApoB mice to the old B6 mice revealed that the old ApoB mice responded more markedly at low and medium doses (Figure 3-14).

3.5.5 DEA NONOate

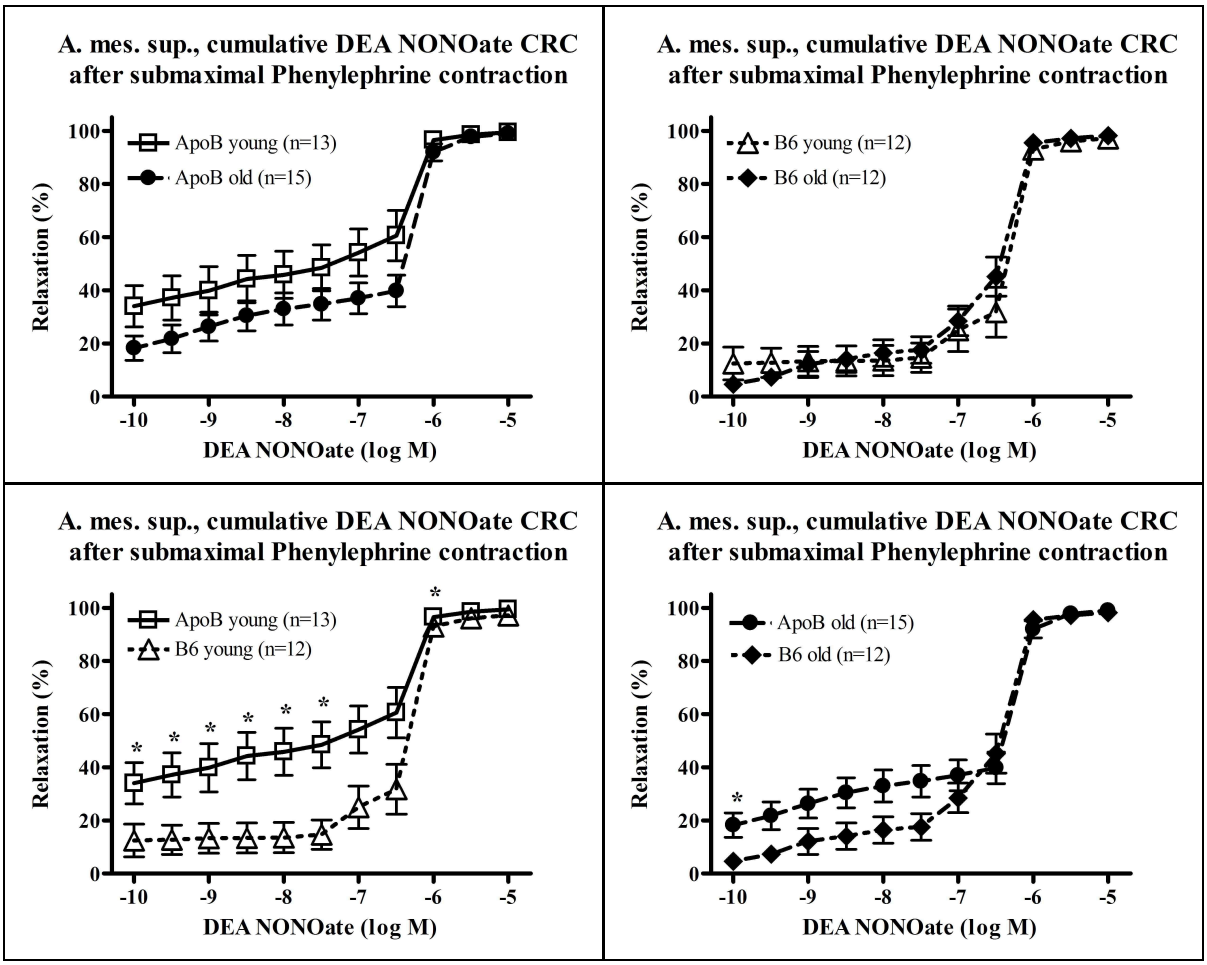

Figure 3-15: A. mes. sup., DEA NONOate CRCs; *, $p<0.05$ between groups; the EC_{50} was not significantly different for any of the groups

Table 3-9: A. mes. sup., EC_{50} (log M) of DEA NONOate CRCs

Mouse type	EC_{50} (log M) ± SD
ApoB young	-6.41 (0.03)
ApoB old	-6.29 (0.02)
B6 young	-6.34 (0.01)
B6 old	-6.44 (0.02)

There was no significant difference between the EC_{50} for any of the groups (Figure 3-15 and Table 3-9). The ANOVA showed a significant difference between the curves of the young ApoB and the young B6 mouse groups and also between the old ApoB and the old B6 mouse groups. The young ApoB mouse group relaxed more deeply than the young B6 group at both the low and medium doses, whereas the reaction to the highest doses was similar. Comparing the old ApoB mice to the old B6 mice revealed that the old ApoB mice responded more markedly at only the lowest dose of 1.0E-10 (log M) (Figure 3-15).

3.5.6 Acetylcholine after incubation with indomethacin

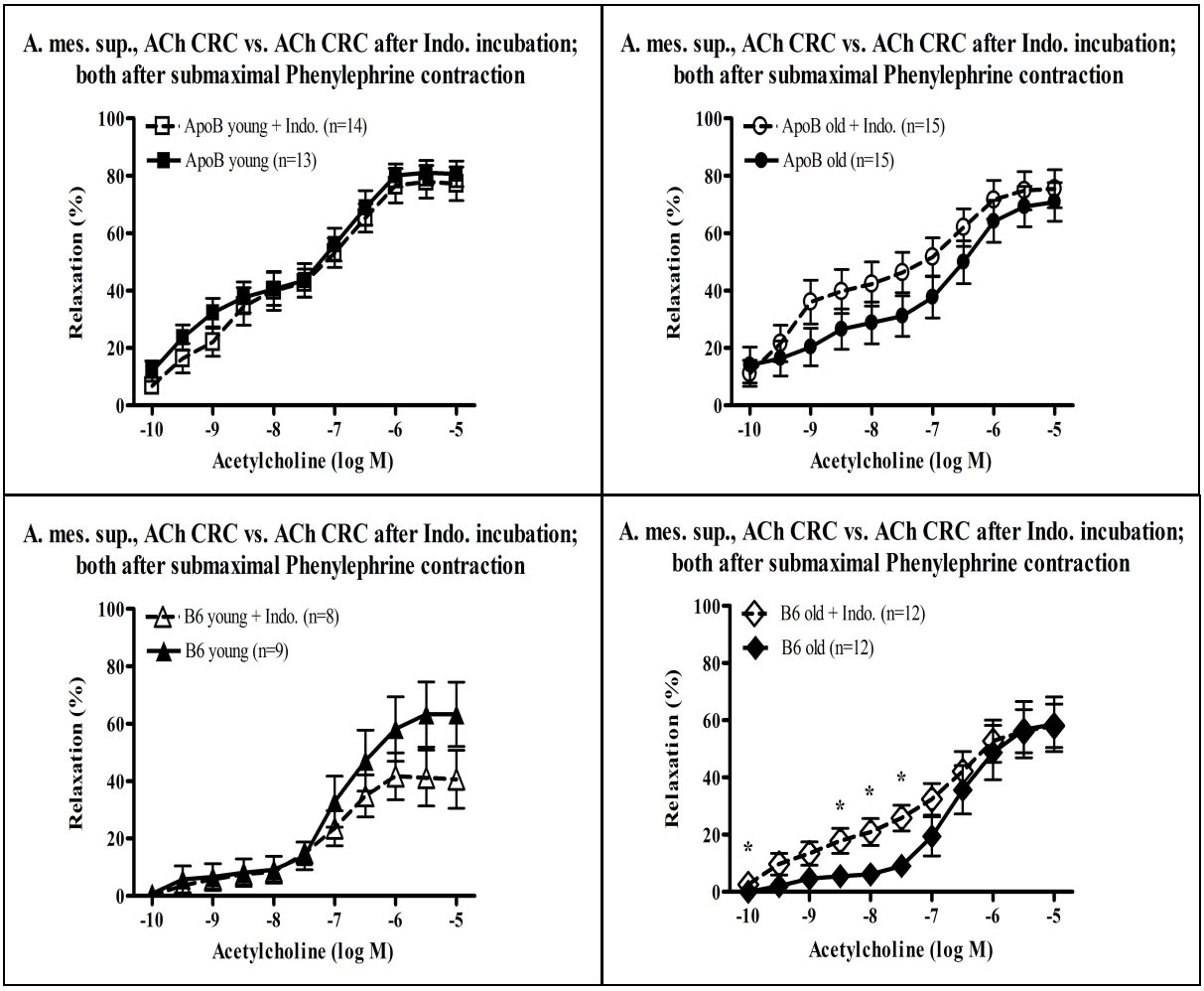

Figure 3-16: A. mes. sup., intragroup comparison of ACh CRCs with and without indomethacin for ApoB groups; *, $p<0.05$ between groups; the EC_{50} was not measurable for the ApoB old and did not show any significant differences for the intragroup comparison of the three other groups

Table 3-10: A. mes. sup., EC_{50} (log M) of ACh CRCs after indomethacin incubation

Mouse type	EC_{50} (log M) ± SD
ApoB young	-7.92 (0.12)
ApoB old	not determinable
B6 young	-7,09 (0.03)
B6 old	-6.86 (0.09)

For the EC_{50} of ACh CRC without indomethacin see Table 3-8. There was no significant difference between the EC_{50} for any of the groups (Figure 3-16 and Table 3-8 and Table 3-10). The ANOVA showed a significant difference after indomethacin incubation in the B6 old mice (Figure 3-16). Indomethacin incubation increased the vessel response for low and medium ACh doses in this group.

4 Discussion

4.1 Reaction to vasocontractile substances

4.1.1 Phenylephrine

The ApoB mice in our study showed significantly less contractile response to Phe than the non-atherosclerotic B6 control mice. The aorta and the a. mes. sup. behaved similarly. A remarkable finding was that there was already at the onset a difference in vessel response between the young ApoB and the young B6 animals. The first atherosclerotic vessel damages are known to take place in the intima with the endothelial layer. Thus, the first marker of vessel damage should be an altered response to ACh; however, we could not find a significant difference regarding the vasodilative response to ACh when comparing the two young groups. Therefore, we can assume that the young ApoB mice had not suffered any significant atherosclerotic vessel damage at this point. Furthermore, although atherosclerotic alteration had changed the vessel function in the old ApoB mice group according to the ACh response, we saw no change in the Phe response during the aging process. Indeed, there was no significant difference between the young and old ApoB mice.

The Phe response in the ApoB model seems unaffected by light microscopic atherosclerotic damage and is established in its characteristic pattern already in very young ApoB mice.

Even though young ApoB mice did not show any atherosclerotic alterations, they had one thing in common with the old ApoB mice: their atherogenic serum profile. Although total cholesterol, sICAM-1 and sVCAM-1 levels in the old ApoB mice were significantly higher than the levels in the young ApoB mice, the high levels of LDL did not change with age. Furthermore, there was no significant difference between the triglyceride levels. It could be possible that the high LDL levels together with the high triglyceride levels had an effect on the vasoconstrictivity.

There is a lot of evidence supporting the influence of lipoproteins and fatty acids on vascular reactivity:
It was found that alterations in the vessel function were induced directly by cholesterol levels [22] and that hypercholesterolemia is per se sufficient to impair the vessel reactivity in large

arteries [12]. Even more, a 30 minute in vitro exposure of the healthy rabbit carotid artery to mildly oxidized LDL caused diminished responses to Phe and ACh. It was suggested that oxLDL might impair intracellular mechanisms that mediate contraction [57]. A study by Fujimara et al., in which the authors used the carotid artery of hypercholesterolemic rabbits suggested the vasoconstrictivity to alpha-adrenoceptor agonists is altered by hypercholesterolemia without the accompanying atherosclerotic changes and endothelial dysfunction. In the early stages of atherosclerosis, either the alpha-1-receptor sensitivity might be decreased or the number of receptors might be decreased [28].

In contrast to these findings, the maximum Phe response was increased after the in vitro perfusion of the rat aorta with oxidated chylomicron remnants, likely through interaction with the NO-pathway [30].

In another study, high LDL-levels increased the production of NO in liver cells by upregulating iNOS expression. Here, it was hypothesized that either the high-fat diet or plasma NO impairs the contractile unit of aortic VSMC [46].

The influence found of FFAs is inconsistent. Esenabhalu et al. found that in mice overexpressing smooth muscle cell derived lipoproteinlipase, the FFA uptake in cells and the vascular wall was increased. While the in vitro Phe contraction was unaltered, the frequency of oscillation following Phe application was increased [24]. The oscillations result from cGMP-dependent activation of K^+-channels by endothelium derived NO. Removal of the endothelium abolished Phe induced rhythmic activity [44]. FFAs were also found to impair endothelial function and endothelium-dependent relaxation. The FFAs influenced vessel activity even without changes in the overall amount of lipoproteins [13][24].

Not all of the studies mentioned above used Phe or the same lipids found in the ApoB mouse serum. Some studies even found no influence of hypercholesterolemia on vessel function [16]. Nevertheless, the studies present a lot of evidence that despite the absence of atherosclerotic alterations, high blood lipid levels can have a significant influence on vessel reactivity and are likely to influence vasoconstrictivity.

The aforementioned hypotheses fail to explain why there was no alteration in the Phe response of the aorta of the old ApoB mice compared to the young ApoB mice. Blood lipids levels have increased even further in the old ApoB group and their aorta showed severe atherosclerotic damage. Due to atherosclerosis, the vessel should have lost its elasticity, changes in the VSMCs should have taken place and eNOS is most likely impaired [94]. Although we did not estimate

the influence of these components, it is surprising that they should have no overall effect when comparing the non-atherosclerotic young ApoB mice with the atherosclerotic old ApoB mice. Even more so considering that we have observed a large influence of atherosclerosis on vessel response to ACh, Ang II and K^+ in the ApoB model.

In our study, no iunfluence of atherosclerosis on the EC_{50} was observed. In contrast to the findings in our ApoB model, Vita et al. wrote that in humans the endothelial dysfunction (ACh response) that characterizes early and late atherosclerosis is associated with a marked increase in sensitivity (EC_{50}) to the constrictor effects of catecholamines [86].
Other groups found that because of atherosclerosis, the EC_{50} for catecholamines was decreased [22][44]. For example, Jiang et al. found in atherosclerotic ApoE (-/-) x LDLr (-/-) mice that sensitivity to Phe in the aorta was diminished. The opening of K^+-voltage channels was reduced and they were susceptible to depolarization resulting in Ca^{2+} entry. The authors hypothesized that the VSMCs were dependent on compensatory mechanisms to limit Ca^{2+}entry, which could have led to a decreased sensitivity to Phe [44].

In our ApoB model, there is no way around the fact, that the aorta and the a. mes. sup. showed the exact same response to Phe in all ApoB groups independent from atherosclerotic damage.

Future perspectives
Immuno-histology: To estimate the overall number of alpha-1-adrenoceptor and thus to determine if there has been an up- or down-regulation of mechanisms regulating the receptor number. This could be addressed through immuno-histological research using alpha-1-adrenoceptor antibodies.

Incubation with selective alpha-1-adrenoceptor-subtype antagonist: There are 3 functional alpha-1-adrenoceptor isoforms in blood vessels: alpha-1A, alpha-1B, and alpha-1D. The contribution of the different subtypes varies with species and vascular bed [95]. By incubating the vessels with selective subtype antagonists before Phe application, one could estimate the influence of the three isoforms comparing young non-atherosclerotic to old atherosclerotic ApoB mice.

Incubation with the NOS inhibitor L-NAME to estimate the influence of NO: The endothelial NO-synthetase (eNOS) and / or the inducible NO synthetase (iNOS) might be up- or down-

regulated in this model, thereby counteracting the Phe response, which might be altered in the atherosclerotic aorta of the old ApoB mice in absence of the NOS compared to the other groups.

In vivo experiments: It has been discussed whether the aorta is a valid vessel for functional research. Hof et al. showed that the Phe induced blood pressure increase reflected the function of resistance vessels [36]. Therefore, systemic in vivo administration of Phe in the ApoB mouse could reveal different and more applicable results.

4.1.2 Angiotensin II

The angiotensin II response has to be viewed critically because the vessel reactions were often inhomogeneous and some vessels became desensitive to angiotensin II during the recording of the CRCs.

For the aorta, the ANOVA of all intergroup comparisons showed significant differences. Similar to the Phe response, the aorta of the ApoB strain showed a weaker Ang II response than the B6 strain. Analogous to the observations with the Phe response, we hypothesize strain immanent ApoB features to be responsible for the attenuated reaction, at least for the aorta of the young ApoB mice. However, unlike the Phe response, both the young ApoB and young B6 group showed a more pronounced response than their corresponding old groups. In fact, the vessel tonus even decreased in the old ApoB mouse group as doses increased.

For the a. mes. sup., the intergroup comparison was significant only for the comparison of age in the B6 mouse strain, where the older mice showed more marked reactions for some doses. All other intergroup comparisons were not significant. This is in accordance with our observation that there was no atherosclerotic alteration in this vessel for any of the groups.

Still, the aorta of the old ApoB group with severe atherosclerosis reacted differently than the other groups by relaxing instead of contracting after Ang II stimulation. Atherosclerosis might have led to a decrease in vessel responsiveness in our in vitro experimental design, possibly through an increase of vasodilative mechanisms leading to the production of NO or prostacyclins. Another possibility would be a change in the quality of secondary messenger mechanisms and intracellular signaling, resulting in vasodilation rather than in vasoconstriction. Thirdly, the expression of Ang II receptor subtypes could have changed in the atherosclerotic aorta.

A decrease in vessel tonus to Ang II in aortic rings was also described by Anning et al. They explored the vessel response of 12/15-Lipoxygenase (LOX) knock out mice, which are resistant to atherosclerosis. LOX catalyzes the oxidation of unsaturated fatty acids to hydroperoxides and other bioactive metabolites. In contrast to the wild type mice, which showed a contraction, mice incubated with Ang II showed a decrease in vessel tonus. In this model, Ang II-dependent constriction was fully restored to wild-type-levels after L-NAME incubation, while selective inhibition of nNOS or iNOS did not have any significant effects [2]. Tasaki et al. found similar results. In their study, aortic rings from hyperlipidemic atherosclerotic rabbits showed a significantly lower contractile response to Ang II than strips from control rabbits. When the endothelium was removed, the contractile response to Ang II was greater; however, this increase was more pronounced in the control group than in the atherosclerotic group. Incubation with L-NAME also increased responses to Ang II [77].

Ang II stimulation can lead to the release of vasodilative substances, such as NO and prostacyclin. The origin of these substances is mainly attributed to the vascular endothelium [77]. In our own study, ACh stimulation of NO-mediated vessel relaxation seemed massively impaired in the old ApoB group. Taking this into account, we would actually predict a larger rather than a weaker Ang II response in the old ApoB mice group compared to the other groups.

Since we hypothesized that the influence of the vasodilative AT_2 receptor might be responsible for the weak Ang II responses, we performed some extra experiments. Here, we blocked the AT_2 receptor with PD 123,319 di (trifluoroacetate) salt. No differences in reactivity to Ang II occurred in any of the vessels.

In accordance with our findings, Tasaki and colleagues also reported a hypocontractility to Ang II in an atherosclerotic rabbit model in the presence of the AT_2 receptor antagonist PD 123,319 suggesting that AT_2 receptor-mediated vasodilation is not involved in hypocontractility. The authors suggested that the decrease in the Ang II contraction in their model was due mainly to an abnormality in VSMC contractility rather than caused by changes in the release of vasodilative substances [77].

The findings regarding the alterations of the Ang II response are not consistent [88]. It was found that despite an enhanced pressure response to Ang II in atherosclerotic conscious rabbits [36],

Ang II-induced contraction was reduced in isolated aortic rings and unaltered in the iliac artery [18]. Because of that, Wilfert et al. suggested that the altered cardiovascular response in the atherosclerotic rabbit model might not only be defined by the isolated vessel, but also by alteration of systemic blood pressure regulating mechanisms such as the arterial baroreceptor reflex. Indeed, they found a reduced baroreceptor reflex sensitivity in their atherosclerotic rabbit model [88].

Future perspectives

The incubation of the vessels with NOS inhibitors such as L-NAME before Ang II application could offer further insight into the contribution of NO to the altered Ang II response.

Ang II receptor subtypes: It will also be helpful to quantify the Ang II receptor subtypes in the vessels to find out if there is a relationship between atherosclerotic damage, vessel response and receptor expression. According to studies by other groups, we would expect a massive increase in the AT_1 receptor number [60][88][93]. At the same time, our mouse model might show a decrease in AT_1 receptor number, which could explain the weak vessel responses.

In vivo blood pressure measurements after Ang II administration: Blood pressure and baroreflex experiments can show pathological alterations while in vitro myograph experiments might show nothing despite using the same model. Determining the function of the baroreceptor reflex could help to understand possible changes in the vascular function in the ApoB model.

4.1.3 K^+ response:

For the aorta, we observed no significant strain difference between the two young animal groups; however, when the mice grew older, the strains developed differently – the old ApoB mice showed a significantly weaker contractile response than the young ApoB mice and than the old B6 mice. The old B6 mice also showed a significantly larger response than the young B6 mice. For the a. mes. sup., we observed no intergroup differences.

Some studies showed increased K^+ contractions in hyperlipidemic [24] or atherosclerotic experimental models [44]. Other studies revealed no changes in the K^+ response in atherosclerotic [7][23][54] or hyperlipidemic vessels [75]. Only the study by Wroblewski et al. reported that the K^+ was response reduced in atherosclerotic aortic rings of rabbits. This changed when they added excess FFA blood to the organ chamber – the K^+ responses increased [91].

Although we did not see an effect of age for the a. mes. sup. in our study, part of the increase in K^+ response in the aorta of the old B6 group might be explained by further age related growth of the mice compared to the young B6 mice; however, if this was the case, we would expect to have seen the same thing in the old ApoB group. Furthermore, in the atherosclerotic aorta of the old ApoB animals, the number of VSMCs should have increased as is normally observed with atherosclerosis; however, the contractile response decreased in this group.
On the other hand, we found diminished vessel responses for all applied vasocontractile substances (Phe, Ang II). Thus, the K^+ response has to be viewed in context with the Phe and Ang II responses.
A simple explanation for the weaker K^+ response could be the increased stiffness of the vessels due to atherosclerotic plaques and calcifications. This stiffness could make it harder for the VSMCs to establish an appropriate vessel contraction. In comparison to the studies mentioned above, that found either increased or unaltered K^+ responses in other animal models, this effect seems to be very pronounced and typical in the ApoB mouse strain.

It is important to mention that the increased stiffness cannot be the reason for the weaker responses to the other vasoactive substances tested in our ApoB model. The strength of the Phe and Ang II responses in our study are expressed as a percentage of the K^+ response (i.e., Phe response divided by K^+ response, and Ang II response divided by K^+ response, respectively). Therefore, an effect of vessel stiffness would be nullified since it should affect all vessel contractions equally.

The same can be hold for the weak ACh response. The precontraction is done with Phe. A stiff vessel would only contract little, thus ACh would only need to relax the vessel a little bit in order to achieve complete vessel relaxation. The stiffness would not make it harder for ACh to induce relaxation because the level of precontraction would be quite low.

4.2 Reaction to vasodilative substances

4.2.1 Acetylcholine

The young ApoB mice and the B6 mouse strain both young and old did not show atherosclerotic alterations in our study, suggesting that their endothelial function was not impaired. That was

confirmed by the CRCs. The vasodilation was massively impaired in the severe atherosclerotic aorta of the old ApoB group compared to the young ApoB group and to the old B6 group.
For the EC_{50} in the aorta, the comparison young ApoB mice / old ApoB mice and old ApoB mice / old B6 mice was significant.
Along with the impaired endothelial function seen in the CRC, the old ApoB mice showed less sensitivity than the young ApoB mice and the old B6 mice, which might be due to a downregulation in the processes regulating ACh sensitivity or due to NO degradation by ROS.

For the a. mes. sup., the comparison young ApoB mice / young B6 mice and old ApoB mice / old B6 mice was significant. In both cases, the ApoB mice strain showed stronger ACh responses than the B6 mice strain for low and medium doses. At the same time, the comparison young ApoB mice / old ApoB mice was not significant.
None of the groups showed any histological atherosclerotic alterations in the a. mes. sup.
We conclude that the altered ACh response of the ApoB strain compared to the B6 strain was not related to atherosclerosis in the a. mes. sup. but was caused by ApoB mice strain and vessel immanent features. No atherosclerotic alterations were observed, we found stronger instead of weaker ACh responses in the ApoB mice strain and there was no significant difference between the young and old ApoB mice.

Our findings for the aorta agree with the results of most other studies. It is well established that ACh induced endothelium-dependent relaxation is impaired in atherosclerotic animal models [16][17][23][26][44][64][75]; however, results concerning ACh sensitivity are inconsistent. In some models, an increase in sensitivity was reported [17], while in other the sensitivity was unchanged [45] or even decreased [23].

The finding of impaired endothelium-dependent relaxation in the old ApoB mouse aorta is important for the evaluation and interpretation of all other substances tested. ACh is a very sensitive substance for functional atherosclerotic alterations. Therefore, we have shown that the only vessel affected by atherosclerosis is the aorta of the old ApoB mice.
Although we hypothesize hypercholesterolemia as the underlying reason for the altered response to Phe in the ApoB mouse, this argument cannot be used to explain the ACh relaxations. Despite hypercholesterolemia, the young ApoB mice do not show an attenuated ACh response.

The reasons for the decrease in ACh-relaxations can be numerous, such as:

1. Attenuated superoxide dismutase (SOD) activity and diminished sensitivity of VSMCs to NO because of oxidative degradation of NO [44][92]
2. A diminished capacity to release NO [45]
3. Immanent vascular bed differences [57]
4. A loss of endothelial cells [47]
5. oxLDL inhibition of endothelium dependent relaxation [27]
6. Unopposed ACh activation of muscarinic receptors on VSMC in atherosclerotic blood vessels provoking vasoconstriction [9][11]

Future perspectives

The SOD activity and NO degradation might be an interesting avenue to explore in future studies with the ApoB mouse model. The application of SOD prior to ACh application could restore, or at least improve, the impaired vessel relaxation in situations where ROS-mediated degradation of NO occurs.

Incubation with L-NAME before ACh application in our ApoB mouse model might reveal the singular role for NO in vasodilation; however, it is possible that other substances such as EDHF may also play an important role in atherosclerotic vessels.

Furthermore, a reason for the impaired ACh response might be the decrease in NO production rather than in NO degradation. Thus, we could measure iNOS and eNOS activity and uncoupling following stimulation with ACh.

4.2.2 DEA NONOate

For the aorta, there was no significant intergroup difference for the CRCs. For the a. mes. sup., the comparison of the young ApoB group with the young B6 group revealed a deeper relaxation for small and medium doses in the young ApoB group. The EC_{50} did not show any significant intergroup differences.

Through the application of DEA NONOate we aimed to examine whether the atherosclerotic alteration in the ApoB model causes changes of the NO response in VSMCs. We hypothesized that due to atherosclerosis, VSMC NO sensitivity could be decreased, which could explain the impaired ACh induced relaxation. Another possibility could be the VSMC upregulation of NO sensitivity to compensate for the impaired atherosclerotic endothelium. Neither possibility was

supported by our findings. Neither in "healthy" nor in atherosclerotic vessels did we observe any differences in NO sensitivity. The fact that we found an increase in vessel relaxation in the a. mes. sup. of the young ApoB mice compared to the young B6 mice was remarkable; however, we cannot draw any useful information from this finding. The ApoB mice did not develop atherosclerotic alterations in the a. mes. sup.

The unchanged DEA NONOate response agrees with the results of many other studies. In most models, the relaxation to NO-donors was unchanged [17][26][36][44][45][64][75][79].
Only in some atherosclerotic models was the relaxation to endothelium-independent NO-donors impaired [16][23]. For example, d'Uscio et al. found relaxations to DEA NONOate reduced in their atherosclerotic ApoE (-/-) mouse model. At the same time, the sensitivity was decreased [23]. In some models, the NO sensitivity was decreased while the maximum relaxation was unaltered [47].

A possible explanation for the unaltered DEA NONOate response was given by Yaghoubi et al. They found that in most studies of atherosclerosis, the relaxation of isolated arteries or in vivo vasodilation as a result of NO-donor application was reported to be normal, or approximately normal. This was often taken as evidence that attenuated endothelium-dependent vasodilation was caused by diminished NO-production [92]. They hypothesized that enhanced ROS production could also contribute to the impaired response to NO either released from the endothelium or administered exogenously. They tested this hypothesis by measuring responses to NO gas as well as to sodium nitroprusside (SNP, a NO donor like DEA NONOate) in isolated aortas of ApoE (-/-) mice, which are susceptible to developing atherosclerosis. In atherosclerotic vessels, impaired sensitivity to NO gas was found while the SNP response remained unaltered. The authors hypothesized that the response to SNP might be unaltered because the NO release of SNP happens predominantly intracellularly. In this regard, the extracellular space, which is susceptible to accumulating high levels of ROS, is avoided [92].

Future perspectives

When applying DEA NONOate as a substance similar to SNP, we might have excluded the NO degradation by extracellular ROS. By applying NO gas in future studies, the involvement of ROS could be resolved. The application of SOD together with NO gas should then reestablish the vessel relaxation.

4.2.3 ACh CRC after incubation with indomethacin

For the aorta, the young ApoB mice and the young B6 mice intragroup comparisons were significant after incubation with indomethacin. Without indomethacin, the young B6 mice showed weaker responses for the lower ACh doses and deeper relaxations for higher ACh doses. After indomethacin incubation, the young ApoB mice showed weaker responses for high ACh doses. The EC_{50} was significantly different for the intragroup comparison to the young ApoB mice and the young B6 mice. In these groups, incubation with indomethacin decreased sensitivity to ACh. The old ApoB mice, which was the only group that had suffered from atherosclerosis and thus should have shown functional alterations after incubation with indomethacin, did not show a significant difference in EC_{50}.

For the a. mes. sup., only the old B6 mice showed significant intragroup differences. Here, the responses for low and medium doses were increased after indomethacin incubation.

We have to assume that in the ApoB model, the COX products do not play a major role for endothelium-dependent relaxations in the a. mes. sup. and in the atherosclerotic aorta. Additionally, it appears that in the ApoB mouse model the influence of COX products in "healthy" vessels is seemingly positive for the endothelial function, since COX inhibition resulted in a decreased sensitivity and weaker responses to ACh in the aorta of the young ApoB mice.

Future perspectives

A means to further elucidate the influence of COX activity in the ApoB model could be through the selective inhibition of COX subtypes with COX-1 inhibitors such as dexketoprofene or COX-2 inhibitors such as rofecoxib or celecoxib, since COX subtypes are known to have different effects on the vascular tone [51].

Furthermore, it has been found that ROS have a significant influence on NO mediated vessel responses. Indomethacin has been shown to exert COX-independent influences on ROS production in animal models [20]. Therefore, we could apply SOD and indomethacin before ACh application to see if the COX inhibitory effects of indomethacin, which could possibly lead to better vasodilation after ACh stimulation, is attenuated by its stimulation of ROS synthesis in atherosclerotic vessels.

4.3 Lipid profile

The ApoB strain showed a very atherogenic lipid profile. Total cholesterol and triglyceride serum levels were significantly increased. Furthermore, the total cholesterol level of the old ApoB mice was significantly higher than that measured in the young ApoB animals. Taking a closer look at the distribution of cholesterol in the different lipoprotein fractions, we observed large differences between the ApoB and B6 strains. The B6 strain carried most of its cholesterol in the atheroprotective HDL fraction, whereas the ApoB strain had only a small HDL fraction and a massive increase in the LDL fraction. In addition, the old ApoB mice also had an increased VLDL fraction compared to the young ApoB mice. Due to the genetically induced perturbations in lipid metabolism associated with this strain, these findings were not surprising. Indeed, they agree with the histological atherosclerotic findings in the aorta of the old ApoB mice and the previous work by Veniant et. al. [85].

The increased plasma cholesterol in the old ApoB group compared to the young ApoB group was presumably transported in the VLDL fraction. It appears that the progress of enduring hyperlipidemia has resulted in not only functional damages of the aorta but also in further alterations in lipid metabolism. The VLDL uptake seems to be impaired in the old ApoB mice. This is remarkable considering that the young ApoB mice had a comparably low VLDL level. The VLDL particles, which are produced in the ApoB mouse liver, do not contain ApoB48 and thus no ApoE **[73]**. Physiological clearance via the LRP **[85]** or the VLDLr [74] seems impossible, since both require ApoE as a receptor ligand.
Nevertheless, when the ApoB mice are young, mechanisms for clearing VLDL seem to be partially sufficient. As Tacken et al. hypothesized, backup mechanisms for clearing VLDL particles could be effective in situations where ApoE-mediated clearance is compromised [74]. This backup mechanism could be impaired during the process of aging and enduring hyperlipidemia, leading to higher VLDL serum levels.

4.4 Adhesion molecules

sICAM-1 and sVCAM-1 levels were measured as inflammatory markers in order to establish a correlation with the histological and functional vessel alterations.

The sICAM-1 levels in both groups of old mice were markedly increased compared to young mice. Since only the old ApoB mice developed severe atherosclerosis, we would have expected

only the old ApoB mice to show high sICAM-1 levels. Increased sICAM-1 levels were described in other studies as a predictive marker for the development of atherosclerosis in healthy subjects [6]. As such, the high sICAM-1 levels in the old B6 mice might be explained by the fact that these mice actually have some risk of developing atherosclerosis in old age.

The sVCAM-1 levels showed a significant strain difference. Both the young and old ApoB groups had higher levels than the B6 groups. The atherosclerotic old ApoB group had significantly higher sVCAM-1 levels than the young ApoB and the old B6 mice. Thus, in contrast to sICAM-1, sVCAM-1 seems to be a more appropriate marker for atherosclerotic alterations (at least for the aorta) in the ApoB mouse model. The predictive role that other studies using initially healthy human cohorts found for sICAM-1 but not for sVCAM-1 regarding the development of atherosclerosis [6] appears to be applicable to sVCAM-1 in the ApoB mouse model. In young ApoB mice, which have not suffered atherosclerotic alterations but are at high risk for it, the high sVCAM-1 levels might already indicate the disease onset. In the established atherosclerotic alteration of the aorta in the old ApoB group, sVCAM-1 levels have increased further, possibly reflecting the severity of the disease. This is in agreement with most other studies [6].

4.5 Conclusions

Atherosclerotic alteration of the aorta of the ApoB mice coincides with a diminished vasoreaction to major vasoactive substances (K^+, angiotensin II, and acetylcholine). The phenylephrine response was attenuated already to the same degree in the non-atherosclerotic aorta of the young ApoB mice as in the atherosclerotic aorta of the older ApoB mice. The endothelium-independent vasodilative capacity was unchanged (DEA NONOate). An involvement of COX-products in the altered function of the atherosclerotic aorta could not be observed (acetylcholine response after incubation with indomethacin).

For the arteria mesenterica superior, which was consistently non-atherosclerotic, the principal finding was the diminished phenylephrine response in both ApoB mice groups compared to the B6 mice.

The diminished response to phenylephrine in both the aorta and the a. mes. sup. in the ApoB strain seems to be the most remarkable finding of our study. We hypothesize that the attenuated

response to phenylephrine could be attributed to characteristics of the ApoB mouse strain such as the permanent state of hypercholesterolemia.

5 Summary

5.1 Introduction and derivation of the question

Atherosclerosis is a major health problem. It is the most important underlying cause of cardiovascular disease, which is the main cause of death among European men under 65 and the second most common cause of death among European women. Atherosclerosis is a chronic, slowly progressing, non-reversible disease affecting the blood vessel walls leading to a "hardening" or "stiffening" of the arteries. The etiopathological connection between atherosclerosis and high blood cholesterol levels has been established in many studies.

Different animal models have been used to explore the underlying mechanisms and characteristics of this disease and have generated inconsistent results. The lipid profiles of these models were often different to that observed in the human atherogenic lipid profile and thus did not appropriately reflect the pathogenesis of human atherosclerosis.

Recently, the B6;129S-ApoBtm2SgyLdlrtm1Her mouse (ApoB mouse) was developed. It is an atherosclerotic double-knock-out low-density-lipoprotein-receptor-deficient apolipoprotein-B-100-only mouse. This mouse model has a lipid profile similar to most humans suffering from atherosclerosis.

Using the ApoB mouse model, we explored the changes in vasoreactivity during the process of atherosclerotic alteration. For a further characterization of this mouse model, lipid profile and adhesion molecules were measured and morphometric evaluation of the research vessels was performed.

5.2 Materials and methods

Male ApoB mice and B6129SF2/J mice (B6 wild type, control mice) were examined at age 4 months (young group) or 18 months (old group). 2 mm ring preparations of the root of the aorta thoracica and of the main branch of the arteria mesenterica superior were mounted on tungsten wires into separate organ chambers of a myograph (Mulvany apparatus). Vessels were kept at

36.7°C in a physiological Krebs-Henseleit solution. Contraction was measured under isometric conditions.

Phenylephrine, angiotensin II, acetylcholine (with and without indomethacin incubation), and DEA NONOate (NO donor) were applied in a cumulative concentration response manner. Contractile responses to phenylephrine and angiotensin II were presented as a percentage of K^+ response. Responses to the vasodilators acetylcholine and DEA NONOate were investigated after submaximal phenylephrine contraction.

Cholesterol and triglyceride serum levels were measured using enzymatic colorimetric assays. The distribution of cholesterol among the lipoprotein subclasses was determined using a Superose 6 column. sICAM-1 and sVCAM-1 were examined by immunoassay.

The stenosis rate of the vessel lumen was quantified after Giemsa staining of paraffin sections with the assistance of a digitizing morphometry image-analysis system.

5.3 Results

Morphometric analysis showed that all old ApoB mice had developed severe atherosclerosis in the aorta while the arteria mesenterica superior was unaffected. More than half of the lumen of the aorta thoracica of the old ApoB mice was covered with atherosclerotic plaques. Young ApoB mice as well as B6 wild type mice generally did not show signs of atherosclerosis using light microscopy.

In the atherosclerotic aorta of the old ApoB mice, vessels had a reduced response to K^+ and angiotensin II compared with the respective old B6 wild type mice and compared with the young ApoB mice. The vessel reactivity to these agonists was unchanged in the non-atherosclerotic arteria mesenterica superior of the young and old ApoB mice. In contrast, phenylephrine reactivity was diminished to the same degree in both aorta and arteria mesenterica superior of young and old ApoB groups when compared to the B6 wild type mice.

Acetylcholine-induced, endothelium dependent dilation was reduced in the aorta of the old ApoB mice compared to young ApoB mice and wild type controls. The arteria mesenterica superior of the ApoB mice strain responded stronger to acetylcholine than the B6 mice strain.

In the aorta, endothelium independent dilation to DEA NONOate was alike in all groups. The arteria mesenterica superior of the young ApoB mice dilated more strongly in response to low dose DEA NONOate compared to young B6 mice. No other groups differed in the DEA NONOate response.

Acetylcholine-induced vasodilation was slightly impaired after indomethacin incubation in the young ApoB mice. Indomethacin treatment did not influence reactions to acetylcholine in the arteria mesenterica superior of the ApoB strain.

Serum parameters showed a rise in total cholesterol and triglycerides in the ApoB strain compared to B6 control mice. The LDL fraction within the lipoprotein profile was markedly increased. The old ApoB group also showed an increase in the total serum cholesterol and VLDL compared to the young ApoB and B6 wild type mice. sICAM-1 and sVCAM-1 were increased in the old ApoB group compared to the young ApoB mice. sVCAM-1 concentration was also greater in both ApoB groups compared to their respective B6 wild type mice. Further, sICAM-1 was increased in the old B6 compared to young B6 mice.

5.4 Conclusions

The atherosclerotic ApoB mouse model demonstrated severe atherosclerotic changes in the aorta but not in the arteria mesenterica superior. This genetic model also demonstrates the importance of age, since young ApoB mice did not show morphological alterations of the aorta using light microscopy.

The study shows that atherosclerotic alterations of the aorta of ApoB mice coincide with a diminished vasoreaction to K^+, angiotensin II, and acetylcholine. Remarkably, the phenylephrine response was attenuated already to the same degree in the non-atherosclerotic aorta of the young ApoB mice as in the atherosclerotic aorta of the ApoB old group. Moreover, the phenylephrine-induced constriction was also reduced in the non-atherosclerotic arteria mesenterica superior. This suggests changes in receptor expression and/or signaling pathway for adrenergic receptors in both types of vessels, which may develop before the occurrence of structural changes in the arterial wall of ApoB mice. Concurrently, the endothelium-independent vasodilative capacity was unchanged (DEA NONOate).

Our data supports the idea of a special role of the adrenergic system for atherosclerosis related changes of vessel function in ApoB mice.

Reduced endothelium dependent relaxation to acetylcholine in the aorta of ApoB old mice underlines the morphological finding of impairment of the endothelium in this vessel of ApoB mice during aging.

We conclude from the vessel reactions that of all applied pharmacological substances acetylcholine is the most useful substance to test atherosclerotic alterations in the ApoB mice model.

The data further indicates no involvement of COX-products in the altered function of the atherosclerotic aorta.

This study provides original data about vessel function, morphology, and serum parameters in ApoB mice, thereby giving a first insight into the pathogenesis of atherosclerosis related impairment of arterial vessel function in this model.

6 Zusammenfassung

6.1 Einleitung und Herleitung der Fragestellung

Atherosklerose ist ein großes Gesundheitsproblem und die wichtigste Ursache kardiovaskulärer Erkrankungen. Diese stellen die häufigste Todesursache europäischer Männer unter 65 und die zweithäufigste Todesursache europäischer Frauen dar. Bei der Atherosklerose handelt es um eine chronische, langsam voranschreitende und irreversible Erkrankung, die zur Verhärtung der ateriellen Blutgefäßwände führt. Die ätiopathologische Verbindung zwischen Atherosklerose und insbesondere hohem Blutcholesterol wurde in vielen Studien gezeigt.

In der Vergangenheit wurden unterschiedliche Tiermodelle verwendet, um die zugrundeliegenden Mechanismen und Eigenschaften dieser Erkrankung zu beschreiben. Häufig waren die Ergebnisse inkonsistent. Die verwendeten Tiermodelle wiesen oft stark abweichende Lipidprofile im Vergleich zum atherogenen humanen Lipidprofil auf und ermöglichten nur deutlich eingeschränkte Aussagen zur Pathogenese humaner Atherosklerose.

In jüngerer Zeit wurde die B6;129A-ApoBtm2SgyLdlrtm1Her-Maus (ApoB-Maus) entwickelt. Es handelt sich hierbei um eine LDL-Rezeptor-defiziente, „apolipoprotein-B-100-only" doppel-knock-out Maus. Durch die genetische Modifikation hat diese Maus ein Lipidprofil, das dem der meisten Menschen ähnlich ist, die an Atherosklerose leiden.

In der vorliegenden Arbeit wurde mit dem ApoB-Mausmodell die Veränderung der Gefäßansprechbarkeit während der Entwicklung einer Atherosklerose untersucht. Zur weiteren Beschreibung der ApoB-Maus wurden das Lipidprofil sowie die Adhäsionsmoleküle bestimmt. Des Weiteren wurde eine morphometrische Evaluation der untersuchten Gefäße vorgenommen.

6.2 Materialien und Methoden

Männliche ApoB-Mäuse und B6129SF2/J-Mäuse (B6-Wildtyp, Kontrollmäuse) wurden im Alter von 4 Monaten (junge Gruppen) oder 18 Monaten (alte Gruppen) untersucht. Jeweils eine 2 mm Ring-Präparation der Aortenwurzel und des Hauptastes der Arteria mesenterica superior wurden auf Wolfram-Drähten in zwei getrennte Myographen-Kammern eingebracht (Mulvany-Apparat).

Die Blutgefäße befanden sich im Myographen in 36,7°C warmer physiologischer Krebs-Henseleit-Lösung. Die Gefäßkontraktionen wurden unter isometrischen Bedingungen gemessen.

Phenylephrin, Angiotensin II, Acetylcholin (mit und ohne Indomethacin-Inkubation) und DEA NONOate (NO-Donor) wurden im Sinne einer kumulativen Konzentrations-Wirkungs-Kurve in die Badlösung appliziert. Die Stärke der kontraktilen Antworten auf die Phenylephrin- sowie Angiotensin II-Gabe wurden in Prozent der K^+-Antwort angegeben. Die Reaktionen auf die Gefäßdilatoren Acetylcholin sowie DEA NONOate wurden nach der Auslösung einer submaximalen Phenylephrin-Kontraktion untersucht.

Die Cholesterol- und Triglyzeridkonzentrationen im Serum wurden mit Hilfe enzymatischer kolorimetrischer Assays bestimmt. Die Verteilung des Cholesterols auf die Lipoprotein-Subklassen wurde mit einer Superose-6-Säule ermittelt. sICAM-1 und sVCAM-1 wurden mit einem Immuno-Assay gemessen.

Der Stenosierungsgrad der Gefäßlumen wurde nach Giemsa-Färbung an Paraffinschnitten mit Hilfe eines digitalen morphometrischen Bildanalysesystems quantifiziert.

6.3 Ergebnisse

Die morphometrische Analyse ergab starke atherosklerotische Veränderungen der Aorten aller alten ApoB-Mäuse, während die Arteria mesenterica superior stets unbetroffen war. Mehr als die Hälfte des Lumens der Aorta thoracica dieser Mäuse war mit atherosklerotischen Plaques bedeckt. Sowohl junge ApoB-Mäuse als auch der B6-Wildtyp zeigten keine lichtmikroskopischen atherosklerotischen Veränderungen.

In der atherosklerotischen Aorta der alten ApoB-Mäuse war die Antwort auf K^+ und Angiotensin II im Vergleich zu den alten B6-Mäusen und den jungen ApoB-Mäusen verringert. Die Gefäßreaktivität auf diese Agonisten war unverändert in der nicht-atherosklerotischen Arteria mesenterica superior der jungen und alten ApoB-Mäuse. Im Gegensatz hierzu war die Reaktion auf Phenylephrin sowohl in der Aorta als auch in der Arteria mesenterica superior der jungen sowie alten ApoB-Mäuse im Vergleich zum B6-Wildtyp vermindert.
Die acetylcholininduzierte, endothelabhängige Dilation war in der Aorta der alten ApoB-Mäuse im Vergleich zu den jungen ApoB-Mäusen und dem B6-Wildtyp reduziert. Die Arteria

mesenterica superior der ApoB-Mäuse zeigte nach Acetylcholin-Gabe stärkere Antworten als der B6-Wildtyp.

In der Aorta war die endothelunabhängige, durch DEA NONOate ausgelöste Vasodilation in den ApoB-Gruppen ähnlich und unterschied sich nicht vom B6-Wildtyp. Die Arteria mesenterica superior der jungen ApoB-Mäuse dilatierte bei geringen DEA NONOate-Dosen stärker als die des B6-Wildtyps. Die anderen Gruppen zeigten keine Unterschiede.

In der Aorta der jungen ApoB-Mäuse führte die Indomethacin-Inkubation zu einer leichten Hemmung der Acetylcholin-Antwort. In der Arteria mesenterica superior der ApoB-Mäuse hatte die Inkubation keinen Einfluss.

Die Serumparameter zeigten einen Anstieg im Cholesterol- und Triglyzeridspiegel im ApoB-Stamm im Vergleich zum B6-Wildtyp. Innerhalb des Lipoproteinprofiles stieg besonders die LDL-Fraktion an. Die alten ApoB-Mäuse zeigten darüber hinaus einen weiteren Anstieg des Gesamt-Serum-Cholesterols sowie der VLDL-Fraktion im Vergleich zu den jungen ApoB-Mäusen und zum B6-Wildtyp. sICAM-1 und sVCAM-1 waren in der alten ApoB-Gruppe im Vergleich zur jungen ApoB-Gruppe erhöht. Darüber hinaus war sVCAM-1 in beiden ApoB-Gruppen im Vergleich zum B6-Wildtyp erhöht. sICAM-1 zeigte außerdem einen Anstieg in der alten B6-Gruppe im Vergleich zur jungen B6-Gruppe.

6.4 Schlussfolgerungen

Das atherosklerotische ApoB-Mausmodell zeigte starke pathologische Veränderungen in der Aorta und keine in der Arteria mesenterica superior. Dieses genetisch modifizierte Modell lässt darüber hinaus eine Altersabhängigkeit erkennen, da junge ApoB-Mäuse keine lichtmikroskopischen Veränderungen der Aorta aufwiesen.

Die Studie zeigt, dass atherosklerotische Veränderungen der Aorta der ApoB-Maus mit verminderter Gefäßreaktivität auf K^+, Angiotensin II sowie Acetylcholin einhergehen. Bemerkenswerterweise war die Phenylephrin-Antwort in der nicht-atherosklerotischen Aorta der jungen Apo-Gruppe sowie in der atherosklerotischen Aorta der alten ApoB-Gruppe in gleichem Maße stark vermindert. Des Weiteren war die phenylephrininduzierte Kontraktion ebenso in der nicht-atherosklerotischen Arteria mesenterica des ApoB-Stamms reduziert. Dies weist darauf

hin, dass es Veränderungen in der Rezeptor-Expression und/oder den Signalwegen für adrenerge Rezeptoren in beiden Blutgefäßtypen gibt, die bereits vor dem Auftreten struktureller Veränderungen in der arteriellen Wand der ApoB-Maus auftreten. Gleichzeitig war die endothelunabhängige vasodilative Kapazität unverändert (DEA NONOate).

Die Daten weisen darauf hin, dass das adrenerge Sytem in Bezug auf atherosklerose induzierte Veränderungen der Gefäßfunktion der ApoB-Maus eine besondere Rolle spielt.

Die reduzierte endothelabhängige Acetylcholinantwort in der Aorta der alten ApoB-Mäuse und die morphologischen Befunde des atherosklerotischen Befalls weisen auf eine Beeinträchtigung der Endothelfunktion dieses Gefäßes während des Altersgangs in diesem Tiermodell hin.

Wir schließen aus den Gefäßreaktionen, dass von den verwendeten pharmakologischen Substanzen Acetylcholin am besten zur Charakterisierung der atherosklerotischen Veränderungen der ApoB-Maus geeignet ist.

Die Daten ergeben keine Anhaltspunkte für eine Beteiligung von COX-Produkten in der atherosklerotisch veränderten Funktion der Aorta.

In dieser Studie wurde erstmals die Gefäßfunktion in Gegenüberstellung zu Serumparametern und Morphologie der Aorta und Arteria mesenterica superior von ApoB-Mäusen untersucht. Sie gestattet erste Einblicke in die atherosklerosebedingte Pathogenese der arteriellen Gefäßfunktion in diesem Mausmodell.

7 Bibliography

[1] Alvaro-Gonzalez LC, Freijo-Guerrero MM, Sadaba-Garay F. Inflammatory mechanisms, arteriosclerosis and ischemic stroke: clinical data and perspectives. Rev Neurol. 2002;35:452-62

[2] Anning PB, Coles B, Bermudez-Fajardo A et al. Elevated endothelial nitric oxide bioactivity and resistance to angiotensin-dependent hypertension in 12/15-lipoxygenase knockout mice. Am J Pathol. 2005;166:653-62

[3] Auto Dual Wire Myograph System – 510A. Aarhus N, Denmark: Danish Myo Technology A/S, 2001 (Accessed 2001, at http://www.dmt.dk/specifications/510A.pdf)

[4] Auto Dual Wire Myograph System Model 410 A, Version 2.00, User Manual. Aarhus N, Denmark: Danish Myo Technology A/S, 2003 (Accessed July 7, 2006, at http://www.dmt.dk/user_manuals/usermanual410a.pdf)

[5] Binder CJ, Shaw PX, Chang MK et al. The role of natural antibodies in atherogenesis. J Lipid Res. 2005;46:1353-63

[6] Blankenberg S, Barbaux S, Tiret L. Adhesion molecules and atherosclerosis. Atherosclerosis. 2003;170:191-203

[7] Brizzolara AL, Stewart-Lee A, Burnstock G. Responses of rabbit basilar arteries to vasoconstrictor and vasodilator agents: the effects of Atherosclerosis, age and sex. J Vasc Res. 1994;31:106-13

[8] Brown MS, Goldstein JL. Lipoprotein receptors: therapeutic implications. J Hypertens Suppl. 1990;8:33-6

[9] Brunner H, Cockcroft JR, Deanfield et al. Endothelial function and dysfunction. Part II: Association with cardiovascular risk factors and diseases. A statement by the Working Group on Endothelins and Endothelial Factors of the European Society of Hypertension. J Hypertens. 2005;23:233-46

[10] Chait A, Han C, Oram JF, Heinecke JW. Thematic review series: The immune system and atherogenesis. Lipoprotein-associated inflammatory proteins: markers or mediators of cardiovascular disease? J Lipid Res. 2005;46:389-403

[11] Chataigneau T, Feletou M, Huang PL et al. ACh-induced relaxation in blood vessels from endothelial nitric oxide synthase knockout mice. Br J Pharmacol. 1999;126:219-26

[12] Chilian WM, Dellsperger KC, Layne SM et al. Effects of Atherosclerosis on the coronary microcirculation. Am J Physiol. 1990;258:529-39

[13] Christon R,Marette A,Badeau M et al. Fatty acid-induced changes in vascular reactivity in healthy adult rats. Metabolism. 2005;54:1600-9

[14] Corti R, Hutter R, Badimon JJ, Fuster V. Evolving concepts in the triad of atherosclerosis, inflammation and thrombosis. J Thromb Thrombolysis. 2004;17:35-44

[15] Cotran RS, Mayadas-Norton T. Endothelial adhesion molecules in health and disease. Pathol Biol (Paris). 1998;46:164-70

[16] Crauwels HM, Van Hove CE, Holvoet, P et al. Plaque-associated endothelial dysfunction in apolipoprotein E-deficient mice on a regular diet. Effect of human apolipoprotein AI. Cardiovasc Res. 2003; 59:189-99

[17] Crespo MJ, Altieri PI, Escobales N. Altered vascular function in early stages of heart failure in hamsters. J Card Fail. 1997;3:311-8

[18] Dam JP, Vleeming W, Riezebos J, Post MJ, Porsius AJ, Werner J. Effects of hypercholesterolemia on the contractions to angiotensin II in the isolated aorta and iliac artery of the rabbit: role of arachidonic acis metabolites: J Cardiovasc Pharacol. 1997;3:118-23

[19] Daugherty A. Mouse models of Atherosclerosis. Am J Med Sci. 2002;323:3-10

[20] De Angelis A, Rinaldi B, Capuano A, Rossi F, Filippelli A. Indomethacin potentiates ACh-induced vasodilation by increasing free radical production. Br J Pharmacol. 2004;142:1233-40

[21] Degrace P, Moindrot B, Mohamed I, Gresti J. Clouet P. Moderate consumption of beer reduces liver triglycerides and aortic cholesterol deposit in LDLr(-/-) ApoB (100/100) mice. Atherosclerosis. 2006:Article in Press (accepted January 16, 2006)

[22] Dowell FJ, Hamilton CA, Lindop GB, Reid JL. Development and progression of Atherosclerosis in Aorta from heterozygous and homozygous WATANABE HERITABLE HYPERLIPIDEMIC rabbits. Effects of simvastatin treatment. Arterioscler Thromb Vasc Biol. 1995;15:1152-60

[23] D'Uscio LV, Barton M, Shaw S, Luscher TF. Chronic ET(A) receptor blockade prevents endothelial dysfunction of small arteries in apolipoprotein E-deficient mice. Cardiovasc Res. 2002;53:487-95

[24] Esenabhalu VE, Cerimagic M, Malli R et al. Tissue-specific expression of human lipoprotein lipase in the vascular system affects vascular reactivity in transgenic mice. Br J Pharmacol. 2002;135:143-54

[25] Farese RVJr, Veniant MM, Cham CM et al. Phenotypic analysis of mice expressing exclusively Apolipoprotein B48 or Apolipoprotein B100. Proc Natl Acad Sci U S A. 1996;93:6393-8

[26] Finta KM, Fischer MJ, Lee L et al. Ramipril prevents impaired endothelium-dependent relaxation in arteries from rabbits fed an atherogenic diet. Atherosclerosis. 1993;100:149-56

[27] Froese DE, McMaster J, Man RY, Choy PC, Kroeger EA. Inhibition of endothelium-dependent vascular relaxation by lysophosphatidylcholine: impact of lysophosphatidylcholine on mechanisms involving endothelium-derived nitric oxide and endothelium derived hyperpolarizing factor. Mol Cell Biochem. 1999;197:1-6

[28] Fujiwara T, Chiba S. Alterations of vascular alpha 1-adrenergic contractile responses in hypercholesterolemic rabbit common carotid arteries. J Cardiovasc Pharmacol. 1993;22:58-64

[29] Ghazalpour A, Doss S, Yang X et al. Thematic review series: The pathogenesis of Atherosclerosis. Toward a biological network for Atherosclerosis. J Lipid Res. 2004;45:1793-805

[30] Grieve DJ, Avella MA, Elliott J, Botham KM. The influence of chylomicron remnants on endothelial cell function in the isolated perfused rat Aorta. Atheroslerosis. 1998;139:273-81

[31] Guzik TJ, Black E, West NE et al. Relationship between the G894T polymorphism (Glu298Asp variant) in endothelial nitric oxide synthase and nitric oxide-mediated endothelial function in human Atherosclerosis. Am J Med Genet. 2001;100:130-7

[32] Hansson GK. Inflammation, Atherosclerosis, and coronary artery disease. N Engl J Med. 2005;352:1685-95

[33] Harrison DG, Freiman PC, Armstrong ML, Marcus ML, Heistad DD. Alterations of vascular reactivity in Atherosclerosis. Circ Res. 1987;61:II74-80

[34] Hees H, Sinowatz F. Allgemeine und spezielle Pathologie; Deutscher Ärzte-Verlag. 1993;2:142-50
[35] Hirano K, Young SG, Farese RV Jr et al. Targeted disruption of the mouse Apobec-1 gene abolishes Apolipoprotein B mRNA editing and eliminates Apolipoprotein B48. J Biol Chem. 1996;271:9887-90
[36] Hof RP, Hof A. Vasoconstrictor and vasodilator effects in normal and atherosclerotic conscious rabbits. Br J Pharmacol. 1988;95:1075-80
[37] Hof RP, Tapparelli C, Weinstein DB. Hemodynamic, antivasoconstrictor, and antiatherosclerotic effects of calcium antagonists in animal models of Atherosclerosis. J Cardiovasc Pharmacol. 1990;15 Suppl 1:7-12
[38] Huo Y, Ley K. Adhesion molecules and atherogenesis. Acta Physiol Scand. 2001;173:35-43
[39] Ishibashi S, Brown MS, Goldstein JL, Gerard RD, Hammer RE and Herz J. Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenovirus-mediated gene delivery. J Clin Invest. 1993;92:883-93
[40] Jaffe EA. Cell biology of endothelial cells. Hum Pathol. 1987;18,3:234-9
[41] Jax® Mice Data Sheet. Bar Harbor, Main, USA: The Jackson Laboratory, 2006 (Accessed May 22nd, 2006, at http://jaxmice.jax.org/strain/003000.html)
[42] Jax® Mice Data Sheet. Bar Harbor, Main, USA: The Jackson Laboratory, 2006 (Accessed May 22nd, 2006, at http://jaxmice.jax.org/strain/101045.html)
[43] Jay MT, Chirico S, Siow RC et al. Modulation of vascular tone by low density lipoproteins: effects on L-arginine transport and nitric oxide synthesis. Exp Physiol. 1997;82:349-60
[44] Jiang J, Thoren P, Caligiuri G, Hansson GK, Pernow J. Enhanced Phe-induced rhythmic activity in the atherosclerotic mouse Aorta via an increase in opening of KCa channels: relation to Kv channels and nitric oxide. Br J Pharmacol. 1999;128:637-46
[45] Johansson ME, Hagg U, Wikstrom J et al. Haemodynamically significant plaque formation and regional endothelial dysfunction in cholesterol-fed ApoE-/- mice. Clin Sci (Lond). 2005;108:531-8
[46] Kim JW, Kang KW, Oh GT et al. Induction of hepatic inducible nitric oxide synthase by cholesterol in vivo and in vitro. Exp Mol Med. 2002;34:137-44
[47] Kolodgie FD, Virmani R, Rice HE, Mergner WJ. Vascular reactivity during the progression of atherosclerotic plaque. A study in Watanabe heritable hyperlipidemic rabbits. Circ Res. 1990;66:1112-26
[48] Lacy PS, Pilkington G, Hanvesakul R et al. Evidence against potassium as an endothelium-derived hyperpolarizing factor in rat mesenteric small arteries. Br J Pharmacol. 2000;129:605-11
[49] Lebuffe G, Boullier A, Tailleux A et al. Endothelial derived vasorelaxation is impaired in human APO A-I transgenic rabbits. Biochem Biophys Res Commun. 1997;241:205-11
[50] Lieu HD, Withycombe SK, Walker Q et al. Eliminating atherogenesis in mice by switching off hepatic lipoprotein secretion. Circulation. 2003;107:1315-21
[51] Linton MF, Fazio S. Cyclooxygenase-2 and Atherosclerosis. Curr Opin Lipidol. 2002;13:497-504
[52] Lu LF, Fiscus RR. Interleukin-1beta causes different levels of nitric oxide-mediated depression of contractility in different positions of rat thoracic Aorta. Life Sci. 1999;64:1373-81

[53] Luksha L, Nisell H, Kublickiene K. The mechanism of EDHF-mediated responses in subcutaneous small arteries from healthy pregnant women. Am J Physiol Regul Integr Comp Physiol. 2004;286:R1102-9

[54] Maguire JJ, Wiley KE, Kuc RE et al. Endothelin-mediated vasoconstriction in early Atherosclerosis is markedly increased in ApoE-/- mouse but prevented by atorvastatin. Exp Biol Med (Maywood). 2006;231:806-12

[55] Minor RL Jr, Myers PR, Guerra R Jr, Bates JN, Harrison DG. Diet-induced Atherosclerosis increases the release of nitrogen oxides from rabbit Aorta. J Clin Invest. 1990;86:2109-16

[56] Napoli C, Ignarro LJ. Nitric oxide and Atherosclerosis. Nitric Oxide. 2001;5:88-97

[57] Napoli C, Paterno R, Faraci FM et al. Mildly oxidized low-density lipoprotein impairs responses of carotid but not basilar artery in rabbits. Stroke. 1997;28:2266-72

[58] Napoli C. Nitric oxide and atherosclerotic lesion progression: an overview. J Card Surg. 2002;17:355-62

[59] Navab M, Ananthramaiah GM,Reddy ST et al. The oxidation hypothesis of atherogenesis: the role of oxidized phospholipids and HDL. J Lipid Res. 2004;45:993-1007

[60] Nickenig G, Sachinidis A, Michaelsen F et al. Upregulation of vascular angiotensin II receptor gene expression by low-density lipoprotein in vascular smooth muscle cells. Circulation. 1997;95:473-8

[61] Pfister SL. Aortic thromboxane receptor deficiency alters vascular reactivity in cholesterol-fed rabbits. Atherosclerosis. 2006

[62] Powell-Braxton L, Veniant M, Latvala RD et al. A mouse model of human familial hypercholesterolemia: markedly elevated low density lipoprotein cholesterol levels and severe Atherosclerosis on a low-fat chow diet. Nat Med. 1998;4:934-8

[63] Purcell-Huynh DA, Farese RV Jr, Johnson DF et al. Transgenic mice expressing high levels of human Apolipoprotein B develop severe atherosclerotic lesions in response to a high-fat diet. J Clin Invest. 1995;95:2246-57

[64] Rabelo LA, Cortes SF, Alvarez-Leite JI, Lemos VS. Endothelium dysfunction in LDLr knockout mice: a role for H2O2. Br J Pharmacol. 2003;138:1225-20

[65] Rader DJ, FitzGerald GA. State of the art: Atherosclerosis in a limited edition. Nat Med. 1998;4:899-900

[66] Sanan DA, Newland DL, Tao R et al. Low density lipoprotein receptor-negative mice expressing human Apolipoprotein B-100 develop complex atherosclerotic lesions on a chow diet: no accentuation by Apolipoprotein(a). Proc Natl Acad Sci U S A. 1998;95:4544-9

[67] Schulman IH, Zhou MS, Raij L. Interaction between nitric oxide and Ang II in the endothelium: role in Atherosclerosis and hypertension. J Hypertens Suppl. 2006;24:45-50

[68] Simonet S, Rupin A, Badier-Commander C et al. Evidence for superoxide anion generation in Aortas of cholesterol-fed rabbits treated with L-arginine. Eur J Pharmacol. 2004;492:211-6

[69] Song K, Shiota N, Takai S et al. Induction of angiotensin converting enzyme and angiotensin II receptors in the atherosclerotic aorta of high-cholesterol fed cynomolgus monkeys. Atherosclerosis. 1998;138:171-82

[70] Srisawat S, Phivthong-Ngam L, Unchern S et al. Improvement of vascular function by chronic administration of a cyclo-oxygenase inhibitor in cholesterol-fed rabbits. Clin Exp Pharmacol Physiol. 2003;30:405-12

[71] Steinberg D. The pathogenesis of Atherosclerosis. An interpretive history of the cholesterol controversy, part IV: the 1984 coronary primary prevention trial ends it—almost. J Lipid Res. 2006;47:1-14

[72] Steinberg D. Thematic review series: the pathogenesis of Atherosclerosis. An interpretive history of the cholesterol controversy: part I. J Lipid Res. 2004;45:1583-93

[73] Sun Q, Wang A, Jin X et al. Long-term air pollution exposure and acceleration of Atherosclerosis and vascular inflammation in an animal model. JAMA. 2005;294:3003-10

[74] Tacken PJ, Teusink B, Jong MC et al. LDL receptor deficiency unmasks altered VLDL triglyceride metabolism in VLDL receptor transgenic and knockout mice. J Lipid Res. 2000; 41:2055-62

[75] Tagawa H, Tomoike H, Nakamura M. Putative mechanisms of the impairment of endothelium-dependent relaxation of the Aorta with atheromatous plaque in heritable hyperlipidemic rabbits. Circ Res. 1991;68:330-7

[76] Takeuchi, K. [Signal transduction systems of Ang II receptors]. Nippon Rinsho. 1999;57:1070-7

[77] Tasaki K, Wakabayashi I, Shishido T et al. Diminution of Ang II-induced contraction of the abdominal Aorta isolated from Watanabe heritable hyperlipidemic rabbits. J Smooth Muscle Res. 2005;41:87-97

[78] Thorsgaard M, Lopez V, Buus NH, Simonsen U. Different modulation by Ca2+-activated K+ channel blockers and herbimycin of ACh- and flow-evoked vasodilation in rat mesenteric small arteries. Br J Pharmacol. 2003;138:1562-70

[79] Toda N, Miyazaki M, Hazama F. Functional and histological changes in mesenteric arteries and Aortas from monkeys fed a high cholesterol diet. Jpn J Pharmacol. 1988;48:441-51

[80] Touyz RM, Berry C. Recent advances in Ang II signaling. Braz J Med Biol Res. 2002;35:1001-15

[81] Usui M, Egashira K. Angiotensin II receptor and oxidative stress. Nippon Rinsho. 2002;60:1893-7

[82] Vanderlaan PA, Reardon CA. Thematic review series: the immune system and atherogenesis. The unusual suspects:an overview of the minor leukocyte populations in Atherosclerosis. J Lipid Res. 2005;46:829-38

[83] Veniant MM, Sullivan MA, Kim SK et al. Defining the atherogenicity of large and small lipoproteins containing Apolipoprotein B100. J Clin Invest. 2000;106:1501-10

[84] Veniant MM, Withycombe S, Young S G. Lipoprotein size and Atherosclerosis susceptibility in Apoe(-/-) and Ldlr(-/-) mice. Arterioscler Thromb Vasc Biol. 2001;21:1567-70

[85] Veniant MM, Zlot CH, Walzem RL et al. Lipoprotein clearance mechanisms in LDLr-deficient "Apo-B48-only"and "Apo-B100-only" mice. J Clin Invest. 1998;102:1559-68

[86] Vita JA, Treasure CB, Yeung AC et al. Patients with evidence of coronary endothelial dysfunction as assessed by ACh infusion demonstrate marked increase in sensitivity to constrictor effects of catecholamines. Circulation. 1992;85:1390-7

[87] Warnholtz A, Nickenig G, Schulz E et al. Increased NADH-oxidase-mediated

superoxide production in the early stages of Atherosclerosis: evidence for involvement of the renin-angiotensin system. Circulation. 1999;99:2027-33

[88] Wilfert K, Drischel K, Unbehaun, A et al. Vascular response to Ang II in Atherosclerosis: role of the baroreflex. Hypertension. 2000;35:685-90

[89] Williams JK, Kaplan JR, Suparto IH, Fox JL, Manuck SB. Effects of exercise on cardiovascular outcomes in monkeys with risk factors for coronary heart disease. Arterioscler Thromb Vasc Biol. 2003;23:864-71

[90] Wohlgemuth B. Allgemeine und spezielle Pathologie; J. A. Barth, Leipzig. 1990;2:134-40

[91] Wroblewski T, Witanowska A. The contractile response of the normal and atherosclerotic aortic strips to noradrenaline, and potassium ions. Acta Physiol Pol. 1982;33:353-60

[92] Yaghoubi M, Oliver-Krasinski J, Cayatte AJ, Cohen RA. Decreased sensitivity to nitric oxide in the Aorta of severely hypercholesterolemic apolipoprotein E-deficient mice. J Cardiovasc Pharmacol. 2000;36:751-7

[93] Yang BC, Phillips MI, Mohuczy D et al. Increased Ang II type 1 receptor expression in hypercholesterolemic Atherosclerosis in rabbits. Arterioscler Thromb Vasc Biol. 1998;18:1433-9

[94] Yang Z, Ming XF. Recent advances in understanding endothelial dysfunction in Atherosclerosis. Clin Med Res. 2006;4:53-65

[95] Zacharia J, Hillie Chris, MacDonald A. Alpha1-adrenoceptor subtypes involved in vasoconstrictor responses to exogenous and neurally released noradrenalin in rat femoral resistance arteries. Br J Pharmacol. 2004;141:915-24

8 List of abbreviations

A. mes. sup.	Arteria mesenterica superior
ACE	Angiotensin I converting enzyme
ACh	Acetylcholine
Ang II	Angiotensin II
ANOVA	Analysis of variance
ApoB mouse	B6;129S-ApoBtm2SgyLdlrtm1Her mouse
ApoB100	Apolipoprotein B 100
ApoB48	Apolipoprotein B 48
ApoE	Apolipoprotein E
B6 mouse	B6129SF2/J mouse
Ca^{2+}	Calcium
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
COX	Cyclooxygenase
CRC	Concentration response curve
CVD	Cardiovascular Disease
DEA NONOate	2-(N,N-Diethylamino)-diazenolate-2-oxide
EC_{50}	Effective concentration 50
EDHF	Endothelium-derived hyperpolarizing factor
eNOS	Endothelial nitric oxide synthase
FFA	Free fatty acid
HDL	High density lipoprotein
ICAM-1	Intercellular adhesion molecule 1
IDL	Intermediate density lipoprotein
indo.	Indomethacin
iNOS	Inducible nitric oxide synthase
K^+	Potassium
KH	Krebs Henseleit
LDL	Low density lipoprotein
LDLr	LDL receptor
L-NAME	N-nitro-l-arginine methyl ester
LOX	12/15-Lipoxygenase
LRP	LDL-receptor-related protein
MWU-test	Mann-Whitney U-test
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
NOS	Nitric oxide synthase
$O_2^{\bullet -}$	Oxygen radical
oxLDL	Oxidated LDL
PGI_2	Prostacyclin
Phe	Phenylephrine
RAS	Renin-angiotensin system
ROS	Reactive oxygen species
SD	Standard deviation

SEM	Standard error of mean
sICAM-1	Soluble ICAM-1
SNP	Sodium nitroprusside
SOD	Superoxide dismutase
sVCAM-1	Soluble VCAM-1
VCAM-1	Vascular-cell adhesion molecule 1
VLDL	Very low density lipoprotein
VLDLr	Very low density lipoprotein receptor
VSMC	Vascular smooth muscle cell
WHHL rabbit	Watanabee Heritable Hyperlipidemic rabbit

9 List of publications

Andreas Patzak, Andreas Steege, Enyin Lai, Jan Ole Brinkmann, Eckehardt Kupsch, Nadine Spielmann, Adrian Gericke, Angela Skalweit, Johannes Stegbauer, Pontus B. Persson, Erdmann Seeliger.

"Angiotensin II response in afferent arterioles of mice lacking either the endothelial or neuronal isoform of nitric oxide synthase"

American Journal of Physiology – Regulatory, Comparative and Integrative Physiology; accepted October 24th 2007

10 Curriculum Vitae

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

11 Eidesstattliche Erklärung

Ich, Jan Ole Brinkmann, erkläre, dass ich die vorgelegte Dissertationsschrift mit dem Thema "Vessel function of the atherosclerotic low-density-lipoprotein-receptor-deficient apolipoprotein-B-100-only mouse" selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt, ohne die (unzulässige) Hilfe Dritter verfasst und auch in Teilen keine Kopien anderer Arbeiten dargestellt habe.

Datum Unterschrift

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