Supply-demand balance in the atrium

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Kelly Anna Wilhelmina Maria van Bragt
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PROMOTOR:

Prof. Dr. U. Schotten

CO-PROMOTOREN:

Dr. S. Verheule
Dr. J.J. Luiken

BEOORDELINGSCOMMISSIE:

Prof. Dr. H.J.G.M. Crijns (voorzitter)
Prof. Dr. D.J.G.M. Duncker
Prof. Dr. F.W. Prinzen

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I looked for the dust in the air,
for that is where the words live,
tumbling lazy, remaining
just out of reach, and staying, staying,
staying, until something,
an unseen waft of air
causes them to drift right up to
your reach, gather into sentences,
one sentence, two sentences,
that's all you need to get started.

-Jimmy Breslin

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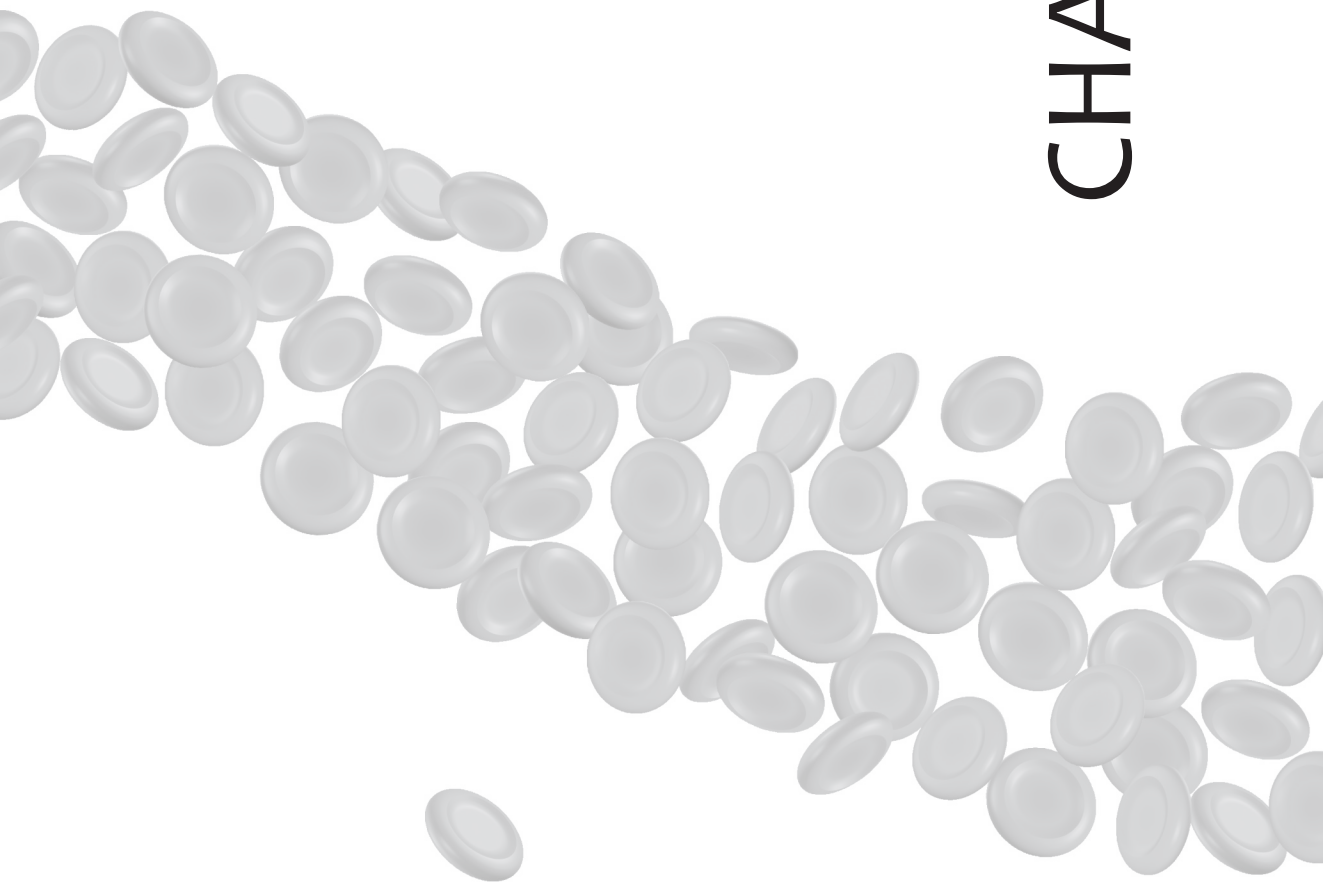
CHAPTER 1

General introduction

Kelly van Bragt



CHAPTER 1

1. GENERAL INTRODUCTION

Atrial fibrillation (AF) is the most common arrhythmia in clinical practice, affecting 1% of the total population. The prevalence of AF increases with age (to ~5% in people over 65 and ~10% in people over 75 [1-3]). The socio-economic burden related to AF is thus expected to increase with the aging of the general population. Chronic Ischemic heart disease is, amongst others, an important risk factor for AF [1, 4-10]. In acute myocardial infarction (AMI), about 10-15% of patients develop new-onset AF [11-16], a phenomenon that seems to be dependent on the involvement of atrial coronary branches [16-20]. As an experimental correlate, the short- and long-term effects of the complete occlusion of an atrial artery on AF were investigated in-vivo [21, 22] and ex-vivo [23, 24]. Several mechanisms, e.g. shortening of the action potential, slowing of conduction velocity and changes in calcium handling, were identified as potential mechanistic contributors to AF.

While these studies describe the role of atrial ischemia/infarction in the development of a substrate for AF, in this thesis, we describe another possible interrelation between ischemia and AF. We propose that AF in itself can cause (supply-demand) ischemia in the atria. This form of ischemia may form an important trigger for the remodeling processes that have been described as a result of AF. There is circumstantial evidence that supply-demand ischemia does indeed occur in the atria as a consequence of AF. The expression of hypoxic and angiogenic markers (e.g. $HIF_{1\alpha}$, $HIF_{2\alpha}$, VEGF) was shown in a goat model of AF [25] and in atrial biopsies of AF patients [26-28]. Some of the changes (electrical, contractile and structural remodeling) observed during chronic AF can be viewed as adaptive processes to conserve energy and lower the atrial oxygen demand to restore the atrial supply-demand balance. One of these processes, called myocardial hibernation was first described for fibrillating atria by Ausma [29-33], and is reminiscent to ventricular remodeling in response to chronic low-flow ischemia.

2. AIM OF THIS THESIS

How the atrial supply-demand balance is controlled and under which circumstances a mismatch will occur is still to be investigated. The general aim of this thesis is to

characterize the different regulatory mechanism of atrial oxygen supply-demand balance and determine whether or not there is supply-demand mismatch during acute and long-term AF. Atrial blood supply can be regulated at two levels: atrial coronary resistance and atrial oxygen extraction. Both are investigated in this thesis.

A detailed literature overview and background to the studies presented in this thesis are given in CHAPTER 2. The supply-demand mismatch during AF could in itself contribute to the self-perpetuation of AF and could therefore be a useful target for AF treatment.

We aimed to visualize and describe the atrial coronary anatomy and the regional distribution of atrial myocardial blood supply (CHAPTER 3). We secondly aimed to investigate the dynamic coronary flow regulation in time using Doppler flow probes around an atrial artery in healthy animals (CHAPTER 4). Here, dynamic atrial flow regulation was investigated during changes in atrial demand, such as an increase in atrial rhythm and atrial fibrillation.

In addition to the regulation of atrial flow, atrial oxygen supply is also determined by oxygen extraction. We investigated the contribution of the flow reserve vs the extraction reserve in maintaining the atrial supply-demand balance during sinus rhythm and acute changes in atrial rhythm in healthy animals (CHAPTER 5).

Finally, we aimed to address the long-term effect of AF on supply-demand balance in a model of AF in pigs (CHAPTER 6). In this model, 5 weeks of rapid atrial pacing mimics persistent AF and allows electrical, structural and contractile remodeling to take place. These forms of atrial remodeling may influence atrial demand and supply both in a negative and positive fashion. Whether or not this will disturb or maintain the total supply-demand balance is investigated here. In addition, a first analysis of vascular remodeling (perivascular fibrosis and capillary density) related to AF, an important aspect of structural remodeling in supply-demand ischemia, is described.

The final chapter of this thesis (CHAPTER 7) summarizes our findings and discusses the possible impact of these studies for future research and potential AF therapies.

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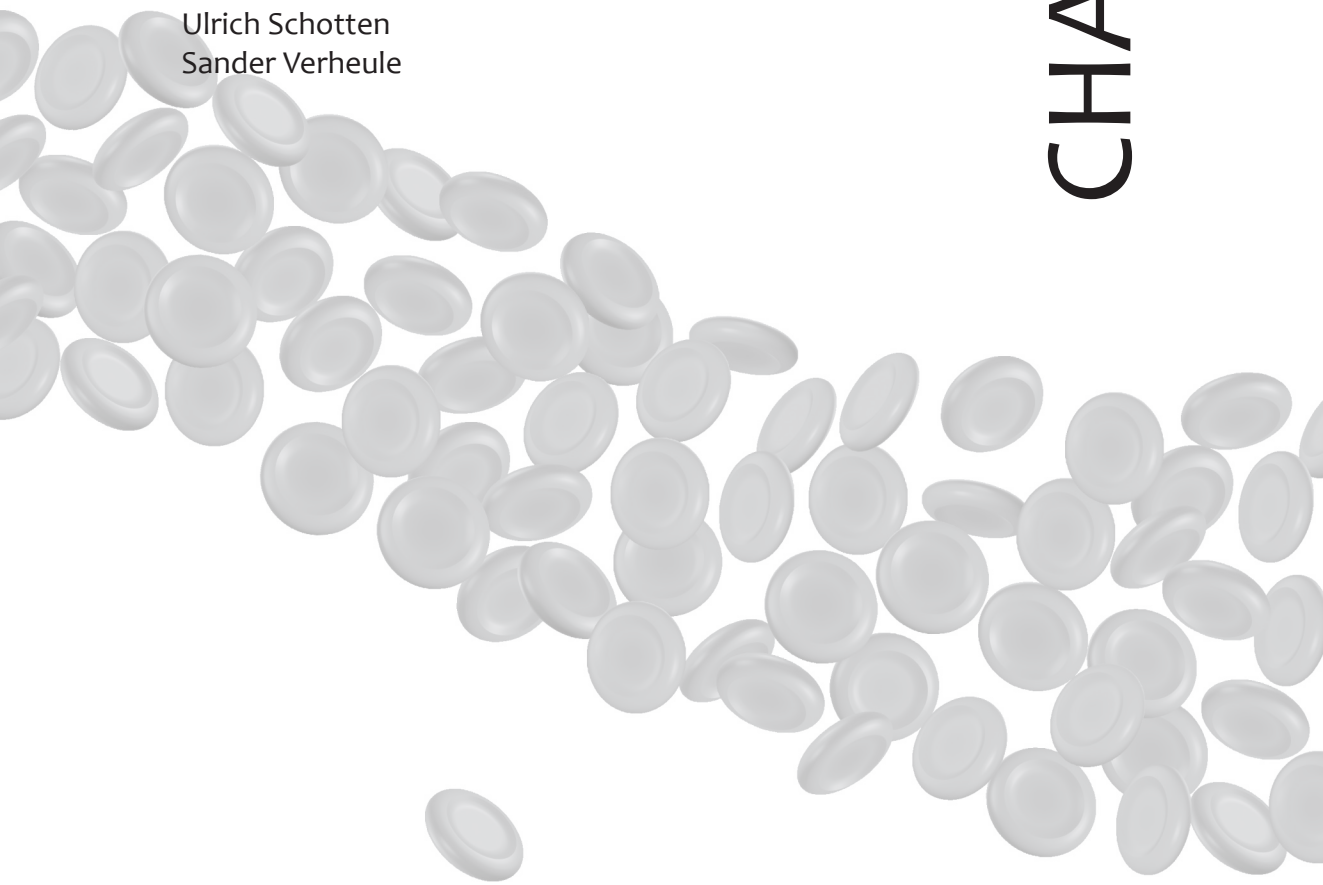
CHAPTER 2

Atrial tissue perfusion and ischemia in atrial fibrillation

Kelly van Bragt
Hussein M. Nasrallah
Ulrich Schotten
Sander Verheule



ABSTRACT

Atrial fibrillation (AF) is associated with atrial ischemia. The precise relationship, and specifically the causal relationship between ischemia and the remodeling, process during AF remains unclear. This review aims to gives an overview of the regulation of atrial blood supply and the effect of AF on atrial supply-demand balance. The atria are supplied by branches from the left and right coronary arteries, forming a very extensive capillary network. In order to maintain supply-demand balance, blood flow and oxygen extraction are regulated to match atrial oxygen demand. A reduction in atrial blood supply (e.g. myocardial infarction, coronary artery disease, vascular remodeling) or an increases in atrial demand (e.g. increased cardiac rhythm, increased blood pressure) can cause a disturbance in the balance, resulting in atrial ischemia. Many of these aspects have been under investigation, often with severe ischemia being the cause of atrial fibrillation. We propose a subtler role of supply-demand ischemia caused by AF. This supply-demand ischemia induces electrical, structural, vascular and metabolic remodeling, which might contribute to the perpetuation of AF. Further research is warranted to identify possible treatment options.

1. INTRODUCTION

Atrial fibrillation (AF) is the most common sustained arrhythmia in clinical practice. Both in animal models and in many AF patients, the duration of AF episodes gradually increases with time. Over the past decades, it has become clear that AF itself causes electrical and structural changes in the atrial myocardium that result in stabilization of AF. Electrical remodeling consists of a decrease in action potential duration (APD), AF cycle length (AFCL), effective refractory period (ERP) and a reversal in the rate-dependence of the ERP. Electrical remodeling is a fast process. It takes place in the first few days of AF and a new steady state is reached after 3-5 days [1] and is on itself is not sufficient for AF to become persistent. Structural remodeling takes weeks to months to develop and AF becomes more stable during this time. It comprises a variety of changes in cellular ultrastucture (e.g. mitochondrial swelling, glycogen accumulation, myolysis) [2, 3], connexin distribution [4] and increased extracellular matrix volume[2]. Overall, atrial myocytes dedifferentiate to a more neonatal phenotype [5, 6]. These changes, also referred to as 'hibernation' are reminiscent of changes in ventricular myocytes during chronic low-flow ischemia. Indeed, many aspects of atrial remodeling can be interpreted as adaptations to energy shortage [7]. This is also compatible with observations in AF models in goats and in AF patients, where markers of hypoxia ($HIF_{1\alpha}$) and angiogenesis (VEGF) were detected [8-12]. We propose that supply-demand ischemia is a possible stimulus for atrial remodeling during AF. It will act on the atrial cardiomyocytes until a new steady-state supply-demand balance is created. Some factors of atrial supply-demand balance, e.g. atrial myocardial blood supply, have been under investigation since the early 1900's. This review aims to give a literature overview of the atrial coronary arteries and regulation of myocardial blood flow and describe the relationship between atrial fibrillation and atrial ischemia.

2. ATRIAL CORONARY ANATOMY

The atrium, like the ventricle, is supplied by branches from the left and right coronary arteries [13-19] and has a very extensive capillary network (CHAPTER 3). The human atrial coronary anatomy was first described in the early 1900's by Keith and Flack, Gross, Crainicianu and Spalteholz [13, 14, 20, 21]. Several techniques, including the intracoronary injection of radiopaque substances or plastics, were used to visualize the

atrial anatomy post-mortem [14, 22 15, 23-27]. In the early studies, nomenclature was not conclusive. Spalteholz referred to the atrial coronary arteries as rami (ramus is Latin for "branch") atrialis [13]. They were specified as dexter or sinister, depending on the origin from the left circumflex (LCx) or the right coronary artery (RCA) respectively, followed by anterior, intermediate or posterior depending on their origin from the LCx or RCA. However, not all hearts show well developed anterior, intermediate and posterior arteries. Therefore, many others chose for specific names for the most commonly observed atrial coronary arteries. For example, Gross used names that referred to the area an artery is supplying [14].

2.1. *Number and size of the atrial coronary arteries in humans*

The RA is mainly supplied by the RCA, while the LA is mainly supplied by the left coronary artery [19, 28, 29]. The number and size of the atrial arteries, their branching points and the area they supply show considerable inter-individual variation [17]. Hromada et al reported 5 to 8 branches from both the left and right coronary artery supplying left and right atrium [17]. Busquet counted only major branches and found 2 to 6 on the right side [18]. Atrial branches are mostly 0.5-1 mm in diameter and their points of origin are found along the whole length of the coronary arteries [19, 30]. Atrial branches are often classified according to their regional origin as anterior, intermediate and posterior atrial branches [15, 16, 29]. However, not in all cases, branches will originate in all three regions of the coronary arteries [15]. Larger atrial arteries (diameter >1 mm) will originate mostly in the anterior portion of the coronary arteries and less in the intermediate and posterior parts [30].

2.2. *Most commonly encountered atrial coronary arteries*

2.2.1. The sinus node artery

Because of the large variability in atrial branches, most authors choose to describe the course of the largest and most consistently found arteries. The artery supplying the sinoatrial node (SAN) area is one of the largest and most consistently found atrial arteries in man [15]. Since its first description, it has received many different names, for instance ramus ostii cavae superioris [14], Keith-Flack artery [21], the main atrial branch [30]

or most commonly the sinus node/ sinoatrial node artery [18, 31-33]. The origin of the SAN artery is a branch of the RCA in approximately 60% of all cases. In 35% of the cases, the origin is exclusively from the left coronary artery (LCA) and in a small percentage (5%), both the right and left coronary artery supply a branch to the SAN area. The SAN artery branched off in the proximal part (first 3 cm) of the RCA or LCA in 90% of the cases [15, 18, 25, 31, 34, 35]. In cases where the SAN artery originates from the left, its origin was almost always from the LCx and in rare cases from the left main stem [36, 37]. In addition to the branches from the right or left coronary artery, also branches from the rami bronchiales (arteries that supply the bronchi) have also been reported to supply the SAN [17, 29, 38, 39].

2.2.2. Intermediate right atrial artery

Usually there is at least one large artery originating from the area of the acute margin of the right ventricle [15, 18, 40]. The artery runs over the epicardium, and then divides into 2-3 smaller branches that continue intramurally through the atrial wall [18]. It may form anastomoses with branches from the sinus node artery in the area of the superior vena cava [15, 40].

2.2.3. Left atrial circumflex

The left atrial circumflex artery (LACx) is an artery that branches off from the proximal LCx [15, 37, 40]. In a rare case, the LACx originates from the left main stem or the proximal left descending artery (LAD) [37]. The LACx runs superior and parallel to the LCx, along the margin of the atrial free wall and terminates in the posterior LA (Figure 1) [15, 40]. Occasionally, it is nearly the size of the LCx itself [40]. The LACx gives off numerous branches to the LA, thereby supplying most of the LA wall [15, 37, 40]. In 5-10 % of the cases, it also supplies the sinus node [15]. The LACx was found in 83% of the human hearts investigated by Tjandrawidjaja et al [37]. According to Gensini, on the other hand, most often the atrial branches originate directly from the LCx itself, not from a LACx [40]. An explanation for this discrepancy might be the definition of the LACx. For example, when the branch runs parallel to the LCx, but the diameter of the potential LACx is rather small and an additional branch is found from the LCx, it might be discarded as LACx.

2.3. *Anastomoses*

An anastomosis is a connection between two vessels that does not contain capillaries. They can connect arteries with other arteries, veins with veins or even arteries with veins. Coronary anastomoses can occur within the myocardial wall (intramural anastomoses). Atrial arteries or veins that connect directly to the cardiac chambers, bypassing the capillary bed, are called cardiac luminal anastomoses. They are sometimes classified as a separate group, while other authors classify them as intramural anastomoses [16,30]. In addition, there are anastomoses that can connect coronary arteries to the extracardiac circulation (extracardiac anastomoses). Bronchial arteries and internal thoracic arteries supply the posterior pericardium or enter the pericardium as vasa vasorum of aorta, vena cava and pulmonary veins to form anastomoses with coronary arteries [16, 17, 19, 29, 39, 41]. As mentioned above, arteries from the bronchial circulation are, for example, reported to supply the SAN [29, 39]. Coronary anastomoses are formed during fetal development and persist under normal, non-pathological condition. However, these preexisting coronary anastomoses will develop into larger arteries under hypoxic stimulation, for example during coronary artery disease (CAD)

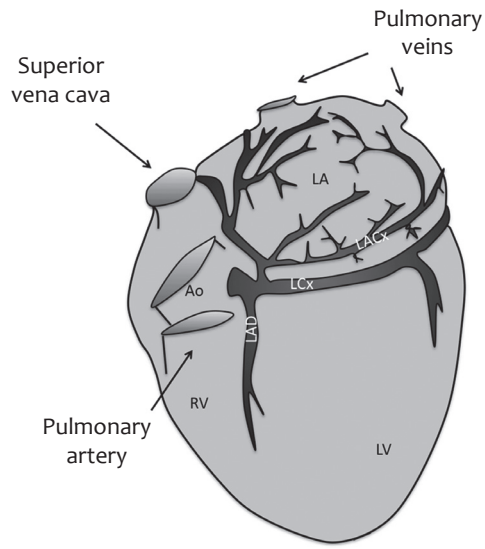


Figure 1: Representation of a left atrial circumflex artery modified from James and Burch, circulation, 1958 [15]

or after a myocardial infarction (MI) through a process called arteriogenesis and thus contribute to the collateral circulation [16, 42-44].

2.4. *Atrial coronary anatomy in animals*

Descriptions of the atrial coronary anatomy can be found for many animal species such as Angora rabbits [45], sheep [46], North American beaver [47], ostrich [48], bonnet monkey [49] or yak [50]. Of special interest are the animal species that are frequently used as models for cardiovascular disease, such as pig, dog and sheep. Often, only a very general description is given for the atrial coronary anatomy. Major anterior, intermediate and posterior branches were described, branching off from the RCA and LCx [51-54]. The right and left coronary arteries give off branches that supply the right and left atrium, respectively [47, 53, 54]. The size of the atrial arteries is reported to be somewhat smaller in pigs than in dogs [53]. In addition, dogs have an extensive collateral network [55-57]. The number of collaterals found in swine hearts is more comparable to humans [51, 52, 55, 56]. For this reason, pigs are often used as a model for chronic ischemia [55]. As in humans, the SAN in pigs, dogs and sheep is supplied by a branch from the RCA in the majority of cases [46, 51, 52, 54] and usually originates at the anterior portion of the RCA [15, 18, 25, 31, 34, 35, 46, 51]. In the atria of sheep [58] and pigs (CHAPTER 3) vessels do not seem to have overlap in perfusion area, indicating a high sensitivity for tissue damage during partial or complete blockade of an atrial vessel. In the dog it was shown, like in humans, that the area of the sinoatrial node can be supplied by more than one artery [59].

2.5. *Role of atrial coronary arteries in AF treatment*

One of the strategies to treat AF is radiofrequency catheter ablation, creating transmural atrial lesions to prevent conduction of ectopic activity and to interrupt reentry pathways. At least two studies have shown that major atrial coronary arteries run through radiofrequency ablation lines in the pulmonary vein area, the LA roof, the LA anterior wall and the mitral isthmus of all patients investigated [25, 60]. Damaging these atrial arteries could produce atrial infarctions and possibly result in an arrhythmogenic substrate. Some new ablation strategies take into consideration the course of mayor arteries in the atrial wall to prevent damage to atrial coronary arteries [25, 61, 62]. In addition to atrial infarction, radiofrequency ablation can cause endothelial

dysfunction in atrial coronary arteries in the vicinity of the radiofrequency ablation line in pigs [63]. Also in patients, microvascular dysfunction and a proinflamatory reaction were observed after radiofrequency catheter ablation and this mechanism was linked to early recurrence of AF [64, 65].

3. ATRIAL BLOOD FLOW MEASURING TECHNIQUES

Several techniques are available to measure atrial blood flow. These techniques give information of either spatial aspect or time aspects of atrial coronary flow, but not both. Microsphere analysis gives information about the myocardial distribution of blood flow. The measurement is an average over the time of the microsphere injection. Techniques like electromagnetic flow probes [66], Doppler flow probes [67, 68] and Doppler guide wires [69] have made it possible to image real time blood flow through a vessel. This allowed researchers to see variations in blood flow pattern within one heartbeat to obtain insights into the mechanical forces acting on the atrial myocardial bed. Using a guide wire for flow measurements is a minimally invasive technique that can be used in patients. To measure blood flow, the vessel should be entered. Doppler and electromagnetic flow probes allow blood velocity measurement from the outside of a vessel. These flow probes can be used acutely after preparation of a vessel, or chronically implanted to measure blood flow in awake and standing or exercising animals [67].

4. PHASIC ATRIAL FLOW PATTERNS

Coronary blood flow has a phasic pattern. This pattern is well studied in the ventricle. The atrial blood flow pattern, however, has hardly been investigated. Some atrial recordings were made in patients [69, 70], pigs [71] and dogs [68]. During the atrial systole, a sharp decrease in atrial flow velocity has been observed. Atrial contraction, rather than the increase in atrial pressure, is responsible for this phasic decrease in the atrial flow [68, 72] (CHAPTER 4). The phasic nature of the LA arterial blood flow resembles the aortic pressure pattern. The atrial flow signal starts to increase after the atrial contraction and just before the start of ventricular contraction. A small decrease was seen at the moment of aortic valve opening and closure. LA flow patterns are highly similar in shape between humans, dogs and pigs (Figure 2A, B and C). To our know-

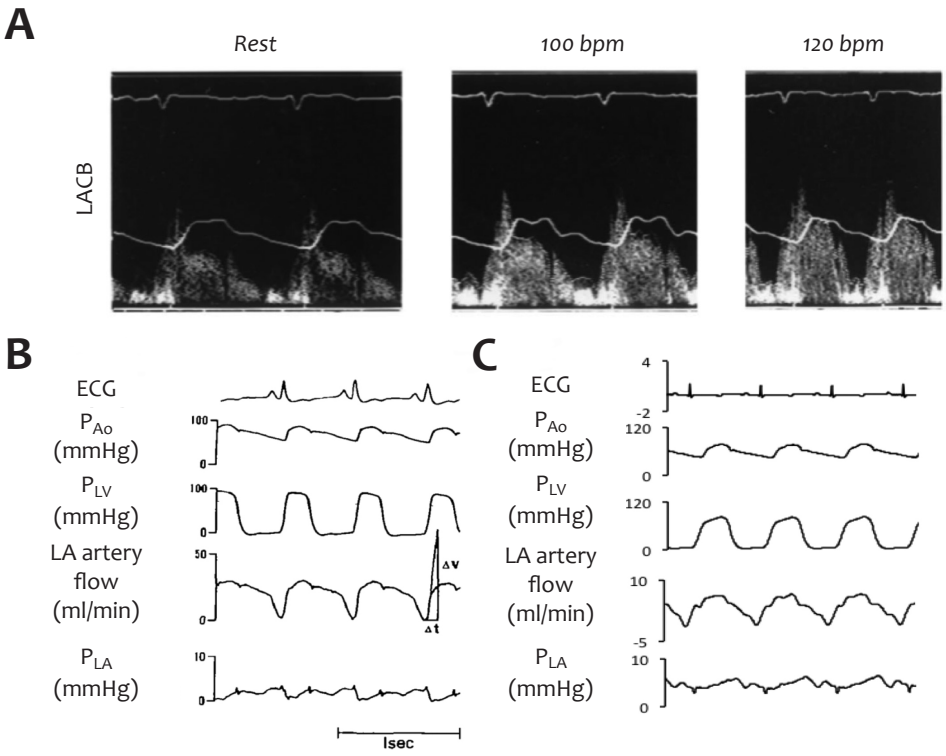


Figure 2: Representative example of the flow pattern in a left atrial branch of the circumflex artery (A) during sinus rhythm and pacing in patients under anaesthesia using a 15-MHz Doppler guide wire (From Skalidis et al, 2003 [69]), (B) during sinus rhythm in the dog heart using a laser Doppler flow probe with an optical fiber during an open chest experiment (From Kajiya et al, 1989 [68]) and (C) during sinus rhythm in the pig using a Doppler flow probe during an open chest experiment (From van Bragt et al,CVR [71]). Example of blood flow measurements in a right ventricular branch and right atrial branch (Sinoatrial node of SAN artery) in an anesthetized dog (D) (From White et al, 1986 [73]).

ledge, data on the right atrial phasic flow pattern is limited. White et al described the flow patterns of sinus node artery, branching off from the RCA [73]. Also in the RA, atrial flow is out of phase with right ventricular flow and increases after atrial contraction (Figure 2D).

5. REGIONAL DISTRIBUTION AND REGULATION OF ATRIAL MYOCARDIAL FLOW

Most knowledge of atrial coronary flow distribution and regulation has been obtained in dogs. There is also some data available from miniature pigs [74], horses [75] and baboons [76]. In the dog, the weight per atrium is about 10g and together they constitute about 10% of the total heart weight [77-80]. During sinus rhythm, the atria receive about 5% of total coronary blood flow [77, 81, 82]. Thus, the atria receive less flow than the ventricles per gram of tissue. Myocardial blood flow is higher in the LA than in the RA and in the LV than in the RV [59, 73, 75, 81-84]. Table 1 shows myocardial blood flow distributions in different studies during normal sinus rhythm in animals under anesthesia or in awake animals at rest. A large variation in baseline values can be observed. Possible explanations for these variations are baseline differences in heart rate and mean aortic pressure, differences in experimental procedure (awake vs anesthetized) and species differences. The influence of heart rate and mean aortic pressure on myocardial blood flow distribution is explained in the following section.

5.1. Flow distribution to the different chambers of the heart

Blood flow through a vessel is dependent on the pressure difference between the two ends of a vessel and on the vascular resistance. Like ventricular coronary arteries, the atrial myocardium is supplied by arteries that branch off from the aorta and the venous blood drains in the coronary sinus that drains into the RA lumen. Some of the atrial veins also drain directly into the LA and RA lumen as thebesian veins (CHAPTER 6). Therefore, aortic pressure and atrial pressure directly influence atrial myocardial perfusion. Atrial coronary artery resistance is regulated by atrial demand. The next section deals with the effect of changes in pressure and in atrial workload (atrial pacing, atrial fibrillation and exercise) on regional atrial myocardial perfusion.

	Species	Anesthetized / Awake	HR (bpm)	Mean P_{Ao} (mmHg)	Myocardial blood flow (ml/(min*100g))			
					RA	LA	RV	LV
Neill et al, 1973 [81]	Dog	Anesthetized	71±5	95±7	18±3	30±4	59±8	72±4
White et al, 1977 [60]	Dog	Anesthetized	175±6	-	83±7	-	116±12	150±10
Neill et al. 1983 [82]	Dog	Anesthetized	103±20	91±9	27±13	47±24	46±14	67±17
Smiseth et al, 1986 [83]	Dog	Anesthetized	162±10	157±11	-	77±1	-	148±10
White et al, 1986 [84]	Dog	Anesthetized	154±3	104±5	45±4	75±11	46±4	84±7
Laughlin et al, 1988 [74]	Minipig	Awake	120±16	136±4	82±20	89±26	-	-
Bauman et al, 1993 [85]	Dog	Awake	78±7	110±5	27±4	35±4	47±5	90±9
Monohar et al, 1994 [75]	Horse	Awake	42±1	118±4	19±4	26±3	44±6	74±10

Table 1: Myocardial blood flow (ml/(min*100g)) in the right (RA) and left (LA) atrium and right (RV) and left (LV) ventricle. HR= heart rate (sinus rhythm) in beats per minute, Mean P_{Ao} = mean aortic pressure in mmHg.

	Species	HR (bpm)	Mean PAo (mmHg)	Myocardial blood flow (ml/(min*100g))			
				RA app	RA body	LA app	LA body
Bauman et al, 1989 [78]	Dog	pacing 120	-	20±3	39±5	38±7	57±7
Bauman et al, 1993 [85]	Dog	78±7	110±5	15±3	33±4	30±5	37±4
Hoit et al, 1993 [87]	Dog	88±20	104±5	-	-	148±48	81±67

Table 2: Myocardial blood flow distribution in the right (RA) and left (LA) atrial appendage (app) and non-appendage (body) region. HR= heart rate (sinus rhythm) in beats per minute, Mean P_{Ao} = mean aortic pressure in mmHg.

5.1.1. Effect of changes in atrial and aortic pressure on atrial myocardial blood flow

Neill et al have described the effect of pressure manipulations on atrial flow in an open chest experiment in dogs [82]. They reported an increase in myocardial blood flow to all chambers when LA pressure increased from 8 to 12 or 20mmHg during mild and moderate mitral regurgitation. Most importantly, there was a redistribution of flow and the atria received a larger percentage of flow when LA pressure increased. Hypotension, established by controlled bleeding, resulted in a decrease in flow in all chambers. The relative distribution of total flow to the separate chambers was the same as during baseline. During atrial fibrillation, LA pressure increases [73, 85, 86]. Even when LA pressures were increased by volume loading to levels comparable to AF, LA myocardial blood flow was still higher during AF than during volume expansion [85]. This indicates that in addition to the effect of increased atrial pressure, other factors, such as the increase in atrial energy expenditure, contributes to a higher atrial myocardial blood flow.

5.1.2. Effect of changes in atrial workload on atrial myocardial blood flow

Atrial pacing increases atrial workload (CHAPTER 4). Creating AV block allows investigation of atrial flow during atrial pacing with controlled and low ventricular rate and visa versa. Atria pacing at rates above 200 bpm resulted in an increase in atrial flow with no change in ventricular flow. As expected, ventricular pacing resulted in increased ventricular blood flow with no changes in atrial flow [77, 87]. Atrial blood flow was also measured in conscious horses and dogs during rest and exercise. During exercise, the heart rate increases, with an increase in both atrial and ventricular workload. Exercise resulted in an increase of total myocardial blood flow depending on exercise intensity, as expected [75, 84]. Interestingly, the increase in RA myocardial blood flow was much larger than in the other heart chamber. With this redistribution of total coronary flow, the differences in myocardial perfusion between LA and RA seen at rest disappear during exercise [79, 84].

During AF, the overall contractility is diminished, but locally very rapid electrical and mechanical activation rates increase the energy demand within the atrial myocyte. AF increases atrial blood flow even further than atrial pacing, with no (or only a mo-

dest) effect on ventricular flow [73, 85, 87]. When a decrease in mean aortic pressure was observed during AF, the increase in atrial flow was more modest and AF was not more potent than atrial pacing in increasing atrial blood flow [86].
Results mentioned above show that atrial vascular resistance is regulated separately from ventricular coronary resistance to match local myocardial blood supply to myocardial demand under different conditions.

5.2. *Flow distribution within the atria*

Since myocardial workload is an important factor in blood flow supply, regional difference in flow distribution within the atrium were investigated in a small amount of studies (Table 2). Bauman showed that myocardial blood flow in the atrial appendage was lower than in the non-appendage region [59, 84]. In a supine and standing position, both RA and LA appendage flow was lower than in the respective atrial body. During mild (heart rate 173±10) and moderate exercise (heart rate 209±8), appendage flow exceeded myocardial flow in the non-appendage regions in both LA and RA [84]. As Bauman suggested, the appendage regions might be involved to a greater degree in atrial pump function and therefore acquiring more coronary blood flow than non-appendage regions during exercise [79, 88]. In contradiction with the other results, Hoit et al found that LA appendage flow was higher, instead of lower, than in the LA body in sinus rhythm [86]. In addition, values for LA appendage flow were higher compared to other studies with a similar heartbeat. The contradictory findings may be explained by differences in experimental set up that have caused the appendage to call on its vasodilator response in the dogs examined by Hoit. During AF, a difference between right and left atrium was noticed. The different regions of the LA responded to AF as was seen during exercise. For the RA, total myocardial blood flow increased during AF, but appendage flow never exceeded flow in the non-appendage regions [78].

6. ATRIAL ISCHEMIA AND AF

Ischemia occurs when oxygen supply is not sufficient to match oxygen demand. This may be due to a reduction in oxygen supply or an increase in demand. Atrial blood supply is dependent on atrial coronary arteries as described above. Involvement of atrial coronary arteries in chronic coronary artery disease or acute myocardial

infarction will cause atrial blood supply to decrease, resulting in a supply-demand mismatch. The complex interaction between AF and ischemia has been investigated experimentally and several mechanisms for the role of ischemia in AF and visa versa have been identified.

6.1. *Acute severe atrial ischemia is a risk factor for AF*

6.1.1. Acute myocardial infarction

Acute myocardial infarction (MI) is an important risk factor for atrial fibrillation [89-92]. AF is present in 10-20% of patients that are hospitalized with acute MI of which 7.2-11.3% is new onset AF [93-98]. In addition to AF, also atrial flutter and sinus arrhythmias were observed after MI [96, 99-101]. Most cases of new onset AF occur within the first few days after acute MI and the episodes are often transient [95, 98, 102]. However, 10% of the patients that develop new-onset AF post-MI do not convert to sinus rhythm spontaneously and they leave the hospital in AF [95]. Proposed mechanism by which acute MI causes AF are atrial ischemia and atrial MI, or right atrial overload due to right ventricular acute MI [98]. The number of occluded coronary arteries involved is significantly higher in the patients that do develop AF compared to non-AF patients after MI [95, 98, 103]. There are even a few case reports that describe an immediate cardioversion of AF to sinus rhythm after restoration of blood flow through angioplasty [104, 105]. A study by Hod et al included 214 patients of which 7 (3%) developed AF after MI. Their data indicated that all AF patients had a coexisting occlusion of 2 atrial arteries (AV node artery and proximal left circumflex artery), suggesting that acute left atrial ischemia could be the pathophysiologic mechanism of new onset AF after MI [106]. In addition, atrial infarctions are regularly observed in post mortem studies after MI (17-42%) [107-110]. Most atrial infarctions are seen in the right atrium (RA), especially the right atrial appendage, but also the left atrium (LA) or both atria can be affected [108, 111, 112]. Since the atria are very thin, atrial infarctions are usually transmural. A study by Cushing et al showed that 43% (10 out of 23) of patients with proven atrial infarctions were in AF.

6.1.2. Identifying mechanisms of ischemia-AF interaction

To investigate the mechanism by which atrial ischemia is linked to AF, acute ischemia

was experimentally induced in various animal models. Atrial ischemia resulted in an IK_{ATP}-dependent shortening of the action potential duration (APD) in isolated rabbit myocardial tissue strips [113] and in both the ischemic and non-ischemic zone of isolated sheep hearts [114]. In addition, slowing of conduction velocity in the ischemic zone [114-117] and an increase in dominant frequency [114], conduction heterogeneity index [115, 117] and AF duration [115, 116, 118] were seen both in isolated hearts and whole animal experiments. Some studies show a slight increase in ERP at 3h [115] and 5h of atrial ischemia [115, 116], but other studies report no change in ERP [118]. Also, an increase in ectopic activity was seen after occlusion of a LA branch in isolated sheep hearts [114], but this was not confirmed during acute RA branch occlusion in anaesthetized dogs [116].
The chronic effects of coronary artery occlusion and myocardial infarction were investigated. Cushing et al reported atrial extrasystole in addition to wandering pacemaker, atrial tachycardia and flutter after atrial infarction in dogs [108]. RA artery occlusion (atrial infarction, 1week) in dogs resulted in spontaneous atrial ectopic activity [119]. Underlying disturbances in calcium (Ca^{2+}) handling were recorded in isolated atrial myocytes, such as an increase in spontaneous Ca^{2+} sparks (confocal microscopy) during β-adrenergic stimulation, faster decay of caffeine-evoked Ca^{2+} transients and enhanced (by ~73%) Na^{2+} - Ca^{2+} exchange (NCX) current were found in dog with an atrial infarction compared to control animals. In addition to abnormal Ca^{2+} handling, also fibrosis was seen at the border zone of the infarcted area, resulting in conduction disturbances and reentry.

6.2. *Chronic low-flow ischemia is a risk factor for AF*

AF and coronary artery disease (CAD) often coexist. In fact, AF patients are more likely to have underlying chronic coronary artery disease than their aged-matched controls in sinus rhythm [90, 120]. The prevalence of coronary artery disease in AF patients is 6.4-19.2% [92, 121, 122], and even higher (50%) in patients who were originally diagnosed with "lone" AF [120]. Coronary artery disease, but also myocardial infarction and angina resulting from CAD, are identified as an important risk factor for AF [89, 123]. In addition, the presence of AF in patients with CAD is associated with a worse cardiovascular outcome [37, 124].

6.3. *Subtle supply-demand ischemia caused by AF*

6.3.1. Evidence for supply-demand mismatch in AF

During AF, the atrial oxygen demand is increased due to local rapid electrical and mechanical activation [85]. A decrease in cellular phosphocreatin (PCr) levels is a tell-tale sign of supply-demand ischemia [125, 126]. Indeed, in AF maintained by burst pacing in goats leads to a marked decrease (60%) in PCr. This decrease was already seen at one week of AF, and persisted during the ensuing months. However, after 4 months, PCr had returned to baseline levels. Throughout this entire time course, ATP levels remained similar to the baseline value, indicating that no severe ischemia occurred [127]. In the same animal model, Thijssen et al confirmed a role for early ischemic stress in AF, showing an increase in gene expression of hypoxia inducible factor 1α ($HIF_{1\alpha}$) only in the first week of atrial tachypacing –induced AF [8]. High levels of LA Vascular Endothelial Growth Factor (VEGF) were observed in patients with paroxysmal AF, but not in persistent AF patients. This supports a transient supply-demand mismatch during the stabilization of AF [11]. Other patient studies indicate a more chronic supply-demand mismatch in AF. Analysis of right atrial appendage biopsies of persistent AF patients revealed an increase in hypoxic and angiogenic markers compared to sinus rhythm controls [9]. An increase in right atrial appendage $HIF_{1\alpha}$ was also observed with an increasing stabilization of AF from paroxysmal to persistent [12]. Protein levels of cytoplasmic $HIF_{1\alpha}$, $HIF_{2\alpha}$, vascular endothelial growth factor (VEGF), VEGF receptors (e.g. KDR) and phosphorylated KDR (pKDR) proteins were increased [9]. Ogi et al pooled data of paroxysmal and persistent AF patients and observed that $HIF_{1\alpha}$ and VEGF were predominantly produced and co-localized in the endothelium of atrial arteries in the AF group [10].

6.3.2. Identifying mechanisms of ischemia- AF interaction

Several ion-channels were investigated to determine the interaction between AF and supply-demand ischemia. A first candidate is the ATP-regulated potassium channels (K_{ATP}), involved in atrial action potential duration and the effective refractory period. However, blocking K_{ATP}-channels did not increase the AF interval in the chronic AF goat-model [128]. This can be explained by the fact that ATP levels remained completely

normal during AF in the chronic AF goat model, while phosphocreatine was reduced in the first week of AF [127]. Another candidate ion-channel involved in atrial ischemia and AF is the sodium/proton exchanger (NHX). During a supply-demand mismatch, protons accumulate, which is known to activate the NHX [129]. Extrusion of protons from the cell leads an inward current of sodium (Na^{2+}), triggering on its turn the opposing NCX that will increase intracellular Ca^{2+} [130]. Intracellular calcium overload is thought to be the cause of the downregulation of the L-type calcium current (ICa_L). NHX blockade prevented AF-induced shortening of the ERP [131] and AF-induced contractile dysfunction after 5h of AF in the dog [132]. In a study in goats, however, AF-induced shortening of the ERP could not be prevented by NHX blockade [133]. In addition, different mechanisms seem to be involved in short-term vs. long-term atrial remodeling. In chronic AF models, NHX blockade could not prevent AF-induced ERP shortening in dogs after 7 days of AF [134] or reverse electrical remodeling in paroxysmal AF (no effect on AF duration) or persistent AF (no effect on AF cycle length) in goats [133]. It can be concluded that the mechanisms involved in acute, severe ischemia are different from the chronic, subtle supply-demand ischemia induced by AF and blocking NHX or KATP-channels is not effective.

6.3.3. Atrial remodeling as a consequence of supply-demand ischemia

6.3.3.1. Myocardial stunning

One of the first signs during acute ventricular ischemia is 'stunning', this is the fast decline of ventricular contractility [125]. Similar findings are reported in the atria after an AF episode. A rapid decline in atrial contractility was seen already after 5 min of AF in goats [135] and after 1h in the atria of pigs [136]. Contractile function can still be restored after restoration of myocardial perfusion [3, 7, 49, 125, 137, 138]. The presence of atrial stunning in AF supports the hypothesis of supply-demand ischemia during AF.

6.3.3.2. Hibernating myocardium

When ventricular low-flow ischemia becomes chronic, e.g. in CAD patients, hibernating myocardium becomes apparent [4]. The ventricular myocardium remains viable and cells dedifferentiate as part of "programmed cell-survival", a compensatory mechanism to decrease myocardial energy demand to match the decrease in blood sup-

ply. It prevents necrosis or apoptosis.
Ausma et al have indicated an important resemblance between the structural changes that occur as a result of AF and the structural changes in the ventricles as a result of chronic low-flow ischemia [2, 3, 137]. The time course and degree of structural changes were investigated in a model of tachypacing induced AF in the goat. Glycogen accumulation, loss of myofibrils, changes in mitochondrial shape, dispersion of nuclear chromatin and remodeling of the contractile apparatus into a more neonatal phenotype 5 were observed progressively in a goat model of lone AF [2, 3, 8, 137, 139], without any signs of myocardial degeneration [2] or apoptosis [138].

6.3.3.3. Vascular remodeling

Vascular remodeling is a component of atrial structural remodeling during AF that is getting more and more attention. As described earlier, hypoxic and angiogenic markers (VEGF, $HIF_{1\alpha}$) were increased in atrial tissue of AF patient [59, 11, 12, 140] and co-localized in the endothelium of atrial arteries in the AF group [10]. $HIF_{1\alpha}$ and VEGF result in atrial angiogenesis and new, stable vessels will improve atrial perfusion. Atrial microvessel size and capillary density were investigated both in experimental AF (CHAPTER 6) and AF patients[9, 141]. The capillary density was investigated in the LA posterior wall of control patients, patients with mitral regurgitation (MR) and patient with MR and AF. Patients with MR + AF had a significantly lower capillary density than MR patients without a history of AF and control patients. Similar results were recently observed in our group showing a decrease in capillary density in the left and right atrium after 5 weeks of atrial tachypacing-induced AF in pigs (CHAPTER 6). In the RA appendage, Gramley et al showed opposing results, reporting that microvessel size and density were increased in AF patients [9]. The stabilization of $HIF_{1\alpha}$/VEGF-induced vessel formation is dependent on the balance between the growth factors angiopoietine 1 (Ang-1) and 2 and (Ang-2). When the balance is in favor of Ang-1, this increased endothelial stability. Ang-2 promotes new vessel sprouting and facilitates the actions of VEGF. Plasma levels of Ang-2 are increased in AF patients shifting the balance to endothelial destabilization [140]. In that case, an increase in capillary density does not necessarily increase functional perfusion. Some functional measurements were performed in clinical AF [70]. Skalidis et al reported coronary flow reserve impairment in lone AF patients when measured during sinus rhythm. They concluded that

there is microvascular dysfunction [70].

Endothelial dysfunction is also shown by the release of inflammatory biomarkers from the endothelial tissue. Upregulation of adhesion molecules (e.g. Vascular Cell Adhesion Molecule 1 (VCAM-1), monocyte chemotactic protein-1 (MCP-1), Intercellular Adhesion Molecule 1 (ICAM)) is seen in AF patients [142, 143] and in experimental AF [144]. These adhesion molecules attract immune cells and start an immune response in the atrial tissue. The activation of the immune system was associated with post-operative AF in patients undergoing cardiac surgery [145]. In addition, they are associated with thromboembolic events or death in AF patients [143].

6.4. Conclusion

The relationship between AF and ischemia is complex. Distinction can be made between acute (e.g. MI) and chronic (e.g. CAD) ischemia. Especially the role of acute ischemia in AF is obvious and well investigated. In both cases, a sudden or partial decrease in the atrial supply is the cause of an atrial supply-demand mismatch. In addition, AF itself can cause a disturbance of the supply-demand balance. Hypoxic and angiogenic markers are increased in atrial myocardium of AF patients and animal models of AF. In AF, several types of atrial remodeling have been observed being electrical, contractile, structural[1 46], and vascular [9, 70]. They can be viewed as energy saving mechanisms in order to restore the supply-demand balance. Atrial remodeling as a result of AF-induced supply-demand ischemia could on its turn contribute to a substrate for AF. This will result in a vicious circle in which AF causes ischemia causes AF and contribute to the self-perpetuating character of AF. More insights on the regulation of the supply-demand balance could prevent ischemia and further atrial remodeling and the stabilization of AF.

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CHAPTER 3

Anatomy of the atrial vasculature in pigs

Kelly van Bragt
Pepijn van Horssen
Jeroen P. van den Wijngaard
Hussein M. Nasrallah
Piotr Podziemski
Jos A. Spaan
Ulricht Schotten
Maria Siebes
Sander Verheule

Submitted to Cardiovasc Res.

ABSTRACT

BACKGROUND. We have previously reported that acute Atrial Fibrillation (AF) leads to supply-demand ischemia and chronic AF leads to vascular remodeling. The atrial coronary vasculature has not been investigated as extensively as the ventricular circulation. Here, we have investigated porcine atrial vascular anatomy.

METHODS. The atrial vasculature was studied by ink injection (n=18) and corrosion casts (n=5). In a third set of animals, fluorescent microspheres were injected in open chest experiments to assess regional flow distribution during sinus rhythm and AF and 3-dimensional reconstructions of the vasculature were made using videomicrocryotomy (n=5)

RESULTS. Both atria possess a dense capillary network, even in the thinnest parts of the walls. Most of the right (RA) and left atrium (LA) is supplied by branches of the right coronary artery (RCA) and circumflex artery (LCx), respectively, with limited overlap in perfusion territories. In a majority of pigs, the LA free wall was supplied by an 'atrial circumflex', running parallel to the LCx. In addition, bronchial arteries contributed to perfusion of the posterior LA/ superior RA. During AF, LA flow increased by factor 2.5±0.6 (p=0.02), without significant regional differences in response. Venous drainage in the LA was conferred by a combination of veins draining into the coronary sinus and Thebesian veins draining into the LA cavity.

CONCLUSIONS. Both atria possess a dense vascular bed from the coronary circulation, with a contribution of the bronchial circulation in some regions. Assessment of the atrial vascular anatomy may be relevant for cardiac surgery and development of ablation strategies.

1. INTRODUCTION

Atrial fibrillation (AF) is the most common arrhythmia in clinical practice. We have previously shown that acute AF leads to an increase in lactate production (CHAPTER 4). In addition, markers of hypoxia and angiogenesis have been identified in experimental [1] and clinical [2-5] AF. An increase in atrial high-energy phosphates in short term AF also indicates a mismatch in supply-demand balance [6, 7]. Animal models are required to identify the exact role of ischemia in AF. The coronary arterial circulation in pig and humans show strong similarities, both with respect to anatomy and vascular regulation [8, 9]. Earlier studies provide a general introduction on atrial coronary anatomy [10], extracoronary circulation [11-13] and specifically the supply of the sinus node [14, 15] and AV node [14, 16]. Here, we give a detailed overview of atrial coronary arteries and extracoronary blood supply in pigs.

2. METHODS

All animal procedures were in accordance with national and institutional guidelines. In this study, 28 healthy Dutch Landrace pigs where used with a weight of 68±4kg.

2.1. Ink injections in the coronary circulation

The anatomy of arterial branches of the left atrium was investigated using ink injections in eleven pigs. The hearts were quickly excised after euthanizing the pigs with Euthasol (20% pentobarbital 200mg, 200mg/kg I.V.). A catheter was placed into the left coronary circumflex artery (LCx) and blue ink was injected. Branches were investigated by dissection of the LCx.

2.2. Ink injection in the extracoronary circulation

To investigate the extracardiac supply, 7 pigs were euthanized using Euthasol and the heart was excised with the closed pericardium, lungs and descending aorta attached. The aorta was closed off above the level of the coronary arteries in order to exclude them from the ink injection. The three major branches in the aortic arch (the brachiocephalic artery, the left common carotid artery and the left subclavian

artery) were closed off as well to prevent ink leakage. A catheter was placed into the descending aorta and blue ink was injected in the aorta. The only route that ink could leave the aorta was via small aortic ostia other than the coronary arteries. The most prominent of these is the ostium of the bronchial circulation, found in the inner curve of the aortic arch (Figure 5A), with an approximate diameter of 0.5-1 mm.

2.3. *Corrosion cast of the coronary circulation*

A first 3D visualization of the atrial vasculature was performed in two pigs. The hearts were quickly excised after administration of Euthasol. Cannulas were inserted in the left and right coronary ostia and fixated with purse string sutures. Vessels were maximally dilated with 100ug/l adenosine in PBS, followed by injection of a red low viscosity polymer resin (Batson's #17 anatomical corrosion kit, Polyscience Inc, Warrington, PA, USA). The heart was left overnight on ice and afterwards all tissue was dissolved in 4M KOH.

2.4. *Corrosion cast of the extracoronary circulation*

Hearts from three other pigs where excised after administration of Euthasol together with the intact pericardium and the lungs. The coronary arteries and bronchial artery were cannulated and injected with a red and blue resin, respectively (Batson's #17 anatomical corrosion kit, Polyscience Inc, Warrington, PA, USA).

2.5. *3D atrial vasculature and flow distribution*

In five Dutch landrace pigs, anesthesia was induced with Zoletil (5-8mg/kg I.M.) and Thiopenthal (5-15 mg/kg I.V.) and maintained with Midazolam (1mg/kg/h), Sufentanyl (4mg /kg/h) and Propofol (10 mg/kg/h). After intubation and the start of mechanical ventilation, pigs were instrumented with a pigtail catheter (7F Cordis injection catheter) into the left ventricle, to infuse microspheres via the blood stream into the heart tissue. These microspheres (Life Technologies Corporation, Carlsbad, CA, US, 15µm polysterene fluospheres in scarlet, green, yellow and carmine) were used to determine regional blood flow distribution in the heart. Microspheres (4ml*10^6 microspheres/ml) were gradually injected during sinus rhythm and AF. For AF induction,

an ablation catheter (RFmarinr, Medtronic, Inc., Minnesota, MA, USA) was placed in the right atrium and used for atrial pacing continuously at 20Hz, four times stimulation threshold. After instrumentation, a left lateral incision was made, the fifth rib removed, and the pericardium opened to expose the left side of the heart. After the microsphere injections, the heart was excised. The coronary arteries of the explanted heart were cannulated and vessels were maximally dilated by infusion of 100ug/L adenosine in PBS, followed by injection of resin (Batson's #17 anatomical corrosion kit, Polyscience Inc, Warrington, PA, USA) with UV Blue dye (2-5% VasQtec, Zürich, Switzerland). The heart was placed on ice overnight and afterwards the cavities were filled with carboxymethylcellulose sodium solvent mixed with 5% Indian ink and placed in the freezer (-20 degrees Celsius). Episcopic fluorescent imaging using a cryomicrotome, as described earlier by van Horssen et al. [17, 18] and van den Wijngaard et al [19], was used to gather 3D information on atrial vasculature and flow distribution. In brief, the frozen heart was mounted in a custom developed cryomicrotome system with its long axis perpendicular to the cutting plane. The specimen was sliced by a fully automated cutting mechanism at a set thickness, fixed in the range between 27-30µm from atrium to ventricular base. After each slice, a black and white reflectance image was taken of the remaining bulk material with an Alta Apogee U16 camera (Apogee Imaging Systems Inc., South Windsor CT, USA 4096x4096 pixels 16 bit greyscale) as well as images from the emission of the four different colors of microspheres and the vasculature filled with UV-blue dye. From the separate images, a high-resolution 3D representation of the entire heart and coronary vessels was generated through interpolation (Amira 3.1, Visage Imaging, Fuerth, Germany and OsiriX 5.9, Pixmeo SARL, Bernex, Switzerland). The location of each microsphere was determined in relation to the vascular structure and anatomical landmarks at high spatial accuracy. The post-processing of 3D data and images was done in Blender 2.71 (Stichting Blender Foundation, Amsterdam, NL).

2.6. *Atrial veins*

The atrial drainage of blood was investigated by injecting ink in the LCx or in the Coronary sinus (CS). The CS is a large vein that collects most of the ventricular blood and drains into the right atrium. To visualize the atrial veins that drain into the CS, retrograde ink injections in the CS ostium were performed and atrial veins were in-

vestigated by dissection. To assess the presence of Thebesian veins, that would drain directly into the atrial cavity, an inside-out atrial preparation was produced, and ink was injected into the LCx after all ventricular branches had been ligated.

3. RESULTS

3.1. Main atrial branches of the left circumflex artery

In two pigs, a corrosion cast was made to visualize the atrial coronary anatomy. Both the left and right atria possessed a dense capillary network, also in the thin epicardial layer overlaying the trabeculated parts of the wall (Figure 1A and 1B). Several branches of 0.5-1 mm were found to originate from the LCx and RCA supplying the respective atria (indicated with a red dot in Figure 1). In the 11 pigs investigated with ink injection, no atrial branches were observed originating from the left main stem or LAD to the left atrium. In total, one to five branches ranging from 0.5-1 mm in diameter originated from the LCx. Most of the branches originated from the proximal part of the LCx, dividing into smaller branches shortly after their origin from the LCx and disappearing into the wall of the left atrial appendage (LAA) supplying the epi- and endocardial tissue of the LAA. The first LA branch (>0.5 mm) was found on average at 10±2mm from the origin of the LCx. The distal part of the LCx often showed almost no atrial branches. The average distance between all the LCx branches was 14±2mm. In 9 out of 11 pigs, an atrial branch was found, originating around the level of the obtuse marginal ventricular artery (Indicated with a large red arrow in Figure 1C and 2C). This branch followed a course parallel to the AV groove, giving numerous smaller branches to the LA, and continued into the left atrial ridge towards Bachmann bundle (Figure 1E). In two pigs, this branch originated closer to the left coronary ostium, but continued along the same course as in the other nine pigs. In one of those two pigs, the branch originated immediately after the origin of the LCx and no other branches were found using the ink injection (Figure 1D).

3.2. 3D reconstruction of the atrial vasculature

Figure 2 A, B and C are aspects of the casts of LA arteries in pigs. In all casts, one or two very early branches were found to ascend towards the LA roof. In one cast, a

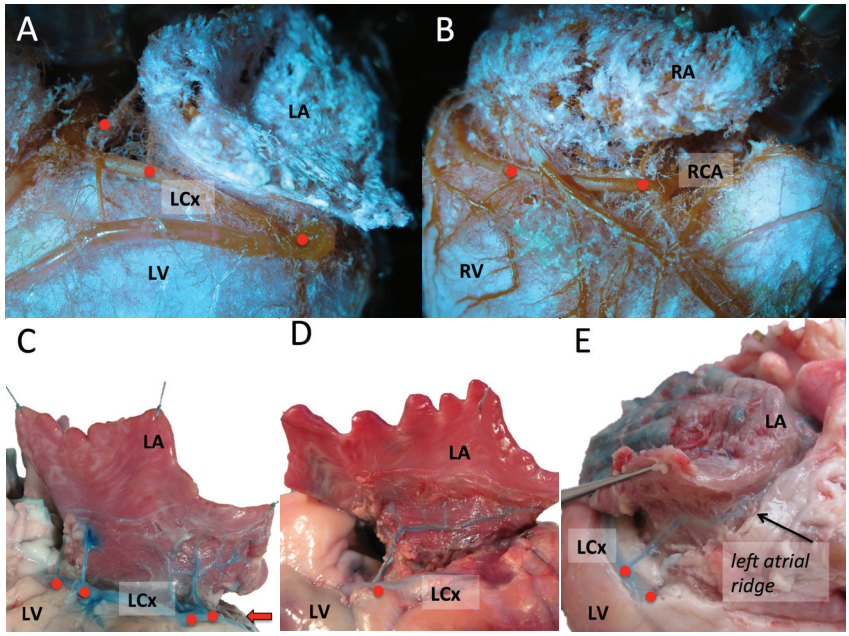

Figure 1: Atrial coronary anatomy.
Representative example of a corrosion cast of the left (A) and right (B) coronary artery and the microvascular network in the respective atria. Representative examples of the ink injections into the left circumflex artery, showing the variability of left atrial branches in number and branching point (C,D,E). Atrial branches are indicated with a red dot. The obtuse marginal ventricular artery is indicated with a large red arrow.
LA =left atrium, RA = right atrium, LV = left ventricle, RV = right ventricle, LCx = left circumflex artery, RCA = right coronary artery.

first branch to the LA originated from the left main stem (Figure 2C). In addition, there was an atrial branch running through the LA ridge in all five pigs. This branch always supplied the same atrial region, supplying the posterior LA free wall and giving off several branches to the left atrial wall along its course, but the exact point of origin was variable. The more distal this large artery originated from the LCx, the more atrial branches could be found directly from the LCx to supply the atrial appendage and anterior free wall (Figure 2C). If this artery originated earlier from the LCx, it supplied the atrial appendage and anterior free wall and few branches were observed that originated directly from the LCx (Figure 2A and B). Tracing of vascular trees

revealed that in general, the perfusion territories of individual atrial branches did not show extensive overlap (Figure 2F). However, in some left atrial regions, arteries do intermingle or form anastomoses, especially in the posterior atrial free wall, indicated with an asterisk in Figure 2A, B and C.

In the right atrium, a consistent branch was found, branching off from the proximal part of the RCA (Figure 2D and E). In four pigs, the branch was relatively large and ran towards the superior vena cava, likely supplying the sinus node. In one pig, this branch was smaller, and covered a smaller territory, approximately reaching halfway

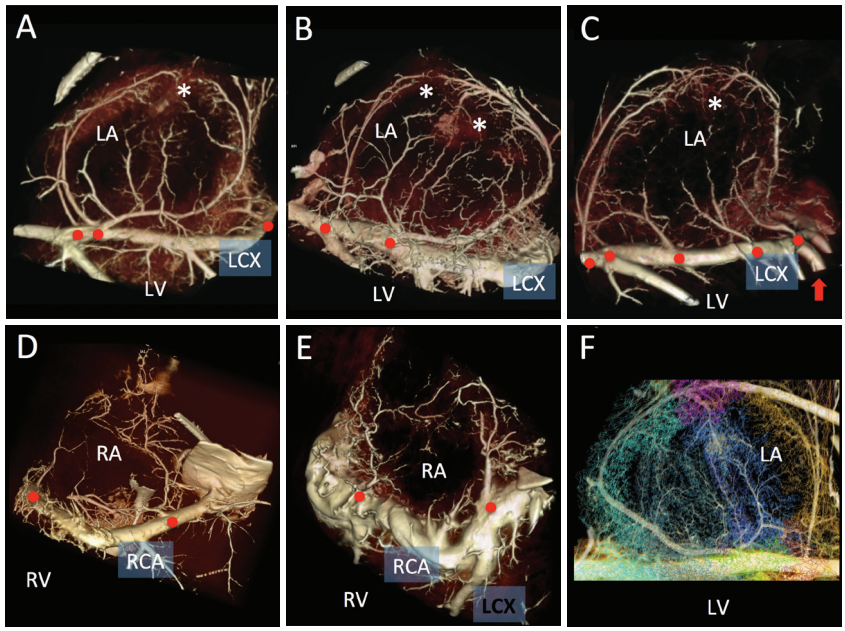

Figure 2: 3D atrial coronary anatomy.

Representative examples of a 3D digital reconstruction of the corrosion cast of the left atrium (A, B and C) and the right atrium (D and E), showing the variability of left atrial branches in number and branching point. Atrial branches are indicated with a red dot. The obtuse marginal ventricular artery is indicated with a large red arrow. The asterisk indicates an area of anastomosis on the LA roof. Color representation of the perfusion territory of the different left atrial branches (F).

LA =left atrium, RA = right atrium, LV = left ventricle, RV = right ventricle, LCx = left circumflex artery, RCA = right coronary artery.

the right atrial appendage. In this pig, a large left atrial branch from the proximal LCx was more pronounced, running towards the right atrial roof, to the area of the superior vena cava and sinus node. A second large branch was found in two pigs (Figure 2E) originating from the intermediate RCA and coursing over the RA free wall. In the other pigs, the intermediate branch was small (Figure 2D). Most of the RA branches were relatively small and inconsistent in number and branching point. Arteries originating from the RCA seemed to supply a similar area as the branches from the LCx in the posterior atria, the interatrial septum and sinus node area and might form anastomoses here (Figure 3).

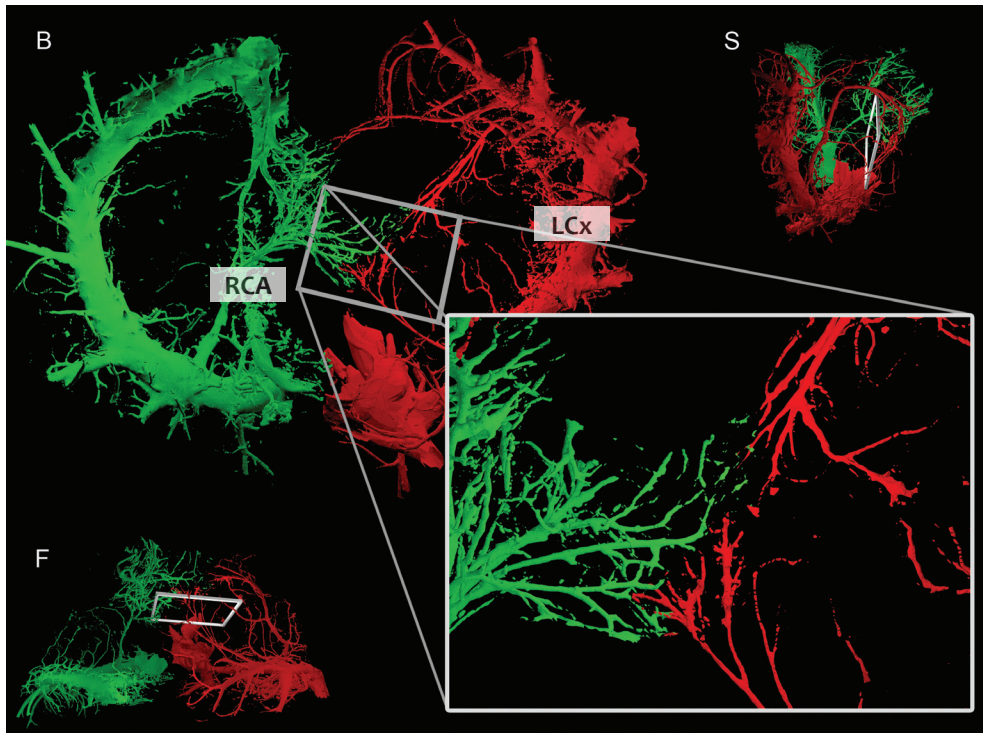

Figure 3: Atrial anastomosis.
Representative example of the anastomoses between the left (red) and right (green) atrial arteries in a bottom (B), front (F) and side (S) view and zoom (white square). LCx = left circumflex artery, RCA = right coronary artery.

3.3. *Regional atrial flow distribution*

The regional flow distribution was assessed in the five pigs used in the 3D reconstruction of the coronary anatomy, by determining the position of individual microspheres (Figure 4A). Several regions in the heart where selected and the number of microspheres per voxel was calculated. The % increase from sinus rhythm to AF is shown for the ventricle (Figure 4B) and atrium (Figure 4C). In all areas investigated, there was an increase in microsphere deposition during AF. Although all areas show a similar effect size during AF, some areas showed a large variability between animals in flow increase, and the increase in flow was not significant as a consequence. A significant

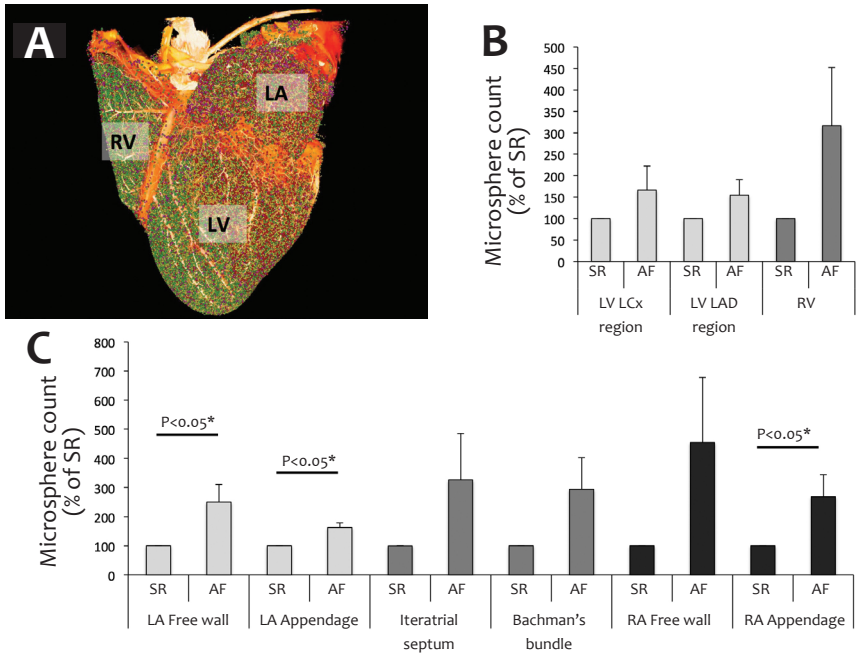

Figure 4: Atrial microsphere distribution.
(A) Representative example of microsphere visualization in the 3D digital reconstruction of the heart. Microsphere deposition expressed as % of increase compared to sinus rhythm in different regions of the ventricles (B) and the atria (C). LA =left atrium, LV = left ventricle, RV = right ventricle, SR = sinus rhythm, AF = atrial fibrillation, LCx = left circumflex artery, LAD = left descending artery. * p<0.05 is significantly different vs. sinus rhythm.

increase from SR to AF was found in the LA free wall, the LA appendage and the RA appendage ($P<0.05$, Figure 4C). In the ventricles, a non-significant trend towards an increase in flow was observed (Figure 4B).

3.4. *Extracoronary contribution to the atrial supply*

In seven pigs, the extracoronary contribution to the atrial vasculature was investigated using ink injections into the descending aorta with blockade of the coronary arteries. Non-coronary branches of the aorta, most importantly the bronchial circulation, supply the conducting airways, pericardium, esophagus, thymus, trachea and aortic wall. Also in the atria, part of the myocardial sleeve of the superior vena cava was stained in about half of the hearts (four out of seven). In addition, part of the posterior LA, the region in between the pulmonary veins was stained in four out of seven animals (Figure 5A and B). In one pig, part of the endocardium of both LA and RA free wall was stained. In a second pig, the LA free wall showed a staining of both endocardium and epicardium, but no staining of RA free wall. In two pigs, an artery was found containing ink and running over the Bachmann's bundle in the epicardium of the RA appendage to form an anastomosis with the proximal RCA (indicated with a black arrow in Figure 5C).

After identifying the bronchial artery as a supplying vessel for the atrial myocardium, its ostium in the inner aortic arch was cannulated and a corrosion cast was made. The combined corrosion casts of the coronary (red) and bronchial (blue) circulations showed that these circulations are closely apposed at the posterior LA (Figure 5D). Removal of the main parts of the lungs revealed a network of small vessels supplied by the bronchial artery covering the posterior left atrium (Figure 5E). Also, anastomoses with the proximal and intermediate RCA and posterior LCx were found.

3.5. *Atrial veins*

Retrograde ink injections into the CS showed that the left atrium can have several veins (indicated by blue arrows in Figure 6A and B) that drain into the CS and blood is subsequently drained into the right atrial cavity. Another route of left atrial venous drainage is via Thebesian veins. Thebesian veins in the left atrium were visualized by

ink injections into the LCx, illustrating that ink leaked from several small orifices directly into the left atrial cavity (indicated by yellow arrows in Figure 6C).

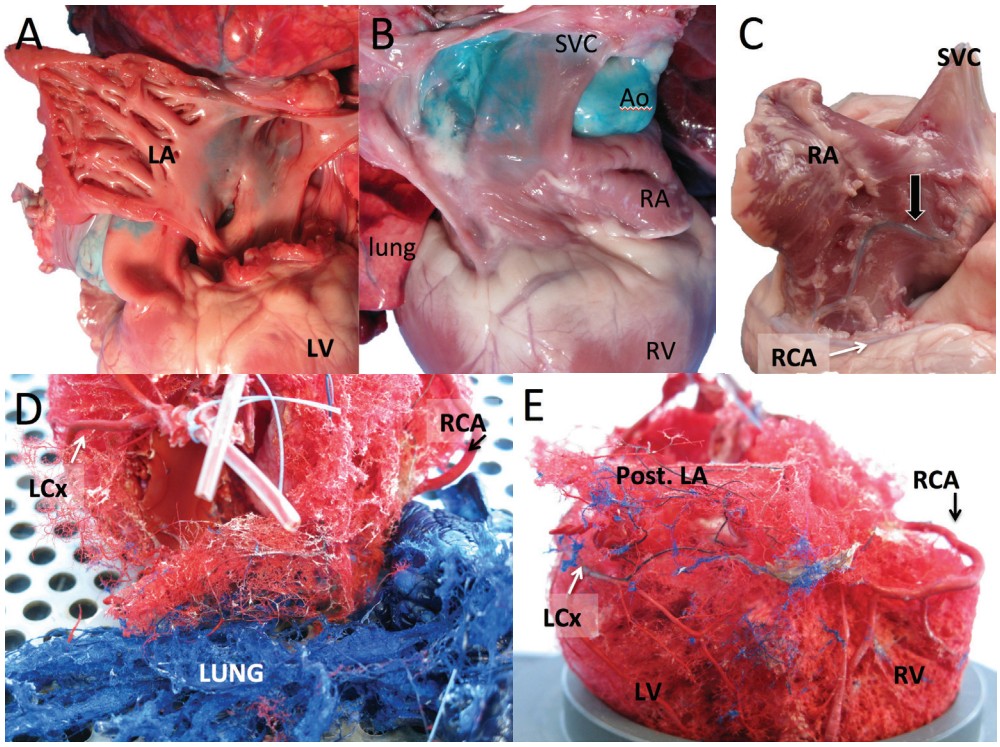

Figure 5: Extracoronary circulation to the atrium.
Representative example of an ink injection in the descending aorta. (A) Representative picture of the left atrium and pulmonary veins, (B) representative picture of the right atrium, superior vena cava and aorta, and (C) picture of the anastomoses between extracoronary circulation and right coronary artery.
Representative example of combined corrosion cast of the coronary (red) and bronchial (blue) circulation with (C) and without (D) the lungs attached. LV = left ventricle, RV = right ventricle, LCx = left circumflex artery, RCA = right coronary artery, SVC = superior vena cava, Ao = aorta

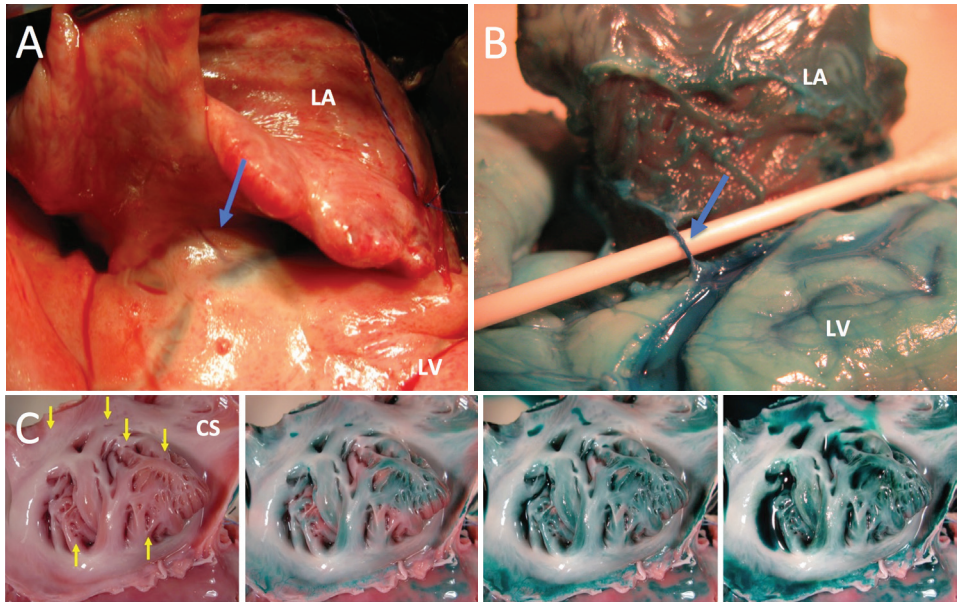

Figure 6: Atrial veins.
(A) LA drainage into the CS in situ, left lateral thoracotomy, (B) explanted heart, after ink injection into CS and dissection, (C) thebesian veins.
Veins that drain into th CS are indicated with a blue arrow, thebesian veins are indicated with a yellow arrow. LA = left atrium, LV = left ventricle, CS = coronary sinus.

4. DISCUSSION

4.1. Comparison of atrial vascular anatomy in pigs and humans

In general, the left atrium is supplied by branches form the LCx. We show that in 16 out of 18 pigs, an artery can be found that follows (completely or partially) a course parallel to the LCx, and ascends through the LA ridge. James et al have also described an artery with a highly similar course in humans as one of two atrial arteries in the atria being less variable than others [20]. While Spalteholz described an origin at the intermediate LCx [21], James and Burch found an origin for this artery often splitting off from the LCx early after the bifurcation of the left main [20]. This phenomenon was de-

scribed earlier as a "left atrial circumflex"(LACx), a branch originating from the first few mm of the LCx or in a rare case from the left main stem or even proximal LAD. James and Gensini have suggested that a LACx is present in most of the hearts, without describing the incidence [20, 22]. Tjandrawidjaja found a LACx in 83% of patients (N=454). Patients with a compromised perfusion of the LACx had a higher incidence of early atrial arrhythmia's, which supports the role of ischemia in the development of atrial arrhythmias [23]. In our study, we observed that the artery running through the left atrial ridge could originate from the whole length of the LCx; proximal, intermediate or more distal. In our study, two out of 11 pigs had a single LA artery splitting off directly at the origin of the LCx, running parallel to the LCx and giving off smaller branches to the LA supplying the parts of the LA free wall, atrial septum and posterior LA wall. According to the original definition of James, these arteries could be considered as a true LACx. In the other pigs, the origin was intermediate, but the artery followed a similar course along the LCx and up through the LA ridge and could also be considered a LACx. In one pig, the origin was very distal and the artery went straight up through the LA ridge. When it originated more proximal, it supplied more branches to the LA wall and less branches were found that originate from the LCx. The more distal its origin, the more branches from the LCx originated from the proximal part of the LCx.

In man, the large artery originating at the LCx and running through the LA groove supplies the sinus node in approx. 10% of the patients investigated and forms important inter- and intracoronary anastomoses [24, 25]. We also observed in at least four out of five 3D reconstructed hearts that this artery intermingled with proximal and distal branches of the LCx and with branches from the RCA in the atrial roof. In addition, we showed that arteries within the LA wall did not show extensive overlap in perfusion territories. Similar results were found in the left atrium of sheep, where several vessels were injected with ink or ethanol. The ischemic area (after ethanol injection) and ink-stained areas where clearly delineated without significant overlap in perfusion territories [26, 27].

In humans, a large artery is usually present that branches off from the RCA and traverses the RA free wall while dividing into two or three branches [20, 21]. This intermediate right atrial artery was not consistently seen in the five pigs in this study. In two pigs, a large intermediate artery was present. In the other pigs, a small branch split

from the intermediate RCA. Sahni et al has also reported that the arteries branching from the RCA are rather small in pigs [15].

4.2. *Atrial ischemia in ablation strategies and myocardial infarction*

Several authors have proposed that knowledge of the atrial coronary anatomy may be an important determinant for the success rate of atrial ablation strategies [24, 25, 28, 29]. Specifically, the large artery over the LA roof through the left atrial ridge observed in both pigs and humans was implicated in these considerations. In addition to this artery, this LA ridge also contains the remnant of the vein of Marshall and abundant autonomic nerve bundles [29]. This artery is very close to ablation lines as a treatment for atrial fibrillation. Major arteries were also found in the mitral isthmus and LA anterior wall [24, 28]. The presence of such large vessels can act as a source of convective heat loss to the blood flow. This could create difficulties in obtaining a transmural lesion and thus electrical isolation of the pulmonary veins. On the other hand, radiofrequency ablation may cause vascular damage, potentially causing coronary artery occlusion and atrial infarction, affecting a much larger atrial region than the direct target of the ablation [30-33]. Meissner et al reported a transient reduction in functional atrial flow reserve in approximately 20% of patients after radiofrequency ablation [34]. The correlation between coronary artery occlusion and AF can also be observed in myocardial infarctions (MI). In MI patients, 7-11% suffer from new-onset AF [35-38] and post-mortem studies show an atrial infarction in 17- 42% of MI cases [39-42]. The anatomical variability in atrial vascular anatomy could explain the variability in ablation outcome. Therefore, an anatomical examination of large atrial arteries (e.g. by angiography) could help in designing a tailor-made ablation strategy in AF patients.

4.3. *Extracoronary supply to the left atrium*

In man, the bronchial circulation is described as a source of extracoronary blood supply to the atria. Bronchial arteries supply the posterior atrial pericardium or enter the pericardium as vasa vasorum of aorta, vena cava and pulmonary veins to form anastomoses with coronary arteries [43-50]. In the pig, the bronchial artery origin and course are similar to humans [12]. In our study in pigs, we show extracoronary supply from the bronchial circulation. Both ink and low viscosity polymer resin injections in

the ostium of the bronchial artery showed a clear supply of vena cava, aorta and pulmonary veins and into the posterior walls of both left and right atrium. In addition, anastomoses where found with the intermediate RCA and posterior LCx. Bronchial anastomoses with coronary arteries in pigs were demonstrated earlier [11-13]. It is not known how large the contribution is under normal conditions or whether this contribution changes during pathological conditions. Gade et al reported communications between the bronchial circulation and both coronary arteries in all cases [12]. Our study and the study of Kotoulas et al [13] report anastomosis in the majority, but not in all pigs, and not always with both coronary arteries. The resin used for the corrosion cast was injected at high pressure. Although we show that overlap and anastomoses between the coronary and bronchial circulation exist, we are unable to determine the functional contribution of the extracoronary supply to atrial perfusion. In fact, the contribution of these anastomoses may be small under normal physiological conditions [11]. The importance of the extracoronary circulation may increase during ischemic conditions. White et al. have described that the extracoronary collateral formation (bronchial and internal mammary artery) with coronary arteries during ischemia in pigs can supply up to 30% of resting myocardial blood flow [11]. It was proposed in studies on cadavers and during cold cardioplegic cardiac arrest, that patients with severe coronary artery disease have the highest likelihood of extracoronary blood supply [51, 52]. The presence of this extracoronary blood flow may cause several problems during open-heart surgery such as the re-excitation of the arrested heart, inhomogeneous cardioplegic delivery, myocardial rewarming, and blood in the operative field [53]. Experiments in dogs, however, suggest that the myocardial perfusion during global ischemia mostly happens through systemic-pulmonary channels and that the contribution of extracoronary myocardial perfusion is low [53, 54].

5. CONCLUSION

Atrial anatomy in pigs is very similar to human atrial anatomy. Recognizing the differences and similarities between pigs and humans is important when the pig is used as a model for atrial flow regulation and atrial ischemia. We have used several techniques, including a 3D reconstruction of heart and vessels, to investigate atrial anatomy and perfusion territories. Even the thinnest parts of the atrial myocardium possess a dense capillary network. The left and right atria are generally supplied by branches

of the LCx and RCA, respectively. In the left atrium, one large artery was consistently found that courses along the LCx and upwards through the left atrial ridge supplying most of the left atrium. It also formed inter- and intracoronary anastomoses. In all but two pigs, additional posterior, intermediate and distal branches were found supplying the LA. In the right atrium, a proximal and intermediate branch were always found, the other branches were relatively small and variable. The bronchial circulation supplies part of the right and left atrial wall, the posterior right and left atrium and forms anastomoses with both coronary arteries.

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CHAPTER 4

Atrial supply-demand balance in healthy adult pigs: Coronary blood flow, oxygen extraction and lactate production during acute atrial fibrillation

Kelly van Bragt
Hussein M. Nasrallah
Marion Kuiper
Joost J. Luiken
Ulrich Schotten
Sander Verheule

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ABSTRACT

AIMS: Little is known about how atrial oxygen supply responds to increased demand, and under which conditions it falls short (supply-demand mismatch). Here, we have investigated the vasodilator response, oxygen extraction and lactate production of the left atrium (LA) and left ventricle (LV) in response to atrial pacing and atrial fibrillation (AF).

METHODS AND RESULTS: Series A (n= 9 Dutch landrace pigs) was instrumented to measure LA and LV vascular conductance in branches of the
circumflex artery. Coronary conductance reserve (CCR) was calculated as the ratio between conductance during adenosine infusion and baseline. Series B (n= 7) was instrumented with sampling catheters in LA and LV veins for determination of blood gases and lactate levels. LA CCR (1.76±0.14) was significantly lower than LV CCR (3.16±0.27, P= 0.002). However, basal oxygen extraction was lower in LA (27±3%) than that in the LV (58±6%, P= 0.0006), indicating a larger extraction reserve in the LA than that in the LV (4.68±0.84 vs. 1.88±0.26, P= 0.01). Atrial pacing caused an increase in LA conductance (Series A) and oxygen extraction (Series B). AF increased LA vascular conductance to 177±14% at 1 min, 168±14 at 5 min, and 164±31% at 10 min of AF (P <0.05 vs. baseline). Atrial oxygen extraction also increased from 26± 3% at baseline to 63±5% (P <0.01) at 5 min and 60±11% (P <0.01) at 10 min of AF. Arterio-venous lactate difference increased significantly (P= 0.02) during AF.

CONCLUSIONS: In healthy pigs, the LA has a lower CCR, but a higher extraction reserve compared to the LV. Although both reserves were recruited during AF, atrial lactate production increased significantly.

1. INTRODUCTION

Atrial fibrillation (AF) is a common arrhythmia characterized by a fast and irregular activation of the atria. During acute AF, the fast rates of excitation and contraction are likely to increase atrial energy and oxygen demand. In sinus rhythm, the atria receive 5% of coronary blood flow, and AF increases atrial coronary blood flow [1-8]. However, it is not known whether this increase in atrial supply is sufficient to meet increased atrial demand. If this supply would be insufficient to meet demand, atrial supply-demand ischemia would ensue. Indirect evidence for supply-demand ischemia are decreased atrial phosphocreatine levels and transiently increased expression of $HIF_{1\alpha}$ in the first weeks of AF in a goat model [9, 10]. In addition, the atrial structural changes after months of AF are very similar to hibernating ventricular myocardium due to low-flow ischemia [11].

Here, we have studied the supply-demand balance by investigating the regulation of oxygen supply (coronary flow, oxygen extraction) and lactate production during short periods of increased atrial demand (atrial pacing and AF) in normal healthy adult pigs. Recruitment of coronary conductance reserve and extraction reserve during these interventions are the main determinants of oxygen supply due to increased demand. We report that the LA has a lower CCR than the LV, but a higher extraction reserve. Conductance and extraction ratio are both increased during short-term atrial pacing and AF to meet the increase in LA oxygen consumption. Vascular conductance and oxygen extraction ratio increased less in the LV than in the LA. There is an increase in LA arterio-venous lactate difference during short term AF, despite the recruitment of both the conductance and extraction reserve, indicating supply-demand ischemia during acute AF. This mismatch could form an important trigger for the slower processes of electrical and structural remodeling that have been observed in animal models of AF and in AF patients.

2. METHODS

2.1. Animal model

Healthy Dutch Landrace pigs weighing 62±2 kg were used. All animal procedures were in accordance with national and institutional guidelines and approved by the lo-

cal ethical committee. For open chest sacrifice experiments, anesthesia was induced with Zoletil (5-8 mg/kg I.M.) and Thiopenthal (5-15 mg/kg I.V.) and maintained with Midazolam (1.0 mg/kg/h I.V.), Sufentanyl (4 mg/kg/h I.V.) and Propofol (10 mg/kg/h I.V.). After intubation and the start of mechanical ventilation, a left lateral incision was made, the 5th rib removed and the pericardium opened to expose the left side of the heart for further instrumentation.

2.2. *Series A: Coronary flow*

In a first group of animals (n=9), we determined LA and LV coronary conductance reserve (CCR) and the recruitment of this reserve during an increase in atrial pacing and acute AF. Doppler flow probes were placed around a LA and LV branch of the left circumflex artery (LCx), as depicted schematically in Figure 1A. An example of the atrial branches of the LCx is given in Figure 1B and 1C. To calculate vascular conductance, pressures in the right atrium (P_{RA}) and aorta (P_{Ao}) were measured using a Millar microtip pressure sensor (Millar Instruments, Houston, TX) and a Sentron pressure catheter (Sentron Europe BV, Roden, The Netherlands) respectively. Maximal conductance was measured by infusion of adenosine via a JR6 catheter (Cordis Corporation,Bridgewater, NJ) in the left main coronary artery. A 10ml bolus with an adenosine concentration of 300µg/ml was given to induce maximal dilatation. Adenosine was administered in increasing concentrations to make sure that the maximal effect was reached. Coronary conductance was calculated as flow divided by the pressure difference across the vascular bed (i.e. between P_{Ao} and P_{RA}):

$$C_{LA} = Q_{LA} / (P_{Ao} - P_{RA})$$
$$C_{LV} = Q_{LV} / (P_{Ao} - P_{RA})$$

Maximal conductance (C_{MAX}) was calculated as conductance during intracoronary adenosine infusion ($C_{Adenosine}$) minus the effect of the injection itself ($\Delta Saline = C_{Saline} - C_{Baseline}$). In addition, the corrected coronary conductance reserve (CCR) was calculated as C_{MAX} divided by $C_{Baseline}$ (Figure 2B).

$$C_{MAX} = C_{Adenosine} - \Delta Saline$$
$$CCR = C_{MAX} / C_{Baseline}$$

2.3. *Series B: Oxygen extraction and lactate levels*

A second set of experiments (n=7) was performed to determine extraction reserve and lactate production. Instrumentation is shown schematically in Figure 1D and 1E. Arterial blood was sampled from the LV through a dual Ao/LV Millar pressure catheter (Millar Instruments, Houston, TX). LV venous blood was sampled through an I.V. cannula (Braunule, Braun, Melsungen, Germany) that was introduced into a LV vein in the perfusion territory of the LCx. To sample venous blood from the LA, the coronary sinus (CS) was closed off around an atrial vein, creating a small 'sampling pocket' in the CS. A venous sampling catheter (B Braun, Melsungen, Germany) was introduced in the CS pocket. Any ventricular veins ending in the CS pocket were closed off to ensure that the pocket only contained atrial venous blood. The atrial venous blood collected in this CS pocket drains the part of the LA appendage and free wall similar to the area supplied by the atrial arteries measured in series A. An example of a left atrial vein draining into the CS is shown in Figure 1F. To avoid venous congestion in the ventricles, the great cardiac vein was bypassed to a sheet in the jugular vein. Blood gas analysis was performed on these samples on an i-Stat analyzer (Abbott Laboratories, Illinois, U.S.A). Values were obtained for pH, partial arterial and venous oxygen pressure (PO_2), partial carbon dioxide pressure (PCO_2), hemoglobin (Hb) concentration and arterial and venous oxygen saturation (SO_2). Blood lactate concentration was measured in the same blood samples using a Lactate SCOUT+ (Senslab GmbH, Leipzig, Germany). Arterial and venous total oxygen content (CaO_2 and CvO_2) were calculated using the following formula:

Total oxygen content (ml O_2 /100ml) = oxygen bound to Hb
+ oxygen dissolved in plasma
= (1.36 (ml O_2/g Hb) x SO_2 x Hb (g/100ml))
+ (0.0031 ml O_2/100ml/mmHg x PO_2 (mmHg))

Oxygen extraction was calculated as the difference between arterial and LA or LV venous oxygen saturation:

$$\text{Oxygen extraction (\%)} = (CaO_2 - CvO_2)/CaO_2 * 100$$

The extraction reserve was determined from the arterial oxygen saturation divided by the baseline LA and LV oxygen extraction:

$$\text{Extraction reserve} = CaO_2/(CaO_2\text{-}CvO_2)$$

2.4. Interventions

In both series A and B, measurements were performed during normal sinus rhythm (NSR), right atrial pacing at a basic cycle lengths (BCL) of 500, 450 and 400 ms and during acute AF maintained by burst pacing (10 min). An ablation catheter (RFmarinr, Medtronic Inc, Minnesota, MA, USA) was placed in the RA for pacing and AF induction.

2.5. Statistics

The effect of different pacing cycle lengths on conductance and blood gas parameters was investigated at different time points using a two-way ANOVA, with a Bonferroni post-hoc test to compare the mean during pacing with the mean before pacing. For the parameters during AF, a one-way ANOVA was performed for 3 different time points. A post-hoc test (Dunnett's multiple analysis comparison) was performed to compare the values at each time point to baseline values. All measurements during the interventions were compared to a baseline value obtained just before the intervention. To determine differences between basal atrial and ventricular parameters, a paired Student's t-test was performed. To determine the difference between LA and LV during the interventions, a two-way ANOVA was used. P-values smaller than 0.05 were considered significant.

3. RESULTS

3.1. Series A: Coronary vascular conductance

Corrosion casts of the coronary vasculature show a dense capillary bed in the atrial wall (Figure 1B). In the LA, this network is supplied by branches of the LCx. In series A, flow in LA and LV branches of the LCx artery was measured using Doppler flow

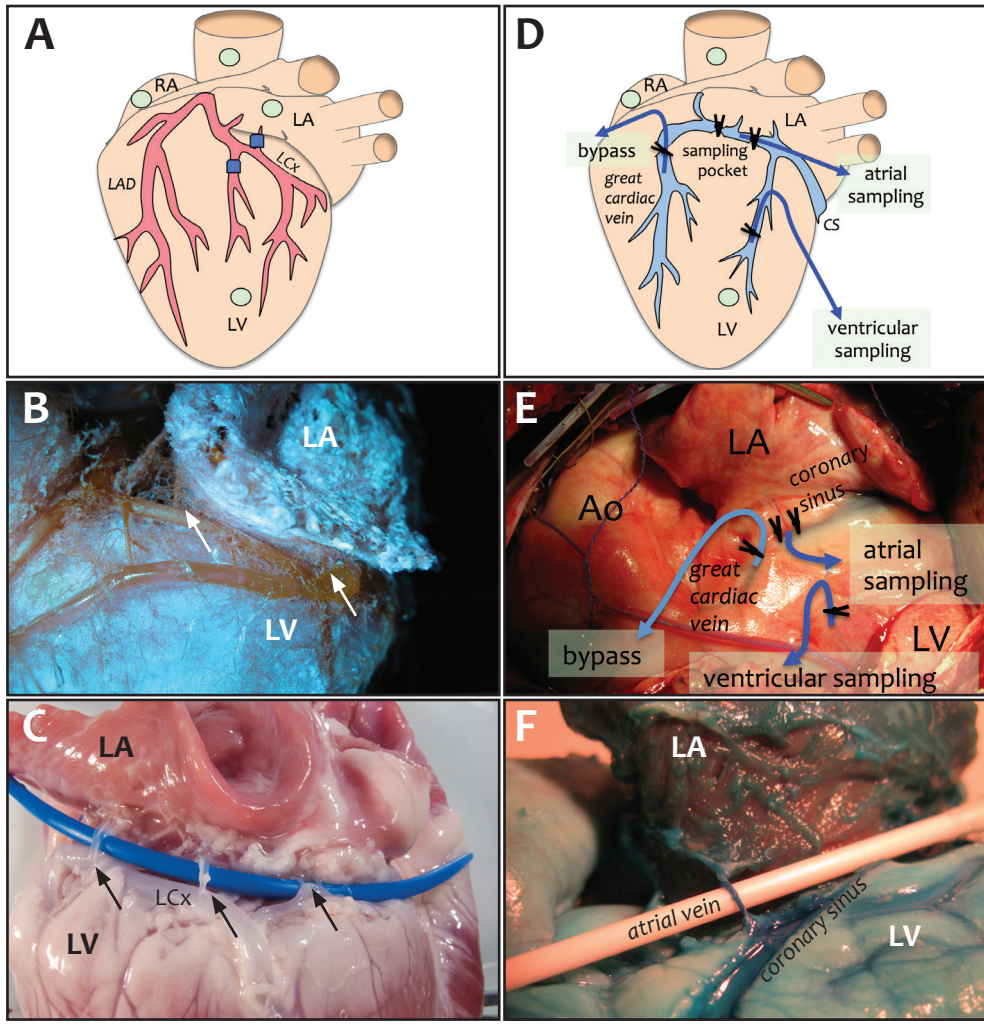

Figure 1: Vascular anatomy and instrumentation. LA=left atrium, RA= right atrium, LV = left ventricle, LCx= left circumflex artery, LAD= left anterior descending artery, CS=Coronary sinus A) Schematic representation of the instrumentation during experiment series A. Arteries in red, dark blue squares indicate Doppler flow probes, green circles indicate pressure catheters. B) Corrosion cast of coronary vasculature, using Batson's #17 resin (Polysciences Inc, Warrington PA) injected into the aorta of explanted heart. Atrial arteries indicated with arrows. C) Dissected preparation of LA arteries (arrows). D) Schematic representation of the instrumentation during experiment series B. Veins in blue. E) In situ example of instrumentation during experiment series B. F) Dissected preparation of a LA vein draining into the CS.

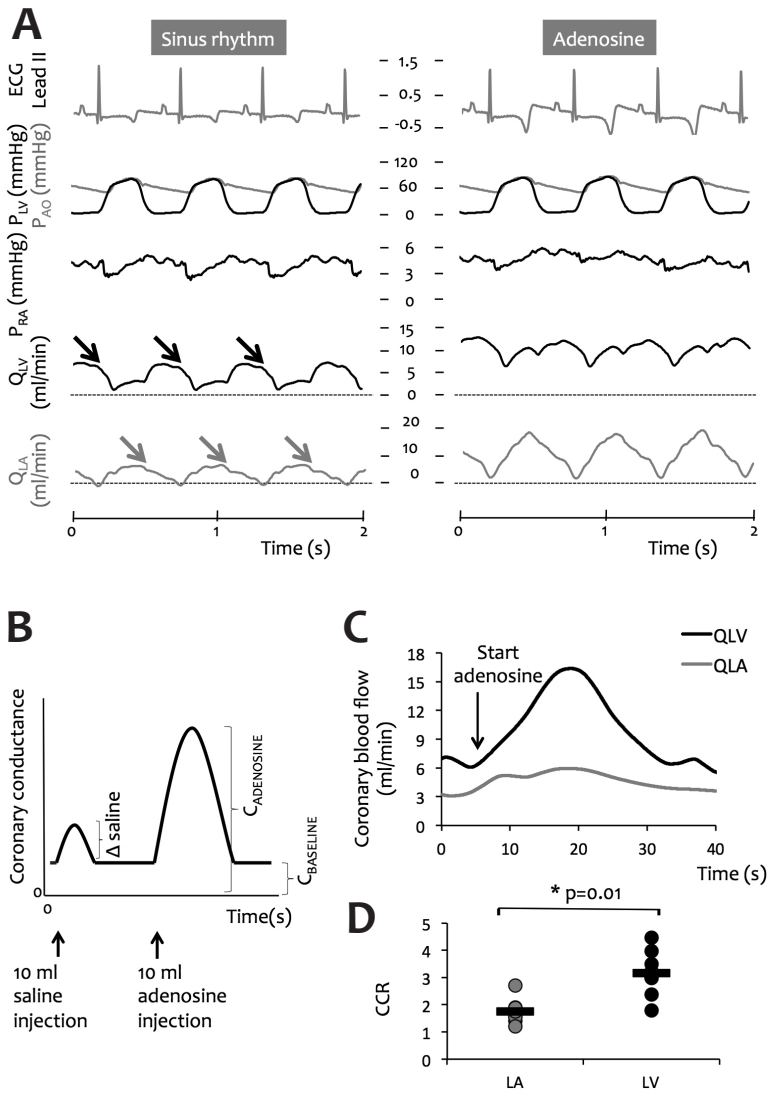

Figure 2: Determination of flow reserve. A) Left ventricular (P_{LV}) and aortic (P_{Ao}), left atrial (P_{LA}) and right atrial (P_{RA}) pressure, left ventricular (Q_{LV}) and left atrial (Q_{LA}) Doppler flow during NSR and adenosine infusion. Arrows indicate the effect of LV (black) and LA (grey) systole on the phasic blood flow pattern. B) For the determination of the coronary conductance reserve (CCR), maximal flow and conductance are corrected for the effect of injection itself (Δsaline). C) Average Doppler flow in a LA and LV branch of the circumflex artery during adenosine infusion. The phasic pattern of the cardiac cycle was eliminated using a 10-level wavelet filter, thereby deriving average flow. D) Left atrial and ventricular CCR for all animals in series A (n=9).

probes. Flow during normal sinus rhythm (NSR) averaged 3.62±0.86 ml/min for LA and 6.31±0.50 ml/min for LV branches, corresponding to a vascular conductance of 0.05±0.01 ml/min/mmHg and 0.09±0.01 ml/min/mmHg, respectively.

The atrial flow pattern showed a consistent decrease during the atrial contraction (grey arrows in Figure 2A), similar to the decrease in LV flow during the ventricular contraction (black arrows in Figure 2A). In most cases, flow in LA arteries reversed (i.e. became negative) during atrial contraction. Because of the asynchrony between atrial and ventricular contraction, the atrial and ventricular flow signals were out of phase.

Intracoronary injection of a bolus of adenosine led to a transient, dose-dependent increase in coronary flow (Figure 2B and 2C). The maximum flow during adenosine infusion, corrected for the small increase observed during infusion of saline, was used to determine the maximal vascular conductance (C_{MAX}) and coronary conductance reserve (CCR). The calculated CCR (Figure 2D) was significantly lower in the LA (1.76±0.14) than in the LV (3.16±0.27, p=0.002).

Coronary flow was also measured during right atrial pacing at different pacing rates. An example of LA and LV flow signals during pacing is shown in Figure 3A (right panel). An increase in rate causes a change in the phasic pattern of LA and LV flow, and an increase in average LA and LV flow. During atrial pacing, LA and LV coronary flow (Figure 3B) reached a steady state within 30 s and the response in coronary conductance was calculated at 1 min after the start of pacing. In the LA, a clear frequency-dependent increase in conductance was seen (Figure 3C). Both at 450 ms (135±9%, p<0.001) and at 400 ms (143±9%, p<0.001), LA vascular conductance was significantly increased compared to baseline. Pacing significantly increased LV conductance at all rates (Figure 3C). The increase in LA vascular conductance was significantly larger than the increase in LV conductance at 450 ms and 400 ms (p<0.05 LA vs LV).

Calculated as % of C_{MAX} (Figure 3C), there was an overall effect of right atrial pacing on LA (p=0.007) and LV (p=0.03) conductance. LA conductance reached approximately 55% of maximal conductance at baseline, which was significantly higher that the ventricle (approx. 33% of C_{MAX}, p<0.01). In addition, LA conductance was significantly higher during all BCLs as well. Thus, the LA used significantly more of the conductance reserve at every BCL than the LV (p<0.001).

Flow during AF was measured for 10 min (Figure 4A). AF resulted in a significant decrease in P_{Ao} after 5 min of AF (Figure 4B, left panel). The effect of AF on aortic and

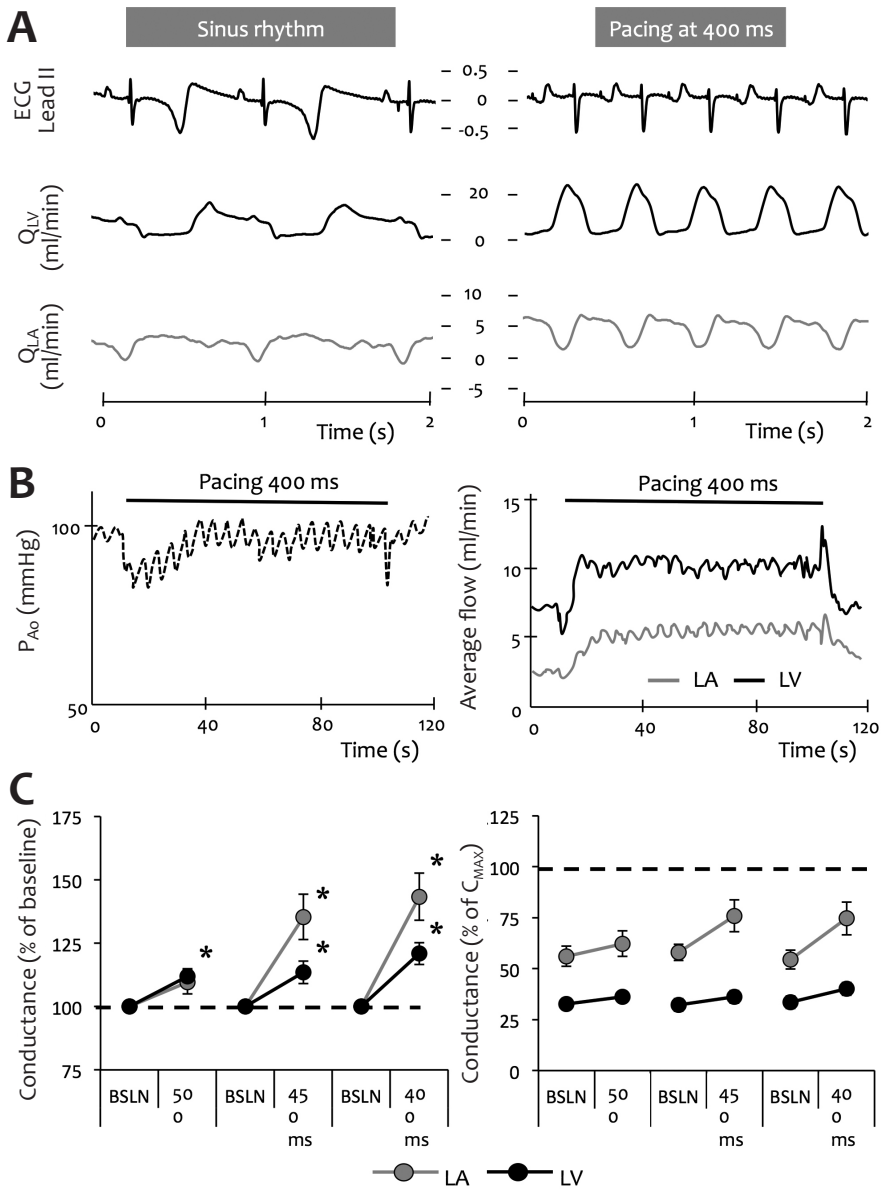

Figure 3: Coronary flow and conductance during pacing. A) ECG lead II and LA and LV Doppler flow patterns during NSR (left) and right atrial pacing at a BCL of 400ms (right). B) Mean aortic pressure (left) and Doppler flow for LA and LV (right) at 400 ms BCL. C) LA and LV conductance at different BCLs expressed as % of baseline conductance (left, n=9) and % of maximal conductance (C_{MAX}, n=8) (right). Values ±SEM. * p<0.05 intervention vs baseline.

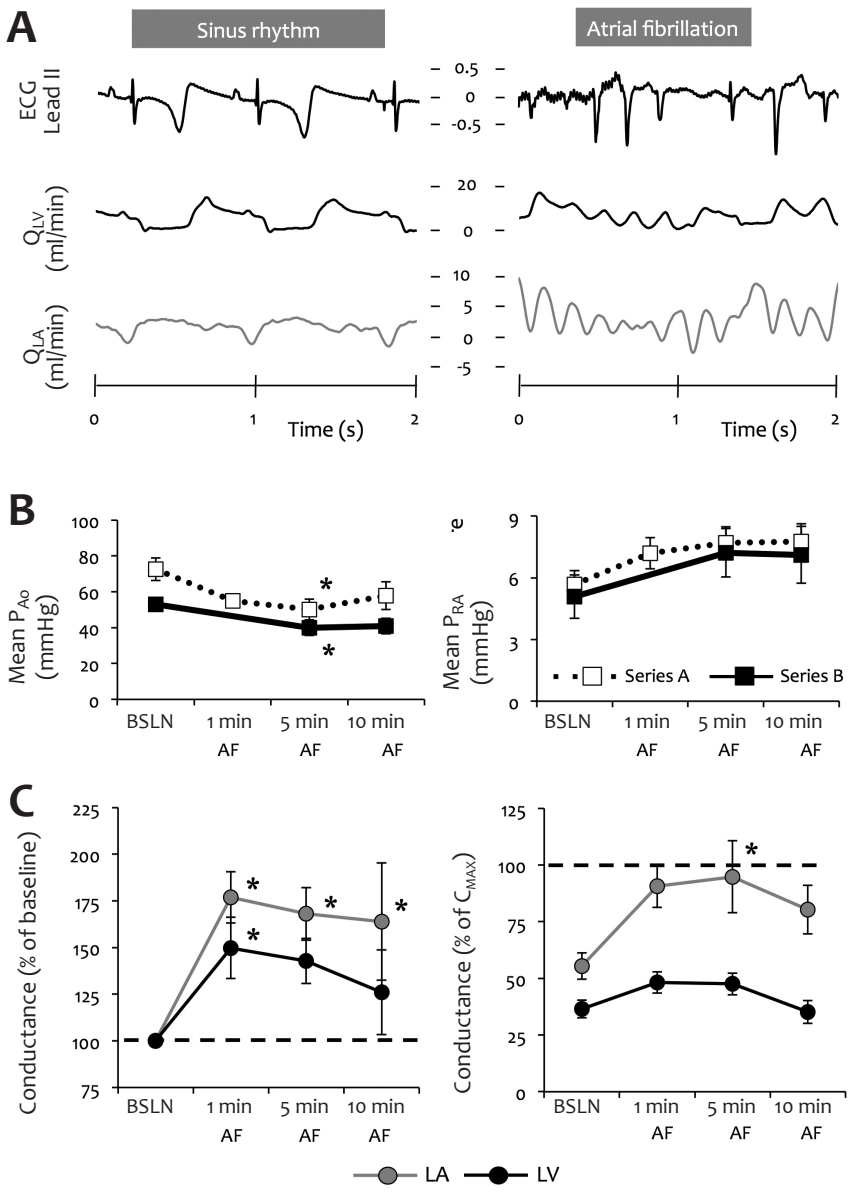

Figure 4: Coronary flow and conductance during AF. A) ECG lead II and LA and LV Doppler flow patterns during NSR (left) and AF (right). B) Mean aortic pressure (P_{Ao}, left) and right atrial pressure (P_{RA}, right) for series A and B during AF. C) LA and LV conductance during AF expressed as % of baseline conductance (left, n=9) and % of maximal conductance (C_{MAX}) (right, n=8). Values ±SEM. * p<0.05 intervention vs baseline.

right atrial pressures was similar between series A and B. (Figure 4B). AF caused a rapid rise in LA conductance (p=0.02, Figure 4C, left panel), with a significant increase to 177±14% at 1 min, 168±14% at 5 min and 164±31% at10 min of AF (p<0.05 vs baseline). In the ventricle, the overall effect of AF on vascular conductance as % of baseline was not significant (p=0.07 n.s.). However, at 1min of AF, LV conductance was significantly higher than baseline (P<0.05). No significant effect of AF was found on LA (p=0.07) or LV (p=0.1) conductance expressed as % of C_{MAX}. LA conductance approached C_{MAX} (Figure 4C, right panel) by 91±9% (n.s.), 95±16% (p=<0.05 vs baseline) and 80±11% (n.s.) at 1, 5 and 10 min of AF, respectively. In contrast, LV conductance did not even reach half of its C_{MAX} at the same time point of AF. Thus, the LA used a substantially larger part of the conductance reserve than the LV at every time point during AF (p<0.01).

3.2. Series B: Oxygen extraction and lactate levels

LA venous oxygen saturation was higher at baseline compared to the LV (74±3% vs 43±7% respectively, p<0.001). Correspondingly, the extraction reserve was larger in the LA than in the LV (4.68±0.84 vs 1.88±0.26, p=0.01). The same interventions as in series A were used to establish to which extent this reserve in oxygen extraction was utilized.

Venous blood gas analysis was performed during pacing. There were no significant overall effects on venous and arterial PO_2 and PCO_2 (Figure 5A and 5B). However, atrial venous PO_2 decreased significantly at 400 ms BCL from 38±7 mmHg at baseline to 24±6 mmHg at 5 min and to 28±6 mmHg at 10 min of pacing (Figure 5A). LA total oxygen concentration (CvO2), but not LV CvO_2 or CaO_2, showed a frequency-dependent decrease (data not shown). LA extraction ratio was strongly increased at 400ms pacing from 25±5% before pacing to 55±12% at 5 min (p<0.05) and 53±9% (p<0.05) at 10 min of pacing (Figure 5C). LV O_2 extraction remained unchanged during pacing at all BCLs.

During AF, LA venous PO_2 significantly decreased at the 5 min, but not the 10 min time point (Figure 5A). AF resulted in a significant increase in LA venous PCO_2 after 10 min (Figure 5B), rising from 43±2 mmHg at baseline to 58±4 mmHg (p<0.05 vs NSR). LV venous and arterial PO_2 and PCO_2 remained statistically unaltered during AF. A large decrease in LA CvO_2 was seen from 10.9±0.5 ml O_2/100 ml just before AF induction to 5.0±0.7ml O_2/100 ml at 5 min (p<0.01) and 5.8±1.7 ml O_2/100 ml at 10 min of AF

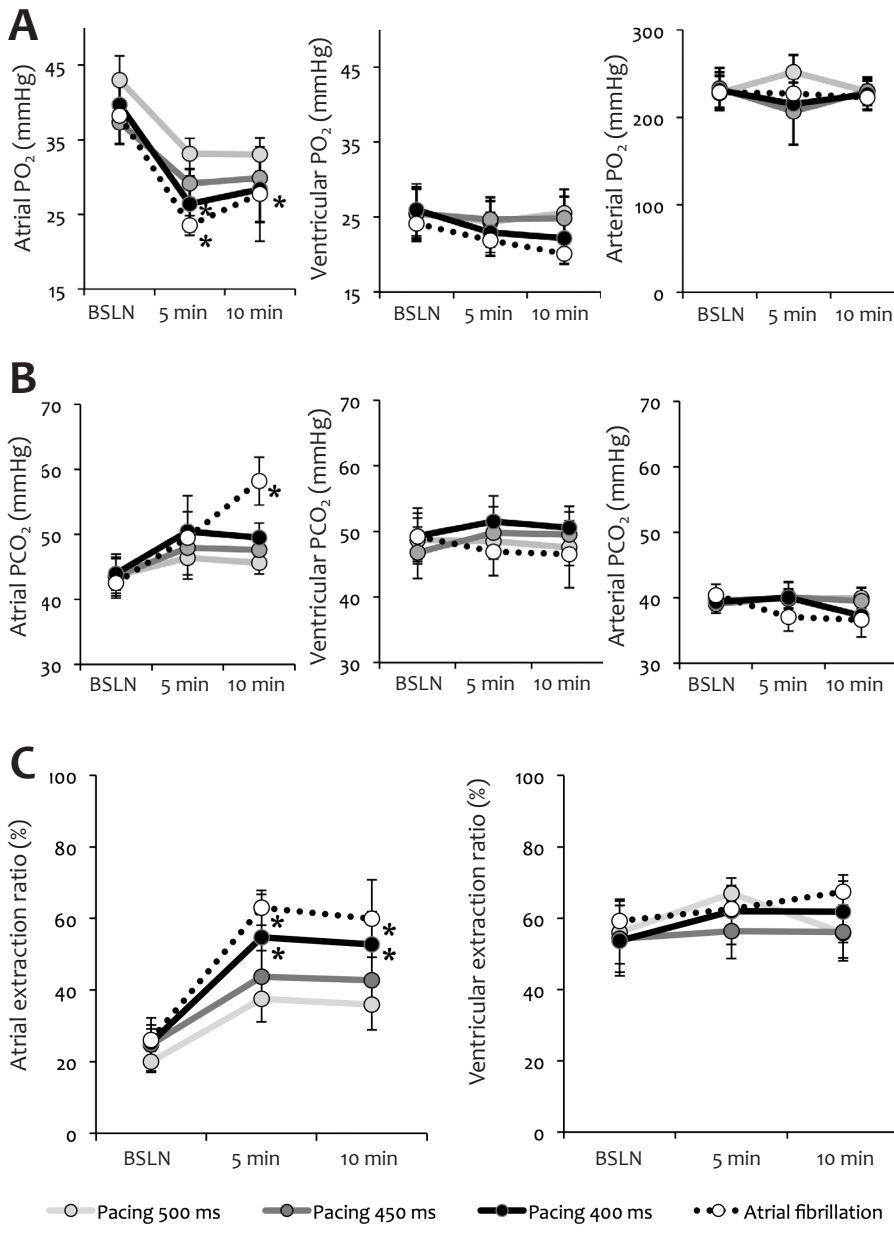

Figure 5: Blood gas analysis during pacing and AF. A) LA venous, LV venous and arterial partial oxygen pressure (PO_2) and B) carbon dioxide pressures (PCO_2) C) LA and LV oxygen extraction ratio (n=6) during pacing at a BCL of 500, 450 and 400 ms and during AF. Values ±SEM. * $p<0.05$ intervention vs NSR.

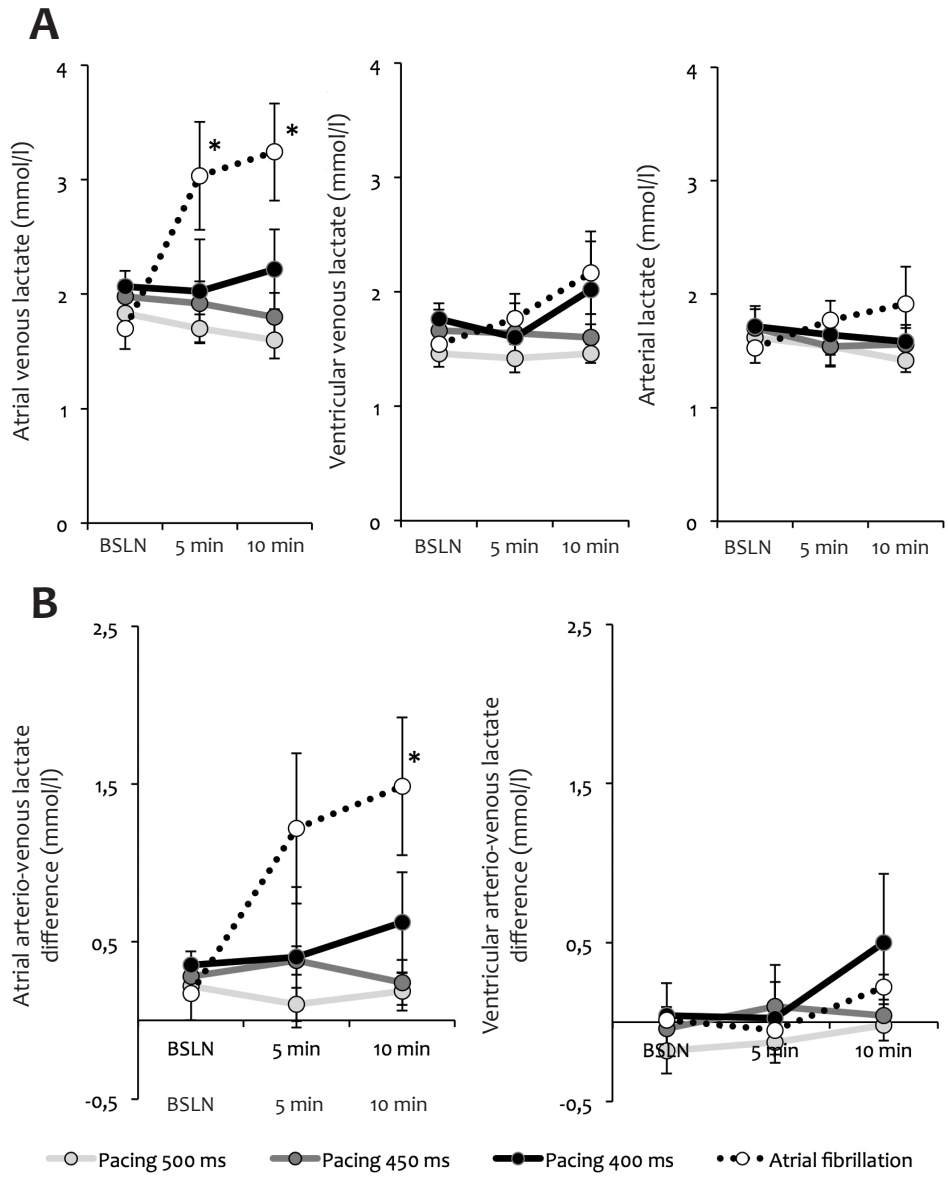

Figure 6: Lactate levels. A) LA venous, LV venous and arterial lactate concentration and B) arterio-venous concentration difference during pacing at a BCL of 500, 450 and 400 ms and during AF (n=6). Values ±SEM. * p<0.05 intervention vs NSR.

(p<0.01, data not shown). Correspondingly, LA oxygen extraction ratio was high during AF (Figure 5C), increasing significantly from 26±3% at baseline to 63±5% (p<0.01) at 5 min and 60±11% (p<0.01) at 10 min of AF. AF did not change LV extraction ratio. In summary, LA oxygen extraction reserve was recruited to a considerable degree both during pacing at 400 ms and during AF.

During NSR, LA venous blood lactate concentration was comparable to LV venous lactate concentration (1.7±0.2 mmol/L vs 1.4±0.1 mmol/L, p=0.11). Arterial lactate concentration was 1.5±0.1 mmol/L. Myocardial arterio-venous lactate difference shows that little lactate is produced or consumed by either the LA or LV during NSR.

Pacing did not significantly affect venous or arterial lactate levels (Figure 6A).

AF caused atrial venous lactate levels to increase from 1.7±0.2 mmol/L before AF to 3.0±0.5 mmol/L at 5min and 3.2±0.4 mmol/L at 10 min of AF without an increase in ventricular venous or arterial lactate levels (Figure 6A). The LA arterio-venous lactate difference showed a significant increase at 10 min of AF (from 0.2±0.2 mmol/L to 1.5±0.4 mmol/L, p<0.05 vs NSR, Figure 6B), but it remained unchanged in the LV during 10 min of AF. Thus, despite the recruitment of the LA conductance and extraction reserve, lactate is produced in the LA during acute AF.

4. DISCUSSION

Because of its obvious relevance to ischemic heart disease, the ventricular vasculature has been extensively studied. In comparison, very little is known about atrial vasculature and atrial balance between oxygen supply and demand.

Most of our knowledge of the regulation of atrial coronary flow derives from experiments on dogs. However, both in (ventricular) coronary flow regulation and vascular anatomy, pigs are more similar to humans than dogs are [12-16].

In this study, we investigate the recruitment of coronary conductance reserve and oxygen extraction reserve of the left atrium and ventricle during pacing and AF in anesthetized healthy pigs.

4.1. Coronary vascular conductance

The coronary conductance reserve was larger in the LV (3.16±0.27) than the LA (1.76±0.14). An increase in RA pacing rate and acute AF resulted in a stronger

increase in coronary conductance in the LA than in the LV. As a result, the LA came closer to its conductance reserve during these interventions than the LV. Our data in series A agree with earlier findings in dogs showing that an increase in atrial rate caused an increase in atrial myocardial blood flow [1, 2, 8, 17]. In dogs, atrial blood flow increased by a factor 1.5 to 3 during the first minutes of AF [1, 2, 8, 17]. Ventricular myocardial blood flow in dogs was not significantly or only slightly increased due to AF, despite an increase in ventricular rate [1, 2, 8].

4.2. Oxygen extraction and lactate production

Few data are available on atrial oxygen consumption and extraction in animal models, and none in humans. In a study in dogs, Wichmann et al reported that AF caused a decrease in coronary sinus (CS) oxygen saturation from 26±2% to 22±1%, but the specific contributions for LA and LV could not be determined [18]. Using a microspectrophotometric technique on frozen vessels, White et al determined the atrial venous and arterial oxygen saturation in tissue samples from anesthetized dogs [1]. They reported an arterial oxygen saturation of 87±1.5% and an atrial venous saturation of 61±1%, corresponding to an LA extraction of approximately 26%. Acute AF in the anesthetized dogs caused a slight increase in atrial oxygen extraction to approximately 31% [1].

Our technique of collecting venous blood samples allows taking repeated samples under various conditions and conventional blood gas analysis. For the LV we found comparable extraction values to other studies in dogs under anesthesia (approximately 60%) [19]. Oxygen extraction ratio during NSR was lower in the LA (25±3%) than in the LV (59±6%, p=0.002). Therefore, atrial oxygen extraction reserve was higher than in the ventricle during NSR (4.68±0.84 vs 1.88±0.26, p=0.01). Atrial pacing and AF caused an increase in LA oxygen extraction ratio, but had a much smaller effect on LV extraction ratio.

In summary, the LA recruited both conductance and extraction reserve to increase atrial oxygen supply during atrial pacing and short term AF. To determine whether this is sufficient to meet atrial oxygen demand, we have measured lactate production, which is a hallmark response to acute supply-demand ischemia [20-23]. Previous studies have shown that ventricular ischemia resulted in a peak lactate production at 5-15 min [20-22]. Ventricular lactate production correlated with the severity of ische-

mia [24]. Our results show a significant increase in LA arterio-venous lactate difference during acute AF. This indicates that during acute AF, atrial oxygen demand increases more than oxygen supply, potentially leading to a supply-demand mismatch. The atrial arterio-venous lactate difference we observed during AF (1.2-1.5 mM) approaches ventricular values during acute moderate ischemia in isolated rat hearts (2 mM after 3 min) [25] and in anesthetized pigs (4.2 mM after 5 min)[26]. Therefore, we conclude that the severity of the atrial supply-demand mismatch induced by AF is comparable to mild-to-moderate ventricular ischemia.

4.3. Relevance

The rapid electrical and mechanical activation rates during AF are likely to increase the energy expenditure of the atrial myocardium. Several studies have shown a concomitant increase in atrial myocardial blood flow [1, 3, 8]. Our study shows that, in addition to an increase in LA coronary conductance, atrial oxygen extraction also increased during AF. Despite the recruitment of both atrial coronary conductance reserve and extraction reserve, the atrial oxygen supply is insufficient to fully match increased atrial oxygen demand during acute AF. This is reflected in the increase in atrial venous lactate levels during short term AF in a healthy heart. Supply-demand ischemia may also explain the rapid decrease in atrial contractility that has been reported after 5 min of AF in goats [17] and after 1 h of AF in pigs [27]. This may be comparable with the response to ischemia in the ventricles, where a fast decline in contractility has been proposed to constitute an active adaptation to downregulate myocardial energy demand[22]. The occurrence of atrial ischemia during AF is further supported by increased expression of hypoxia and angiogenesis markers ($HIF_{1\alpha}$, $HIF_{2\alpha}$, VEGF) that has been found in AF patients [28]. In a goat model of AF, a reduction in phosphocreatine and an increase in $HIF_{1\alpha}$ have been observed [9, 10]. In addition, gene expression profiles in a pig model showed changes indicative of energy depletion after 7 h of AF [29]. Supply-demand ischemia may form the trigger for the slow process of atrial structural remodeling, the first signs of which can be observed at the ultrastructural level after one week of AF in goats [9, 30]. Several aspects of atrial structural remodeling (myolysis and myocyte dedifferentiation) in AF patients and in a goat AF model are consistent with chronic ischemia and are strongly reminiscent of hibernating ventricular myocardium due to chronic low-flow ischemia [11, 31-33].

During prolonged AF, atrial remodeling may reduce the energy expenditure and oxygen demand of the atria. Electrical remodeling, especially the reduction in calcium current, strongly decreases atrial contractility. In the ventricles, low-flow ischemia and heart failure lead to metabolic remodeling, hallmarked by a shift from fatty acid to glucose utilization that increases oxygen-efficiency [34]. Whether this shift also takes place in the atria during prolonged AF is unknown at present, but expression profiles show marked changes in the expression of metabolism-related genes after 24 h of rapid atrial pacing in dogs [35]. In addition, structural remodeling leads to a hibernating phenotype of atrial myocytes that can also be interpreted as an energy-conserving adaptation to ischemia [11, 31, 36]. Because of adaptive electrical/contractile, metabolic and structural remodeling, the phase of atrial supply-demand ischemia may be transient. Indeed, the decrease in atrial phosphocreatine levels, an indicator of supply-demand ischemia and increased expression of $HIF_{1\alpha}$, a common response to oxygen shortage were both observed in the first weeks of AF, but had disappeared at later stages in a goat model of 'lone' AF [9]. Nevertheless, these adaptive processes contribute to the formation of a substrate for AF. In this context, it is relevant that in patients AF initially is often paroxysmal. Typically, the time spent in NSR between paroxysms would be sufficient for full reversal of electrical remodeling [17]. Under such conditions, atrial supply-demand ischemia might occur during successive paroxysms, and any resulting damage to the atrial myocardium could be cumulative.
Prevention of atrial supply-demand ischemia may represent a valuable therapeutic target. Its occurrence would present a window of opportunity for interventions that improve oxygen efficiency (such as trimetazidine or ranolazine) [37], thereby reducing supply-demand ischemia and possibly preventing the progression of AF.

4.4. *Limitations*

We propose that the results of our study are relevant for AF patients, and in this respect, there are several potential limitations.
Firstly, this study was performed in pigs. Ventricular flow regulation in pigs is similar to humans, but the similarity in atrial flow regulation cannot be ascertained at present. Opportunities to measure atrial coronary blood flow in humans are limited. However, one study using a Doppler guide wire has reported phasic flow patterns similar to our recordings [38]. In addition, an elevated heart rate increased atrial blood

flow more than ventricular (LCx) blood flow, in agreement with our data in pigs. Secondly, the recordings were performed under anesthesia. The anesthesia used allowed stable recording of flow and pressure over a time course of several hours. However, the activity of the autonomic nervous system is likely to be lower under anesthesia, which may exacerbate blood pressure fluctuations, for example during acute AF. Nevertheless, our data show that under these conditions, the atria are more vulnerable to supply-demand ischemia than the ventricles. In addition, reported values for LV venous oxygen saturation tend to be somewhat higher in anesthetized animals than in awake animals (40% in our study vs. 20% in awake pigs [39]). No venous oxygen saturation has been reported for the LA in awake animals, but if it were similarly lower, this would actually decrease atrial extraction reserve and thus increase the vulnerability to supply-demand ischemia.

5. CONCLUSION

The LA has a lower coronary conductance reserve than the LV, but a higher oxygen extraction reserve. Although, both reserves are recruited during short term AF, a supply-demand mismatch still arises in the LA. This imbalance may represent a pivotal trigger that induces atrial structural changes on the longer term, thereby contributing to the progressive stabilization of AF.

FUNDING SOURCES

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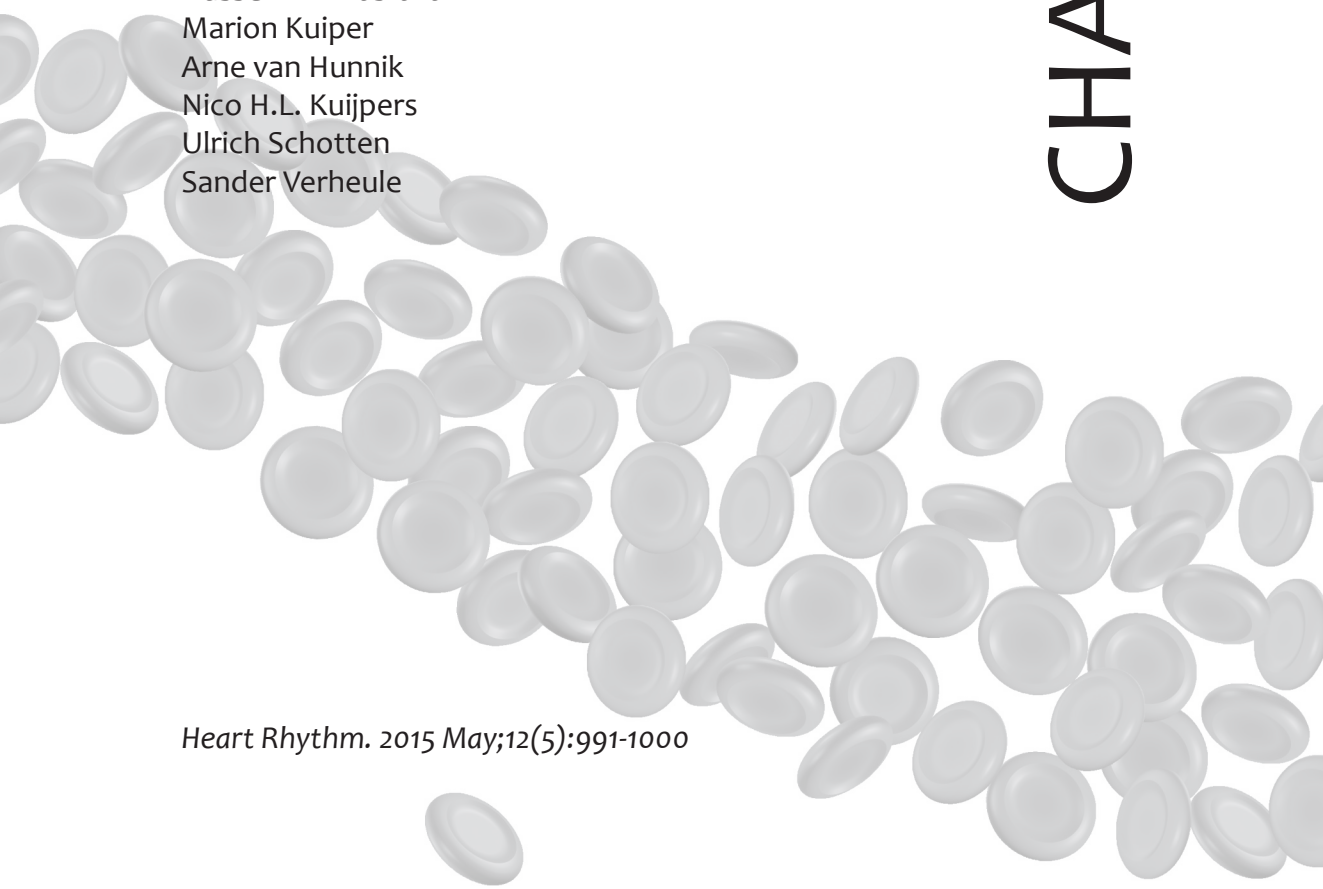
CHAPTER 5

Dynamic regulation of atrial coronary blood flow in healthy adult pigs

Kelly van Bragt
Hussein M. Nasrallah
Marion Kuiper
Arne van Hunnik
Nico H.L. Kuijpers
Ulrich Schotten
Sander Verheule



Heart Rhythm. 2015 May;12(5):991-1000

ABSTRACT

BACKGROUND: There are several indications for a mismatch between atrial oxygen supply and demand during atrial fibrillation (AF), but atrial coronary flow regulation has not been investigated extensively.

OBJECTIVE: This study was designed to characterize the dynamic regulation of atrial coronary flow in pigs. Methods: In anesthetized open-chest pigs, Doppler flow probes were placed around LA and LV branches of the circumflex artery. Pressures and work indices were measured simultaneously. Systolic and diastolic flow contribution, flow response kinetics and relationship between pressures, work and flow were investigated during sinus rhythm, atrial pacing and acute AF.

RESULTS: During atrial systole, LA flow decreased. Only 2% of total LA flow occurred during atrial systole. Pacing with 2:1 AV-block and infusion of acetylcholine revealed that the atrial contraction itself impedes atrial coronary flow. The response to sudden changes in heart rate was slower in LA compared to LV. Both LA and LV vascular conductance were positively correlated with work. After the cessation of acute AF, the LA showed a more pronounced phase of supranormal vascular conductance than the LV, indicating a period of atrial reactive hyperemia.

CONCLUSION: In healthy adult pigs, atrial coronary flow is impeded by atrial contraction. Although atrial coronary blood flow is positively correlated with atrial external work, it reacts more slowly to changes in rate than ventricular flow. The occurrence of a pronounced hyperemic phase after acute AF supports the notion of a significant supply-demand mismatch during AF.

1. INTRODUCTION

Atrial fibrillation (AF), the most common tachyarrhythmia in clinical practice, is associated with increased morbidity and mortality [1-3]. The rapid and irregular rate of excitation and contraction during AF is likely to lead to increased atrial energy demand. Indeed, several studies using microspheres in healthy animals have shown that an increased atrial demand, such as short-term atrial pacing, exercise and atrial fibrillation resulted in increased atrial coronary blood flow [4-11]. However, if this increase in supply were insufficient to meet the increased demand, a state of supply-demand ischemia would ensue. In the goat model of AF, the occurrence of supply-demand ischemia over a longer period is supported by decreased phosphocreatine levels[1 2]. In addition, $HIF_{1\alpha}$ and VEGF expression were increased in atrial biopsies of both goats in AF [13] and AF patients [14]. Because its (ventricular) coronary anatomy and flow regulation are similar to those in humans [15], we have chosen pigs to study atrial coronary flow regulation. We have recently shown that, although atrial arteries dilate and atrial oxygen extraction increases in response to acute AF in healthy pigs, atrial lactate production increases, indicative of supply-demand ischemia [16].

Understanding the dynamic regulation of atrial coronary flow will help in understanding the role of ischemia in the onset and perpetuation of AF. In this study, we have studied the dynamic regulation of atrial coronary blood flow in normal healthy pigs. The phasic coronary flow pattern was analyzed and the kinetics of flow regulation were investigated. To this end, simultaneous measurements of atrial work and atrial flow were performed under different circumstances that influence atrial energy demand.

2. METHODS

2.1. Animal preparation

Eleven healthy Dutch Landrace pigs weighing 66±3 kg were studied. Some aspects of the measurements in these animals have been reported previously [17]. For the present study, three additional animals were included, and additional analysis, interventions and parameters are described. All animal procedures were in accordance with national and institutional guidelines. Anesthesia was induced with Zoletil (5-8 mg/kg I.M.)

and Thiopenthal (5-15 mg/kg I.V.) and maintained with Midazolam (0.8 mg/kg/h), Sufentanyl (6 µg/kg/h) and Propofol (2.5-10 mg/kg/h). A left lateral incision was made with removal of the 5th rib and the pericardium was opened to expose the LA and LV.

2.2. *Instrumentation*

Pigs were instrumented as shown in Figure 1B. To measure atrial and ventricular flow, Doppler flow probes (Transonic Systems Inc., Ithaca, NY) were placed around a left atrial (LA) and left ventricular (LV) branch of the left circumflex artery (LCx). Flow signals were recorded during sinus rhythm (SR), atrial pacing at different pacing cycle lengths (500,450 and 400 ms) and AF. For this purpose, a pacemaker lead (5568, Medtronic Inc, Minneapolis, MN) was placed endocardially in the RA to pace and record local electrograms.

Because the stability of AF was low in healthy pigs, continuous rapid burst pacing at 20 Hz (4x diastolic threshold) was used to artificially maintain the arrhythmia for recordings during AF. AF episodes had approximately the same duration in each ani-

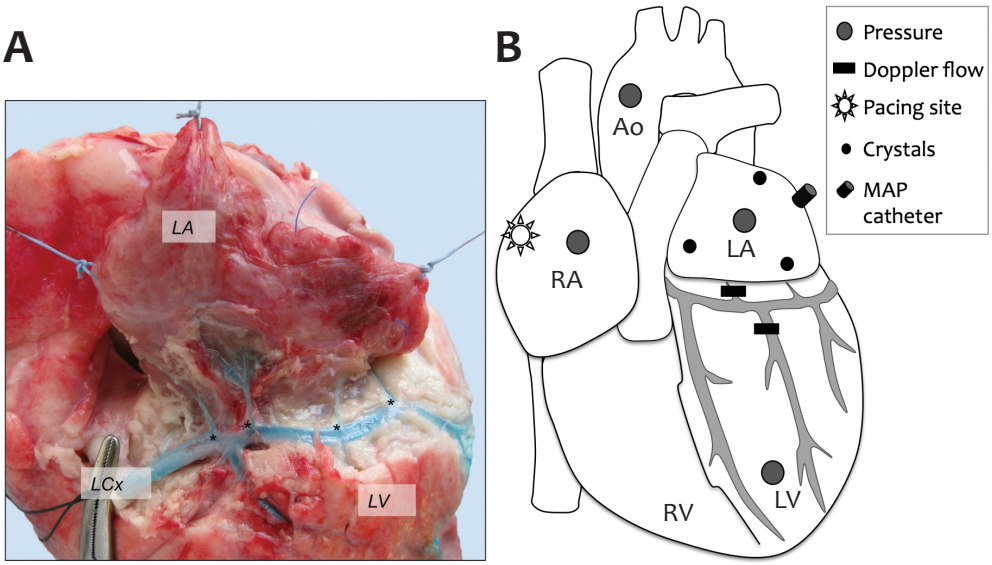


Figure 1: Instrumentation. A) Ink injection into the left circumflex artery (LCx). Atrial branches are indicated with an asterisk. B) Instrumentation in aorta (Ao), left ventricle (LV), left atrium (LA) and right atrium (RA).

mal, because pigs spontaneously converted to sinus rhythm within seconds of cessation of burst pacing.

To analyze the phasic flow pattern, the diastolic and systolic phases of the flow signal were defined (see section "Calculation of diastolic and systolic fraction"). To analyze the trends in the coronary flow pattern, the response kinetics of the flow signal were determined during atrial pacing (See section "Calculation of flow response kinetics"). LA and LV work indices were measured simultaneously with coronary flow signals. Left atrial work index (LAWI) was estimated using LA dimensions and pressure (see section "Calculation of atrial and ventricular work"). Three piezo-electric crystals (Sonometrics, London, Ontario, Canada) were introduced through the LA free wall into the LA lumen in a triangular orientation (Figure 1B) in order to measure atrial dimensions. To measure LA pressures (P_{LA}), a Millar microtip pressure sensor (Millar Instruments, Houston, TX, USA) was inserted through the LA free wall into the LA lumen. Left ventricular work index (LVWI) was determined using a Sentron conductance catheter (Sentron Europe BV, Roden, The Netherlands) to measure LV volume and pressure (P_{LV}). In order to calculate vascular conductance, aortic (Sentron Europe BV, Roden, The Netherlands) and RA (Millar Instruments, Houston, TX, USA) pressures were measured. Conductance was then calculated by dividing coronary flow by the pressure difference over the coronary vascular bed (P_{Ao}- P_{RA}). Atrial work and flow were measured during atrial pacing at a cycle length of 500 ms, 450 ms and 400 ms and during short term AF (10 min). The same parameters were measured during intracoronary infusion of acetylcholine (ACh) via a JR6 catheter (Cordis Corporation, Bridgewater, NJ, USA) in the left main coronary artery. Prior to and during ACh infusion, monophasic action potentials (MAP) were recorded using a monophasic action potential catheter (7F MAP-4801, Harvard Apparatus, Holliston, MA, USA). After 1 min of pacing or steady state ACh infusion, a 10 s period was analyzed to calculate the average atrial and ventricular conductance and work indices.

2.3. *Calculation of diastolic and systolic fraction of flow*

Left ventricular systolic flow was calculated as the flow occurring between the onset of ventricular contraction (i.e. the start of the increase in LV pressure) and the onset of ventricular relaxation (i.e. the start of the decrease in LV pressure, illustrated in

Figure 3A). Left atrial systolic flow was defined as period between the start of the atrial contraction (i.e. the increase in LA pressure following the start of the P wave in the ECG), and the start of atrial relaxation (i.e. the decrease in LA pressure, illustrated in Figure 3B). The time-flow integrals were calculated for 5 consecutive beats during systole (TFIs) and diastole (TFId). The systolic fraction (SF, in %) of total flow per beat was calculated as:

$$SF = (TFIs / (TFIs+TFId))*100$$

2.4. *Calculation of flow response kinetics*

The response kinetics of the average flow signal over time was compared between the LA and LV branches. To this end, the flow signal was filtered using a 10-level wavelet filter to eliminate the phasic pattern of the cardiac cycle, thereby deriving average flow. For 3 different pacing cycle lengths, the time to reach the half-maximal flow increase ($T_{1/2\ MAX}$) from the baseline was calculated. In addition, the time to the half-maximal flow decrease ($T_{1/2\ bsln}$) from the maximum after the cessation of pacing was determined (illustrated in Figure 4A). To quantify the hyperemic phase after cessation of acute AF, the ratio between the time integral of the total conductance and the baseline over the same time period were calculated.

2.5. *Calculation of work indices*

Ventricular work indices were determined by integrating all completed LV pressure-volume loops during periods of 10 s. Atrial work indices were assumed to be proportional to the integral of pressure-distance loops during the atrial contraction [18]. Atrial pressure-distance loops result from both passive and active atrial emptying. The active atrial work starts at the moment of LA pressure rise and followed by shortening of the atrial distance (indicated with a star in Figure 5A). The atrial active work loop ends when the same atrial diameter is reached again. Three distances were recorded on the LA free wall and work index was calculated by the average value from the three pressure-distance loops. LAWI was normalized with respect to heart rate, by multiplying the work per beat times the number of heartbeats per second and was expressed as mm*mmHg/s.

2.6. *Statistical analysis*

Results are expressed as mean± SEM. Statistical analysis was performed using a 1-way ANOVA to assess the effects of different interventions on work indices and conductance. Values were compared to baseline using a post-hoc test (Dunnett's Multiple Comparison). LA and LV values were compared with a 2-way ANOVA followed by a post-hoc Dunnett test. Correlation between work indices and conductance was tested using a linear mixed model analysis. We have applied three linear mixed models and chose the one with the lowest Akaike's Information Criterion (AIC). A p-value<0.05 was considered statistically significant.

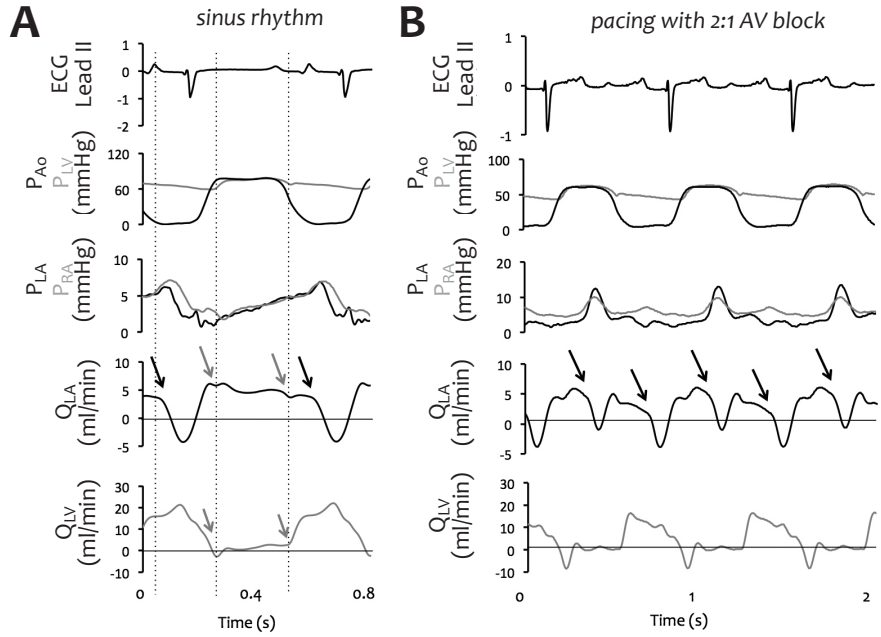


Figure 2: Phasic flow pattern during sinus rhythm (A) and 2:1 AV block (B). Black arrows indicate the decrease in atrial flow during atrial systole. Grey arrows indicate the timing of aortic valve opening and closure.

3. RESULTS

3.1. The phasic atrial flow pattern

The atrial flow signal showed a phasic pattern during every cardiac cycle. Both atrial and ventricular flow patterns consistently showed a small decrease during the opening and closing of the aortic valve (Figure 2A, indicated with gray arrows). As expected, ventricular flow decreased during ventricular systole [19, 20]. Similarly, a consistent decrease in atrial flow was observed during atrial activation (Figure 2A, indicated

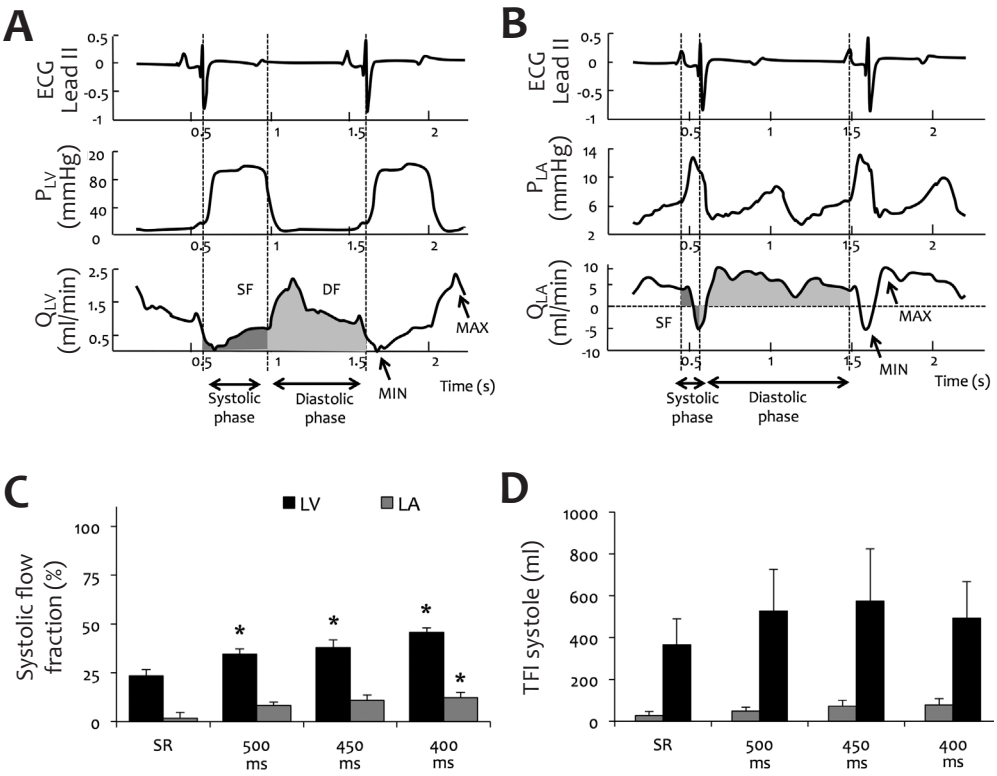


Figure 3: Calculation of systolic and diastolic fraction of flow. Systolic phase in indicated in dark grey, diastolic phase in light gray in the LV (A) and LA (B) phasic flow pattern. Minimum (MIN) and maximum (MAX) flow are indicated in the flow signal. From the flow signal of LV and LA, systolic flow fraction (C) and time-flow integral during systole (TFIs) (D) were calculated. *p≤0.05 vs baseline.

with a black arrow). In principle, the decrease in atrial flow during atrial activation could be caused either by the increase in atrial pressure during the atrial contraction or by the atrial contraction itself. When 2:1 AV block occurred during atrial pacing, the atrial pressure alternated between large increases, when the atria contracted against a closed mitral valve (isovolumetric contraction), and much smaller increases, when the atria contracted with an open mitral valve, as illustrated in Figure 2B. The decrease in atrial coronary flow was comparable for both types of contractions, indicating that the atrial contraction itself impedes atrial coronary blood flow (Figure 2B, black arrows). In 8 out of 11 animals, atrial flow reversed during atrial contraction. The minimum in atrial flow (-0.76±0.64 ml/min during SR) was reached at the end of atrial systole (Figure 3B). In the LV, flow reversed in 4 out of 11 animals (minimum flow during SR -0.2±0.9 ml/min). Both in the LA and LV, maximal flow was reached in the early diastolic phase (Figure 3A and 3B). The atrial systole is short compared to the ventricular systole. In the LA, 1.8±2.9% of the total coronary flow occurred during atrial systole (Figure 3C) and 98.2±2.9% during diastole. In the LV, 23.5±3.3% of total ventricular flow occurs during ventricular systole and 76.5±3.3% during diastole. The TFI during systole is depicted in Figure 3D.

3.2. *Response to atrial pacing*

As the heart rate increases, the diastolic period becomes significantly shorter in both LA and LV. LV diastolic time per heart beat was 52±3% during SR, 41±2% during 500 ms, 37±2% during 450 ms and 35±2% during 400 ms pacing, $p<0.01$ vs SR. The TFI was calculated during diastole and systole (Figure 3D) for both LA and LV. TFIs showed a trend towards an increase in both LA and LV with incremental pacing, but this was not statistically significant. The contribution of the systolic flow fraction gradually increased with pacing rate, both in the LA and in the LV (Figure 3C), and correspondingly, the contribution of diastolic flow decreased.

We have previously shown that the average atrial and ventricular coronary flow increases with increasing pacing rate [16]. Figure 4A and B show the time course with which atrial and ventricular coronary flow respond to a period of pacing at a basic cycle length (BCL) of 400 ms. Left atrial flow reacted to pacing by increasing 120±6%, 143±16% and 146±21% of baseline flow at 500, 450 and 400 ms respectively. Left ventricular flow increased 125±10%, 125±12% and 121±11% at the same respective cycle lengths.

Both after the onset and cessation of pacing, the response of atrial flow appears to be slower than that of ventricular flow (Figure 4A vs. B).
At the start of pacing, the half time was calculated to reach the steady state coronary flow during pacing ($T_{1/2\ MAX}$, Figure 4A). After the cessation of pacing, the half time to return to baseline coronary flow ($T_{1/2\ bsln}$, Figure 4A) was calculated. At all pacing cycle lengths investigated, the response time of flow to pacing was significantly slower in the LA than in the LV, both after the onset and after cessation of pacing (Figure 4C and D).

3.3. Relation between flow and work

To investigate the relation between atrial work and atrial vascular tone, pressure-distance loops were measured simultaneously with coronary flow measurements. The LAWI was 13±4 mm*mmHg/s during SR and showed a cycle length dependent increase (p=0.0059), with a LAWI of 58±16, 93±29 and 154±39 mm*mmHg/s at 500 ms, 450

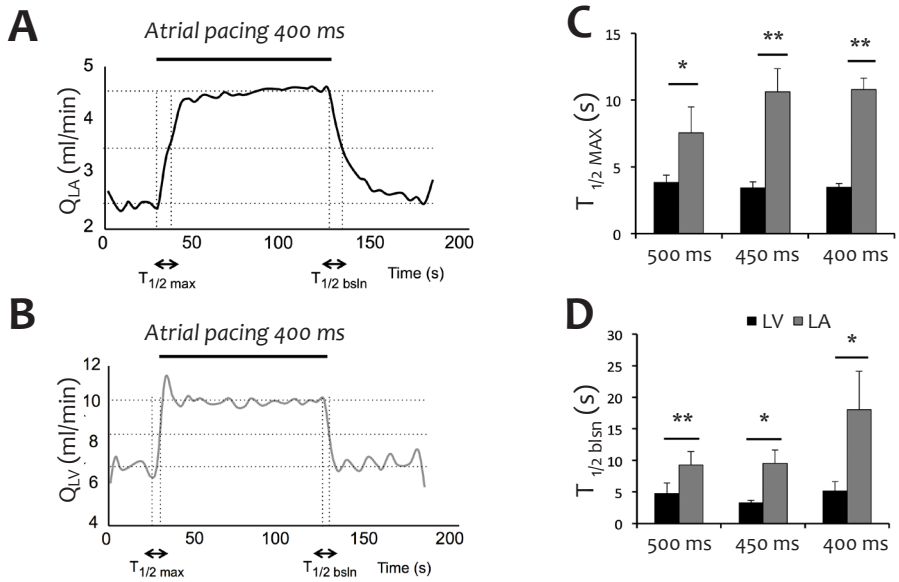


Figure 4: Kinetics of flow during pacing. A) Example of filtered flow signal in the LA (A) and LV (B) upon atrial pacing at 400ms. The time to reach half max ($T_{1/2MAX}$) and half baseline ($T_{1/2bsln}$) of the flow signal are indicated in the signal. Calculation of $T_{1/2MAX}$ (C) and $T_{1/2bsln}$ (D) during pacing at 500 ms, 450 ms and 400 ms cycle length. *p≤0.05, **p≤0.01 vs LA.

ms and 400 ms BCL respectively (Figure 5B left panel). LVWI was significantly higher during SR and at every pacing rate compared to LAWI ($p<0.001$, Figure 5B right panel). The average conductance was 0.042±0.007 ml/min/mmHg in the LA branch and 0.078±0.011 ml/min/mmHg in the LV branch. In agreement with the increase in LAWI, atrial pacing significantly increased LA conductance (data not shown, $p<0.001$) from 100% at baseline to 112±4% at 500 ms ($p>0.05$, n.s.), 140±9% at 450 ms ($p<0.001$) and 156±13% at 400 ms ($p<0.001$). Also LV conductance increased significantly by pacing ($p<0.001$). A positive correlation was found during right atrial pacing between LAWI and the increase in LA conductance (Figure 5C upper panel). The slope 0.052 (95% CI: 0.016, 0.088) is significantly different from zero ($p=0.012$). As expected, LVWI and LV conductance also correlated significantly with a slope of 0.288 (95% CI: 0.112, 0.464, $p=0.003$, Figure 5C lower panel).

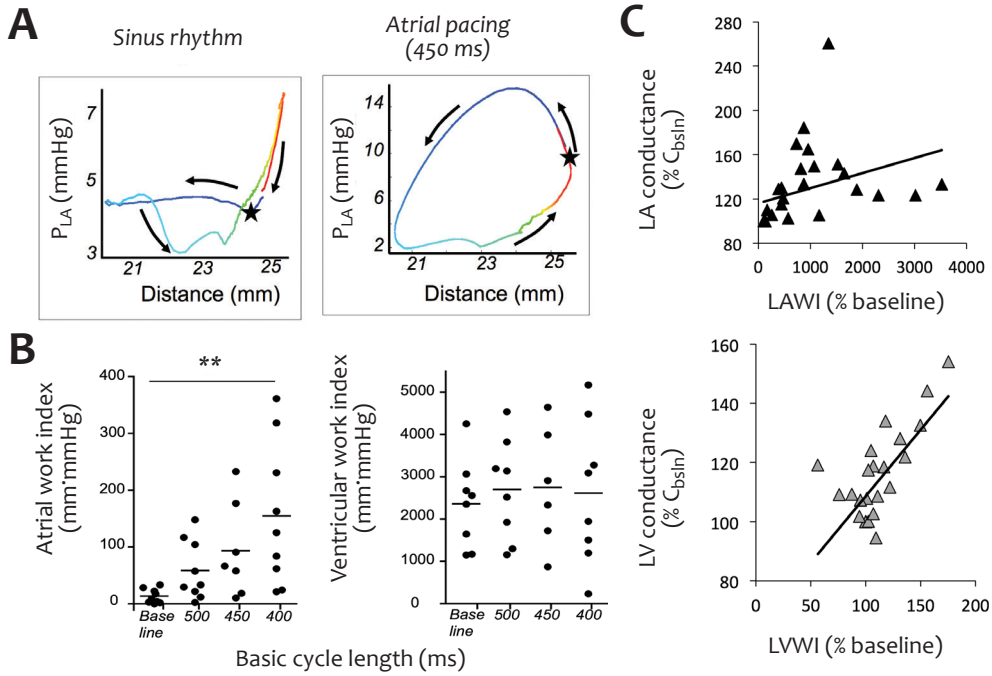


Figure 5: Work-conductance relationship during pacing. A) Example of atrial distance-pressure relationship. B) Atrial work index during baseline and atrial pacing at 500 ms, 450 ms and 400 ms cycle length. C) Correlation between left atrial work index (LAWI) and LA conductance expressed as % of baseline. D) Correlation between left atrial work index (LVWI) and LV conductance expressed as % of baseline. **p≤0.01 vs baseline.

Secondly, the relationship between atrial workload and coronary conductance was investigated by administration of acetylcholine (ACh) during atrial pacing at 450 ms. The ACh-induced reduction in atrial contractility greatly reduced the phasic decrease in the atrial blood flow pattern (Figure 6A). During ACh infusion, atrial monophasic action potentials from the LA endocardial free wall were recorded. ACh administration resulted in a shortening of the action potential (Figure 6B, inset), thereby reducing atrial contractility and the active atrial work. The correlation between atrial work index and conductance had a slope of 1.01 (Figure 6B, 95% CI: 0.83-1.20, $p<0.001$). At the same concentration of ACh, the ventricular action potential duration was not affected, as indicated by the lack of effect on QT time (236±15 ms for baseline vs. 230±18ms during ACh infusion, $p=0.78$). The atrial conductance was compared between baseline condition and the concentration of ACh that resulted in approximately a 50% reduction in LAWI (Figure 6B). A reduction in APD80 to 33±15% of baseline ($p=0.02$) resulted in a reduction in LAWI to 47±2% of baseline (Figure 6C). This was accompanied by a decrease in LA vascular conductance to 24±8% of baseline (Figure 6D). P_{RA} and P_{Ao} did not change significantly, but P_{LA} was increased significantly from 6.20±1.01 mmHg to 7.07±1.11 mmHg ($p=0.03$). The effect of ACh on ventricular systolic function was evaluated by calculating the slope of the positive upstroke of the LV pressure curve (+dP/dt). The +dP/dt was unchanged, as was the LV work index (data not shown). Accordingly, the LV conductance was not significantly influenced by ACh administration, as expected from the lack of effect on LV work.

3.4. Response to atrial fibrillation

During atrial fibrillation, the atrial flow pattern was irregular (Figure 7A). The average atrial conductance increased significantly during the first few minutes of AF. There was a trend towards an increase in LV conductance as well, but this was not statistically significant. The kinetics of the response in average conductance were measured for LA and LV flow after cessation of AF. The LA vascular conductance was transiently elevated above baseline values (i.e. the period preceding the induced AF episode), reflecting a hyperemic phase (Figure 7B). By contrast, the LV vascular conductance did not display a hyperemic phase. The ratio between the integrals of the area above and below baseline conductance was significantly higher for the LA compared to the LV (Figure 7C, $p=0.04$).

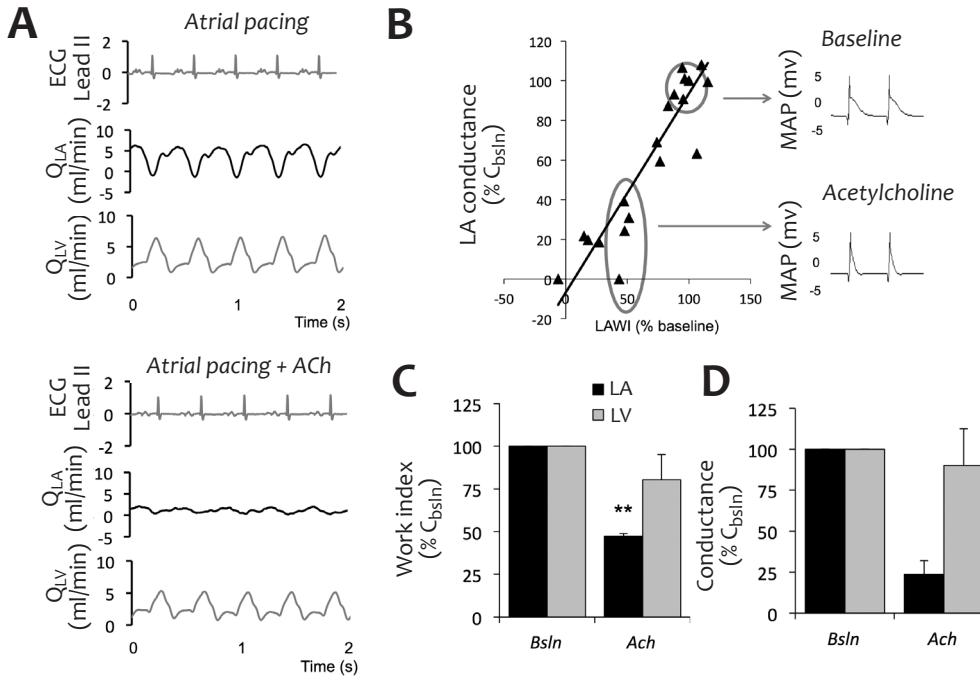


Figure 6: Work-conductance relationship during acetylcholine infusion. A) Correlation between LA work index (LAWI) and conductance during acetylcholine (Ach). Data points were divided in two groups: baseline and 50% reduction in LAWI. Examples of monophasic action potentials (MAP) are given for the two groups. Work index expressed as % of baseline (B), conductance expressed as % of baseline and an example of atrial and ventricular flow (C) in the baseline groups and Ach group. **p≤0.01 vs baseline.

4. DISCUSSION

In this study, we have investigated the dynamic regulation of the atrial coronary blood flow in detail and compared it to the ventricle in normal adult pigs. The atrial coronary flow pattern was assessed during sinus rhythm, atrial pacing and acute AF.

4.1. Atrial flow pattern

A consistent decrease in atrial flow was observed during the atrial contraction. This pattern is similar to the pattern found in other studies on left atrial branches in patients and dogs measured by a Doppler guide wire, Doppler crystals and laser Dop-

pler optical fibers [6,21-23]. In principle, this phasic decrease could be caused to the rise in atrial pressure or by the atrial contraction itself. However, when 2:1 AV block occurred during atrial pacing, every atrial contraction resulted in a similar decrease in atrial flow while atrial pressure alternated depending on whether the atrium contracted against an open or closed mitral valve. In addition, we show that during acetylcholine administration the decrease in atrial contractility led to the disappearance of the decrease in atrial flow during atrial contraction. This is in agreement with studies in dogs where premature ventricular stimulation caused a fluctuation in atrial pressure without affecting atrial coronary flow and where increasing atrial contractility by administration of isoproterenol affected atrial flow, but not atrial pressure [23,24]. In most pigs in our study, atrial flow reversed during atrial contraction. In an earlier study on dogs, reversal of atrial flow during the atrial contraction was only observed during administration of isoproterenol and not under baseline conditions [23].

In the LV, where the decrease of coronary flow during contraction has been investigated extensively, the intramyocardial pump action is the major factor impeding systolic flow [25,26]. Our results are consistent with a similar mechanism affecting flow in the LA, which may have important consequences during AF. We have recently reported that acute AF leads to a supply-demand mismatch, resulting in atrial lactate production. While organized atrial contractility is absent during AF, the local contraction rate increases dramatically. Because atrial energy demand increases during AF, the negative effect of rapid local contractions on local flow may exacerbate the supply-demand mismatch.

The systolic contribution to total coronary flow was calculated for atrium and ventricle. The systolic fraction of total ventricular flow during ventricular contraction was 23.5±3.3%, comparable to values in literature (25%-35%)[21, 27]. In the LA, flow during atrial systole contributes 1.8±2.9% to total atrial flow. Our results on phasic atrial flow show that atrial systole is responsible for the transient decrease in atrial flow, often leading to a reversal of atrial flow and, consequently, it only constitutes a small fraction of total atrial coronary flow.

4.2. Relation between coronary flow and myocardial work

In the LA, the diastolic time was much longer than in the LV, as mentioned above flow was strongly diminished during atrial systole. As a consequence, the relative

contribution of systolic flow to total flow was smaller in the LA, although it increased gradually with an increased heart rate. During atrial pacing, the LAWI increased in a cycle length dependent manner, in agreement with an earlier study in goats using distance-pressure loops for the RA that are similar to the LA loops in our study [28]. The increase in work index during pacing would be expected to increase atrial demand and thus lead to an increase in atrial coronary flow. Indeed, we observed a positive correlation between LA vascular conductance and work index.

The total amount of myocardial work performed is the sum of external and internal work. External work reflects mechanical work during atrial or ventricular contraction to pump blood and is the calculated as the area within a pressure-volume loop. Internal work is used to elongate elastic and viscous elements in sarcomeres and connective tissue and is therefore proportional to wall stress. In addition to mechanical work, energy is used within cells to maintain ion homeostasis.

The response time of coronary flow to an increase in heart rate was significantly slo-

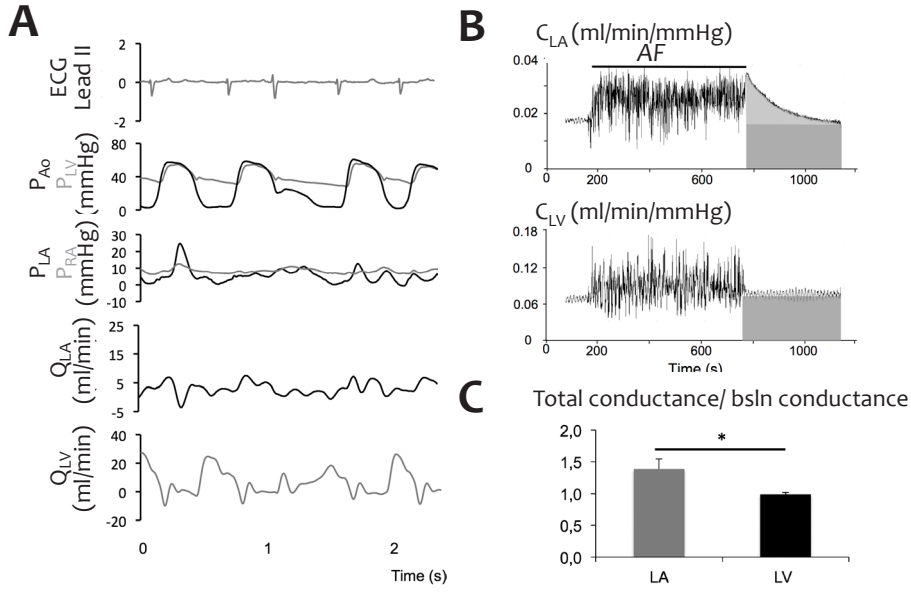


Figure 7: Atrial fibrillation. A) Example of flow signals during atrial fibrillation. B) Conductance in a LA and LV artery before, during and after an episode of acute AF. The hyperemic phase after cessation of AF was determined as the ratio of the time integral of the total flow (light gray + dark gray area) to the baseline flow (dark gray area). C) The hyperemic phase was significantly larger in the LA than in the LV (***** p=0.04).

wer in the LA than in the LV. This may indicate either a difference in the rate of metabolic changes, or a difference in the rate of response to a metabolic change. We have recently shown that compared to the LV, the LA has a lower coronary flow reserve, but a higher oxygen extraction reserve (due to a lower atrial extraction under baseline conditions). Pacing led to an increase in both atrial coronary flow and oxygen extraction [16]. Although the time resolution of oxygen extraction measurements was low, it is conceivable that in response to an increase in atrial demand, atrial oxygen extraction increases more rapidly than atrial coronary flow.

During intracoronary infusion of ACh, ventricular vascular conductance, ventricular contractility and the QT time were not affected, whereas the atrial APD, contractility and coronary flow were reduced. However, in the pig ventricle, ACh has a direct vasoconstrictor effect [29], and we have indeed observed a reduction in ventricular vascular conductance at much higher ACh concentrations. Therefore, our interpretation that the decrease in atrial work during ACh infusion causes the reduction in atrial flow would be incorrect if the atrial vasculature is more sensitive to ACh than the ventricular vasculature.

4.3. Atrial fibrillation

We have recently shown that AF causes an increase in atrial coronary vascular conductance in pigs [16]. Interestingly, White et al have reported that increasing wall stress by volume expansion to the same level observed during AF caused significantly less increase in atrial myocardial blood flow compared to AF [6]. This indicates that the total energy expenditure, rather than wall stress per se, is the major determinant of atrial coronary blood flow during AF. The rapid and irregular contraction of the atrial cardiomyocytes during AF are likely to contribute greatly to atrial energy expenditure, while virtually no useful external work is delivered.

The time required for atrial coronary blood flow to return to baseline values after AF was significantly longer than after atrial pacing with a basic cycle length of 400 ms. After a short episode of AF, atrial vascular conductance remained elevated above the baseline level for a prolonged time. This reflects a hyperemic phase after an episode of AF. We have shown previously that short-term AF causes a supply-demand mismatch, marked by an increase in atrial lactate production [16]. During AF, the active atrial pump function is abolished, but atrial myocytes are contracting rapidly, increasing

energy expenditure. We have showed that atrial contraction in itself impedes atrial flow. It could be expected that the local atrial contractions during AF impede atrial flow by local compression of the vessel wall. In addition, the diastolic phase, which normally provides most of atrial myocardial perfusion, is very short and irregular during AF. This resulting impediment of atrial flow may contribute to the occurrence of oxygen debt during AF and result in a hyperemic period after the return to normal sinus rhythm. After the cessation of AF, the impediment of atrial flow is relieved immediately while the oxygen debt persists, resulting in a transient elevation of vascular conductance.

4.4. Limitations

The experiments are performed in open-chest experiments under anesthesia. Opening of the pericardium may influence atrial wall stress and therefore atrial energy consumption and blood flow, as well as the mechanical interactions between the cardiac chambers [30]. Nevertheless, the waveform characteristics found in this study under open-chest conditions are very similar to the ones measured in humans using a Doppler guide-wire in a closed chest setting.

4.5. Clinical perspective

Regulation of the atrial blood supply is essential in maintaining the supply-demand balance, especially during periods of increased atrial demand such as atrial fibrillation. In this study, we have investigated the dynamic regulation of atrial coronary blood flow in healthy pigs. Atrial coronary flow is positively correlated to atrial workload, but responds more slowly to increased heart rate than ventricular flow. The atrial contraction itself strongly impedes atrial flow. After a period of acute atrial fibrillation, atrial flow shows a phase of reactive hyperemia, consistent with the occurrence of supply-demand ischemia. A fuller understanding of the conditions under which atrial supply falls short of demand may lead to novel strategies to prevent atrial remodeling and the progression of AF.

5. CONCLUSION

The atrial flow pattern showed a consistent decrease during atrial systole. This was caused by the atrial contraction itself rather than atrial pressure. Not only did atrial systole cause flow to decrease, it even reversed in most animals. The systolic phase contributes to only 2% of total atrial flow. However, the contribution of systolic flow fraction to total flow increased with increasing heart rate in both atrium and ventricle. Atrial vascular conductance is correlated with the performed external work. The flow response to acute changes in heart rate, however, was slower in the atrium compared to the ventricle. In addition, the occurrence of hyperemia after the cessation of short term AF in healthy pigs supports our earlier findings of a supply-demand mismatch in AF.

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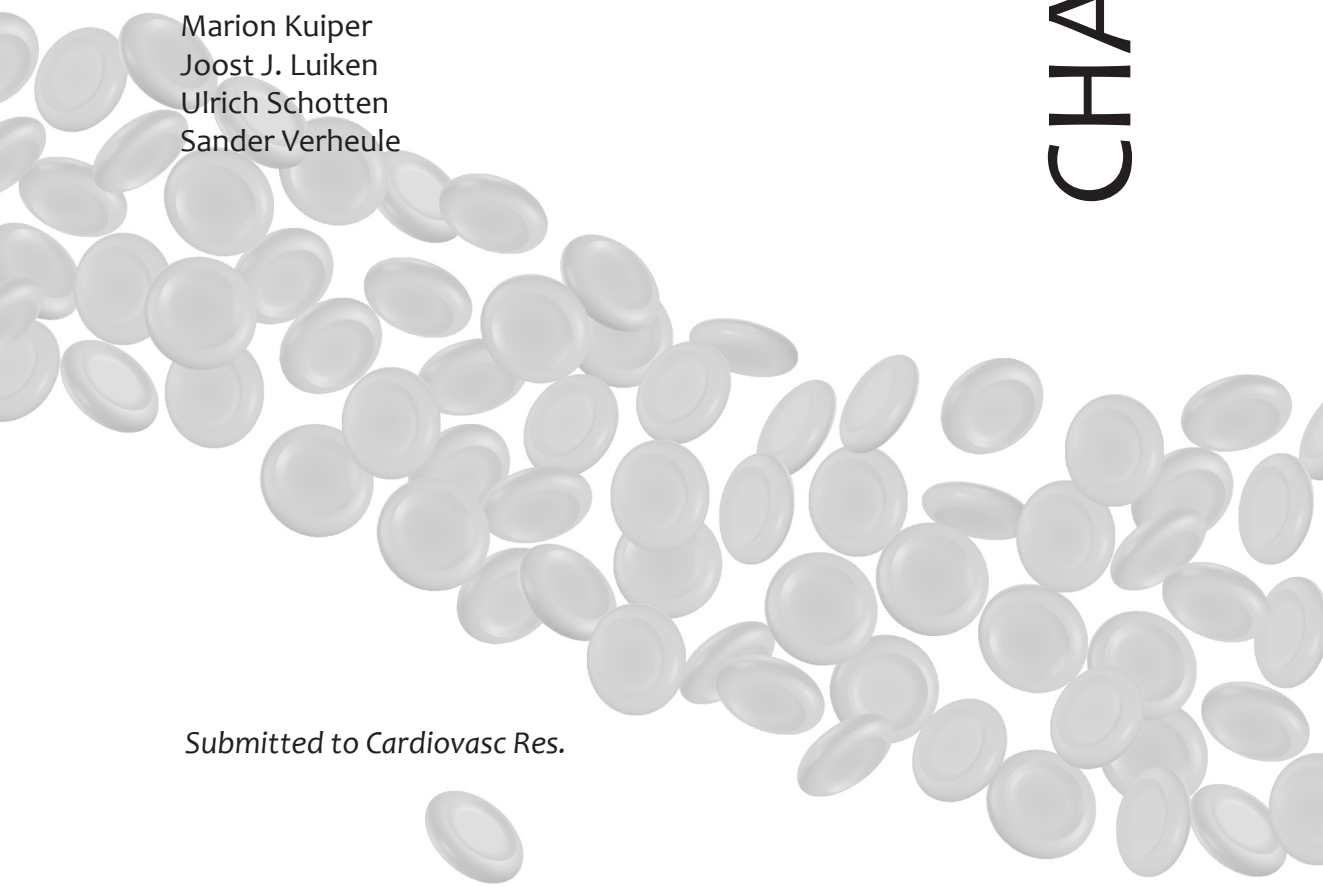
CHAPTER 6

The impact of persistent atrial fibrillation on the atrial supply-demand balance

Kelly van Bragt
Hussein M. Nasrallah
Marion Kuiper
Joost J. Luiken
Ulrich Schotten
Sander Verheule



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ABSTRACT

BACKGROUND: Atrial fibrillation (AF) leads to atrial remodeling that contributes to the perpetuation of the arrhythmia. We have recently shown that acute AF leads to supply-demand ischemia in pigs. Some aspects of atrial remodeling processes can be viewed as adaptation to energy shortage. Here, we have compared atrial coronary flow regulation, oxygen extraction and lactate production between control pigs and pigs with chronic AF.

METHODS AND RESULTS: AF was induced by rapid atrial pacing (RAP, using digoxin to control ventricular rate) in twelve Dutch Landrace pigs (62±3 kg) and compared to fifteen controls (61±2 kg). To characterize the model, alterations in AF stability, hemodynamics and tissue structure were assessed. Atrial and ventricular extraction, conductance and lactate were measured. Five weeks of RAP resulted in an increase in AF inducibility and duration. Myocyte hypertrophy in left and right atria (LA and RA) and increased interstitial fibrosis in the RA were observed. The capillary density was decreased by 51% in the LA and 57% in the RA. Coronary conductance reserve (determined as the response to intracoronary adenosine infusion) was not different between the groups. The response of atrial vascular conductance and oxygen extraction to atrial pacing and AF was similar between RAP and control pigs. The increase in lactate production that was observed after 5 minutes of AF in control animals was not present in the RAP group.

CONCLUSIONS: At 5 weeks of sustained AF in pigs, the supply-demand balance is restored and atrial ischemia during AF is prevented. Atrial remodeling can be seen as a successful adaptation to energy shortage.

1. INTRODUCTION

AF is the most common tachyarrhythmia in clinical practice. AF is self-perpetuating since it causes atrial remodeling processes that in turn increase AF stability. Rapid atrial pacing (RAP), either with or without ventricular rhythm control, has been widely used in animal models to represent AF without preexisting structural heart disease. In these models, different aspects of atrial remodeling have been described. Electrical remodeling, i.e. a shortening of the atrial action potential duration (ADP) and effective refractory period (AERP) [1, 2], is a relatively fast process, developing over 1-2 days. In a goat model of AF, electrical remodeling increased the duration of induced AF episodes from seconds to minutes. The underlying downregulation of the L-type calcium current (ICa_L) not only contributes to APD shortening, but also to the observed reduction in atrial contractility, because entry of calcium through ICa_L initiates the contraction [3,4]. Structural remodeling is a much slower process, evolving gradually over a time course of weeks to years [5,6]. The AF-induced structural changes include myocyte hypertrophy, glycogen accumulation, loss of sarcomeres (myolysis), alterations in connexin expression, changes in mitochondrial shape, fragmentation of sarcoplasmic reticulum, redistribution of nuclear chromatin and changes in quantity and localization of structural cellular proteins [7]. Ausma et al have described these changes as 'hibernation', proposing that they reflect dedifferentiation into a more fetal phenotype, and resembling the changes in ventricular myocytes during chronic low flow ischemia [6, 8, 9].

We have recently shown that acute AF in pigs leads to an increase in atrial coronary flow and oxygen extraction. Nevertheless, supply-demand ischemia occurred within minutes, as evidenced by increased atrial lactate production (CHAPTER 4) [10]. On the longer term, the occurrence of atrial ischemia during AF is supported by decreased phosphocreatine levels in the first weeks of AF in goats [11,12]. In addition, markers of hypoxia and angiogenesis ($HIF_{1\alpha}$, $HIF_{2\alpha}$, VEGF) are increased in goats with AF and in AF patients [13,14]. Some aspects of atrial remodeling can be viewed as adaptation to energy shortage, i.e. decreased APD and contractility and hibernation of myocytes.

The effect of chronic AF on atrial vascular structure and atrial supply-demand balance has not been investigated extensively. Here, we have investigated the effect of 5 weeks sustained AF on atrial vascular structure, function, oxygen extraction and lactate production.

2. METHODS

2.1. *Model*

Twelve Dutch Landrace pigs (62±3 kg), were anesthetized as described below, intubated and mechanically ventilated. An endocardial lead (J-Leads Capsurefix 5568 - 53 cm, Medtronic Inc, Minneapolis, Minnesota) was implanted in the right atrium and connected to a subcutaneous pacemaker (Itrel II, Medtronic, Minneapolis, MN). Pigs were allowed one week recovery after implantation before the pacemaker was switched on at a rate of 10 Hz for 5 weeks (rapid atrial pacing, RAP). Ventricular rate was controlled by digoxin 10 gμg/kg for 1 week, followed by 5 μg/kg for 4 weeks (Timeline Figure 1A). Digoxin was stopped 3 days before the sacrifice experiment in order to reach plasma levels <0.5 μg/ml during the measurements. Fifteen healthy control pigs (61±2 kg) served as a control group. All animal procedures were conducted in accordance with institutional and national guidelines.

2.2. *Study protocol*

For open-chest sacrifice experiments, anesthesia was induced with Zoletil (5-8 mg/kg I.M.) and Thiopenthal (5-15 mg/kg I.V.) and maintained with an intravenous infusion of Midazolam (1.0 mg/kg/h), Sufentanyl (4 mg/kg/h) and Propofol (2.5-10 mg/kg/h). Cardiac output was measured during normal sinus rhythm (NSR) using thermodilution with a closed chest to compare control and RAP animals. Also aortic pressure (P_{Ao}) and left ventricular pressure (P_{LV}) were recorded during NSR with a closed chest. From these signals, end-diastolic pressure, heart rate, dP/dt max and mean P_{Ao} were calculated offline using custom software.

All RAP pigs converted to NSR spontaneously during the induction of anesthesia. A left lateral incision was made, the 5^{th} rib was removed and the pericardium was opened to expose the left side of the heart for further instrumentation. Subsequently, monophasic action potentials were recorded using a MAP-4801 monophasic action potential and stimulation catheter (7 FR, Hugo Sachs Elektronics, Harvard Apparatus, March-Hugstetten, Germany) and the action potential duration (APD) was calcula-

ted. The APD50 and APD80 were calculated to determine the degree of electrical remodeling in the RAP pigs. They reflect the time for the action potential to repolarize to 50% and 20% of the maximum amplitude respectively. In addition, atrial epicardial conduction was measured on the LA and RA free walls using a round high density mapping electrode array of 4 cm in diameter, consisting of 234 unipolar recording electrodes with an interelectrode distance of 2.4 mm, connected to a 256-channel amplifier (sampling rate 1 kHz, filtering bandwidth 0.5-500 Hz). AF was induced 5 times by 5 s 20 Hz burst pacing at 4 times stimulation threshold in the RA. Sustained AF was defined as a rapid, irregular atrial activation pattern lasting more than 5 min. AF was terminated by electrical cardioversion if it lasted > 30 min. Using custom software, time points of maximum negative dV/dt were determined for each electrode to determine local activation times. After epicardial mapping, pigs were randomly assigned to experimental series A or B or both experiments were performed in succession. In both series A and B, an ablation catheter in the RA was used for pacing and AF induction. During NSR, pacing at basic cycle lengths (BCL) of 500, 450 and 400 ms and during acute AF, atrial and ventricular coronary blood flow (series A) or oxygen extraction and lactate production (series B) were investigated.

Series A: Coronary vascular conductance

RAP (N=8) and control (N=9) animals were instrumented with a Doppler flow probe (Transonic Systems Inc, Ithaca, NY) around a left atrial (LA) and left ventricular (LV) branch of the left circumflex artery (LCx), as previously described (CHAPTER 4) [10]. Conductance was calculated by dividing the flow in the atrial (Q_{LA}) and ventricular (Q_{LV}) branch by the pressure difference over the vascular bed (P_{Ao}-P_{RA}). To calculate the pressure difference, a right atrial pressure (P_{RA}, micro tip pressure sensor, Millar Instruments, Houston, TX) and aortic pressure (P_{AO}, pressure catheter, Sentron Europe BV, Roden, The Netherlands) were recorded simultaneously with the Doppler flow signals. In addition, left ventricular pressure (P_{LV}) was measured with a Sentron conductance catheter (Sentron Europe BV, Roden, The Netherlands). The maximal vascular conductance was calculated during a 10 ml intracoronary bolus of 300 µg adenosine infusion as described previously (CHAPTER 4) [10] and was used to determine the coronary conductance reserve (CCR): CCR = maximal conductance (C_{MAX}) / baseline conductance (C_{BSLN})

Series B: Extraction and lactate levels

Blood was collected from an atrial and ventricular vein in RAP (N=9) and control (N=7) animals as was previously described (CHAPTER 4) [10]. Arterial blood was sampled from the LV through a double AO/LV Millar pressure catheter (Millar Instruments, Houston, TX). An i-Stat analyzer (Abbott Laboratories, Illinois, U.S.A, with EG7+ cartridges) was used for blood gas analysis of venous and arterial blood samples. Blood lactate levels were measured in the same blood samples using a Lactate SCOUT+ (Senslab GmbH, Leipzig, Germany). Total arterial and venous oxygen content (CaO_2 and CvO_2), oxygen extraction ratio, extraction reserve and lactate production were calculated using the following formulas:

- CaO_2 / CvO_2 (ml O_2/100ml) = (1.36 x O_2 saturation (%) x Hemoglobin (g/dL)) + (0.0031 x partial O_2 pressure (mmHg))
- Extraction ratio = (CaO_2 –CvO_2)/CaO_2 *100
- Extraction reserve = CaO_2/(CaO_2-CvO_2)
- Lactate production = arterial lactate concentration - venous lactate concentration

2.3. *Histology*

After the open chest experiment, the heart was rapidly excised and rinsed with saline solution. Tissue samples of the LA and RA were stored in 4% formaldehyde for at least 24h, embedded in paraffin and cut into 5 µm tissue sections. Heamatoxilin & Eosin (H&E), and Sirius Red staining were performed to measure cell diameter and interstitial fibrosis, respectively, and to provide information on the degree of atrial structural remodeling. Cell diameter was analyzed on one H&E section (3 to 4 locations per slide) at a 400x magnification in transversely cut cardiomyocytes at the level of the nucleus. Overall fibrosis was measured in 3 to 4 locations per Sirius Red-stained slide at 200x magnification. For this analysis, the epicardial and endocardial fibrous layer and perivacular fibrosis were excluded, and a color threshold was set to determine the relative surface area of red pixels (fibrosis) per tissue area. From the same Sirius Red stanings, larger vessels were photographed at higher magnification. In these images, the degree of perivascular fibrosis was quantified as the area of fibrous

tissue around a vessel to the area of the vessel wall. Griffonia Simplicifolia I (GSI) staining was used to label vessels and thus quantify capillary density. Vessels were detected using a trainable Weka segmentation software, and counted using 'particle analysis' function in Fiji (www.fiji.sc/Fiji). All other histological analyses with ImageJ (http://fiji.sc/ImageJ).

2.4. *Statistical analysis*

To compare mean values for pressures, heart rate, body and heart weight, AF parameters, CCR and extraction reserve between control and RAP animals, a t-test was used. Histological parameters were analyzed with a mixed model analysis. AF inducibility was tested with a χ^2 test. AF duration was analyzed using a Mann-Whitney U test. Values are expressed as mean ±SEM.

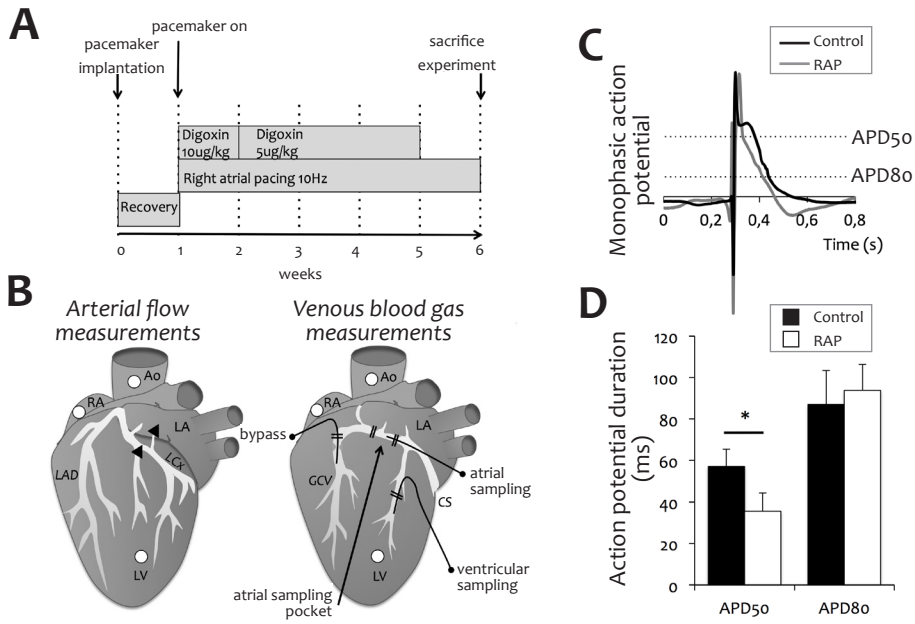

Figure 1: Rapid atrial pacing model. A) Time line of the protocol. B) Graphic representation of arterial flow measurements (left) ad venous blood gas measurement (right). Ao= aorta, RA=right atrium, LA=left atrium, CS=coronary sinus, GVC=great cardiac vein, LV=let ventricular, white circles indicate pressure catheters, black triangles represent flow probes. C) Representative action potential of control (black) and RAP (grey) pig and D) Calculation of action potential duration (APD) at 50% and 80% recovery in control (black, N=5) and RAP (white, N=6).

3. RESULTS

3.1. Characterization of the RAP model

3.1.1. Hemodynamic parameters

A comparison of hemodynamic parameters in control and RAP pigs is provided in Table 1. There were no significant differences between the two groups in body weight, heart weight, cardiac output, heart rate and end-diastolic pressure. Mean aortic pressure and left ventricular dP/dt max were significantly lower in the RAP group compared to control.

3.1.2. Electrophysiological parameters

The morphology of monophasic action potentials differed between control and RAP animals (Figure 1C). Although the APD80 was similar, the plateau phase was less pronounced in the RAP group, reflected in a significant decrease in APD50 (Figure 1C and D; 36±9 ms in RAP (N=6) vs. 57±18 ms in control (N=5), p=0.03).
In the RAP group the average for the longest AF episode observed in each animal was significantly higher than in the control group (Figure 2A and 2B). AF was not

	Control	RAP	P-value
Body weight (kg)	61 ± 2	62 ± 3	ns
Heart weight (g)	352 ± 21	362 ± 20	ns
Heart weight/ body weight (g/kg)	5.8	5.8	ns
Heart rate (bpm)	78 ± 6	69 ± 2	ns
Cardiac output during NSR (ml/min)	4.93 ± 0.48	4.24 ± 0.26	ns
Mean aortic pressure (mmHg)	83.0 ± 3.8	63.9 ± 1.5	p<0.001
dP/dT_{max} (mmHg/s)	1861 ± 127	1053 ± 21	p<0.001
End diastolic pressure (mmHg)	11.8 ± 1.4	12.0 ± 1.3	ns

Table 1: Characteristics of control (N=15) and RAP (N=12) pigs. Values expressed as mean±SEM. *P<0.05 control vs. RAP.

sustained in any of the animals in the control group, but was sustained (i.e. lasting > 5 min) in 11 out of 12 RAP pigs (Figure 2B). Although AF was sustained in the RAP group, the average AF cycle length was longer in the RAP group than in the control group, both in the LA (219±10 vs 153±18 ms respectively, p= 0.002) and RA (208±14 vs 159±26 ms respectively, p=0.046) as shown in Figure 2C.

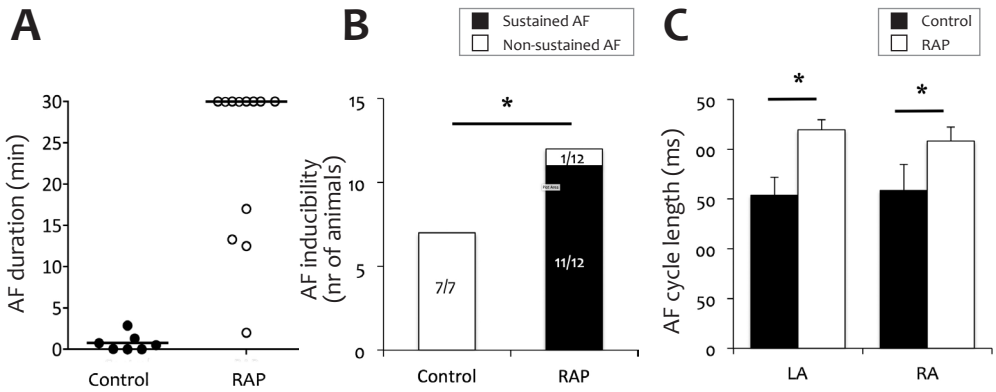

Figure 2: Characteristics of atrial fibrillation (AF). A) AF duration in control (black, N=7) vs. RAP (white, N=12). B) AF inducibility in control (N=7) and RAP (N=12). C) AF cycle length in control (black, N=5) and RAP (white, N=9). Values for AF cycle length in mean±SEM, * p<0.05 control vs. RAP
0.05 control vs. RAP

3.1.3 Histological parameters

RAP resulted in atrial hypertrophy (Figure 3A) in both the left and right atrium. Cell diameter was increased significantly from 10.8±0.6 µm (N=10) in the LA of control animals to 12.5±0.7µm (N=11) in the LA RAP animals (p=0.032). In the RA, cell diameter was 10.9±1.9µm (N=8) in the control group compared to 16.2±1.2µm (N=11) in the RAP group (p=0.009). RAP resulted in the induction of fibrosis formation (Figure 3B). Interstitial fibrosis (%) per myocardial area in the LA of AF animals was not significantly different from control (p=0.35). However, in the RA, a significant increase in interstitial fibrosis was found in the AF animals (23±2%, N=11) compared to control animals (16±2%, N=8, p=0.01).

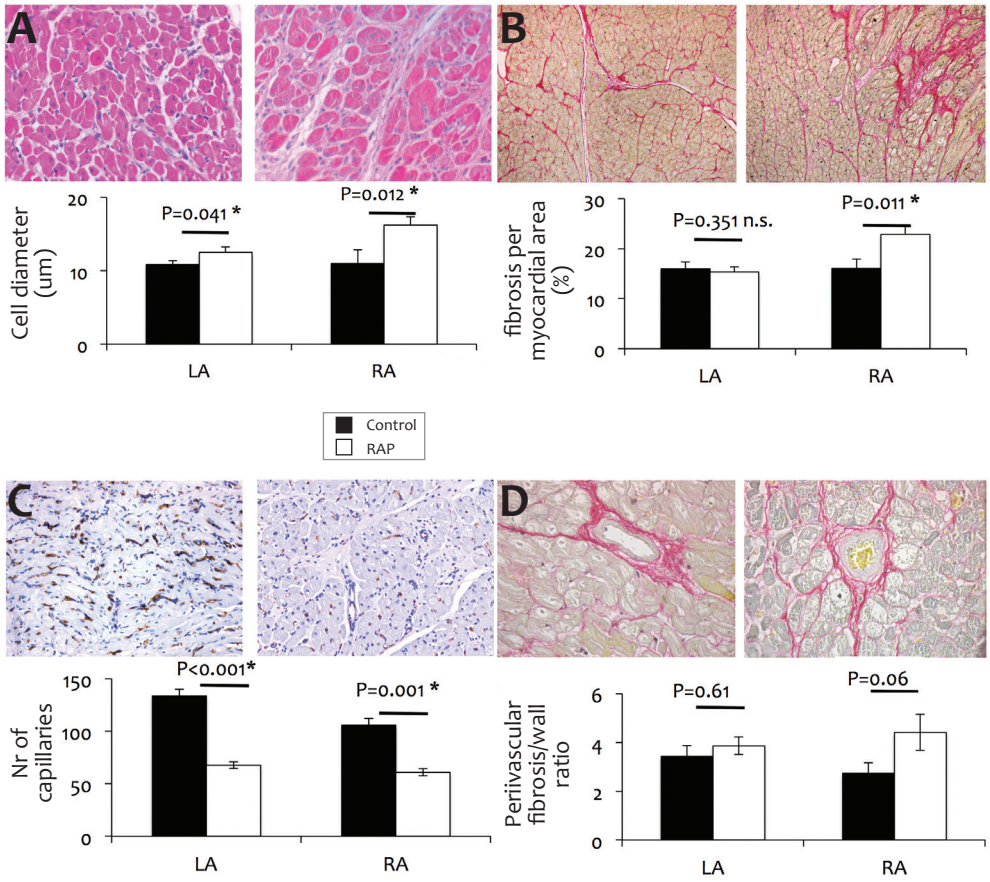

Figure 3: Histology. A) Representative images of Hematoxiline & Eosine staining in the right atrial (RA) of control (left) and RAP (right) and calculation of cell diameter (µm) in left (LA) and right (RA) atrium. B) Representative images of Sirius Red staining in the right atrial (RA) of control (left) and RAP (right) and calculation of interstitial fibrosis per myocardial area (%) in left (LA) and right (RA) atrium. C) Representative images of CD36 staining in the right atrial (RA) of control (left) and RAP (right) and calculation of capillary density per field of view in left (LA) and right (RA) atrium. D) Representative images of Sirius Red staining in the right atrial (RA) around small vessels of control (left) and RAP (right) and calculation of perivascular fibrosis/wall area ratio in left (LA) and right (RA) atrium. P<0.05 control vs. RAP

3.2. *Vascular structure and function*

3.2.1. Vascular remodeling

A histological analysis was performed to identify changes in vasculature structure induced by long-term AF. There was a significant decrease in the capillary density (the number of vessel per field of view) in both the LA and RA of RAP pigs compared to control (Figure 3C $p<0.001$). Larger arteries with sizes in the range of conduit arteries and resistances arterioles were photographed separately at higher magnification to quantify perivascular fibrosis (Figure 3D). The ratio between the areas occupied by perivascular fibrosis and vascular wall was not different between the groups for the LA ($p=0.61$), although there was a trend towards an increase in the RA ($p=0.06$).

3.2.2. Atrial coronary flow regulation

In both groups, the phasic pattern in the atrial coronary flow showed a decrease during atrial activation (Figure 4A and B). However, this decrease was smaller in the RAP group than in the control group. In 7 out of 9 control animals, atrial flow reversed during atrial contraction, whereas a reversal of atrial flow was only observed in 2 out of 7 RAP pigs. In accordance with this observation, the systolic flow fraction (i.e. the relative contribution of systolic flow to total flow) was higher in the RAP group than in the control group (Figure 4C, $p=0.048$).

Flow measurements with Doppler flow probes were performed in comparable atrial branches supplying the LA free wall in all animals. The baseline flow during NSR was not different between the control and RAP groups (3.5±0.9 ml/min vs. 3.3±0.8 ml/min, $p=0.43$). Flow during maximum vasodilatation (i.e. infusion of adenosine into the circumflex artery) was similar between the control and RAP pigs (7.2±1.5 ml/min vs. 11.0±3.9 ml/min, $p=0.16$). The coronary conductance reserve was calculated from the LA and LV vascular conductance (i.e. $Q/(P_{Ao}-P_{RA})$ at baseline and maximum dilatation. Both the LA (1.76±0.14 vs. 2.41±0.57 ml/min/mmHg respectively, $p=0.3$) and LV CCR (3.16±0.27 vs. 4.24±0.64 ml/min/mmHg, $p=0.2$) were not different between the control and RAP group (Figure 4D).

We have shown before that in normal pigs, the external work performed by the atrium increases during atrial pacing (CHAPTER 5) [15]. To investigate the effect of

increased workload on vascular conduction in both groups, the atrium was paced at 500, 450 and 400 ms. Normalized to the vascular conductance during NSR, there was a significant increase at 450 and 400 ms in both the control and RAP groups (Figure 5A). This response was not different between the groups (p=0.4). In the LV, pacing had a larger effect on the conductance in RAP animals compared to control (p<0.0001). In both RAP and control animals pacing induced a significant increase in LV conductance at every pacing rate (Figure 5B). During AF, atrial conductance in RAP animals showed a similar increase as in control animals (p=0.2, Figure 5C). In the LV, vascular conductance increased during AF in the RAP group, but not in the control group (Figure 5D).

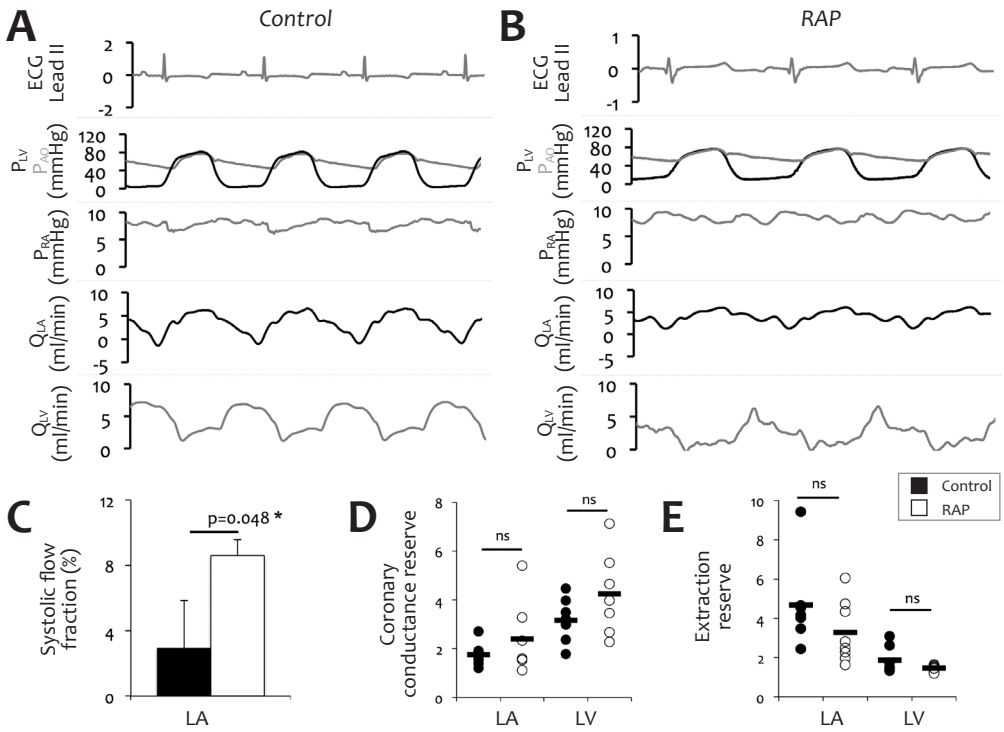

Figure 4: Analysis of coronary flow, conductance and extraction. Representative flow signals of A) control and B) RAP pigs. PAO=aortic pressure, PLV=left ventricular pressure, PRA=right atrial pressure, QLA= left atrial flow, QLV=left ventricular flow. C) Calculation of the contribution of flow during systolic flow to total flow in control (black, N= 9) and RAP (white, N=7). Values in mean±SEM, *p<0.05 control vs. RAP. D) Coronary conductance reserve and E) extraction reserve in control (black) and RAP (white) in the left atrium (LA) and left ventricle (LV)

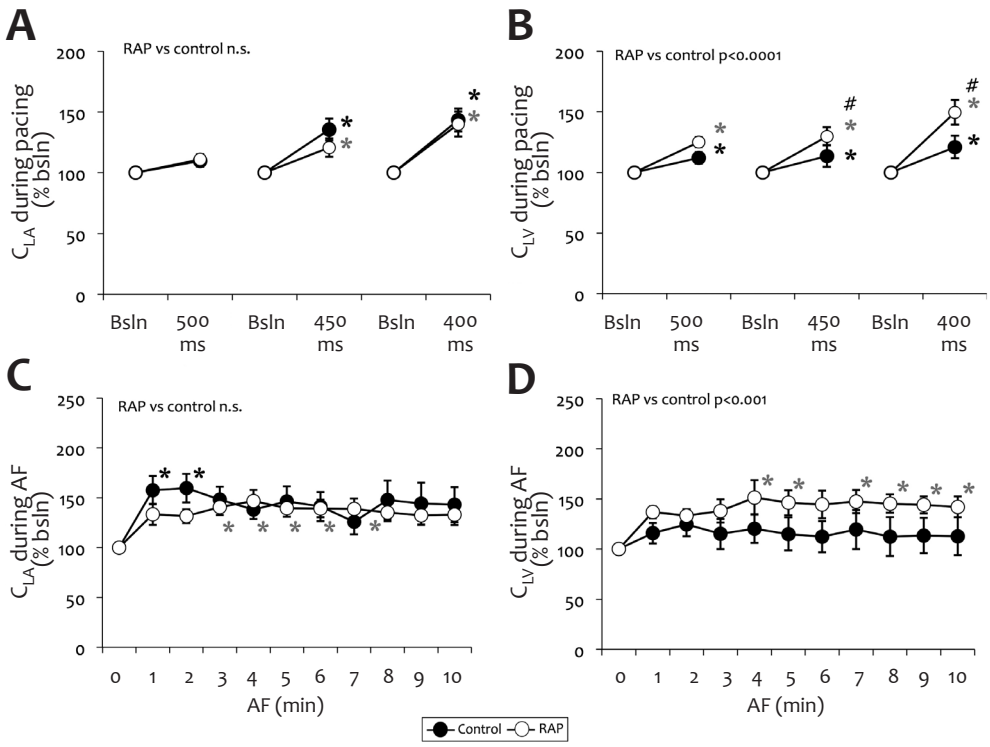

Figure 5: Coronary conductance during pacing and AF. A) Left atrial conductance (C_{LA}) and B) left ventricular conductance (C_{LV}) during pacing at 500, 450 and 400 ms expressed as % of baseline in control (black, N= 9) and RAP (white, N=7). C) C_{LA} and D) C_{LV} during 10 min of AF expressed as % of baseline in control (black, N=9) and RAP (white, N=6). Values are expressed as mean ±SEM, black * p<0.05 vs. baseline for control, grey * p<0.05 vs. baseline for RAP, # p<0.05 control vs. RAP

3.2.3. Atrial oxygen extraction and lactate production

As depicted in Figure 4E, the oxygen extraction reserve tended to be higher in the control group than in the RAP group in the LA (4.68±0.84 vs. 3.29±0.55, p=0.2) and LV (1.88±0.26 vs. 1.47±0.05, p=0.2).

Oxygen extraction was also measured in the LA and LV during pacing at 500 ms, 450 ms and 400 ms and during AF (Fig. 6A and 6B). At 400 ms BCL and AF, a significant increase in atrial extraction was seen in control animals at 5 and 10 min. In the RAP animals, there was also a trend towards an increase in atrial extraction, but this was

not significant at any intervention. There was also no significant difference found between the two groups. Ventricular extraction was not affected in control nor in RAP animals.

Lactate concentrations were measured during the same interventions. During AF, the arterio-venous lactate difference was significantly increased in the LA of control animals from 0.17±0.17 mmol/l to 1.22±0.48 mmol/l at 5 min and 1.48±0.44 mmol/l at 10 min (Figure 7A). This was significantly lower in the atrium of RAP pigs. In the RAP group, arterio-venous lactate difference was 0.20±0.12 mmol/l at baseline and did not significantly increase at 5 min (0.28±0.07 mmol/l) and 10 min (0.43±0.18 mmol/l) of AF. In the ventricle, no increase in arterio-venous lactate difference was observed in control or RAP group under any intervention (Figure 7B).

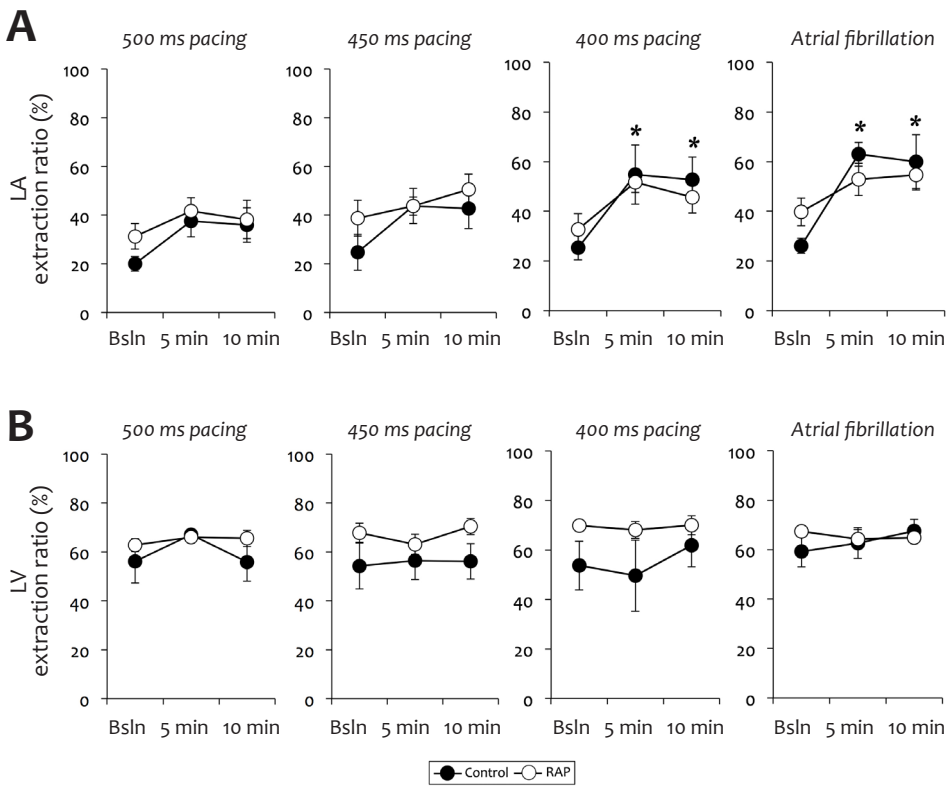

Figure 6: Extraction during pacing and AF. A) Left atrial (LA) and B) left ventricular (LV) extraction in control (black, N=7) and RAP (white, N=8). * Values are expressed as mean ±SEM, p<0.05 vs. baseline.

4. DISCUSSION

4.1. RAP in pigs as a model for AF

In this study, we have used rapid atrial pacing in pigs as a model for AF without preexisting structural heart disease. Although dogs and goats with RAP have been more extensively characterized as models for AF, atrial coronary vessels are poorly accessible in goats and there are significant differences in (ventricular) coronary anatomy and regulation between dogs and humans [16-18]. Pigs were chosen as a species because the anatomy and regulation of the coronary vasculature is known to be similar to humans [18-20]. RAP in goats without ventricular rate control does not lead to congestive heart failure (CHF) within 6 months [21]. By contrast, dogs [22] and pigs [23] do develop CHF as a result of RAP, evidenced by a decrease in LV ejection fraction, cardiac output, an increase in LV end diastolic, LA and RA pressures, along with clinical signs of CHF [23, 24]. The heart weight to bodyweight is reported to increase from 4.9±0.6 to 7.3±0.4 after RAP in dogs [23]. CHF by itself, in the absence of AF, can also cause structural changes in the atrium [25]. In order to prevent the development of CHF, pigs received oral digoxin to inhibit AV conduction. In the RAP group, two pigs showed mild ascites, while all other clinical signs were normal in all pigs. Cardiac output was measured with closed chest and was not decreased compared to the control group. Heart weight to total body weight ratio was 5.8 in the control group, and not altered in the RAP group. These parameters indicate that the development of CHF is at most modest. However, mean arterial pressure and the ventricular dP/dt max, a measure for LV contractility, were significantly decreased in the RAP group, indicating a degree of ventricular systolic dysfunction, although the cardiac output was maintained.

In several species, AF (artificially maintained by RAP) leads to electrical and structural remodeling, resembling the remodeling processes in AF patients [5]. In goats, the relatively fast process of electrical remodeling (1-2 days) leads to a decrease in APD, ERP and a concomitant decrease in AFCL [1,2,26]. Similarly, APD, ERP and AFCL decreased in a dog model of RAP with controlled ventricular rate (AV block + ventricular pacing) [27]. The decrease in APD during electrical remodeling coincides with a down-regulation of ICa_L and I_{to} currents [1,28,29] but also an upregulation of I_{K1} current [30]. Mathematical modeling suggests the latter is the most important determinant of APD shortening[30].

The decrease in atrial contractility during electrical remodeling closely parallels the decrease in ICa_L current density [3]. The relatively slow process of structural remodeling as a result of RAP has also been characterized in several species. The presence of fibrosis is different in different models of AF. In dogs, 6-8 weeks of RAP with a controlled ventricular rate (AV block + ventricular pacing) did not lead to an increase in overall fibrosis [27]. However, an increase in LA and RA interstitial fibrosis and extracellular matrix proteins was described previously in a pig model after 6 wks of RAP with controlled ventricular rate (digoxin) [25]. RAP without controlled ventricular rate did lead to degenerative structural remodeling in dogs, including fibrosis [31]. In goats, overall fibrosis does not increase as a result of RAP, but interstitial fibrosis in the epicardial layer does increase [32]. Also in the goat model atrial structural changes

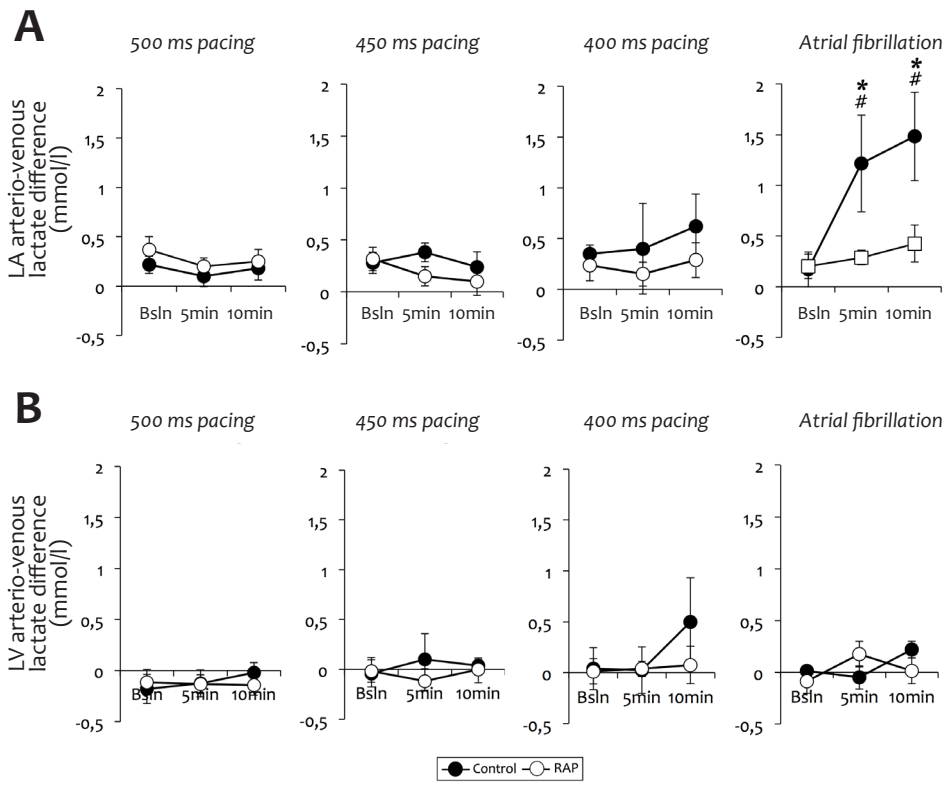

Figure 7: Lactate production during pacing and AF. A) Left atrial (LA) and B) left ventricular (LV) arterio-venous lactate control in control (black, N=7) and RAP (white, N=8) during pacing and AF. * Values are expressed as mean ±SEM, $p<0.05$ vs. baseline, #$p<0.05$ control vs RAP.

seem to depend to some extent on a rapid ventricular response [22].
In this study, we show a change in the action potential morphology, a decrease in the plateau phase, reflected in a decrease in APD50, without a significant change in APD80. AF episodes were very short in the control group, but sustained AF was seen in all but one RAP animals, and AF inducibility was increased in the RAP group, as in a previous study in pigs [33]. Increased inducibility of AF and AF duration were also previously shown in dogs [27,33] and goats [2] as a result of RAP. Despite the increase in AF stability and inducibility, AFCL was increased. Our data indicates that electrical remodeling is not a major factor in AF stabilization in pigs within 6 weeks of RAP. A discrepancy in time course of electrical remodeling and AF stabilization was already shown earlier by Wijffels et al [2]. Structural remodeling was also seen to some extend in our 6 months RAP model. Myocyte hypertrophy was present in both atria and interstitial fibrosis was significantly increased in the RA, but not in the LA. As atrial fibrosis seems to be related to ventricular rate, the level of digoxin in the pig model could influence the degree in atrial fibrosis found.

4.2. Atrial supply-demand balance

We have previously shown that acute AF in normal, anesthetized pigs leads to supply-demand ischemia, as evidenced by an increase in atrial lactate production within 5-10 min of AF (CHAPTER 4) [10] although atrial vessels dilated and oxygen extraction increased. Apparently, the increased demand caused by rapid rates of electrical activation and mechanical contraction during acute AF cannot be accommodated by the increase in supply. Several studies in other species support the occurrence of atrial ischemia during AF. Atrial creatine phosphate levels are decreased in the first weeks of AF [11, 12]. In addition, the atria produce markers of hypoxia and angiogenesis (e.g. $HIF_{1\alpha}$, VEGF, KDR) [14, 34-36]. During AF, a more indirect argument is that many aspects of atrial structural remodeling resemble ventricular hibernating myocardium resulting from chronic low-flow ischemia [6,9].
Adaptation to supply-demand ischemia may involve a decrease in atrial energy/oxygen demand and/or an increase in atrial energy/oxygen supply. In this study, we have investigated both factors by measuring vascular remodeling, coronary blood flow, oxygen extraction and lactate production.
The atrial flow signal in the RAP model was similar to that in controls. However, the

decrease in atrial flow during atrial activation was smaller in the RAP group compared to control. The phasic decrease in the atrial flow pattern is caused by the atrial contraction itself (CHAPTER 5) [15, 37, 38]. AF causes contractile remodeling, resulting in a decrease in contractile force [3, 39, 40]. Although APD80 did not decrease in RAP pigs, the decrease in APD50 reduced plateau potential is consistent with a decrease in calcium current amplitude and thus a reduced contractile force. This would explain the alteration in atrial flow pattern and increased contribution of flow during atrial systole in the RAP group. As an important consequence, a reduction in atrial contractility would decrease the atrial demand.

To assess atrial coronary supply, we have investigated both atrial vascular structure and regulation. Fibrosis around conduit/resistance arterioles may reduce their capacity to dilate. However, the degree of perivascular fibrosis around conduit/resistance arterioles was not significantly different between the control and RAP groups. The capillary density was significantly lower in both atria in the RAP group. Clinical studies on capillary density showed a decreased capillary density in AF patients compared to controls in the sinus node area [41], LA appendage, free wall [42] and posterior wall [43]. A decreased capillary density increases oxygen diffusion distances [42] and could exacerbate atrial ischemia during AF. The decrease in capillary density may seem at odds with the increase of angiogenic markers that has also been observed in AF patients. However, the stability of newly formed capillaries is influenced by growth factors such as angiopoietin-1 and -2 (Ang-1 and Ang-2). In $HIF_{1\alpha}$/VEGF-induced vessel formation, Ang-1 favors endothelial stability by interaction with pericytes, whereas Ang-2 promotes new vessel sprouting and facilitates the actions of VEGF [44]. Plasma levels of Ang-2 were found to be increased in AF patients, which would shift the balance to endothelial destabilization and capillary rarefaction [36].

Our functional measurements indicate that vascular regulation is intact after 5 weeks of RAP. The vasodilator response to adenosine and coronary conductance reserve were not different between the control and RAP groups. During pacing and AF, the LA vascular conductance and oxygen extraction both increase, indicating that these interventions cause an increase in atrial demand and a concomitant increase in supply. In control pigs, the mobilization of conductance and extraction reserve was insufficient to prevent supply-demand ischemia, and atrial lactate production increased within 5-10 minutes of acute AF. By contrast, a similar recruitment of conductance and extraction reserve in RAP is sufficient to prevent lactate production. This

supports the hypothesis by Ausma et al that the remodeling processes taking place in the atria during prolonged AF represent energy saving mechanisms [6, 8]. Indeed, in a goat model of AF, the phosphocreatine level is decreased and $HIF_{1\alpha}$ expression is increased in the first weeks of AF, but they subsequently recover [12, 13]. Remodeling processes in the atrium thus lead to an amelioration of supply-demand ischemia, and can be viewed as a successful adaptation to energy shortage during AF. The loss of capillary density may become particularly relevant in patients with paroxysmal AF. Paroxysms of AF may cause vascular remodeling and capillary rarefaction. In a study on AF patients (with 2 or more episodes of AF in the previous year), Skalidis et al did indeed observe a decrease in coronary reserve compared to controls [45]. In between paroxysms, the atria rapidly recover from electrical remodeling and atrial contractility recovers to a large degree [3], which would increase atrial energy expenditure.

5. CONCLUSIONS

Five weeks of chronic AF in pigs results in atrial structural remodeling. Electrical remodeling was investigated and indicates that the decrease in APD and AFCL is not a prerequisite for increased AF stability. Functional flow measurements show that vascular regulation is intact after 5 weeks of RAP, despite the observed decrease in capillary density. The coronary conductance reserve was comparable between the control and RAP groups. During pacing and AF, the LA vascular conductance and oxygen extraction both increase similarly to control pigs. As opposed to acute AF in controls, chronic AF does not result in lactate production with a similar increase in atrial flow and extraction. Atrial remodeling can be seen as an energy saving adaptation to supply-demand ischemia.

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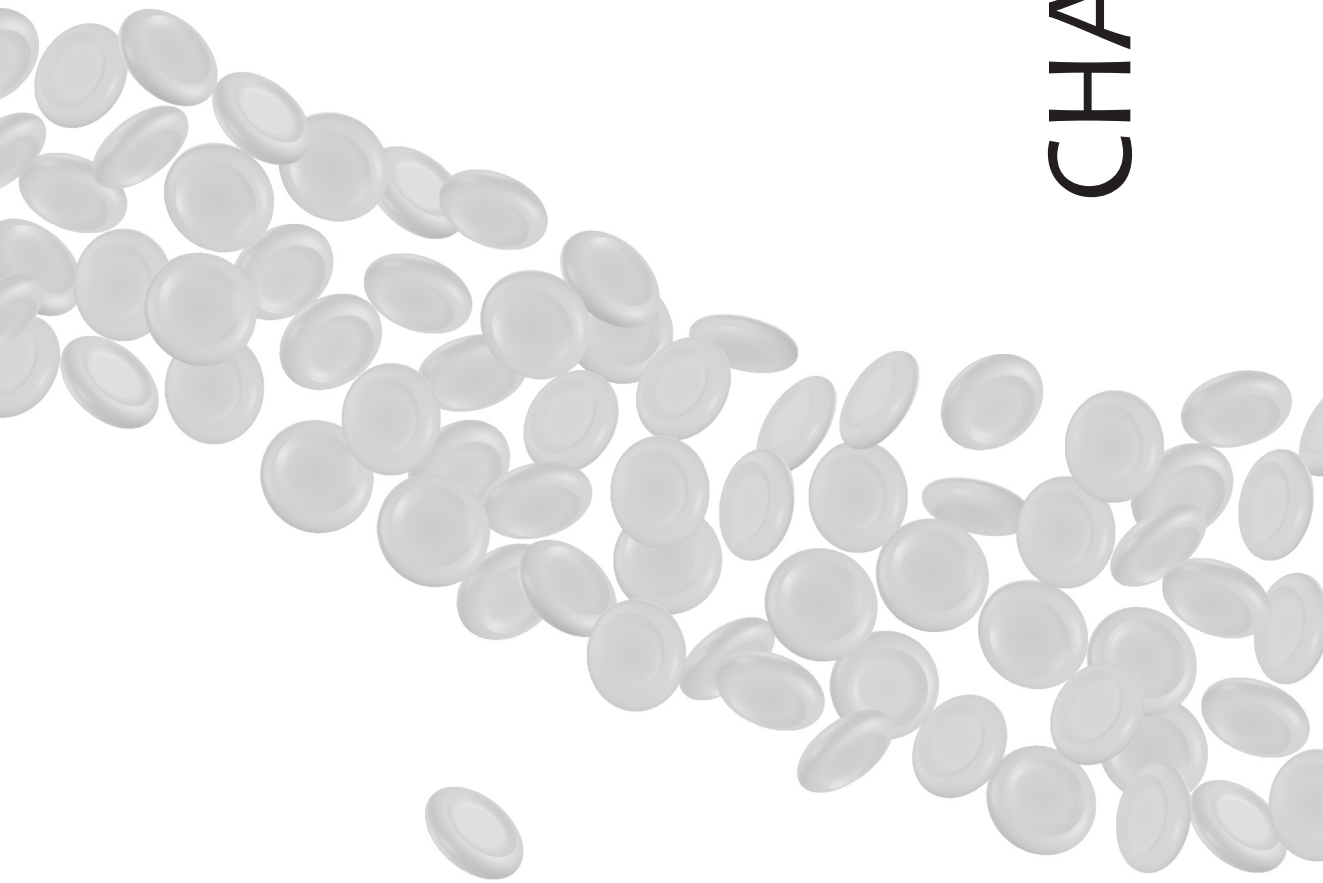
CHAPTER 7

General discussion

Kelly van Bragt



1. INTRODUCTION

Atrial fibrillation is the most common cardiac arrhythmia in clinical practice. It is a self-perpetuating disease. In AF patients, structural, electrophysiological and contractile changes are observed. Action potential duration and effective refractory period (ERP) are shortened, and the rate-dependent effect on ERP is lost in human atria during AF [1]. In addition, short-term AF (minutes) in humans was shown to be sufficient to cause contractile dysfunction [2]. Fibrosis may [3-5] or may not [6] be significantly increased in AF patients. Other structural changes as a result of AF include e.g. mitochondrial abnormalities, glycogen accumulation, change in structure of the sarcoplasmic reticulum, loss of myofibrils [7, 8]. The structural remodeling process is an important determinant in AF stability [9, 10]. However, the origin of these structural changes is not completely understood.

Ausma et al were the first to point out that the changes in atrial ultrastructure resemble the changes seen in hibernating myocardium during ventricular low-flow ischemia [4, 11-13]. Cells return to a more fetal phenotype, which can be interpreted as an adaptive mechanism to energy shortage [14]. Further circumstantial evidence is available that suggests a role for supply-demand ischemia in AF. Markers of hypoxia (e.g. Hypoxia Inducible Factor 1α ($HIF_{1\alpha}$)) and angiogenesis (e.g. Vascular Endothelial Growth Factor (VEGF)) were found in AF patients [15-18]. Also in animal models of AF, evidence for supply-demand ischemia was found. After 1 week of AF, an increase in gene expression of $HIF_{1\alpha}$ [19] and a 60% decrease in the high-energy phosphate phosphocreatine (PCr) [20] were observed. However, both $HIF_{1\alpha}$ gene expression and PCr levels return to baseline after a few weeks of AF. These data suggest that the atrial supply-demand ischemia occurs in an early phase and is transient, suggesting a successful adaptation to restore supply-demand balance. In this thesis, we have aimed to describe regulation of the atrial supply-demand balance during acute and chronic AF. In addition, we have provided a comprehensive description of the atrial vascular anatomy and the changes in vascular structure that are caused by AF.

2. ANIMAL MODELS OF ATRIAL FIBRILLATION

In order to understand the processes resulting in the perpetuation of AF and investigate the relation to atrial ischemia, there is a need for appropriate animal models. In the past, pigs, dogs and goats have been used as models for chronic atrial fibrillation induced by rapid atrial pacing (RAP). Atrial electrophysiological, structural and contractility parameters were extensively investigated in the goat and dog model of AF and were comparable to the pathophysiological changes identified in AF patients [10]. Electrical remodeling, i.e. shortening of the effective refractory period and shortening of the action potential, was shown to be complete within the first hours to days [21-23]. It was also shown that the downregulation of the L-type calcium current (ICa_L) not only contributes to shortening of the action potential, but also resulted in a decrease in atrial contractility. This aspect was further elucidated in the goat model and it was shown that atrial contractility was almost completely abolished after 2 days of AF [24]. The identified electrical and contractile changes were fully reversible after restoration of sinus rhythm [21]. Structural remodeling like myocyte hypertrophy, loss of sarcomeres, accumulation of glycogen, and mitochondrial abnormalities were observed in the goat model [12]. Total fibrosis was not increased in the atria of AF goats [12] and dogs [22], but an increase was observed in the amount of extracellular matrix per myocytes (interstitial fibrosis/microfibrosis) [12] and in transverse intermyocyte distances within bundles (endomysial fibrosis) [25]. In contrast to electrical and contractile remodeling, reversal of structural remodeling after restoration to sinus rhythm was slow and incomplete [26].

In order to investigate the role of supply-demand atrial ischemia, an animal model is needed with a coronary anatomy similar to humans. Coronary and bronchial arteries supplying the pig heart are very similar to humans [27-29]. A characterization of atrial pathological changes during AF is given in CHAPTER 6 of this thesis. From earlier studies in pigs it became evident that rapid atrial pacing caused a significant increase in ventricular rate, with congestive heart failure (CHF) as a major consequence [30-32]. As discussed in CHAPTER 6, pigs were subjected to rapid atrial pacing with a controlled ventricular rate by the administration of oral digoxin. In this pig model of AF, we observed significant interstitial fibrosis in the left atrium and myocardial hypertrophy

in left and right atrium. The action potential duration did not shorten as was seen in the goat, but the action potential morphology changed as the plateau phase disappeared in AF pigs. AF episodes were longer and easier to induce than in controls. The development of CHF was modest at most. The pig was chosen as species to further elucidate the relationship between AF and ischemia (CHAPTERS 4-6) and a detailed description of the atrial blood supply in the pig is provided in this thesis (CHAPTER 3).

3. ATRIAL CORONARY ANATOMY IN PIGS

In the early 1900's, scientists have identified the presence of arteries branching off from the left and right coronary arteries into the atrial myocardium [33-38]. Also the extracoronary circulation (e.g. bronchial circulation) appeared to play a role in atrial blood supply, forming anastomoses with large atrial vessels [36-41]. With more and more circumstantial evidence for a role of atrial ischemia in the perpetuation of AF, a detailed and comprehensive investigation of the anatomy of the atrial vasculature was necessary. In CHAPTER 3, we have used new techniques and experiments to elucidate the anatomy of the atrial blood supply. In this chapter, a detailed overview was given of pig atrial coronary anatomy. Large vessels branching off from the coronary and bronchial circulation were identified as described earlier. Knowledge of the anatomy of atrial arteries may be relevant during heart transplants and AF ablation strategies. Ablation lines running through (or in very close proximity of) major atrial arteries could cause ischemia, thereby increasing the chance of AF recurrence. In addition, we have visualized the extensive atrial capillary network (CHAPTER 3). Despite the fact that the atrial wall is very thin and in close contact with the blood in the atrial cavity, there is a considerable blood-supply network in place that is tightly regulated to maintain supply-demand balance in the atria.

4. ATRIAL FLOW REGULATION

Changes in vascular diameter, especially in the small arteries and arterioles (resistance vessels), determine the total amount of blood flow (thus oxygen delivery) to the tissue downstream. Vascular resistance is partly regulated by the autonomic

nervous system and the circulating vasoactive hormones like angiotensine II, (nor-) epinephrine, vasopressin, atrial natriuretic peptide and endothelin. Changes in these vasoactive hormones are observed in AF. For example, increased atrial protein and gene-expression levels of Endothelin-1 are associated with atrial dilatation, fibrosis, hypertrophy and AF in patients [42, 43]. In addition, elevated baseline plasma levels of Endothelin-1 are a predictor for recurrent AF after ablation [44, 45]. Another important factor is the local regulation of the vascular resistance. Vasodilators and vasoconstrictors are secreted by the cardiomyocytes to meet their metabolic needs and by the vascular endothelium. During contraction of the heart muscle, extravascular forces cause a compression of the vasculature, which is an additional determinant of total vascular resistance.

4.1. Phasic pattern of atrial flow

Earlier microsphere measurements provide information on average blood flow per region [46-51]. To obtain detailed information on blood flow regulation over time, we measured coronary flow with Doppler flow probes, as described in CHAPTER 4 and 5 of this thesis. The atrial flow pattern in pigs was described in CHAPTER 4 and was very similar to that in humans [52, 53]. During atrial systole, a decrease, or even a reversal of flow, is observed. Both our study (2:1 AV block and acetylcholine administration) and earlier studies [54, 55] (isoproterenol) confirm that it is atrial contraction, rather than the increase in atrial pressure during systole, which causes the decrease in atrial flow. Atrial systole is short and 98% of total flow takes place during the atrial diastole. During short-term AF, the active pump function of the atria is abolished, but the local activation and contraction are very fast and irregular. These irregular and local contractions result in a very irregular atrial flow pattern during AF, but did not reverse atrial flow in any of the pigs as was seen during synchronous atrial contraction in sinus rhythm or pacing. However, they could continuously impede local flow, thereby aggravating the supply-demand mismatch.

4.2. *Regional regulation of atrial flow*

CHAPTER 2 includes a literature overview about myocardial blood flow distribution measured with microspheres. Blood flow is higher in the left side of the heart compared to the right side during sinus rhythm [47, 51, 56-60]. Changes in myocardial workload result in a redistribution of myocardial blood flow. An increase in atrial pressure or rhythm results in a larger portion of total flow going to the atria [56, 57, 60]. Atrial fibrillation is an even more potent stimulus than atrial pacing to increase atrial flow [46, 51, 61]. During AF, ventricular flow was not significantly affected in these microsphere experiments. Within the atria, differences in flow distribution also exist. Myocardial blood flow in the atrial appendages is lower than in the atrial free wall during rest [59]. During AF [49] and exercise [60], this flow distribution changes and the appendages will receive more myocardial flow. We have found similar results in healthy pigs. AF caused in increase in all area's investigated (atrial free walls, appendages, interatrial septum and Bachmann's bundle), but was only significant in LA free wall, LA appendage and RA appendage (CHAPTER 3).

4.3. *Dynamic regulation of atrial flow*

Atrial flow regulation was further investigated in CHAPTER 4 and 5 of this thesis in healthy pigs. A first set of experiments showed results consistent with microspheres studies. During pacing, conductance in a LA and LV branch of the left circumflex artery (LCx) increased. Since Doppler flow measurements plot flow against time, kinetics of flow regulation could be further investigated (CHAPTER 5). An immediate change in atrial and ventricular rhythm results in immediate increase in atrial conductance and the opposite was found when atrial pacing ceased. However, the atrial response to a quick change in rhythm was slower than in a ventricular artery. Atrial vascular conductance during AF increased even more than during atrial pacing and almost reached maximal conductance of the atrial vasculature. In the LV, an early increase (first minutes) was also observed during short-term AF, but this slowly decreased back to baseline values over time. In CHAPTER 5, we show that atrial conductance is positively correlated with atrial workload, as was shown in an earlier goat study,

but the correlation is weaker than in the ventricle. The atrial work calculated here, determined by calculating the area of the atrial distance-pressure loops, reflects the mechanical atrial work. In addition to mechanical work, cellular processes like ion homeostasis also require energy and thus contribute to the total energy demand in the atria. Although the distance-pressure loops collapse during AF, individual cells are contracting at a very high rate and atrial work and energy expenditure per cell are high. This could explain the large increase in atrial conductance and flow during AF.

4.4. Atrial flow as a limiting factor during AF

Cessation of AF did not result in an immediate decrease, but rather an increase of conductance compared to the period before and during AF, which was not seen in the LV artery (CHAPTER 5). This reactive hyperemia is another indicator for atrial supply-demand ischemia during and shortly after atrial fibrillation. The diastolic period in AF is very short and the constantly contracting tissue could exert mechanical stress on the atrial arteries and limit atrial blood flow, even if they are fully dilated. A decrease in mechanical force on the vascular wall after restoration of sinus rhythm would still result in an increase in atrial coronary conductance if the need for oxygen supply would remain high.

5. ATRIAL OXYGEN EXTRACTION

In addition to the regulation of atrial blood flow, the amount of oxygen extracted from the blood also plays an important role in maintaining the supply-demand balance. Oxygen extraction in the atrium was low (~25%) during normal sinus rhythm, whereas the values found for the ventricle (~60%) were very comparable to values in other studies under general anesthesia (CHAPTER 5). During pacing (150 beats per minute) and AF, we observed a significant increase in atrial extraction, while ventricular extraction remained unchanged. To our knowledge, this is the first study to measure atrial arterial and venous oxygen saturation in fresh blood samples. White et al measured saturation in frozen vessels and found an atrial arterial saturation of only 87±1.5% and a venous saturation of 61% [61]. Oxygen extraction during sinus rhy-

thm (~26%) was very comparable to our results and was increased slightly, but not significantly during AF (~31%).

6. ATRIAL ISCHEMIA DURING ATRIAL FIBRILLATION

An overview of literature on AF and ischemia was given in CHAPTER 2 and will be further discussed in terms of new findings in this chapter. CHAPTER 3, 4 and 5 aimed to describe the regulation of atrial supply-demand balance in healthy animals. During atrial fibrillation, oxygen demand is high, but the coronary conductance and extraction reserve are used to increase oxygen supply to the atrial myocardial tissue. The atrial conductance reserve turned out to be a limiting factor in oxygen supply and the hyperemic phase after the cessation of AF indicated a supply-demand mismatch. To determine whether a mismatch in supply-demand balance existed in short-term AF, atrial lactate production was measured in CHAPTER 4 of this thesis. Lactate production is an indicator for tissue supply-demand mismatch. The main fuel for ATP production in the heart are fatty acids. A second, but smaller source for ATP production in the ventricle is glucose that is converted through glycolysis and lactate into pyruvate that can enter the mitochondria for oxidation. Fatty acid oxidation is a strong inhibitor of glycolysis. However, when oxygen shortage arises, fatty acid oxidation is slowed down and glycolysis is stimulated. During oxygen shortage, mitochondria are not able to burn all pyruvate and the excess is converted to lactate in the cytoplasm. Subsequently, lactate is released and can be measured in the venous blood. Atrial arterio-venous lactate differences in healthy pigs showed a significant production of lactate after 5 and 10 minutes of AF, indicating supply-demand ischemia (CHAPTER 5). To investigate the effects of AF-induced remodeling on this supply-demand mismatch, a second group of animals was tested after 5 weeks of AF (CHAPTER 6). The atria had undergone significant electrophysiological and structural remodeling. Flow regulation was similar to control animals and oxygen extraction was even slightly lower in RAP pigs during sinus rhythm. However, during AF, no lactate was produced in the atria, showing that the adaptive mechanism are to some extent able to restore supply-demand after 5 weeks of continuous AF in pigs.

7. VASCULAR REMODELING

In 2008, Skalidis et al reported that patients with lone recurrent AF suffered from microvascular dysfunction. They observed a significant impairment of atrial coronary flow when measured during sinus rhythm [53]. Vascular remodeling in pigs was further elucidated in CHAPTER 6 of this thesis. In the pigs that underwent rapid atrial pacing for 5 weeks no functional impairment in coronary conductance reserve was observed. In addition, capillary density and perivascular fibrosis were calculated to assess the effect of 5 weeks AF on atrial vasculature. Capillary density was significantly decreased in both left and right atrium in pigs subjected to 5 weeks of rapid atrial pacing compared to control pigs. Perivascular fibrosis was, however, not increased in both left and right atrium compared to controls. Decreased capillary density could result in a decreased oxygen delivery capacity. Similar results were found in patients: AF patients showed a significantly lower capillary density than in patients without a history of AF [62, 63]. However, Gramley et al reported an opposing observation. In AF patients, capillary density and microvessel size were increased compared to patients without a history of AF [15]. It should be noted that these patients were all in persistent AF and paroxysmal AF patients were excluded from the study. It is likely to assume that capillary destabilization is also an early and transient phase that is dependent on the balance of circulating factors like $HIF_{1\alpha}$, VEGF, angiopoietin-1 and angiopoietin-2 [64]. During this process, the time point of measurement and type of AF will determine the effect of AF on capillary density.

8. ATRIAL ISCHEMIA IN PAROXYSMAL AF VS. PERSISTENT AF

Our conclusions and speculations on the atrial supply-demand balance during acute, paroxysmal and persistent AF are represented in Figure 1. We propose that atrial supply-demand ischemia plays an early role in AF (CHAPTER 4), but supply-demand balance is restored after a few weeks as a result of atrial remodeling (CHAPTER 6). Atrial ischemia could thus be an important driver for early electrical and contractile remodeling. If AF episodes last for days to weeks, this supply-demand mismatch could also drive structural remodeling processes (e.g. fibrosis and vascular remode-

ling). These remodeling processes can be considered as energy-saving, adaptive mechanisms, reducing the atrial energy demand.

The interactions between these processes may be particularly relevant for paroxysmal AF patients. As discussed in paragraph 2 of this chapter, electrophysiological changes and contractile dysfunction quickly recover after restoration of sinus rhythm. However, structural changes like loss of capillaries and increased fibrosis may recover much more slowly or not at all. In patients with paroxysmal AF, episodes of AF may induce progressive vascular (e.g. rarefaction) and structural remodeling. In ensuing periods of sinus rhythm, electrical and contractile remodeling are known to reverses quickly [24]. This would increase atrial work and energy/oxygen demand. Subsequent periods of AF would then again lead to supply-demand ischemia. In these patients, every AF episode would thus lead to ischemia in the atria and the associated damage would be cumulative. To some extent, this view is supported by the observation of an increase in Vascular Endothelial Growth Factor (VEGF) in patients with paroxysmal AF, but not in persistent AF patients [16]. In a study on goats with AF, the increase in $HIF_{1\alpha}$ was also transient and $HIF_{1\alpha}$ gene expression was increased only in the first week of rapid atrial pacing [19]. Interestingly, in AF patients, $HIF_{1\alpha}$ was still increased after (longstanding) persistent AF, suggesting that the phase of ischemia may be much longer in those patients than in the goat model of AF [15, 17].

9. FUTURE PERSPECTIVES

Our data show that there is a transient phase of atrial ischemia during AF. Understanding the changes in supply-demand balance during AF is merely the tip of the 'metabolic iceberg'. Although it is plausible that atrial ischemia could drive the electrical, contractile and structural remodeling processes, this is not yet shown. Also the role of atrial ischemia in the stabilization of AF should be further elucidated.

Reducing the supply-demand mismatch could be clinically relevant in the prevention of the subsequent remodeling processes and the perpetuation of AF. To improve oxygen efficiency in the atrium during AF, it is important to further investigate the underlying processes like metabolic and vascular remodeling. In the ventricle it is shown that low-flow ischemia and heart failure lead to metabolic remodeling, hall-

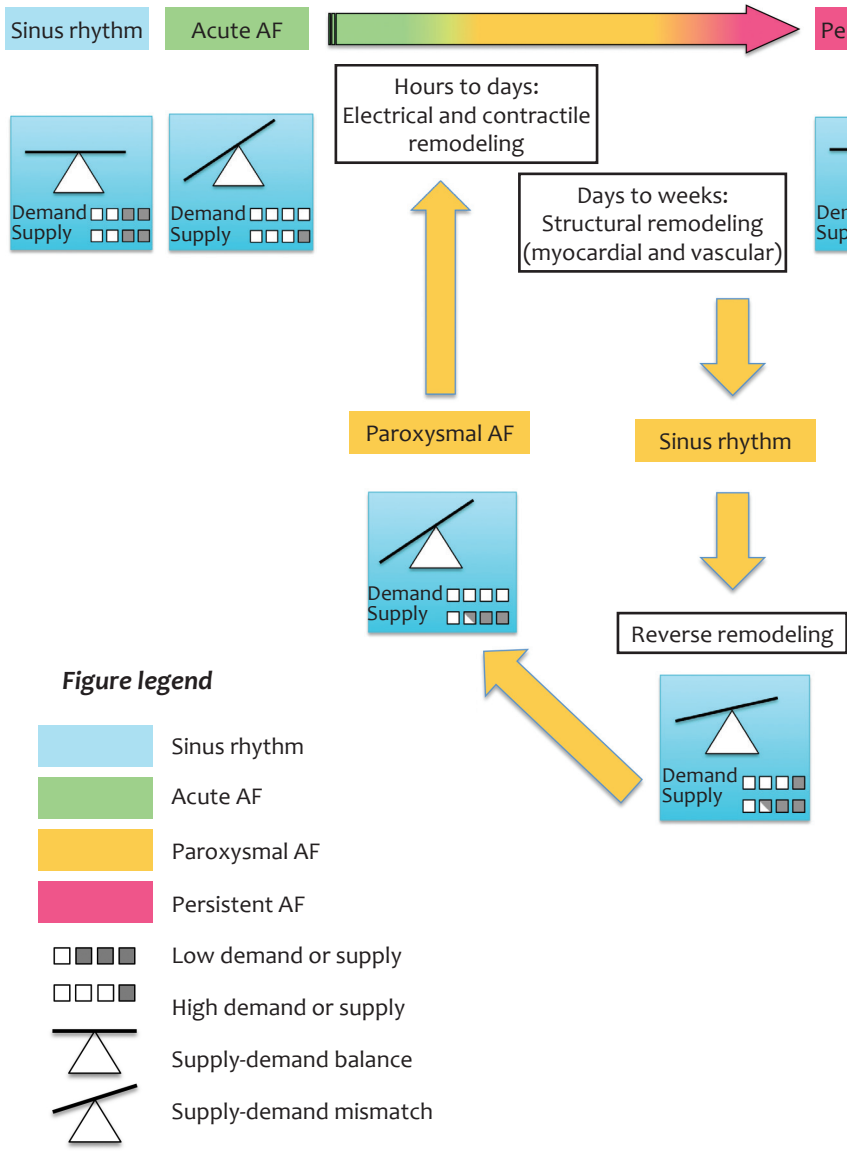


Figure 1: Conclusions and speculations on atrial supply-demand balnce in acute, paroxysmal and persistent atrial fibrillation (AF)

marked by a shift from fatty acids to glucose utilization [65]. Whether or not metabolic remodeling plays a role in AF is unknown, but gene expression profiles show marked changes in metabolism related genes after 24h of rapid atrial pacing in dogs [66]. The use of metabolic shifters like ranolazine and trimetazidine [67] could be useful interventions to improve oxygen efficiency. As discussed earlier in of this chapter, AF is associated with microvascular dysfunction and decreased capillary density that could aggravate ischemia during AF. Identifying new potential targets in the vascular remodeling process could also contribute to improved oxygen delivery and the prevention of supply-demand mismatch during AF. Furthermore, knowledge of atrial coronary anatomy and specifically the location of large atrial vessels may be important in the development of ablation strategies.

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APPENDIX

APPENDIX

SUMMARY

Atrial fibrillation (AF) is a self-perpetuating arrhythmia characterized by fast and irregular activation of the atria. Structural, electrophysiological, contractile and metabolic remodeling takes place in the atrial tissue as a result of AF. The exact trigger for these atrial changes is unknown, but indirect evidence suggests a role for energy shortage in the perpetuation of AF. The ultrastructural changes seen in the atria during AF resemble hibernating ventricular myocardium due to low flow ischemia. In addition, markers of hypoxia and angiogenesis are increased in animal models of AF and in AF patients.

To prevent a supply-demand mismatch during AF, atrial supply should be increased or atrial demand should be decreased. This thesis aims to describe the different aspects of supply-demand balance in the atrium during sinus rhythm and acute and chronic AF. The atrial vascular anatomy of the pig was investigated in CHAPTER 3 and an extensive atrial vascular network was observed. Through this vascular network, the atria receive about 5% of total coronary blood flow during sinus rhythm. Very limited overlap in atrial perfusion territories was observed, which makes the atrium susceptible to ischemia. Both atrial coronary blood flow and atrial oxygen extraction can be recruited to match changes in atrial oxygen demand and maintain supply-demand balance. In CHAPTER 5, we show that an increased atrial rate resulted in an increased atrial workload. Atrial pacing and the induction of AF in healthy pigs resulted in an immediate increase in atrial coronary blood supply. After the cessation of AF, atrial flow did not immediately return to baseline values, which is a first indication for a shortage of oxygen during AF. An increase in atrial oxygen extraction was also observed during acute AF (CHAPTER 4). Despite recruitment of both flow reserve and extraction reserve in the atrium during AF, lactate was produced, showing that a supply-demand mismatch occurred.

In a goat model of AF, the increase in gene expression of hypoxia marker $HIF_{1\alpha}$ was temporary and returned to baseline values after 1 week of AF. Also phospocreatine, a tell-tale sign for supply-demand ischemia, was only increased in the first week of AF

in goats. In addition, angiogenesis marker VEGF was only increased in patients with paroxysmal AF, but restored in permanent AF patients. To further determine the effect of chronic AF, a pig model of 5 weeks (chronic) AF was developed and supply-demand balance was investigated (CHAPTER 6). In the pig model of chronic AF, electrical and structural remodeling was observed and AF stability was increased compared to healthy controls. Coronary flow reserve and extraction reserve were comparable to healthy controls. In addition, flow and extraction increased in similar amounts during AF. As opposed to acute AF in controls, chronic AF did not result in increased lactate production, indicating that the AF-induced remodeling processes are to some extent able to restore the supply-demand balance. The most likely mechanism for this adaptation is probably a decrease in demand during AF resulting from loss of contractility and myocyte hibernation. The capillary density was markedly decreased after 5 weeks of AF. This process of atrial 'vascular remodeling' may maintain atrial susceptibility to ischemia, limiting the capacity of the atrial myocardium to respond to fluctuations in demand.

APPENDIX

NEDERLANDSE SAMENVATTING

Boezemfibrilleren of atriumfibrilleren (AF) is een hartritmestoornis die gekarakteriseerd wordt door een snelle en onregelmatige activatie van de boezems. Structurele, elektrofysiologische, contractiele en metabolische veranderingen in de atria worden geobserveerd in de atria van AF patiënten. De exacte oorzaak voor deze veranderingen is onbekend, maar er is indirect bewijs voor een energie tekort in de atria tijdens AF. De ultrastructurele veranderingen in atriale cellen tijdens AF zijn vergelijkbaar met hibernerende cellen tijdens lage-perfusie ischemie in de ventrikels. Ook zijn er aanwijzingen van hypoxie en angiogenese in diermodellen van AF en in AF patiënten.

De disbalans tussen zuurstof vraag en zuurstof aanbod wordt supply-demand mismatch of supply-demand ischemie genoemd. Om supply-demand ischemie te voorkomen tijdens AF, moet het aanbod stijgen of de vraag dalen. In deze thesis werden de verschillende aspecten van de supply-demand balans in het atrium besproken tijdens sinus ritme en acuut en chronisch AF. De anatomie van de atriale vaat-boom werd beschreven in HOOFDSTUK 3 en een dicht netwerk van vaten werd geobserveerd. Ongeveer 5% van de totale bloedstroom naar het hart gaat via dit dichte netwerk naar de atria. Aangezien er weinig overlap is tussen de perfusie gebieden van de atriale vaten, zijn de atria erg gevoelig voor ischemie. Zowel de atriale bloedstroom als de atriale zuurstof extractie kunnen worden aangesproken indien de vraag naar zuurstof stijgt in de atria. In HOOFDSTUK 5 toonden we aan dat de atriale arbeid toeneemt als het ritme in de atria stijgt. Een verhoging van het atriale ritme en de inductie van AF doormiddel van een pacemaker in de atria resulteerde in een directe toename van de atriale bloedstroom. Zodra het atriaal ritme omsloeg van AF naar sinus ritme, had dit niet direct een daling in de bloedstroom tot gevolg. Dat was een eerste indicatie voor een zuurstof tekort tijdens AF (HOOFDSTUK 4). Ondanks de toename in bloedstroom en zuurstof extractie tijdens AF, werd een toename in melkzuur (lactaat) aangetoond in de atria tijdens AF. Dit is een directe indicatie voor supply-demand ischemie tijdens AF.

In het geitenmodel van AF werd een tijdelijke toename in $HIF_{1\alpha}$ gezien, die terugkeertde naar de originele waarden na 1 week AF. Ook werd er een toename in phosphocreatine aangetoond in het geitenmodel na 1 week AF. Een toename in de angiogenese marker VEGF werd gezien in paroxysmaal AF en niet in persistent (chronisch) AF patiënten. Om het chronische effect van AF verder te onderzoeken, hebben we een model van 5 weken chronisch AF in varkens ontwikkeld (HOOFDSTUK 6). In dit varkensmodel werden structurele en elektrische veranderingen gevonden en AF was meer stabiel dan in controle dieren. De bloedstroom reserve en extractie reserve waren vergelijkbaar in beide modellen. Daarnaast was de toename in bloedstroom en extractie tijdens AF even groot. In tegenstelling tot acuut AF in controle dieren, zagen we geen toename in lactaat in het 5-weken AF model tijdens AF. Dit toont aan dat de veranderingen in het atriaal weefsel die optreden tijdens AF tot op zekere hoogte de supply-demand balans in het atrium kunnen herstellen, waarschijnlijk doordat de vraag naar zuurstof afneemt bij bijvoorbeeld cellulaire hibernatie of een daling in de contractiliteit. De capillaire dichtheid was sterk verlaagd na 5 weken AF. Door de veranderingen in de vaatboom is het atriale weefsel juist extra vatbaar voor een zuurstoftekort en is er in het atrium een beperkte capaciteit om te reageren op fluctuaties in zuurstofvraag.

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OPPORTUNITIES FOR VALORIZATION

The aim of this chapter is to identify opportunities to valorize the research findings of this thesis. Knowledge valorization refers to the "process of creating value from knowledge, by making knowledge suitable and/or available for social (and/or economic) use, and by making knowledge suitable for translation into competitive products, services, processes and new commercial activities" (adapted definition based on the National Valorization Committee 2011:8). According to this definition, scientific research is valued by its direct socio-economic impact. Although the socio-economic impact of basic research is difficult to quantify, increasing knowledge of complex (patho)physiolocial processes is pivotal to future innovations.

1. Societal conditions

As described in CHAPTER 1 of this thesis, atrial fibrillation (AF) is the most common arrhythmia in clinical practice and the prevalence of AF increases with age [1-3]. AF significantly increases the risk for all cause mortality, cardiovascular mortality, stroke and congestive heart failure [3]. In the Netherlands, an estimated 300.000 people suffer from AF. With the ageing of the population, this number is expected to increase to 1.000.000 in 2050. Thus, AF represents a significant socio-economic burden. Current AF treatment involves maintaining patients with paroxysmal and persistent AF in sinus rhythm (rhythm control) or controlling the ventricular rate (rate control). In addition, patients are treated with anti-coagulation therapy to decrease the risk of stroke [4]. However, anti-coagulation therapy has potentially harmful side effects, such as an increased risk of bleeding, and therefore needs to be carefully controlled. Except for anti-coagulation therapy, so far there is no treatment available that substantially improves the prognosis of AF patients [5]. In AF patients, the arrhythmia is often progressive, with a gradual increase in the duration of AF episodes. AF causes structural remodeling of the atrial myocardium, and it is generally accepted that this process is responsible for AF progression. By causing structural remodeling that increases the stability of the arrhythmia, AF is self-perpetuating. Although various aspects of structural remodeling, e.g. fibrosis, myocyte hypertrophy and hibernati-

on, have been characterized extensively, the factors leading to structural remodeling are still poorly understood. Insight into these pathogenic factors is essential for the development of successful upstream therapy, i.e. therapy preventing structural remodeling and thereby inhibiting AF progression.

2. Novelty of the concept

Several potentially important pathogenic factors have been proposed in AF progression, e.g. atrial dilatation/ stretch, calcium overload, oxidative stress, inflammation and altered neurohumoral signaling. Although atrial ischemia has been mentioned as a possible contributor [6-12], its occurrence and role has not been investigated systematically. Nevertheless, it is likely that the rapid rates occurring during AF represent a substantial increase in atrial energy expenditure. If this increase cannot be met by an increase in atrial coronary supply, a state of supply-demand ischemia will develop. In this thesis, we have investigated the structure of the atrial vasculature (CHAPTER 3), the regulation of atrial coronary blood flow (CHAPTER 5) and the occurrence of supply-demand ischemia (CHAPTER 4 and 6). Several remodeling processes observed in AF (e.g. myocyte hibernation) can be viewed as energy saving mechanisms to restore the supply-demand balance. To our knowledge, this is the first study to show atrial supply-demand ischemia induced by acute AF (CHAPTER 4). We have also shown that after a few weeks of AF, atrial supply-demand balance is restored, although accompanied by potentially detrimental vascular remodeling (CHAPTER 6).

3. Road to product

A logical next step to evaluate the role of atrial ischemia as a pathogenic factor leading to structural remodeling is to test interventions that would reduce supply-demand ischemia. Both inventions that affect atrial metabolism (leading to conservation of energy) and the atrial vasculature (leading to improvement of supply) would be suitable candidates for upstream therapy. These would first have to be tested in a suitable large animal model of AF. If such a therapy would prove successful in inhibiting structural remodeling and AF progression in an animal model, they

could be considered a treatment option for patients with AF or patients at risk for developing AF. Targeting supply-demand ischemia may prove particularly suitable in patients with paroxysmal AF, where episodes of AF and sinus rhythm alternate, and where each AF paroxysm may represent an ischemic insult that leads to an accumulation of damage to the atrial myocardium.

4. Conclusions

On the short term, the research findings in this thesis about supply-demand balance in the atrium will be of value to researchers in directly related fields. In addition, knowledge about the atrial vascular anatomy may be relevant in cardiac surgery and the development of new ablation strategies, as described in CHAPTER 3 of this thesis in the paragraph "atrial ischemia in ablation strategies and myocardial infarction". On the long term, these findings may lead to the development of new strategies for upstream treatment of AF. If atrial supply-demand ischemia indeed proves to be an important pathogenic factor in the development of a substrate for AF, then it is likely that vascular or metabolic interventions can inhibit the progression of AF.

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ABOUT THE AUTHOR

Kelly van Bragt was born on the 23rd of January 1985 in Roermond. In 1999 she moved with her family to Maasmechelen, Belgium where she graduated from Heilig Hart College high school in 2004 with the profile Science and Mathematics.

She started at Maastricht University in 2004 to follow the Bachelor program Molecular Life Sciences. After obtaining her Bachelor degree in 2007, she attended a two-year Research Master program in Cardiovascular Biology and Medicine organized by the Cardiovascular Research Institute Maastricht (CARIM). During this Master program she became interested in electrophysiology of the heart and did two internships at the Department of Physiology under supervision of Prof. Schotten and Dr. Verheule. After her internships, she presented her work and future perspectives to a jury of university staff members and CARIM board members and was rewarded with a PhD grant within CARIM. She joined the Physiology department as a PhD in 2009, supervised by Prof. Schotten en Dr. Verheule, where she was involved in determining the project plan and developing a variety of new techniques. In 2010 and 2011, the author joined the organizing committee of the Dutch Association for Physiology. The obtained data from her research at Maastricht University resulted in several publications in peer- reviewed scientific journals. With the presentation of her work, she won two poster awards and an oral presentation award on international symposia.

After her PhD, she started working as a Clinical Research Specialist at the Medtronic Bakken Research Center.

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The impact of persistent atrial fibrillation on the atrial supply-demand balance

To be submitted

Nasrallah HM, **van Bragt KA**, Kuiper M, van Hunnik A, Schotten U, Verheule S. Left atrial oxygen supply in a pig model of congestive heart failure: vascular remodeling, atrial contractile dysfunction and reduced oxygen efficiency

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