Mechanisms of functional tricuspid valve regurgitation in ischemic cardiomyopathy

Thomas M. Joudinaud

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Mechanisms of Functional Tricuspid Valve Regurgitation in Ischemic Cardiomyopathy

By

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Mechanisms of Functional Tricuspid Valve Regurgitation in Ischemic Cardiomyopathy.

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In heart failure, the presence of functional mitral regurgitation secondary to a myocardial infarction (MI) is known to double mortality. While the mechanism of functional mitral regurgitation has been extensively studied, the mechanism of a concomitant functional tricuspid regurgitation has been neglected. We hypothesized that MI alters not only the geometry of the left ventricle but also of the right ventricle leading to both ischemic mitral and tricuspid regurgitation (IMR & ITR). We also hypothesized that these geometric alterations modulate mechanical stresses resulting in molecular changes. To answer these hypotheses, the following Aims were designed: 1) complete an anatomy study of the normal tricuspid valve following the established principles applied to the mitral valve; 2) perform an in vivo sonometric study of the functional anatomy of the atrioventricular valves in order to clarify their relationships; 3) compare in sheep the data obtained with transthoracic echocardiography (TTE) with those acquired with sonomicrometry. Evaluation of the reliability of the echo geometric data is essential given its value because of its non-invasiveness. 4) develop a reproducible animal model of IMR and ITR and analyze the resulting geometrical changes 5) search for variations in eNOS and nNOS expression at increasing distance from the MI as an indicator of cardiomyocyte stretch.

Results 1) An original terminology of the tricuspid valve useful to surgeons and cardiologists was developed; 2) we showed how the mitral and tricuspid valves are linked by the septum; 3) TTE in sheep provides precise information needed for the non invasive follow up of the LV remodeling; 4) an original animal model of IMR and ITR was developed with the percutaneous selective injection of ethanol. Specific geometrical distortions of both ventricles that resulted in IMR and ITR were found; 5) we showed that the expression of eNOS was correlated with the distance from the MI and thus the amount of stress.

Conclusion: This data suggest that IMR and ITR are caused by a geometrical distortion of both atrioventricular valves and that this macroscopic remodeling was associated with an up-regulation of eNOS that may be correlated with an adapative process of the stretched cardiomyocytes.
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List of Abbreviations

A, AP and P: Anterior, anteroposterior and posterior tricuspid annulus.
ALV: crystal on the LV wall at the level of M1 and M2.
AM: Anterior mitral annulus
AML: Anterior mitral leaflet
Ao: Aorta
Ca\(^{2+}\): Calcium ion
cAMP: Cyclic adenosine monophosphate
cGMP: Cyclic guanosine monophosphate
D: Diastole
EF: Ejection fraction
Ej: Ejection
EKG: Electrocardiogram
eNOS: Endothelial nitric oxide synthase
ES & ED: End-systole & end-diastole
EtOH: Ethanol
FAD & FMN: Flavin adenine dinucleotide & flavin mononucleotide
i.v.: Intra venous
IMR: ischemic mitral regurgitation
ITR: Ischemic tricuspid regurgitation
IVC: Isovolumic contraction
IVR: Isovolumic relaxation
KCl: potassium chloride
LA: Left atrium
L-NMMA: L-NG-monomethyl-arginine
LV & RV: Left & right ventricle
LV: Left ventricle
LVEDD: Left ventricle end diastolic dimension
LVESD: Left ventricle end systolic dimension
M1 and M2: Anterior and posterior papillary muscles in the LV.
MI: Myocardial infarction
midD: Mid diastole
MMPs: Matrix metalloproteinases
MR: Mitral regurgitation
Na\(^+\): Sodium ion
NADP & NADPH: Nicotinamide adenine dinucleotide phosphate & reduced form
nNOS: Neuronal nitric oxide synthase
NO: Nitric oxide
NOS: Nitric oxide synthase
OM: Obtuse marginal
P1 and P2: Left and right lateral mitral annulus
PA: Pulmonary artery
PBS-T: Phosphate buffer saline containing tween
PM tip: Papillary muscle tip
PM: Posterior mitral annulus
PM1 & PM2: Right and left part of postero medial mitral leaflet
PML: Posterior mitral leaflet
PMSF: Phenylmethlysulfonyl fluoride
S, AS and PS: Septal, anteroseptal and posteroseptal tricuspid annulus
SDS-PAGE: Sodium dodecylsulfate polyacrylamide gel electrophoresis
SERCA: Sarco-Endoplasmic Reticulum Calcium ATPase
sono: Sonomicrometry
Spm, Apm and Ppm: Septal, anterior and posterior papillary muscles in the RV.
SR: Sarcoplasmic reticulum
T1 and T2: Left and right trigone
TBH4: Tetrahydrobiopterin
TBS-T: Tris-buffered saline containing 0.1% Tween
TIMPs: Tissue inhibitors of metalloproteinases
TR: Tricuspid regurgitation
TTE: Trans-thoracic echocardiography
vol: Volume
WT: Wild type
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**Introduction**

The clinical symptoms of heart failure are due to inadequate performance of the heart. Heart failure develops gradually following a myocardial infarction, as the myocardium is progressively remodeled [1]. The degree of myocardial remodeling determines the alterations in ventricular size, shape and function [2]. Left ventricular remodeling may be associated with the presence of a functional mitral regurgitation that following an ischemic event is known to double mortality [3]. While functional mitral regurgitation has been extensively studied the presence of a concomitant functional tricuspid regurgitation has been generally ignored. In a retrospective study of patients with functional mitral regurgitation from ischemic origin, we observed a concomitant functional tricuspid regurgitation in 30% of the cases. The mechanisms at the origin of functional ischemic tricuspid regurgitation remain unknown.
Background

1: Ischemic Heart Failure with Mitral and Tricuspid Regurgitation

1-1: Heart Failure: A Major Health Problem.

The clinical syndrome of heart failure is a major health problem in the western countries. It is estimated that 4.6 million Americans are currently treated for heart failure and that 550,000 new cases are diagnosed each year [4]. Heart failure is the reason for at least 20 percent of all hospitalizations among persons older than 65. The rate of hospitalizations for heart failure has increased by 159 percent during the nineties. An estimated $5,501 was spent for every hospital discharge diagnosis of heart failure, and another $1,742 per month was required to care for each patient after discharge [2]. In France, 85 to 93% of the economic cost of heart failure is attributable to hospitalizations, most of them avoidable [5]. Substantial efforts have been made to identify and treat the factors that predict recurrent hospitalizations.

Mortality associated with heart failure remains high in spite of advances in therapy. Symptomatic heart failure continues to confer a worse prognosis than the majority of cancer, with one-year mortality of approximately 45 percent [6,7]. Senni et al. reported, in a community-based survey, 76 percent and 35 percent survival rates at one-year and five-years, respectively [8]. Large epidemiologic surveys have not documented any meaningful change in overall death rates.

Heart failure is clinical syndrome of signs and symptoms caused by inadequate performance of the heart. Currently, complex blends of structural, functional, and biologic alterations are evoked to account for the progressive nature of heart failure. Only
the combination of several animal models of heart failure can explain more fully the cascade of mechanisms: the hemodynamic model is focused on the altered load of the ventricle while the neurohumoral model recognizes the importance of the renin-angiotensin-aldosterone axis and the sympathetic nervous system in the progression of cardiac dysfunction. Some studies have scrutinized myocytes from failing hearts in an attempt to detect abnormal signaling, gene expression, or contractile protein structure.

Microbiologic changes and increased levels of circulating neurohormones are only part of the response seen after initial insult to the myocardium. Jessup et al. defined the left ventricular remodeling as the process by which mechanical, neurohormonal and possibly genetic factors alter ventricular size, shape and function [2]. Remodeling occurs after several clinical conditions such as myocardial infarction, cardiomyopathy, hypertension, and valvular heart disease. Its hallmarks include loss of myocytes, hypertrophy and increased interstitial fibrosis [9,10].

1-2: Ischemic Heart Failure

The effect of the underlying cause of heart failure on survival is unclear. In general, patients with heart failure due to left ventricular dysfunction are classified broadly in two groups: those with cardiomyopathy due to ischemic causes and those with cardiomyopathy due to non-ischemic causes. Misclassification of causes of heart failure may result when cardiomyopathy is diagnosed clinically because one-third of patients with cardiomyopathy due to non-ischemic causes have clinical angina [4]. In France, one in two hospitalized patients with heart failure is classified as an ischemic cardiomyopathy [11]. In an analysis of 3,787 patients with left ventricular dysfunction who underwent
coronary angiography, an underlying cause of ischemia was a significant independent predictor of mortality [12]. Five-year survival for patients with ischemic heart disease was 59 percent.

Ischemic heart failure is mostly due to coronary atherosclerosis. During the 1980s, the age-adjusted mortality due to coronary artery disease decreased considerably in the United States [13] and in other countries [14]. This favorable trend was due partly to a decrease in the rate of fatal myocardial infarction and also to the decline of risk factors for coronary disease [13], which strongly influence the severity of atherosclerosis. Severity of atherosclerosis is a major predictor of survival in patients with coronary artery disease [15]. Death seems to have been delayed and occurs a longer time after a myocardial infarction [2]. However Enriquez-Sarano et al. showed that the prevalence of atherosclerosis remained the same from 1980 to 1989 [16]. These findings likely explain the increased prevalence of patients with heart failure symptoms from ischemic cardiomyopathy.

1-3: Ischemic Mitral Regurgitation

1-3-1: Significance of Ischemic Mitral Regurgitation

Mitral regurgitation (MR) in heart failure can be organic or functional. Organic implies a disease of the mitral valve itself and functional implies a “normal” mitral valve. For example, after a myocardial infarction, a rupture of one of the papillary muscle can occur resulting in an organic mitral regurgitation and in acute massive heart failure. Functional means that the mitral valve is normal on macroscopic appearance and MR may be due to annular dilatation (type I in Carpentier’s functional classification of MR)
or alternatively to restricted motion of the leaflets (type IIIb in Carpentier’s functional classification of MR) [17]).

The incidence of functional ischemic mitral regurgitation (IMR) after acute myocardial infarction (MI) has been reported to be between 13% [18] and 39% [19]. Barzilai et al. reported a one-year mortality of 36% compared with 15% in patients without MR [20]. Lehmana reported a one-year mortality of 18.2% for patient with mild MR and 60% for those with moderate or severe MR [18]. In a retrospective analysis of 11,848 patients with significant coronary disease (>75% stenosis of one or more coronary arteries), ejection fraction, age, and number of diseased vessels were also predictors of poor outcome but less significantly compared to presence of mitral regurgitation. An inverse relationship exists between degree of mitral regurgitation and prognosis [21]. In a study of 303 patients, Grigioni et al. showed that the presence of MR doubles the long-term (5-years) mortality [3]. These findings are the same even in cases with mild or moderate regurgitation [22]. Therefore, presence of functional MR is an independent predictor of poor prognosis in ischemic heart failure.

1-3-2: Mitral Regurgitation and Localization of the Ischemic Event

Lehman et al. [18] reported a significant difference (p<0.01) in the presence of MR following an MI. Mitral regurgitation was present in 21% of patients after anterior MI (20/96 patients) compared with 6% of patients after inferior MI (6/106 patients). However, Barzilai et al. [19] reported a MR with 46% of the anterior MI patients (11/24 patients) and 35% with the inferior MI patients (12 of 34 patients).
Localization of MI is not the only parameter that determines the presence of MR. Kumanohoso et al. showed that global LV dilatation and dysfunction were significantly less pronounced in 43 patients with inferior MI compared to 61 patients with anterior MI. However, the percentage of MR was very significant in inferior MI patients compared to those with anterior MI patients (p<0.0001) [23]. Kono et al. reported that the left ventricular shape was the primary determinant of functional MR in heart failure [24]. Change of ventricular shape, size and function or “remodeling” after myocardial infarction is associated with functional mitral regurgitation.

1-3-3: Mechanisms of Ischemic Mitral Regurgitation

The mechanism responsible for functional ischemic mitral regurgitation (IMR) remains uncertain, although specific anatomic abnormalities of the left ventricle (LV) have been proposed. They are mitral annulus dilatation, chamber enlargement, dysfunction of the papillary muscle and associated dysfunction of the ventricular wall [25]. Coexistence of these characteristics in the failing heart makes the identification of the precise abnormality responsible for the IMR difficult.

- Dilatation of the mitral annulus

Experimental models of acute ischemic MR, using sonomicrometry array localization [26], myocardial marker technology or three-dimensional echocardiography [27,28], showed distortions in the geometry of the mitral annulus, just after myocardial infarction. In an animal model of chronic ischemic MR using sonomicrometry array localization, Gorman et al. showed that the end systolic annular area increased from
647±44 mm² to 1,094±173 mm² (p=0.01). The annular dilatation occurred equally along the anterior (47±5.6 mm to 60.2±4.9 mm, p=0.001) and posterior (53.8±3.1 mm to 68.5±8.4, p=0.005) portions of the annulus [29]. Using radiopaque marker technology in the same ovine model of chronic ischemic MR, Tibayan et al. obtained the same results. Mitral annular diameter, septo-lateral and commissure-commissure dimensions increased significantly with the severity of the IMR at seven weeks [30](Figure 1). They also demonstrated the flattening of mitral annulus, which is normally a saddle shaped structure. These experimental results are comparable to what has been observed in the human. In a prospective analysis of the degree of functional IMR in 128 patients with systolic LV dysfunction, the mitral annulus from these patients were compared with 21 normal controls by echocardiography. Diastolic and systolic mitral annular areas were significantly larger than in control group (9.5±1.7 mm and 7.7±1.8 mm versus 6.9±0.8 mm and 4.4±0.7 mm, respectively) [31]. In an analysis of geometric alterations of the mitral apparatus with magnetic resonance imaging, Yu et al. showed that there was a significant difference (p=0.008) in the antero-posterior diameter between patients with a remodeled LV after myocardial infarction without MR (28.7±4.5 mm) and patients with IMR (45.6±13.4 mm) [32]. The consequence of annular dilatation is shortening of the coaptation plane of both the anterior and posterior leaflets. At most, it can result in lack of coaptation and a central leak (type I, Carpentier’s functional mitral regurgitation classification) [17].
Figure 1: Mitral Annulus dilatation in IMR.

The dilatation occurred in antero-posterior and septo-lateral directions.
- Chamber enlargement

Dilatation of the heart after an ischemic event is the first characteristic of myocardial remodeling. The cardio-thoracic index is always increased in heart failure on a chest X-ray. All experimental studies and human observations of IMR showed an increased volume of the LV. Patients without any IMR can also present with LV enlargement. However, enlargement of the LV seems to be more prevalent in cases in which patients present with an IMR. In a comparative analysis of patients after an ischemic event, LV end-diastolic (LVED) and LV end-systolic (LVES) volumes were significantly larger for patients with IMR than patients without IMR (239±84 ml and 148±65 ml versus 202±68 ml and 120±47 ml; p<0.001) [25]. This more pronounced LV enlargement, which is demonstrated in other human [31-33] and animal [34,35] studies, is also associated with a greater index of sphericity (the heart appeared more spherical) [25]. This sphericity of the heart may explain the worsened clinical symptoms and poorer prognosis of these patients according to Torrent-Guasp [36] who described the helical heart as a muscular band scrolled on itself. In case of dilatation of the heart, the longitudinal orientation of the scrolled muscular band becomes more transversal decreasing its twisting during the contraction and thus, the ejection of the blood [36].

- Tethering effect

The greater volume of the heart is associated with increased longitudinal and transverse measurements of the heart. LVED and LVES diameters and inter papillary muscle distances are reported increased in human and animal studies [28,30-32,34,35]. The increased longitudinal diameter also can be measured by the distance between the tip
of the posterior papillary muscle and the anterior part of the mitral annulus. This distance is called the “tethering distance”. The inferoposterior wall bulges outward, displacing the attached posterior papillary muscle outward and apically [27].

During the enlargement of the LV, in the case of IMR, the distance between the tip of the posterior papillary muscle and the mitral annulus increases. Because of the low elasticity of the fibrous tissue of the chords and the mitral leaflet, the chords and more particularly the basal chords, tether the leaflets. Therefore, the enlargement of the heart is responsible for a tethering effect on both mitral leaflets. Leaflets motion is restricted and because of the movement of the coaptation point toward the apex, a central MR results [37](Figure 2).
Figure 2: Tethering effect on the anterior leaflet observed on an ischemic MR patient in systole.

The anterior leaflet shape mimics a tent. The coaptation point of both leaflets is apically displaced. The association of annular dilatation and tethering effect result in massive MR.

LA: Left atrium
LV: Left ventricle
AML: Anterior mitral leaflet
PML: Posterior mitral leaflet

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- Dysfunction of the papillary muscles and associated dysfunction of the ventricular wall:

Damaging the posterior papillary muscle was an early method to produce an experimental model of IMR and study the function of the papillary muscle [38]. In previous human case reports, IMR murmur following myocardial infarction was associated with development of heart failure. Autopsy of two patients showed non-ruptured papillary muscles and chordae. In one case, extensive scars were found in the ventricle wall at the base of the papillary muscle. In the other case, the papillary muscle was infarcted [39]. In the most popular chronic IMR animal model of Llaneras et al., MR appears after infarction of the posterior papillary muscle and the relative left ventricle wall [35]. Messas et al., using an acute ovine model of IMR, showed that inferior ischemia, which did not involve the posterior papillary muscle, was associated with IMR. In their experiment, the ischemia and therefore the dysfunction of the papillary muscle even decreased the amount of MR [28]. The amount of IMR varies with loading conditions [37]. The dynamic variability of IMR is enhanced by the report of Pierard and Lancelloti [40], who described IMR as being responsible for pulmonary edema. A comparative study of patients with left ventricular dysfunction, trivial MR and acute pulmonary edema or non history of acute pulmonary edema showed significant association between exercise-induced changes in the effective regurgitant orifice area and pulmonary edema.

1-4: Functional Tricuspid Regurgitation in Cases of Ischemic Heart Failure

Functional tricuspid regurgitation (TR) has the same definition as functional MR. The valve is leaking, but appears macroscopically normal.
1-4-1: Significance of Functional Tricuspid Regurgitation:

The tricuspid valve is the right-sided atrioventricular valve and its study has always lagged behind those of other cardiac valves. Incidence of tricuspid insufficiency is higher than expected and surgical management does not have the expected clinical results [41]. Patients with severe left ventricular dysfunction who developed functional MR are associated with poor prognosis [18,21,25]. In these patients, right ventricular dysfunction and presence of TR have been shown to be an independent predictor of mortality [42-44].

Greater LV enlargement and higher prevalence of echocardiographic functional MR were associated with functional TR in a comparative study of 117 patients in heart failure. In the same study, estimated 1-year event-free (cardiac transplantation or death) survival was 68% in patients without TR and 30% in patient with TR (p=0.0002). Presence of TR was also a marker of right ventricle (RV) systolic dysfunction, RV dilatation and RV hypokinesia [42]. These criteria were defined as indicators of a poor prognosis in congestive heart failure associated with coronary artery disease by Polak et al. [45].

1-4-2: Mechanism of Functional Tricuspid Regurgitation:

The mechanism of functional TR was never studied with the same accuracy as was applied to study the mechanism of functional MR. Functional TR is the most frequent cause of tricuspid disease. In a retrospective study of 80 patients who presented a hemodynamically significant tricuspid regurgitation, etiology of TR was functional in 85.5% (68) of patients. The most common functional cause was pulmonary artery hypertension (80%) while ischemic (25%) and non-ischemic cardiomyopathy (8%) were
also common [46]. In a postmortem study of patients with pure TR, Waller et al reported that 47% of all cases of TR were functional [47].

The suspected mechanism of functional TR is right ventricular enlargement and abnormal leaflet coaptation. The tricuspid annulus, which is mostly muscular, follows the dilatation of the right cavities. The causes of RV enlargement are ischemic or non-ischemic cardiomyopathy and pulmonary hypertension leading to chronic right ventricular hypertension. Causes of pulmonary hypertension are divided into primary pulmonary hypertension (precapillary) or secondary pulmonary hypertension. Of these causes left-sided valvular disease are common [46]. In the case of IMR, LV pressure filling may develop secondary to pulmonary hypertension [44]. The total perimeter of the tricuspid annulus is 100-120 mm. This annulus circumference can reach 150-170 mm in the case of functional TR [47,48]. Annulus dilatation is not homogeneous. In a postmortem study, Deloche et al. showed that the posterior and anterior portions of the annulus dilated far more than the septal portion [49] (Figure 3). Annulus dilatation results in lack of coaptation of the leaflets and therefore regurgitation.
Figure 3: Dilatation of the tricuspid annulus in the case of functional tricuspid regurgitation (Modified from Deloche et al. [49]).

Dilatation affects mainly posterior and anterior annulus. Numbers indicate the change of length in percentage found by Deloche et al [49].
1-4-3: Alternative mechanisms

Besides the annulus dilatation, another mechanism was cited by Carpentier et al.:

"Excessive dilatation of the right ventricle maintains an abnormal tension of the chordae, impairing the free motion and therefore perfect coaptation of the leaflets" [50].

Anterior displacement of the tricuspid leaflet tips was described 10 years later by Mikami et al. [51]. However, in a study of eleven patients, Come and Riley showed that in 5 patients with failure of systolic leaflet coaptation, tricuspid annular dilatation appeared to play a major role [52]. Severity of tricuspid regurgitation was correlated with the degree of apical displacement of the leaflets in an extensive echocardiography study of Sagie et al [53]. In summary, mechanisms of functional regurgitation in the case of ischemic TR (ITR) remain unclear. Right ventricular enlargement, tricuspid annulus dilatation and restrictive motion of the leaflets as in IMR are three possible origins.

1-5: Surgical Approach of Ischemic Heart Failure

1-5-1: Repair of Ischemic MR

The optimal management of moderate IMR remains controversial. Some authors argue that revascularization alone without any mitral maneuvers is adequate therapy. Potential reversibility of ischemic MR following revascularization alone may not justify longer surgery mixing mitral repair or replacement and revascularization [54].

While functional IMR is related to the location and extent of the infarct, successful reperfusion alone does not reduce mortality, nor does it reliably restore valvular competence [33] [55].
Mitral annuloplasty is performed in nearly 80% of patients with ischemic mitral regurgitation [56]. Undersizing the mitral ring is the technique of choice in cases of IMR (type IIIB dysfunction, restricted leaflet motion in systole only) [54]. Authors who worked on an ovine model advocated a complete and undersized ring as a prosthetic annuloplasty. The dilatation of the mitral annulus occurs in all the annulus, therefore the ring has to be complete and sizing the ring on the dilated intertrigonal distance may result in selecting a ring that is too large [29,30]. The insertion of an undersized ring on a dilated annulus may lead to ring dehiscence because of excessive tension on the sutures. Multiple overlapping sutures around the annulus can reinforce the sutures and prevent this complication [57].

However, mitral annuloplasty is sometimes inadequate. In 30% of patients recurrent MR occurs following annuloplasty for IMR. In a retrospective study of 585 patients who underwent annuloplasty alone for repair of IMR, 28% of patients had 3+ or 4+ MR 6 months after the operation. In this study the use of a Peri-Guard annuloplasty ring, higher grade preoperative MR, jet direction or complex and more severe preoperative LV dysfunction were associated with a higher risk of recurrence of high grade MR than Cosgrove bands and Carpentier rings [58]. In a retrospective study of 100 patients who underwent mitral annuloplasty and revascularizations for ischemic MR, the incidence of recurrent MR was 29% [59]. From 48 patients who underwent revascularization and ring annuloplasty for IMR and left the operating room with <1+ MR, Matsunaga et al. reported 15 patients who had recurrent IMR. In these patients, LV dysfunction and enlargement and papillary muscle displacement were greater than in the
other 33 patients who did not have any recurrent IMR [60]. In a retrospective analysis of 126 patients who underwent mitral annuloplasty in case of IMR, analysis showed no clear survival benefit conferred by the surgery [61].

All these studies showed that annuloplasty alone in cases of ischemic MR seemed to be insufficient. One of the reasons was that the annulus prosthesis addressed only the mitral annulus dilatation, but did not influence the other mechanisms responsible for ischemic MR. Other procedures, which involve the sub-valvular mitral apparatus, have been described.

- Chordal cutting

In cases of functional IMR, the laterally displaced postero-lateral papillary muscle [27] tethers the anterior mitral leaflet (Figure 1) through the stay chords. The anterior mitral leaflet has a characteristic tent-shape and this angulated bend limits its ability to coapt effectively. Messas et al. reported experimentation on sheep with reduction of the amount of IMR by chordal cutting compared with controls [62]. Several patients were operated on with this procedure (personal observation of cases in Paris, France), however no human studies have been published. Only two cases with no follow-up were reported in 2004 in a Japanese journal [63]. Experimentation on sheep showed the importance of these chords as essential for the valvular-LV geometry. Rodriguez et al. showed that cutting second order chords did not prevent acute IMR and resulted in LV systolic dysfunction in sheep [64] [65]. Goetz et al. showed that the anterior mitral stay chords, which connect the papillary muscle to the fibrous trigones, suspend the aorto-mitral
Transection of these chords increased the papillary muscles – fibrous trigones distances and narrowed the aorto-mitral angle in systole and diastole by 5 degrees [66].

The true function of the anterior mitral basal “stay” chords is not yet known. Collagen fiber orientation of anterior mitral leaflet suggests that local stress be directed from papillary muscles over these chords and to fibrous trigone [67,68]. It seems that the stay chords are like pillars that support the anterior mitral leaflet as a vault. Therefore cutting these chords could be dangerous.

- Surgical relocation of the papillary muscle

Kron et al., aware of mitral annuloplasty failure in ischemic mitral regurgitation, reported 18 patients with previous inferior myocardial infarction who underwent posterior papillary muscle relocation associated with revascularization and annuloplasty. This new technique consisted of adding a suture between the left trigone and the posterolateral papillary muscle associated with an annuloplasty. This suture brings the papillary muscle tip closer to the annulus and therefore allows coaptation of the mitral leaflets. This surgical procedure is performed in patients whose LV end-systolic diameter is moderately dilated (<6 cm) [56]. In case of larger dilatation (>6 cm end-systolic LV diameter), surgical relocation of the posterior papillary muscle can be done with a Dor procedure. A Dor procedure consists of a surgical resection of the ventricular wall at the level of the infarction. A suture is placed on the collar of the myomectomy, which reduces the neck of the scars and an endoventricular patch is secured inside the ventricle. This procedure is designed to re-shape the LV and relocate the posterior papillary muscle by giving a more ellipsoid shape to the heart [69,70]. Hvass et al. reported 10 patients
with ischemic mitral regurgitation who underwent a “sling procedure” associated with a mitral annuloplasia. The sling procedure consisted of a Gore-tex tube placed around the two papillary muscles through the trabeculations of the heart. The tube was tightened until the papillary muscles were in close contact. Results showed significant improvement in echocardiography and clinical data [71]. All these surgical relocations of the displaced papillary muscle are associated with an annuloplasty. The results involve only small numbers of patients and further clinical studies have to be reported to draw conclusions.

- Mitral Valve Replacement

In a comparative review of 223 patients, short-term outcome showed that mortality rate was doubled for patients who underwent mitral valve replacement versus repair (30 days mortality 20% versus 10%). Five-year complication-free survivals were 64% for repair and 47% for replacement. However, the statistical analysis suggested an outcome linked primarily to the NYHA functional class and independent from the type of surgery. The authors concluded in this study that replacement and repair were both possible choices and could be left up to the surgeon, the acuteness of the presentation and the pathophysiology involved. In cases where the predominant presentation was a large annulus dilatation, annuloplasty and mitral valve reconstruction had excellent results [72]. Gillinov et al. studied, retrospectively, 482 patients who underwent mitral valve replacement (n=85) or mitral valve repair (n=397) during a 12-year period. Their findings were similar to the results of Grossi et al. in that most patients benefited from mitral valve repair. For the sickest patients, survival after repair and replacement were similar.
According to Miller, a role remains for mitral valve replacement using a bioprosthesis in cases of complex and long predictable repair and for surgeons who do not often do repair procedures and that mechanical valves, given the limited life expectancy of these patients, would not provide any benefit [74].

1-5-2: Repair of functional TR: Critical annulus diameter:

Repair of functional TR is based on the idea that the cause of regurgitation is the lack of leaflet coaptation due to annulus dilatation. Therefore the search for a "critical annulus diameter" beyond which the tricuspid regurgitation should not be ignored by the surgeon is very important. In 1983, Ubago et al. used a right ventriculogram to quantify TR. They showed that the indexed mean maximum diameter for the tricuspid annulus was 21 mm/m² among patients without regurgitation, 31 mm/m² among patients with mild regurgitation and 37 mm/m² among patients with moderate or severe TR. The authors concluded that the critical annulus diameter was 27 mm/m² [48]. In a transthoracic echocardiography study of 11 patients with functional TR, Come et al. reported a mean diastolic annulus diameter of 51 mm in the four-chamber view and 54 mm in the short axis view. Fifteen control patients had a mean annulus diameter of 34 mm in the four-chamber view and 33 mm in the short axis view [52]. Using intraoperative transeosophageal echocardiography, Goldman et al. measured the largest tricuspid annulus diameter from the base of the septal leaflet to the insertion of the anterior leaflet in the free right ventricular wall. When TR was 0-2+, annulus diameter was 26 mm in 36 patients. When TR was 2-4+, annulus length was 39 mm [75].
Therefore, 30 mm appears to be the critical length for these authors who did not describe differences in systolic and diastolic diameter.

Differentiation between organic and functional disease can be done preoperatively by echocardiography. Analysis of leaflet thickness, irregularities, doming and the presence of a transvalvular gradient is a clear indication of organic disease. Annulus dilatation, present in both organic and functional disease is more pronounced in functional TR. In a postmortem study, Waller et al. showed that patients with functional TR had a much larger annulus than patients with organic disease [47]. However, no hemodynamic and angiographic differences were found between organic and functional disease [57].

- Tricuspid annuloplasty

Surgical techniques for functional TR are based on reduction of the tricuspid annulus. To reduce the tricuspid annulus allows the coaptation of the three leaflets. An early method of plication of the posterior annulus was described by Kay [76]. This method was abandoned because of the difficulty in determining the amount of plication necessary. The remaining annulus was also not supported and could dilate later. A partial suture which encircled and narrowed the annulus was described by De Vega [57] and Cabrol [77]. However, this technique is rarely used today because of the late recurrence of tricuspid regurgitation resulting from the suture cutting through or breaking [57]. Total flexible prosthetic rings can correct dilatation and deformation of the annulus. These rings are sized and shaped and give optimal orifice area and the annulus can be reduced selectively at the point of excessive dilatation [50,78]. Surgical techniques for implanting
a tricuspid annuloplasty ring are very similar for all types of rings. The dangerous part of this surgery is the proximity of the atrio-ventricular node along the septal leaflet. In a retrospective study of 790 patients with functional TR, McCarthy et al. [79] showed that tricuspid valve annuloplasty did not consistently eliminate functional TR. Four techniques of annuloplasty were used and regurgitation severity remained stable with Carpentier-Edwards ring (p=0.7), increased with the Cosgrove-Edwards band (p=0.05) and remained unstable with the De Vega (p=0.002) and Peri-Guard (p=0.0009) procedures during the follow-up. Risk factors for TR recurrence included higher preoperative regurgitation grade, poor LV function, permanent pacemaker and repair type different from annuloplasty. At one-month postoperatively, prevalence of 3+ or 4+ TR was 15% for each of the different procedures and the size of ring was not a factor in the failure.

**- Tricuspid valve replacement in case of functional tricuspid regurgitation**

Presence of functional TR is not an indication for tricuspid valve replacement [57]. Tricuspid valve replacement is a high-risk procedure. McCarthy [79] reported a 37% mortality rate in cases of reoperation for functional TR. However, patients were referred late to the surgeon with hepatic and renal dysfunction. Poveda et al. reported a 39% hospital mortality rate in patients with rheumatic disease between 1974 and 1993 [80]. If the replacement is performed after a subsequent open-heart procedure, Hornick et al reported a mortality of 50% for patients operated between 1985 and 1993 [81].

Tricuspid annuloplasty has a better prognosis than tricuspid valve replacement. However,
failure of annuloplasty in a number of patients questions whether this technique is appropriate or not.

2: Ultrastructure of the Myocardium

2-1: The Sarcomere

The myocardium is composed of fibers arranged in parallel. The fiber is made up of longitudinally arranged cardiomyocytes, which are the contractile cells of the heart. The cardiomyocytes are made up of hundreds of myofibrils, which contain many mitochondria and an elongated centrally located nucleus. Cardiomyocyte size varies from 10 to 20 μm in diameter and from 50 to 100 μm in length. The longitudinally oriented myofibrils are formed by interdigitating myosin and actin filaments, which are the contractile elements of the cell. The repeating units of contractile elements, the sarcomere (from 1.5 to 2.2 μm in length), produces a regular pattern of dark and light areas. The dark Z bands are localized at the extremities of the sarcomere and support the attachment of the actin filaments. In the middle of the sarcomere are the thick myosin filaments. The interdigitating intersections produce dark and light zones within the sarcomere under microscopy (Figure 4). They contain an intricate sarcotubular system of tubules, vesicles and cisternae. The sarcoplasmic reticulum (SR) is a plexiform labyrinth of vesicles that tend to be oriented parallel to the myofibrils, which they surround. The periodic invagination of the sarcolemma by transversely oriented tubules constitutes the T-tubules. At the level of Z band, both T-tubules and SR present dilatation—the cisternae of the SR—creating a triadic junction. The sarcotubular system plays an important role in both electrical impulse conduction and electromechanical coupling [82].
Figure 4: Electron microscopy of human cardiomyocyte (freeze fracture of the SR surrounding the myofibrils, x 26,000).

The sarcomere between two Z bands (Z) presents two light I bands (I) constituted by thin actin filaments attached to Z bands. The A band (A) is the dark area between two I bands that contains both actin and myosin filaments. In the center of the A band is the lighter H zone constituted of myosin filament only. The M line (M) constituted by excrescence of the myosin forms the dark thin line in the middle of the A band. SR: sarcoplasmic reticulum (Modified from Nicholas J. Severs, The cardiac muscle cells BioEssays 22:188-199)
2-2: Myocardial Excitation-contraction Coupling

The calcium ion (Ca\(^{2+}\)) plays a central role in the action potential generated along the surface of the myocyte to initiate contraction in the cardiomyocyte. The initiation of the action potential corresponds to the opening of sodium (Na\(^{+}\)) channels that initiates a very rapid influx of Na\(^{+}\), which produces the electrical spike during phase zero of the action potential. During the plateau phase of the action potential, there is a slow inward flux of Ca\(^{2+}\) through Ca\(^{2+}\)-channels in the sarcolemma (myocardial cell membrane) into the intracellular fluid (sarcoplasm). The action potential spreads from the sarcolemma down the T-tubules, which allows the entire activation of the myocyte. At the level of the triadic junction, the calcium release channels of the SR or ryanodine receptors are opened by the Ca\(^{2+}\) that enters through voltage gated L-type calcium channels in the sarcolemma. The SR releases a large amount of Ca\(^{2+}\) in the sarcoplasm. These Ca\(^{2+}\) ions binds to troponin C (protein in the actin filament that inhibits the interaction between actin and myosin) and allows the interaction between actin and myosin, permitting the sarcomere to contract. Relaxation occurs when the rise in sarcoplasmic Ca\(^{2+}\) increases the uptake of Ca\(^{2+}\) into the SR by the calcium pump known as the Sarco-Endoplasmic Reticulum Calcium ATPase (SERCA) located mainly along longitudinal tubules of the SR. The strength and velocity of tension developed by the sarcomere are directly related to the amount of Ca\(^{2+}\) available to induce contraction. Stimulation of sarcolemmal β-adrenergic receptors increases the intracellular concentration of cyclic AMP (cAMP), which augments contractility by increasing Ca\(^{2+}\) entry through calcium channel and also facilitates relaxation by phosphorylation of Troponin I (reduces Ca\(^{2+}\) affinity) and of
phospholamban. This protein when phosphorylated increases the rate of Ca\(^{2+}\) uptake into the SR [83].

2-3: Sarcomere Stretch and Cardiac Function

The preload, the afterload, the contractility, the compliance and the heart rate regulate the contraction of the heart. The preload corresponds to the Frank-Starling law of the heart; the mechanism by which any increase in diastolic volume causes an increase in systolic performance. It establishes the initial muscle length of the cardiac fibers prior to contraction. The afterload is the sum of all the loads against which the myocardial fibers must shorten during systole. The contractility corresponds to the speed and shortening capacity of the heart. The compliance corresponds to the capacity of the heart to fill at any diastolic pressure and the heart rate is the frequency of contraction. These five factors are interrelated and determine the myofibril stretch or length, a principal determinant of cardiac performance [83]. Changes in muscle length not only change the number of actin-myosin bridges that can be formed to develop force but also affect the amount of force produced by the same amount of Ca\(^{2+}\) activation [84]. Lengthening the sarcomere promotes enhanced Ca\(^{2+}\) binding to troponin C and an increase in contractile force in response to the release of Ca\(^{2+}\) from the SR. Length or stretch of the sarcomere modulates the contraction via the excitation Ca\(^{2+}\) release process [85]. The Anrep effect or homeometric autoregulation corresponds to the adaptation of the cardiomyocyte to an abrupt change in length. This change of length in vivo or in vitro increases the Ca\(^{2+}\) concentration in the sarcoplasm and in parallel the development of contractile force [84]. At normal filling pressures, a myocardial sarcomere length of the LV varies from 2.07
µm at end-diastole to 1.8 µm at end-systole [86]. The resting tension of the sarcomere increases markedly between 2.0 and 2.2 µm and the actin and myosin myofilaments are optimally overlapped to provide maximal forces when the myocardial sarcomere measures 2.2 µm after fixation. When the sarcomere is stretched beyond 2.2 µm, the myocardium become very stiff and resting tensions rise greatly while developed tension starts to decline [83]. A number of stimuli such as pressure and volume overload and MI are responsible for the stretch of cardiomyocytes, thus the sarcomeres. Following myocyte loss, a portion of the ventricle is disabled resulting in failure to eject a normal quantity of blood. There is an increase in intraventricular end-systolic volume that is added to the blood entering the ventricle during the diastole. This overload volume creates an increased mechanical stress that stretches the myocardium [83]. The sarcomere lengths increase ultimately to 2.2 µm in endocardial, midwall and epicardial areas, where at this point, further increases in diastolic volume produces a very large increase in diastolic pressure. This limits acute ventricular dilatation and there does not appear to be any overstretch (beyond 2.2 µm) of the sarcomeres. Excessive stretch of the cardiomyocyte may lead to myocyte damage and even death [87]. The adaptations of the heart (or ventricular remodeling) to chronic distending forces are an increase in myofibril length with addition of new sarcomeres in series (hypertrophy) as well as a ventricular dilatation to maintain the cardiac output [88]. The Laplace relationship (T=(P x r)/2h where T=ventricle wall tension, P=LV pressure, r=radius and h=wall thickness) describes the increased workload created by the increased volume for the heart. Wilson et al. [89] showed that, eight weeks after induction of an anterior MI, the ventricle wall stretch measured by the percentage of lengthening of wall segments was heterogeneous. The
lengthening of the wall segments in a remodeled ventricle was directionally greater within the MI region: Further from the MI, less stretch applied on the cardiomyocytes. Following a MI, the mechanical stress exerted by the overload volume associated with ventricle geometrical alterations does not stretch the myocardium homogeneously. The fall of cardiac output that follows the loss of myocytes is also compensated by a variety of physiological mechanisms [2]. These adjustment mechanisms try to preserve the cardiac function within physiological range to maintain the patient symptomatic or minimally symptomatic after the initial event. Heart failure may then develop progressively and at some point the patient will develop symptoms [90]. The activation of the adrenergic nervous and salt-and water-retaining systems preserve pressure and cardiac output, and the activation of vasodilatory molecules, such as natriuretic peptides, prostaglandins and nitric oxide, counteract the systemic vasoconstriction resulting from excessive activation of the renin angiotensin aldosterone and adrenergic systems [91]. These compensatory mechanisms may also participate in the remodeling process and the development of contractile dysfunction [1,2,91,92].
Aim of the Study

The presence of functional mitral regurgitation secondary to an ischemic event is frequent and known to double mortality. While its mechanism has been extensively studied and its surgical treatment standardized the presence, importance and mechanism of a concomitant functional tricuspid regurgitation has been generally neglected. The interest and therefore knowledge of the tricuspid valve has always lagged behind the mitral valve. Because of the paucity of clinical signs when diseased and the imprecision of available diagnostic tools, the tricuspid valve has been generally ignored by cardiologists and surgeons. In a previous retrospective study of patients with mitral regurgitation from ischemic origin, we observed the presence of a concomitant and significant functional TR in 30% of cases. Based on this finding, we hypothesized that infarction alters not only the geometry of the LV, but also of the RV, leading to both IMR and ITR. These geometric alterations also modulate mechanical stresses resulting in molecular changes. To test these hypotheses, the following experimentations were performed.

Before analyzing the geometrical dimensions of both ventricles in heart failure, it was necessary to complete three experiments in normal hearts in order to understand the common elements of both atrioventricular valves. The tricuspid valve presents many variable anatomical characteristics [93] compared to the mitral valve. Therefore, to analyze the constant anatomical characteristics of the tricuspid valve, we performed an anatomical study of the tricuspid valve (including an original terminology) (Experiment 1). Secondly, to clarify the geometrical changes and the relationship of the normal mitral...
and tricuspid valves during the cardiac cycle, we performed an in vivo study of the functional anatomy of both valves using invasive sonomicrometry technology (Experiment 2). Thirdly, following a MI, heart failure develops progressively and repetitive analysis of the ventricle measurement is needed to understand the geometrical changes of the ventricles. The use of the non-invasive trans-thoracic echocardiography seemed more appropriate than the invasive sonomicrometry technology on a chronic heart failure animal model. Therefore, given the lack of published information of trans-thoracic echocardiography in sheep, we compared the trans-thoracic echocardiographic data with the sonomicrometric data (Experiment 3) to assess the reliability of the trans-thoracic echocardiography in sheep. Then, we developed a reproducible animal model of ischemic heart failure leading to IMR and ITR and analyzed the geometrical changes of both atrioventricular valves using trans-thoracic echocardiography (Experiment 4). Lastly, recent advance in molecular physiology of the cardiomyocyte showed that endothelial and neuronal nitric oxide synthase (eNOS & nNOS) contribute to sustain normal excitation/contraction coupling and to response to stretch [94]. The mechanical stress that stretches the cardiomyocyte has a direct impact on the excitation/contraction coupling of the cell as previously seen. Therefore, to analyze the molecular change associated with the geometrical alterations, we searched for regional variations in eNOS and nNOS expressions at increasing distance from the MI given the fact that the further from the MI the less stretch is applied to the cardiomyocytes [89] (Experiment 5).
Normal Heart Experiments

Experiment 1: A Unified Functional Terminology for the Mitral and Tricuspid Valves

The tricuspid valve has been traditionally described as formed by three leaflets and three or more papillary muscles [49] [50] [95], although a recent anatomical study describes it as a bicuspid valve with endless variations in leaflet scallops, chords, and papillary muscles [93]. A precise nomenclature has been developed for the mitral valve, however, no such terminology is available for the tricuspid valve. To understand the tricuspid anatomy and develop a tricuspid valve terminology similar to the existing mitral valve nomenclature, we studied 50 porcine hearts. The use of porcine heart was justified by its availability and the fact that in all higher vertebrates, the morphogenetic programs in the heart are the basically similar [96]. The study was designed as a search for parameters common to both atrioventricular valves that would lead to a unified terminology easy to apply in clinical practice.

Experiment 2: Geometrical Changes of the Mitral and Tricuspid Valves during the Cardiac Cycle.

A simultaneous study of both valves and ventricles in an ovine model allows the understanding of the close anatomic and physiologic relationships between the LV and RV.
Experiment 3: Echocardiography Data versus Sonomicrometrics Data.

Reliability of the use of transthoracic echocardiography (TTE) for the measurement of distances between intracardiac structures was shown by the comparison of sonomicrometric measurements and TTE measurements in normal sheep.

Heart Failure Experiments

Experiment 4: Induction of a Postero-lateral Infarct by Percutaneous Injection of 100% Ethanol and Analysis of LV and RV Geometrical Changes.

We developed an original percutaneous method of inducing MI consisting of the selective injection of pure ethanol in branches of the circumflex artery leading to a posterior-lateral MI that by ± 8 weeks resulted in significant IMR and ITR and analyzed the geometrical change of both ventricles by TTE.

Experiment 5: Change in eNOS and nNOS Expression with the Distance from the Infarct in an Ovine Ischemic Heart Failure Model

The changes of molecular expression and localization of eNOS and nNOS were measured at increasing distances from the postero-lateral MI. A correlation between these changes and the distance from the myocardial infarction was analyzed.
EXPERIMENTS

Experiment 1: A Unified Functional Terminology for the Mitral and Tricuspid Valves

1: Background: Anatomy of the Human Mitral and Tricuspid Valves

1-1: Mitral valve anatomy

The mitral valvular apparatus derived its name from the bishop’s mitre. It is located between the left atrium and the left ventricle (LV) and allows the blood to flow from the left atrium to the left ventricle. The mitral annulus, the leaflets, the chordae tendinae and the papillary muscles are the four elements that constitute the mitral valve.

1-1-1: The mitral annulus

The mitral annulus forms the junction between the left atrium and the left ventricle, and is the insertion of the anterior and posterior mitral leaflets. More an area than a true anatomically identifiable fibrous structure between the left atrium and the left ventricle, the mitral annulus can be defined by its function. It is described by Kumar et al. as “part of the circular area that surrounds the base of the left ventricle and encompasses the inlet, inflow or mitral orifice separated from the outflow orifice by the aortic curtain, which stretches between the two fibrous trigones of the heart” [97]. These two fibrous structures, the right and left trigones, collagenous thickening of the fibrous skeleton of the heart, can be consistently isolated in hearts. The right trigone (T1) is a fibrous
junctional area between the membranous septum, the mitral, the aortic and the tricuspid valves and was considered by Zimmerman et al. as the central body of the heart [98]. The left trigone (T2) is situated between the mitral valve and the left coronary cusp leaflet of the aortic valve. The shape and size of the mitral annulus varies continuously during the cardiac cycle as it was shown in dogs [99] and later in the human by Ormiston et al., who showed that the mitral annular area increases gradually during diastole and then decreases until midsystole. The mitral area starts to increase again during late systole [100].

Sonomicrometry array localization, marker angiography and three dimensional echocardiography were also used to confirm its dynamic changes during the cardiac cycle and to assess the saddle configuration of the mitral annulus with the commissures located at the low point of the saddle [26,101,102]. The hyperbolic paraboloid shape of the saddle configuration decreases the stress on leaflets during systole [101].

1-1-2: The leaflets

Anterior and posterior leaflets constitute the tissue portion of the valve (Figure 5). The anterior leaflet has a semicircular shape and is attached to two-fifths of the circumference of the mitral annulus. The free edge is not indented and there is continuity between the left coronary and non-coronary cusps of the aortic valve and the anterior mitral leaflet between the two trigones (Figure 6). The posterior leaflet is quadrangular and attached to three fifths of the mitral annulus. In an anatomical post-mortem study of 50 mitral valves, Ranganathan et al showed that the division of the mitral leaflets into anterior and posterior leaflets was possible in 98% of the cases by identifying the commissural areas. The posterior leaflet was tri-scalloped in 92% of the hearts with one
middle scallop more prominent in 84% of cases [67]. The posterior leaflet height is half
that of the anterior leaflet height. However, the tissue area of both leaflets is the same and
is twice the size of the orifice area delimited by the mitral annulus. These leaflets are
separated one from the other by the commissures. The commissures are posteromedial
and anterolateral according to their anatomical position in the body. Some leaflet tissue is
delimited at the level of the commissures and can be called commisural leaflets. Distinct
ridges that define the leaflet closure area (or coaptation surface) exist on the atrial side of
the leaflets [67].
Figure 5: The mitral leaflets of a pig heart.

Atrial face after section of the ventricle through the posterior wall.
Anterior leaflet: A,  
Posterior leaflet: P
1-1-3: The chordae tendineae:

The connection between the papillary muscles and the leaflets, the chordae tendineae are classified according to the site of insertion between the free margin and the base of the leaflets. Tandler et al divided the chords in three orders and Lam et al. subdivided the three orders by describing the commissural, strut and basal chordae [68]. Marginal chordae (primary chordae) are inserted into the free margin of the leaflets. They are made up of the commissural chordae, branches of cleft chordae of the posterior leaflet and the rough zone chordae of the posterior and anterior leaflets. Intermediate chordae (secondary chordae) are inserted beyond the free margin of the leaflets. They are made up of those that arise from each papillary muscle and insert into the ventricular aspect on the rough zone of the anterior and posterior mitral leaflet and by the main stems of the cleft chordae of the posterior leaflet. Lam et al. described among them two particularly strong and thick, tendon-like chordae that insert into the undersurface of the body of the anterior leaflet and stretch toward the anterior mitral annulus and called them strut chordae [68]. Leonardo Da Vinci wrote, on his anatomical drawing of the heart, “prima” in front of these presumably chordae (Leonardo da Vinci, Anatomic Drawings, Windsor Collection, Windsor, United Kingdom). These stay chordae remain under the same pressure during the entire cardiac cycle [103] (Figure 6). Basal chordae are limited to the posterior leaflet. They are attached to the leaflet base connecting it to the mitral annulus and the myocardial tissue of the posterior leaflet.
Figure 6: The stay chords.

The strut or stay chords (A & B) and their insertion on the anterior mitral leaflet (ventricular view). C shows the continuity between the left coronary and non-coronary cusps of the aortic valve and the anterior mitral leaflet between the two trigones.
1-1-4: The papillary muscles:

Two papillary muscles arise from the LV wall approximately one-third the length of the LV from the apex. They are anterolateral and posteromedial, according to their anatomical position. They connect the mitral leaflet to the ventricle wall through the chordae tendinae. They are anchored in the LV wall among the trabeculae carneae of the myocardium. Each papillary muscle provides chordae to half of the corresponding mitral leaflet and commissure (Figure 3). The morphology of these papillary muscles is very variable and difficult to describe. Ranganathan et al. described three different types, according to their insertion in the LV wall [104].

- Finger-like: One third of the muscle arises in the LV cavity and there are no trabeculation.

- Tethered: The papillary muscle is hardly seen and only its tip arises in the LV cavity.

- Mixed: The papillary muscle presents both previous characteristics.

This classification was modified in 1996 by Rhamsheyi et al. who described 4 types of papillary muscles from 65 human hearts [105]:

- Type I = Finger-like: Papillary muscles well individualized and non-subdivided corresponded to 63% of the antero-lateral and 41% of the postero-medial.

- Type II: Papillary muscle is divided longitudinally in two parts. The chordae to the posterior leaflet are on one head and the chordae for the commissural and the anterior leaflet are on the other head (7% of the anterolateral and 39% of the posteromedial papillary muscles).
-Type III: Papillary muscle divided into several heads. One head supports all the chordae for the commissural area (15% of the anterolateral and 7% of posteromedial papillary muscle).

-Type IV: Papillary muscle divided in several complex heads with several insertion bases. One thin muscular band supports very short commissural chordae (15% anterolateral and 13% posteromedial papillary muscles).

The vasculature of the papillary muscles is also variable. The anterolateral papillary muscle is vascularized by branches of the left anterior descending artery. The posteromedial papillary muscle is vascularized by branches of the right coronary or of the obtuse marginal artery. This is independent of the dominance of the right or left coronary [106]. Ranganathan et al. described in 10 human hearts the micro-vascularization of the papillary muscles. One or more of long penetrating vessels that originate from the artery on the epicardial surface of the heart supplied each papillary muscle. During their course through the wall, these vessels branched and anastomosed with adjacent arteries. A central artery supplies the free portion of the papillary muscle. In case of many trabecular attachments, the long penetrating intramyocardial vessels were coursing through them [104].

1-1-5: Anatomical and surgical classification

Indentations and scallops of the posterior leaflets associated with relationship with chords and papillary muscles are used to classify the areas of the mitral valve. Two different anatomical nomenclatures are used to analyze the segmental valve (Figure 3 and Table 1). The Carpentier classification delimits both leaflets in three corresponding
areas according to the main scallops of the posterior leaflet and recognizes two commisural areas or leaflets. The Duran classification delimits the anterior leaflet in two parts and the posterior leaflet in four parts and two commissural areas according to their attachment to the papillary muscles [97].

Table 1: Correspondence of Carpentier and Duran nomenclatures of the mitral valve.
A: anterior leaflet, P & PM: posterior leaflet, C: commissural leaflet

<table>
<thead>
<tr>
<th></th>
<th>Carpentier</th>
<th>Duran</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anterior leaflet</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>A1</td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td></td>
<td>A2</td>
</tr>
<tr>
<td>A3</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Posterior leaflet</strong></td>
<td>P1</td>
<td>P1</td>
</tr>
<tr>
<td>P2</td>
<td>PM1, PM2</td>
<td></td>
</tr>
<tr>
<td>P3</td>
<td>P2</td>
<td></td>
</tr>
<tr>
<td><strong>Commissural leaflets</strong></td>
<td>C1</td>
<td>C1</td>
</tr>
<tr>
<td>C2</td>
<td>C2</td>
<td></td>
</tr>
</tbody>
</table>
Figure 7: Classifications of the mitral valve. Black: Duran’s and Grey: Carpentier’s

Carpentier (gray) and Duran classification (black) are represented together. The black lines delimit the chordae repartition while the gray lines delimit the scalloped segmentation of the leaflets.
1-2: Tricuspid valve anatomy

The tricuspid valvular apparatus was named from the predominant presence of three leaflets. It is located between the right atrium and the right ventricle and allows the blood flow to go from the right atrium to the right ventricle. It is, like the mitral valve, constituted of four elements: the tricuspid annulus, the leaflets, the chordae tendinae and the papillary muscles.

1-2-1: The tricuspid annulus

The tricuspid annulus is more an area where the leaflets are attached to the myocardium between the right atrium and the right ventricle than an actual fibrous ring. The absence of an encircling fibrous structure may explain the change in geometry during the cardiac cycle. The geometry changes were demonstrated in a sheep study by Hiro et al [41]. The annulus expanded almost homogeneously during the cardiac cycle from 4.8±0.8 cm² to 6.1±0.9 cm². The septal part of the annulus expanded by 10 %, the anterior by 13% and the posterior by 14%. These findings were also seen in the human by Tei et al. using echocardiography [107]. The tricuspid annulus was described as pear-shaped with its narrower end close to the antero-septal commissure and its wider end corresponding to the midpoint of the posterior leaflet in pressurized post mortem human [108] and sheep hearts [41]. In-vivo, the sheep model of Hiro et al. showed the opposite with the narrow end at the level of the postero-septal commissure. Like the mitral annulus, the tricuspid annulus is a three-dimensional structure with its three highest points at the level of the mid-posterior and mid-anterior annulus and the anteroseptal commissure (Figure 8). Three minimal points were found at the antero-posterior and
postero-septal commissures and the midpoint of the septal leaflet [41]. This complicated
3-D structure of hyperbolic paraboloid-like for the saddle shape of the tricuspid valve
may reduce the stress exerted on the leaflets [101].

Figure 8: The saddle shape of the tricuspid annulus.

The three lowest points are the anteroposterior commissure (AP), the postero-septal
commissure (PS), and the mid of the insertion of the septal leaflet on the annulus (S). A
and P: mid points of the anterior and posterior leaflets on the annulus. AS antero-septal
commissure. (From CMG Duran in Surgery of the chest, p1337 [57])

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1-2-2: The leaflets:

Commonly, the tricuspid valve is described with three leaflets separated by three commissures (the anterior, the septal and the posterior leaflets separated by the anteroseptal, anteromedial and posteromedial commissures). The leaflets are named according to the level of their insertion on the annulus. At the level of the commissures that don’t reach the annulus, can be found small commissural leaflets [57]. The anterior leaflet is the largest, followed by the posterior leaflet with the septal leaflet being the smallest (figure 9 A & B). However, the number of leaflets of the tricuspid valve is highly variable [93]. In a study of 100 normal human hearts, Victor et al. showed numbers of subdivisions for the anterior and posterior leaflets attached to the free right ventricle wall. They described six main different types with a minimum of one and a maximum of five scallops dividing the anterior and posterior leaflets:

- Type A: No division between anterior and posterior leaflets (one scallop) (1%)
- Type B: Division in posterior and anterior leaflets (31%)
- Type C: Type B + antero-posterior commissural scallop (three scallops) (40%)
- Type D: Type C + Presence of a posterocommissural scallop (four scallops) (17%)
- Type E: Type D + Presence of an anterocommissural scallop (five scallops) (10%)
- Type F: Absence of tissue at the antero septal junction. (1%)

The septal leaflet remains constant and is always described by anatomist [93] [50] [57]. However, the anterior and posterior cusps are dominant in tricuspid valve function [109]. Higashidate et al. showed in dogs that resection of the septal leaflet did not result in TR and that the two remaining valves were sufficient for closure of the tricuspid orifice [110].
Figure 9: The Tricuspid valve in a pig heart.

A: Atrial face of the tricuspid valve viewed after section of the anterior wall of the right ventricle. Septal (s), Anterior (a) and posterior (p) leaflets.
B: Tricuspid valve viewed from the atrium.
C: Detail of the finger-papillary muscle and of the marginal chordae tendinae.
**1-2-3: The chordae tendinae and papillary muscles**

In most cases, three tricuspid papillary muscles can be found and identified as anterior, posterior and septal. Anterior and posterior muscles are practically always present, however, the septal muscle might be absent in 20% of patients. The anterior papillary muscle is the longest and sustains the highest number of chordae [57]. In their anatomical work on 100 human hearts, Viktor et al. found that the papillary muscles exhibited endless variations in number, size, shape, fusion and location (Figure 9C)[93]. Marginal and basal chords connect the papillary muscles to the leaflets. Marginal chords are inserted on the free edge of the leaflets and basal chords are inserted into the ventricular surface of the leaflets. The role of the basal chords in the right ventricle is probably the same as in the left ventricle in maintaining the valve and ventricular geometry [57].

The interest and therefore knowledge of the tricuspid valve has always lagged behind the mitral valve. Because of the paucity of clinical signs when diseased and the imprecision of available diagnostic tools, the tricuspid valve has been generally ignored by cardiologists and surgeons. In the case of the mitral valve, more precise nomenclatures have been developed to fit the demands of complex repair maneuvers; however, no such terminology is available for the tricuspid valve.

**2: Materials and Methods**

Fifty pig hearts collected from a local abattoir (Hamilton Packing Inc., Hamilton, Montana, United States) were studied. The pulmonary artery was incised longitudinally. The incision was extended apically between the anterior and right pulmonary cusps and
across the free wall of the right ventricle to ensure that no papillary muscle, muscle band, or chordae was divided. After sectioning the ventricular muscle band, the anterior leaflet was cut from its free edge to the annulus. The tricuspid valve and right ventricle were then fully opened.

To design a terminology for the tricuspid valve, all observations were based on the traditional understanding that the tricuspid valve has three main leaflets and three papillary muscles. Recognizing the high variability of all its structures, an attempt was made to use this classic assumption because it is familiar to surgeons and cardiologists.

The leaflets were classified according to their heights (free edge to base) and their chordal insertions. The three main leaflets were defined as having the largest heights and being supported by chords from two different groups of papillary muscles. They were identified with the classic terms of septal (S), anterior (A), and posterior (P). The remaining leaflets were defined as “commissural” if they had smaller heights and were supported by chords arising from a single papillary muscle group.

The papillary muscles were grouped according to their chordal distribution to a single commissure and two contiguous main leaflets. The group corresponding to the anteroseptal commissure was termed “anteroseptal,” the group corresponding to the anteroposterior commissure was termed “anteroposterior,” and that corresponding to the posteroseptal commissure was called “posteroseptal”. The shape of the papillary muscle within the same group was described as “finger-like” if one-third of the muscle protruded into the ventricular cavity, as “tethered” if the body of the papillary muscle was embedded within the ventricular wall and only its tip could be identified [104], and as “vestigial” when the chords arose directly from the ventricular wall (Figure 10).
Maximum distances between the lateral aspects of the papillary muscles furthest apart within the same group were measured. According to this distance, each papillary muscle group was classified as “small” (1 to 2 cm), “medium” (2 to 3 cm), or “large” (> 3 cm).

The right ventricular band was defined as “atrophic” (< 2 mm width), “medium” (2 to 4 mm), or “large” (> 4 mm).
Figure 10: The three different types of papillary muscles.

F: finger-like; T: tethered; V: vestigial.
3: Results

3-1: The Leaflets and Their Commissures

In all hearts, the tricuspid valve had three main leaflets. As commonly accepted, they were termed septal, anterior, or posterior according to their base attachment to the annulus. Between each main leaflet, one or more commissural leaflets were commonly present. These commissural leaflets did not reach the annulus with more than 5 mm of tissue between its free edge and the annulus. These commissural leaflets were mostly found between the anterior and the posterior leaflet (1.02 per heart, range 0-3) and between the posterior and the septal leaflet (0.98 per heart, range 0-2). The presence of a commissural leaflet between the septal and the anterior leaflet occurred in only eight hearts (0.16 per heart, range 0-1). Every heart studied had at least one commissural leaflet. In one heart, one anteroposterior and one posteroseptal commissural leaflet had the same height as the main leaflet. The number of commissural leaflets present at the anteroposterior, posteroseptal, and anteroseptal commissure is shown in Table 2.

Table 2: Number of commissural leaflets at each commissure.

<table>
<thead>
<tr>
<th>Number of commissural leaflets per commissure</th>
<th>Anteroposterior</th>
<th>Posteroseptal</th>
<th>Anteroseptal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>8</td>
<td>42</td>
</tr>
<tr>
<td>2</td>
<td>35</td>
<td>35</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
3-2: The Papillary Muscles and Chordae Tendinae

Three groups of papillary muscles were found in every heart studied. The three groups were defined according to the distribution of their chords to the two adjacent half-main leaflets and their corresponding commissure. The number of separate papillary muscles per group is reported in Table 3.

In the anteroseptal group, a separate conus papillary muscle was always identified. The other muscles were constantly attached to the ventricular septum below the conus. The shapes of these 89 muscles were finger-like in nine cases, tethered to the wall in 12 cases, and vestigial in 68 cases. The overall width or maximal distance between muscles of the same group was small (< 2 cm) in 22 groups, medium (between 2 and 3 cm) in seven groups, and large (> 3 cm) in three groups.

The anteroposterior group of papillary muscles was better defined than the other groups. In 48 of 50 hearts, it consisted of a single papillary muscle located in the free wall. This finger-like papillary muscle had one head in 25 hearts, two heads in 20 hearts, and was multi-headed in three hearts. The other two hearts presented an anterior group formed by two finger-like papillary muscles in one case and by four in the other. In both cases, the distance between the lateral aspects of the papillary muscles was medium (between 2 and 3 cm).

The posteroseptal papillary muscle group had the highest variability in shape, size, number, and location of the three groups. This posteroseptal group was always attached to the septum, except in one case. In this heart, there was one finger-like papillary muscle attached to the posterior free wall with the remaining papillary muscles anchored to the posterior septum. In all specimens, at least one papillary muscle was
found on the septal wall close to the corner with the free wall. The shapes of the 115 papillary muscles that constituted this group were finger-like in 60 cases (with a single head in 73.3%, double head in 11.6%, and multi-headed in 15.1%). Of the remaining papillary muscles, 50 were tethered to the wall; in five, the papillary muscle was vestigial with the chords inserted directly onto the ventricular wall. The width or maximum distance between papillary muscles in that group was < 2 cm in 10 hearts, between 2 and 3 cm in 22 hearts, and > 3 cm in 12 hearts.

A right ventricular band was always present. Its thickness or diameter was atrophic (< 2 mm) in 14 hearts, medium (2 to 4 mm) in 19 hearts, and thick (> 4 mm) in 17 hearts. In all specimens, this band linked the base of the anterior papillary muscle to the base of the conus papillary muscle.

In two hearts, it was difficult to determine the appurtenance of a single vestigial papillary muscle located on the septal wall between the anteroseptal and posteroseptal groups. Their chordae tendinae were inserted into the midpoint of the free edge of the septal leaflet.

Table 3: Number of separate papillary muscles per papillary muscle group.

<table>
<thead>
<tr>
<th>Number of separate papillary muscles per papillary muscle group</th>
<th>Anteroseptal</th>
<th>Anteroposterior</th>
<th>Postero septal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18</td>
<td>48</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>26</td>
<td>1</td>
<td>22</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Average number per group</strong></td>
<td><strong>1.78</strong></td>
<td><strong>1.08</strong></td>
<td><strong>2.42</strong></td>
</tr>
</tbody>
</table>
3-3: Proposed Terminology and Classification

Careful analysis of the literature and of our findings revealed that the more precise the description of the leaflets, the more confusing and useless it was for the surgeon. Like Kumar et al. [97], who focused on the anatomical elements that are observed by the surgeon through the atriotomy, we divided the structures into three elements: the papillary muscles, the leaflets, and the chords. The three groups of papillary muscles were then defined by the numerals 1 for the anteroseptal; 2 for the posterosptal, and 3 for the anteroposterior.

Each leaflet was divided in two areas that were named according to both the traditional leaflet’s names (S, A, P) and the papillary muscle origin of the chords inserted on it (1, 2, 3). Figure 11 shows the suggested tricuspid valve terminology and Figure 12 shows the combined mitral and tricuspid valve nomenclatures.
Figure 11: Tricuspid valve terminology.

The valve has been opened with a vertical section through the middle of the anterior leaflet. A1 & A3: anterior leaflet; P2 & P3: posterior leaflet; S1 & S2: septal leaflet; C1, C2, & C3: commissural leaflets.
Figure 12: Diagram of the mitral and tricuspid valves as seen by the surgeon through left and right atriotomies.

Circles represent the papillary muscles and straight lines represent the division of the leaflets according to their chordal insertion into the papillary muscles. The shape of both annuli is based on sonomicrometric information.


For the tricuspid valve: A1 & A3: anterior leaflet; P2 & P3: posterior leaflet; S1 & S2: septal leaflet; C1, C2, & C3: commissural leaflets, 1, 2 & 3: papillary muscles.
4: Discussion

4-1: A New Nomenclature for the Tricuspid Valve

Semantics has always been the main stumbling block to progress. A lack of precise definitions results in subjective meanings, variable interpretations, and subsequent confusion. This problem is particularly apparent in heart valve surgery, where more precise anatomic knowledge is required as new techniques are developed. The old terminology soon becomes insufficient to describe pathologic findings, surgical maneuvers undertaken, and evaluation of the results. Also, new surgical procedures demand a common set of anatomical definitions for the reporting of precise pre- and postoperative echocardiographic information between the echocardiologist and surgeon. This situation, which has been largely improved in the case of the mitral valve, is still missing for the tricuspid valve. To clarify this issue, we took the same familiar parameters used by surgeons and echocardiographers for the mitral valve as guidelines and applied them to the tricuspid valve. The aim of the present study was not a strict anatomical description, but a search for parameters common to all atrioventricular valves so that a unified terminology could be applied in clinical practice. Due to the variability in the number of leaflet scallops and number of chords present in the normal valve, we based both terminologies on the papillary muscles. Although the number of papillary muscle heads is also variable (particularly in the tricuspid valve), it was always possible to identify two groups in the mitral valve and three groups in the tricuspid valve. The Duran mitral valve classification was based on the fact that the mitral valve consists of two symmetrical structures supported by two papillary muscles (Figure 3). All elements supported by the anterior papillary muscle (situated to the left of the surgeon) were
identified with the numeral 1; those supported by the posterior papillary muscle (situated to the right of the surgeon) were identified with the numeral 2. Therefore, the papillary muscles were identified as M1 & M2, the trigones were identified as T1 & T2, the commissures were identified as C1 & C2, the anterior leaflets were identified as A1 & A2, the lateral posterior scallops were identified as P1 & P2, and the mid scallop was identified as PM1 or PM2 according to whether its chords arose from M1 or M2 [97]. The earlier classification described by Carpentier [111] was probably based on the fact that the most frequent surgical maneuvers are applied to the posterior leaflet, so he defined its three scallops as P1, P2, and P3 and defined the anterior leaflet into the corresponding opposite A1, A2, and A3. This classification does not encompass the commissures, trigones, and papillary muscles, which must be identified with the old terminology based on anterior/posterior and lateral/medial terms.

The tricuspid valve is more complex than the mitral valve, not only because it is a trileaflet valve but, more importantly, because of its high variability. Traditionally, the tricuspid valve has been described as formed by three very thin leaflets attached to the annulus at their base and to the papillary muscles through the chordae tendinae[49,95]. Although the number of tricuspid leaflets varies according to author [93], it is commonly accepted that the tricuspid valve consists of three leaflets (septal, anterior, and posterior) that are separated by three clefts or commissures [112]. These commissures do not reach the annulus but delineate small commissural leaflets. By defining the main leaflets as tissue receiving chords from two different groups of papillary muscles, we always found three main leaflets associated with a variable number and size of commissural leaflets (interpreted as additional scallops by other authors [93]). Since our focus was on the
surgical classification of the tricuspid valve and not on a precise anatomical report, we did not fully size all leaflets and commissures. The septal leaflet was present in all of the hearts studied, which is always described by others [50,93,112].

As previously reported [93], we found endless variations in the number, size, shape, fusion, and location of the papillary muscles. However, when observing the insertion of the chords into the three leaflets, we were able to identify three different groups of papillary muscles corresponding to the anteroseptal, anteroposterior, and posteroseptal commissures. The anteroposterior muscle was the longest and sustained the highest number of chordae. It was single in 96% of our hearts. The posteroseptal papillary muscle group, although most variable, was always present in our series. Although the anteroseptal muscle has been reported as absent in 20% of patients [112], if we classified it as an anteroseptal group giving rise to chords to the septal and anterior leaflets, we constantly found one papillary muscle on the conus.

The three papillary muscle groups were always present in 1) the middle of the free wall; 2) on the septum close to the corner between the posterior wall and the free wall; and 3) at the level of the conus. Although the main papillary muscle variations were always present on the septal wall, these aberrant muscle heads could always be classified into the posteroseptal or the anteroseptal groups according to the distribution of their chords. It was difficult to classify two septal papillary muscles into either the anteroseptal or the posteroseptal group in only two cases; their chords were inserted exactly in the middle of the septal leaflet.
4-2: Clinical Relevance and Limitations of the Study

The present anatomical study was conducted on pig tricuspid valves, which probably differ from human tricuspid valves. However, the goal of this study was not to provide an exact anatomical description of the porcine tricuspid valve, but rather to attempt to unify the nomenclatures of both atrioventricular valves and find a simple method for surgeons and echocardiologists to describe and record pathologic findings and repair techniques employed. The porcine model was used to determine whether a classification of the different structures of the valve was possible despite the variability between individual hearts. Although tricuspid valve replacement and simple ring annuloplasty do not require detailed knowledge of the valve, newer surgical techniques demand a terminology that identifies the exact location of the pathologic findings and surgery performed. Clear examples of this need include commissurotomy of rheumatic lesions [112], leaflet resection of advanced myxomatous lesions [113], excision of infective vegetations [114], transfer and rotation of leaflet segments in traumatic [115] and bioptome-induced [116] ruptures, and autotransplantation into the mitral valve of whole commissures carrying their corresponding leaflets and papillary muscle [117,118]. In all of these organic lesions, a clear terminology is absolutely required to (at a minimum) record the surgery performed. More recently, the awareness of the high incidence of functional ischemic tricuspid regurgitation [60] and the poor results obtained from standard annuloplasties [79] are promoting studies on its physiopathology and possible new, more complex surgical techniques. Again, precise anatomic definitions become imperative.
The tricuspid valve has been generally ignored by cardiologists and surgeons because of the paucity of clinical signs when diseased and the imprecision of available diagnostic tools. The tricuspid valve has been traditionally described as formed by three leaflets and three or more papillary muscles [49] [50] [95], although a recent anatomical study describes it as a bicuspid valve with endless variations in leaflet scallops, chords, and papillary muscles. This complicated anatomy has resulted in the absence of a systematic classification of the valve. To standardize the description of both atrioventricular valves, we studied the tricuspid valve from 50 pig hearts and described a new terminology for this valve based on the same principles previously applied to the mitral valve. We found that the tricuspid valve may be divided into three elements: three groups of papillary muscles, the chords and the main leaflets. The three groups of papillary muscles were defined by the numerals 1 for the anteroseptal; 2 for the posteroseptal, and 3 for the anteroposterior. Each main leaflet was divided in two areas that were named according to both the traditional leaflet’s names (S, A, P) and the papillary muscle origin of the chords inserted on it (1, 2, 3). Without expecting rapid and universal acceptance of this nomenclature, it is hoped that this attempt will constitute a step toward the goal of more precise data collection, both for particular groups and also between different surgical centers.
Animal Welfare and Anesthesia Protocol for Experiment 2 to 5:

Animal Welfare

All animals were cared for in accordance with the “Principles of Laboratory Animal Care” formulated by the National Society of Medical Research and the “Guide for the Care and Use of Laboratory Animals” prepared by the Institute of Animal Resources, National Research Council, and published by the National Academy Press, revised 1996. The protocol for the use of the animals for this project was also reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of The University of Montana.

Anesthesia protocol:

Targhee sheep (weight range 50 to 80 kg) had a left jugular catheter placed and were premedicated with intravenous (i.v.) administration of ketamine 1.0 mg/kg, atropine 0.03 mg/kg, and propofol 4.0 mg/kg. Artificial ventilation was maintained with a volume regulated respirator (North American Drager, Telford, PA, USA) supplemented with oxygen at 2-4 L/min. Anesthesia was maintained with intermittent propofol and continuous isoflurane (1.5% to 2.5%). A Swan-Ganz catheter was introduced through the left external jugular vein into the pulmonary artery. At baseline, three consecutive sets of measurements of cardiac output and right ventricular (RV) and pulmonary artery (PA) pressures were taken.
Experiment 2: Geometrical Changes of the Mitral and Tricuspid Valves during the Cardiac Cycle

1: Introduction

The following elements constitute both atrioventricular valves: the annulus, leaflets, chordae tendinae and papillary muscles [95]. These elements work together with the ventricles to maintain the competence of the valves. Studies on cadavers showed the remarkable geometry of the valves. Functional studies in animals showed the geometrical changes of the mitral [119,120] and the tricuspid [41] valves. The dynamic interactions between the mitral annulus and the aortic valve were shown Lansac et al. [121]. To understand the relationship of the normal mitral and tricuspid valves, it was necessary to study the normal geometry and dynamic interactions of the tricuspid and mitral valves in the same animal. Therefore, we undertook the following experiment.

2: Materials and Methods

2-1: Definition of the Different Phases of the Cardiac Cycle

The different phases of the cardiac cycle were defined from the LV and aortic (Ao) pressure curve. End-diastole or beginning of systole (isovolumic contraction or IVC) was defined as the beginning of an increase in LV pressure (dP/dt>0). End-isovolumic contraction was defined as the beginning of ejection (Ej) at the point where the LVP curves crosses the aortic pressure curve (gradient AoP/LVP=0). The dichrotic notch on the aortic pressure curve defined end-ejection and the beginning of isovolumic relaxation (IVR). The end of IVR was defined by the lowest pressure point of the LV
pressure curve (D). Mid-diastole point (midD) was defined by the middle-time point between the D and the beginning of systole.

**2-2: Digital Sonomicrometry**

Originally developed by Smith and Vesely [122], the digital sonomicrometer is available from Sonometrics Corporation (London, Ontario, Canada). Sonomicrometry is the measurement of distances in an aqueous medium within soft tissue by using sound energy. Small piezoelectric crystals are utilized as both transmitters and receivers of short pulses of ultrasound energy. The time for a transmitted pulse signal from a transmitter crystal to be received by a receiver crystal is measured. The computer converts this time to distance by using the Doppler formula. When a transmitting crystal is activated, all digital distance counters are reset to zero. The instant that a receiving crystal receives a burst of transmitted ultrasound, its respective digital counter is halted and the resulting numbers are transferred directly to the RAM buffer of the computer. There is no analog conversion process involved in these distance measurements, therefore eliminating the need to calibrate the system.

The circuitry in the digital sonomicrometer (Sonometrics Corporation, London, ON, Canada) allows for up to 32 simultaneous distance measurements to be performed. The range of measurement is software limited from 0 mm to 196 mm. The typical range is from 10 mm to 120 mm. The smallest measurable change in distance is 0.024 μm.

In addition to the digital sonomicrometer readings, a circuit board interfacing directly with the PC-AT bus inside the computer performs the function of digitizing up to 16 external analog signals. The conversion of each analog signal to digital is performed
simultaneously with virtually no phase shift and occurs once per block of sonomicrometry measurements. Analog signal allows the recording of LV and aortic pressures and distances between the crystals during the same time period. The user can select the active transmitters, receivers and analog channels. These parameters including the time and date of data acquisition, are stored along with the raw data.

2-3: Data Acquisition

Sonometrics Digital Ultrasonic Measurement System TRX Series 16 and 1 and 2 mm transmitter/receiver crystals were used to measure displacements. A post-processing program (Sonometrics Corporation, London, Ontario, Canada) was used to examine each individual length tracing between crystals. Data sampling rate was 200 Hz. A filter algorithm eliminates possible signal corruption by analyzing the pattern of both the true distance and the corrupted data. Millar pressure transducer control units TCB 600 and MIKRO-TIP pressure transducers (Millar Instruments, Inc. Houston, TX, USA) were used to obtain the LV and Ao pressures. To ensure stability, the micromanometer catheters were immersed in saline for a minimum of 2 hours before use then placed into a water bath warmed to 37°C and zeroed in the dark. All distances and pressures were displayed and recorded simultaneously on the same screen by the Sonomicrometrics system. This ensures that all data are synchronized and recorded during the same timeline.
2-4: Surgical Preparation

The animals underwent implantation of 19 ultrasonic crystals on the mitral and tricuspid valves using cardiopulmonary bypass. The animal was placed in the right lateral decubitus position on the operating table. The heart was exposed through a T-shaped incision of the pericardium by a standard left thoracotomy into the 4th intercostal space. The heart was then suspended in a pericardial cradle. Heparin at a dose of 300 U/kg i.v. was injected as a bolus in preparation for cardiopulmonary bypass with a target ACT of 480 seconds or more. The ascending aorta was cannulated with a #16 Fr Medtronic arterial cannula. A #32 Fr single venous cannula was inserted into the inferior vena cava and a #24 Fr into the superior vena cava. Cardiopulmonary bypass (CPB) was then instituted. Two cotton umbilical tapes were placed around the superior vena cava and the inferior vena cava. A LV vent line was inserted into the LV apex. The ascending aorta was cross-clamped followed by the infusion of cold crystalloid cardioplegia into the aortic root.

2-4-1: Surgical Implantation of Crystals for the Mitral Valve Study

Ten ultrasonic crystals were used to study the mitral valve complex. The following 2-mm ultrasonic crystals were placed and secured with a 5/0 polypropylene suture on the mitral valve through a left atriotomy. Six crystals were placed on the annulus to delineate the longitudinal (P1, P2) and antero-posterior (AM, PM) diameter, and the intertrigonal distance trigone (T1, T2); 1 crystal on the tip of each papillary muscle at the insertion of the anterior leaflet’s basal chordae (M1, M2); 1 crystal on the anterior wall of the LV at the level of the papillary muscle (ALV); 1 crystal on the apex
of the LV. The crystal electrodes were exteriorized through the ventricular wall for M1, M2 and ALV and through the left atriotomy for the annulus crystals. One high fidelity catheter-tipped pressure transducer (model 510, Millar Instruments, Houston, TX) was placed within the lumen of the proximal ascending aorta and one within the LV cavity through the apex (Figure 13).

2-4-2: Surgical Implantation of Crystals for the Tricuspid Valve Study

Nine 2-mm ultrasonic crystals (Sonometrics Corp, London, Ontario, Canada) were implanted and secured with a 5/0 polypropylene suture through a right atriotomy to study the tricuspid valve complex. Six crystals were located along the tricuspid annulus: at the anteroseptal (AS), the posteroseptal (PS) and the anteroposterior (AP) commissures, and at the relative mid point of the base of the septal (S), anterior (A), and posterior (P) leaflets. Three crystals were located inside the RV on the tips of the septal (Spm), anterior (Apm) and posterior (Ppm) papillary muscles. The crystal sutured at the apex of the LV was used as a reference crystal. The electrodes of crystals inside the RV were exteriorized through the right ventricular wall and the electrodes of the tricuspid annulus were exteriorized through the right atriotomy. High fidelity, catheter tipped pressure transducers (model 510; Millar Instruments, Houston, TX, USA) were placed within the lumen of the pulmonary artery and in the RV.
Figure 13: Surgical implantation of the sonomicrometry crystals.

The posterior part of the heart is in front.
AM: Anterior mitral annulus
PM: Posterior mitral annulus
T1 and T2: Left and right trigone
P1 and P2: Left and right lateral mitral annulus
M1 and M2: Anterior and posterior papillary muscles in the LV. ALV: crystal on the LV wall at the level of M1 and M2.
Spm, Apm and Ppm: Septal, anterior and posterior papillary muscles in the RV.
S, AS and PS: Septal, anteroseptal and postero septal tricuspid annulus
A, AP and P: Anterior, anteroposterior and posterior tricuspid annulus.
After discontinuing the cardio-pulmonary bypass, and after the animal was
hemodynamically stable for at least 15 min, crystal distances on the mitral valve and the
left ventricle were recorded along with left ventricular and aortic pressures. Then, crystal
distances on the tricuspid valve and the RV were recorded along with right ventricular
and pulmonary trunk pressures. Epicardial two-dimensional echocardiography with color
Doppler was used to assess the competence and anatomy of the tricuspid valve. At the
end of experiment the heart was arrested by lethal injection of potassium chloride and
explanted from the body cavity. The correct positions of the crystals were checked.

2-5: Definition of Anatomical Regions

The mitral and tricuspid valve complexes were divided in 3 planes: the annulus,
the annulo-papillary, and the papillary muscle plane. Several lengths and areas defined
each plane. Heron’s formula was used to calculate the area of a triangle with lengths a, b
and c as follows: \( S = \frac{1}{2}(a+b+c) \) and \( \text{Area} = \sqrt{(S-a)(S-b)(S-c)} \).
- Annulus:

The distance changes defined by the intertrigonal distance (T1-T2), the
anteroposterior (AM-PM), commissural-commissural (P1-P2) diameters and by the
distance between each consecutive crystals sutured on the mitral annulus defined the
deformation of the mitral annulus. The distance changes defined by the S-A, the S-AP
and the S-P lengths (the short axis diameters), by P-AS, P-A and A-PS (the long axis
diameters) and by the distance between consecutive crystals sutured on the tricuspid
annulus defined the deformation of the tricuspid annulus. The motion of both annuli was
analyzed at the five time points by computing the changes of both annulus areas and
diameters at the same time. The respective mitral and tricuspid areas were calculated with Heron’s formula using four triangles that characterized the mitral and tricuspid annulus planes.

- Annulo-papillary muscle plane:

  The variations of distances between each papillary muscle and the mitral annulus were explored (M1-Mitral annulus crystals and M2-Mitral annulus crystals). The distances between the apex and the papillary muscle tips (apex-M1 and apex-M2) and between the apex and the crystals of the mitral annulus were analyzed. The change of the average of the distances between the apex and each crystal of the mitral annulus defined the average change of the LV (apex-annulus). Apex-annulus changes were compared with the Apex-M2 and Apex-M2 changes. The annulo-papillary muscle plane of the mitral valve was divided into two functional units: the anterior annulo-papillary apparatus defined by each length of the quadrilateral T1-T2-M1-M2 and the posterior annulo-papillary apparatus defined by each length of the quadrilateral P1-P2-M1-M2. Variations and relationships between these distances were described. The variations of distances between each papillary muscle and the crystals of the tricuspid annulus were explored (Spm-tricuspid annulus, Apm-tricuspid annulus and Ppm-tricuspid annulus). The annulo-papillary muscle plane of the tricuspid annulus was divided into three functional units: the septo-annulo-papillary apparatus defined by each length of the quadrilateral AS-PS-P-S, the anterior-annulo-papillary apparatus defined by each length of the quadrilateral AS-AP-A-S and the posterior annulo-papillary muscle apparatus defined by each length of the quadrilateral AP-PS-P-A. Variations and relationships between these distances were described.
- Papillary muscle plane:

One area (M1-M2-ALV) using Heron’s formula and one length (M1-M2) defined the papillary muscle plane of the mitral valve. In addition, one area (Spm-Apm-Ppm) using Heron’s formula and three lengths (Spm-Apm, Spm P-pm and Ppm-Apm) defined the papillary muscle plane of the tricuspid annulus. The distance between the crystal at the apex and crystals placed on the papillary muscles and both annuli were also studied.

2-6: Measurement and Statistical Analysis Methods

After examination of the data, three consecutive heartbeats that contained the least amount of noise were chosen for analysis for each animal. Once these calculations were complete, summary statistics were reported for each location as mean ± 1 S.D. Between the five time points of the cardiac cycle, the percentages of change were calculated for each sheep and reported as mean ± S.D. The distance changes during the cardiac cycle were analyzed with repeated measures analysis of variance (ANOVA) and distance changes between two time-points of the cardiac cycle were analyzed with Bonferroni/Dunn test. The unpaired student t-test was used to compare the relative change between different distances. A value of p<0.05 was considered significant.

3: Results

Seven Targhee sheep (72 ± 21 kg) underwent implantation of crystals on the mitral valve and the tricuspid valve. Average cardiopulmonary bypass time was 136 ± 9 minutes and average cross-clamp time was 88 ± 6 minutes. The following hemodynamic characteristics were recorded: Arterial pressure 67/40 ± 2/3 mmHg, cardiac output 2.9 ±
0.4 L/min, pulmonary artery pressure 20/11±1/1 mmHg. At the time of recording, there was no mitral or tricuspid regurgitation on epicardial echocardiography. At necropsy all crystals in the mitral and tricuspid annuli, RV and LV were verified to be in the correct position.

3-1: Geometrical changes in Tricuspid and Mitral annuli

3-1-1: Mitral annulus

The main contraction of the mitral annulus occurred during the systole and was mainly dependent of the contraction of the anteroposterior (AM-PM) and the septolateral (P1-P2) diameters. These distances presented the major changes during the cardiac cycle (AM-PM: 15.9 ± 10%, p=0.01, ANOVA and P1-P2: 13.1 ± 4% of change, p=0.013, ANOVA). T1-T2 presented a change of 10.5 ± 5%, but was not significant during the cardiac cycle (p=0.2, ANOVA). The changes of these diameters were different through time. During IVC, AM-PM presented a 4 ± 4% decreased (p=0.17) while T1-T2 and P1-P2 slightly expanded (+2 ± 3% and +2 ± 4%, p=0.3). During the time of ejection AM-PM and T1-T2 increased (+3 ± 5%, p=0.14 and +4 ± 8%, respectively) and P1-P2 significantly decreased (-10 ± 6%, p=0.0012). During IVR, AM-PM and P1-P2 expanded (+9 ± 5%, p=0.2 and +6 ± 1%, p=0.019, respectively) while T1-T2 decreased (-3 ± 2%). Between end of IVR and midD, P1-P2 and AM-PM decreased (-1 ± 3 %, p=0.8 and -3 ± 2%, p=0.3, respectively) while T1-T2 expanded (-2 ± 5%). A significant change for AM-PM was observed between the beginning of IVR and midD (p=0.0033, Bonferroni/Dunn). Figure 14 reports the variations of T1-T2, P1-P2 and AM-PM during the cardiac cycle.
Figure 14: Heterogeneous variations of the intertrigonal (T1-T2), P1-P2 and antero-posterior diameter (AM-PM) of the mitral annulus through the phases of the cardiac cycle in one sheep.

IVC: Isovolumic contraction, Ej: Ejection, IVR: Isovolumic relaxation, midD & D: Diastole, Ao: Aorte, LV: Left ventricle
During the cardiac cycle, T1-AM and AM-T2 (anterior segments of the annulus) presented 11 ± 7% and 12 ± 7% of change, respectively (p<0.05). P1-PM and P2-PM (posterior segments) presented 12.6 ± 4% and 14.3 ± 3.5% of change, respectively (p<0.05). P1-T1 and P2-T2 (lateral segments) presented 10.7 ± 6.5% and 16 ± 6% of change, respectively (p<0.05). The motions of the different segments of the mitral annulus were not homogenous over time (Figure 15). Posterior segment and lateral segment lengths decreased during systole while anterior segment lengths increased. During diastole, posterior and lateral segment lengths increased while T1-AM and AM-T2 lengths decreased.
Figure 15: Variations of the segments of the mitral annulus during the phases of the cardiac cycle in one sheep.

IVC: Isovolumic contraction, Ej: Ejection, IVR: Isovolumic relaxation, midD & D: Diastole, Ao: Aorte, LV: Left ventricle
AM & PM: Anterior and posterior annulus
T1 & T2: trigones
P1 & P2: lateral annulus
3-1-2: Tricuspid Annulus

The antero-posterior (A-P: 13.4 ± 7%, p=0.017, ANOVA), the postero-septal (S-P: 21.6 ± 8.4 %, p=0.001, ANOVA) and anteroseptal-posterior (AS-P: 15.8 ± 5%, p=0.0013, ANOVA) diameters of the tricuspid annulus had significant and major changes throughout the cardiac cycle. The septal-anteroposterior (S-AP) and the septo-anterior (S-A) diameters presented minor non-significant variations during the cardiac cycle (11.1 ± 4.5%, p=0.25 and 11 ± 5%, p=0.2, respectively, ANOVA). The antero-posteroseptal (A-PS) presented the least variation during the cardiac cycle (7 ± 3%, p=0.6, ANOVA). The changes of the tricuspid diameters are reported in Figure 16. The short axis diameters (S-AP, S-A and SP) and A-PS (one of the long axis diameter) had the smallest change and presented the same kind of variation during the cardiac cycle. During IVC, the segments S-AP, S-A, A-PS and S-P trend to decrease (-1 ± 4%, -0.5 ± 4% and -0.5 ± 5% and -4 ± 5%, respectively with for S-P, p<0.05 Bonferoni/Dunn). During most of ejection time, these distances decreased and just before beginning of IVR increased again (percentages of change were respectively: -2 ± 4%, -3 ± 5%, +3 ± 3% and +11 ± 6%, respectively with for S-P, p<0.05, Bonferoni/Dunn). During IVR, these diameters slightly expanded (+2 ± 2, +2 ± 1, +1 ± 2% and +2 ± 4%, respectively). Between end of IVR and the end of diastole, these diameters decreased, reaching their smallest length for S-A and S-AP before midD (S-AP: -3 ± 5%, SA: -4 ± 7%, A-PS -4 ± 5% and SP: -10 ± 5%, respectively with for S-P, p=0.006, Bonferoni/Dunn). Then between midD and beginning of IVC, S-A, S-AP and S-P increased while A-PS decreased (+5 ± 2 %, +3 ± 4%, +1 ± 4% and −1 ± 4%, respectively). The main contraction of the tricuspid annulus occurred
between IVC and end of ejection. The contraction was mainly due to the decrease of the segments between P and the anteroseptal (A, AS and S) part of the annulus.

Figure 16: Variations of the diameters (A-PS, P-AS and A-PS) of the tricuspid annulus during the phases of the cardiac cycle in one sheep.

A, AS & PS: Anterior, anteroseptal & postero septal tricuspid annulus
Dimensions of the antero-septal segments of the annulus (S-AS and A-AS) did not present significant variations during the cardiac cycle (19 ± 13% and 11 ± 7 %, respectively, p>0.05 ANOVA). These distances decreased between IVC and D and then increased during the last part of the diastole. The changes of the antero-posterior part of the annulus (A-AP and P-AP) were not significant during the cardiac cycle (17 ± 10% and 16.6 ± 10 %, respectively, p>0.05 ANOVA). The distances increased between Ej and D before contracting again between the last part of diastole and the IVC. The postero-septal part of the annulus did not contract homogeneously: The P-PS segment (20.5 ±12%, p>0.05 ANOVA) decreased between end of diastole (D) to IVR and increased between IVR and D. The S-PS (20 ± 10% of change, p<0.05 ANOVA) segment followed the motion of P-AP. For S-PS, Bonferroni/Dunn test showed significant expansions between Ej and IVR and between IVR and significant diminution between D and MD (p<0.01). During the cardiac cycle, the different segment changes of the tricuspid annulus were homogenous in proportions, but did not happen at the same time (Figure 17).
Figure 17: Variations of the segment lengths of the tricuspid annulus during the phases of the cardiac cycle in one sheep

IVC: Isovolumic contraction, Ej: Ejection, IVR: Isovolumic relaxation, midD & D: Diastole, PA: Pulmonary artery, RV: right ventricle
A, AS & PS: Anterior, anteroseptal & postero septal tricuspid annulus
3-1-3: Motion of the Mitral and Tricuspid Annuli

Expansion of mitral and tricuspid annulus areas did not occur at the same time. The mitral annulus area expanded significantly from $5.5 \pm 0.9 \text{ cm}^2$ in IVR to $6.08 \pm 1 \text{ cm}^2$ at the end of diastole ($+10\%$) and the tricuspid annulus area expanded significantly from $6 \pm 1.4 \text{ cm}^2$ in Ej to $6.8 \pm 1.4 \text{ cm}^2$ ($+14\%$) in midD. Tricuspid annulus area was maximal while mitral annulus area was minimal. Antero-posterior diameter (AM-PM) of the mitral annulus and long axis diameters (P-AS and P-A) of the tricuspid annulus had the same trend during the cardiac cycle except during the ejection period. P1-P2 changes of the mitral annulus and short axis diameters (S-A, S-AP and S-P) of the tricuspid annulus presented also the same trends during late diastole, IVC and the early ejection period.

During the end of ejection and diastole, tricuspid short axis diameters seemed to expand earlier than P1-P2. Both contracted between D and midD and expanded in late diastole (Figure 18). The lengths of the different segments of both annuli are reported in Table 4. Motions of the septal segments of both annuli were the same in change and timing (S-AS: $19 \pm 13\%$, and T2-P2: $16 \pm 6\%$, maximal contraction in IVR and maximal expansion in end-diastole). Figure 19 shows a reconstruction of both annuli at end-diastole and end-systole.
Figure 18: Motion of the mitral and tricuspid annuli.

Variations of the mitral and tricuspid annulus areas, of the antero-posterior (AM-PM) and commissure-commissure diameters of the mitral annulus and long axis (P-AS) and short axis (S-AP) diameters of the tricuspid annulus. The earlier expansion of the long axis diameter of the tricuspid annulus compared to AM-PM is well represented. Thick tracings correspond to the mitral valve and thin tracing to the tricuspid.

IVC: Isovolumic contraction, Ej: Ejection, IVR: Isovolumic relaxation, midD & D: Diastole, Ao: Aorte, LV: Left ventricle
Table 4: Average measurement of the segments of both annuli during the different phases of the cardiac cycle.

p<0.05 repeated measurement ANOVA. **Bonferroni/Dunn test showed a significant difference between IVC and IVR and between Ej and D (p<0.01).

IVC: Isovolumic contraction, Ej: Ejection, IVR: Isovolumic relaxation, midD & D: Diastole,
S,AS & PS: Septal, anteroseptal and posteroseptal tricuspid annulus,
A, AP & P: Anterior, Anteroposterior, posterior tricuspid annulus
T1 & T2: Trigones, AM & PM: Anterior & posterior mitral annulus, P1 & P2: lateral mitral annulus

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 Mitral | PM-P1* ** | P1-T1* ** | T1-AM | AM-T2 | T2-P2* ** | P2-PM* ** |
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Figure 19: Reconstruction of the mitral valve annulus (left) and of the tricuspid annulus (right).

Variations of segments of both annuli between end-diastole (black) and end-systole (gray). AM: Anterior mitral annulus, PM: Posterior mitral annulus
T1 and T2: Left and right trigone
P1 and P2: Left and right lateral mitral annulus
S, AS and PS: Septal, anteroseptal and postero septal tricuspid annulus
A, AP and P: Anterior, anteroposterior and posterior tricuspid annulus.
3-2: Geometrical Changes in Tricuspid and Mitral Annulo-papillary Muscle Planes

3-2-1: Annulo-papillary Muscle Apparatus of the Mitral Valve

The variations of distances between M1-Mitral annulus and M2-Mitral annulus are reported in Table 5. There was no significant distance change for M1-T1 (3.5 ± 2% of change, p=0.9, ANOVA), M1-P1 (5 ± 2% of change, p=0.07, ANOVA) and M1-PM (5 ± 3% of change, p=0.4, ANOVA) during the cardiac cycle. Significant changes in distances between M1-T2 (11.7 ± 3% of change, p<0.005, ANOVA), M1-P2 (23 ± 6% of change, p<0.005, ANOVA) and M1-AM (6 ± 3% of change, p<0.005, ANOVA) were observed. These significant changes occurred mostly between IVC and D for M1-T2 and M1-P2 (-10 ± 4%, p=0.0008 and -20 ± 5%, p=0.002, Bonferroni/Dunn test, respectively) and between IVR and midD (+6.4 ± 4%, p=0.0007 and 15.5 ± 8%, p=0.004, Bonferroni/Dunn test, respectively). The distance changes for M2-T2 (2.7 ± 2% of change, p=0.24, ANOVA), M2-P2 (6.1 ± 3% of change, p=0.07, ANOVA) and M2-AM (4.2 ± 3% of change, p=0.63, ANOVA) were not significant. However, significant changes in distance for M2-T1 (8±3%, p=0.04, ANOVA), M2-P1 (10.5 ± 6%, p=0.031, ANOVA) and M2-PM (12.6 ± 8%, p<0.005, ANOVA) were observed. These significant changes occurred mostly between beginning of IVC and end of ejection for M2-T1, M2-P1 and M2-PM (-6 ± 5%, p=0.003; -10 ± 6%, p=0.004 and +9 ± 5%, p=0.002, Bonferroni/Dunn test respectively). During the cardiac cycle, Apex-M1 presented 12.9±1% (p=0.021, ANOVA) and Apex-M2 10.5±1% (p<0.005, ANOVA) of change. The averaged distances between the apex and the crystals of the mitral annulus also presented significant changes during the cardiac cycle (8.3 ± 1%, p<0.001, ANOVA). The distance changes for apex-papillary muscle tips and apex mitral annulus crystals are reported in Table 6.
Table 5: Variations in distance between M1-Mitral annulus and M2-Mitral annulus
during the phases of the cardiac cycle.

* = p<0.05 (repeated-measures ANOVA). Significant Bonferroni/Dunn test with p<0.05,
** = IVC versus IVR, *** = IVC versus D, †= Ejection versus isovolumic relaxation, ‡= Ejection versus D, †=Isovolumic relaxation versus mid diastole.
IVC: Isovolumic contraction, Ej: Ejection, IVR: Isovolumic relaxation, midD & D: Diastole, M1 & M2: Papillary muscles of the LV, T1 & T2: Trigones, AM & PM: Anterior & posterior mitral annulus, P1 & P2: lateral mitral annulus

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<td>34.27±1.68</td>
<td>33.39±0.63</td>
<td>32.85±1.63</td>
<td>33.18±1.84</td>
</tr>
<tr>
<td>M2-PM*</td>
<td>31.89±2.79**</td>
<td>34.61±3.33</td>
<td>35.04±3.05</td>
<td>33.40±3.55</td>
<td>31.80±4.03</td>
</tr>
<tr>
<td>M2-P1*</td>
<td>39.75±2.06**</td>
<td>39.34±1.89</td>
<td>36.24±0.52</td>
<td>38.81±1.08</td>
<td>38.84±2.85</td>
</tr>
<tr>
<td>M2-T1*</td>
<td>43.08±0.87**</td>
<td>41.06±1.55</td>
<td>39.98±2.42</td>
<td>40.49±1.62</td>
<td>41.20±0.92</td>
</tr>
<tr>
<td>M2-AM</td>
<td>38.04±2.00</td>
<td>37.78±2.67</td>
<td>38.25±2.35</td>
<td>37.98±2.35</td>
<td>37.50±1.52</td>
</tr>
<tr>
<td>M2-T2</td>
<td>29.72±2.65</td>
<td>30.08±2.63</td>
<td>30.20±2.35</td>
<td>29.81±2.50</td>
<td>29.68±2.58</td>
</tr>
<tr>
<td>M2-P2</td>
<td>26.37±2.77</td>
<td>27.29±2.79</td>
<td>28.03±2.52</td>
<td>26.81±2.60</td>
<td>26.51±2.92</td>
</tr>
</tbody>
</table>
Table 6: Changes through the cardiac cycle of average distances Apex-Annulus, Apex-M1 and Apex-M2

IVC: Isovolumic contraction, Ej: Ejection, IVR: Isovolumic relaxation, midD & D: Diastole, M1 & M2: Papillary muscles of the LV, T1 & T2: Trigones, AM & PM: Anterior & posterior mitral annulus, P1& P2: lateral mitral annulus

<table>
<thead>
<tr>
<th></th>
<th>Apex-Annulus</th>
<th>Average change</th>
<th>Bonferoni/Dunn test</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVC</td>
<td>88.50±4.33</td>
<td>IVC versus Ej</td>
<td>5.2±1% P=0.0001</td>
</tr>
<tr>
<td>Ej</td>
<td>92.46±4.02</td>
<td>Ej versus IVR</td>
<td>-8.3±2% P=0.0001</td>
</tr>
<tr>
<td>IVR</td>
<td>86.19±5.66</td>
<td>IVR versus D</td>
<td>1.5±1% P=0.035</td>
</tr>
<tr>
<td>D</td>
<td>85.21±5.95</td>
<td>D versus midD</td>
<td>-0.9±0.6% P=0.096</td>
</tr>
<tr>
<td>MidD</td>
<td>87.12±5.28</td>
<td>MidD versus IVC</td>
<td>2.1±1% P=0.93</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Apex-M1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVC</td>
<td>46.13±3.21</td>
<td>IVC versus Ej</td>
<td>1±4% P=0.81</td>
</tr>
<tr>
<td>Ej</td>
<td>47.06±3.52</td>
<td>Ej versus IVR</td>
<td>-7±5% P=0.0011</td>
</tr>
<tr>
<td>IVR</td>
<td>43.20±2</td>
<td>IVR versus D</td>
<td>1±3% P=0.6</td>
</tr>
<tr>
<td>D</td>
<td>41.32±3.7</td>
<td>D versus midD</td>
<td>4±3% P=0.035</td>
</tr>
<tr>
<td>MidD</td>
<td>44.13±4.01</td>
<td>MidD versus IVC</td>
<td>2±1% P=0.12</td>
</tr>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Apex-M2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVC</td>
<td>33.82±3.85</td>
<td>IVC versus Ej</td>
<td>0.8±2% P=0.001</td>
</tr>
<tr>
<td>Ej</td>
<td>35.28±3.48</td>
<td>Ej versus IVR</td>
<td>-8±2% P=0.002</td>
</tr>
<tr>
<td>IVR</td>
<td>32.79±3.29</td>
<td>IVR versus D</td>
<td>1±3% P=0.007</td>
</tr>
<tr>
<td>D</td>
<td>31.01±4.03</td>
<td>D versus midD</td>
<td>7±6% P=0.0012</td>
</tr>
<tr>
<td>MidD</td>
<td>33.84±2.5</td>
<td>MidD versus IVC</td>
<td>2±0.5% P=0.91</td>
</tr>
</tbody>
</table>

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Apex-annulus changes were significantly different from Apex-M1 (p=0.002, unpaired t-test) and Apex-M2 (p=0.017, unpaired t-test) changes during the cardiac cycle. During IVC, Apex-Annulus, Apex-M1 and Apex-M2 increased (table 6), however percentages of expansion were significantly different (Apex-annulus: 5.2 ± 1%, Apex-M1: 1 ± 4% and Apex-M2: 0.8 ± 2%, p=0.01 for apex-annulus versus Apex-M1 and for apex-annulus versus Apex-M2). During the ejection period, Apex-annulus reached its maximum value and started to decrease until the IVR period during which it reached its minimum value. Apex-M1 and Apex-M2 followed the same pattern. During these periods of the cardiac cycle, no significant differences were found for the percentages of change between Apex-annulus and Apex-M1 or Apex-M2. Figure 20 shows the variations of the distances between the apex, both papillary muscles and the mitral annulus during the cardiac cycle. The flat curves of the distances between papillary muscles and mitral annulus show that there are almost no variations in distance. Variations of the distances between apex and papillary muscle tips or apex-mitral annulus are also represented.
Figure 20: In one sheep, variations through the phases of the cardiac cycle of the distances apex-Mitral annulus, Apex-M1, Apex-M2, M1-Mitral annulus (PM, P1, T1, AM) and M2- Mitral annulus (PM, P2, T2, AM).

IVC: Isovolumic contraction, Ej: Ejection, IVR: Isovolumic relaxation, midD & D: Diastole, M1 & M2: Papillary muscles of the LV, T1 & T2: Trigones, AM & PM: Anterior & posterior mitral annulus, P1 & P2: lateral mitral annulus, Ao & LV: Aorta and Left ventricle
3-2-2: Annulo-papillary Muscle Units of the Mitral Valve

Between IVC and IVR, the lengths of the quadrilateral T1-T2-M1-M2 that defined the anterior annulo-papillary apparatus and the ones of the quadrilateral P1-P2-M1-M2 that defined the posterior annulo-papillary apparatus remained the same, except for the distance between M1-M2. M1-T1 was almost equal to M2-T2 and that of M1-P1 was almost equal to M2-P2 (Table 7). The ratios between the lengths of the quadrilateral were close to one (Table 8). Because of these consistent lengths and the relationship close to one in systole and diastole, these two quadrilaterals stayed trapezoid-like during systole and diastole.

Table 7: Lengths of the two functional unit and their variations between IVC and IVR

<table>
<thead>
<tr>
<th></th>
<th>IVC</th>
<th>IVR</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1-P2</td>
<td>32.08±6.5</td>
<td>30.57±6.9</td>
<td>5%</td>
</tr>
<tr>
<td>M1-P1</td>
<td>27.68±4.1</td>
<td>28.83±3.9</td>
<td>-4%</td>
</tr>
<tr>
<td>M2-P2</td>
<td>26.61±4.9</td>
<td>28.57±4.1</td>
<td>-7%</td>
</tr>
<tr>
<td>M1-M2</td>
<td>28.11±6.1</td>
<td>21.29±5.5</td>
<td>32%</td>
</tr>
<tr>
<td>T1-T2</td>
<td>25.84±2.15</td>
<td>26.8±3.8</td>
<td>-2%</td>
</tr>
<tr>
<td>M1-T1</td>
<td>28.43±3.3</td>
<td>28.56±3</td>
<td>0.5%</td>
</tr>
<tr>
<td>M2-T2</td>
<td>31.66±2.9</td>
<td>30.65±4.6</td>
<td>3%</td>
</tr>
</tbody>
</table>
Table 8: Relationship between segment of the quadrilaterals of anterior and posterior functional unit.

<table>
<thead>
<tr>
<th>Segment</th>
<th>IVR</th>
<th>IVC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior functional unit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1-T2/M1-M2</td>
<td>0.9±0.1</td>
<td>1.2±0.2</td>
</tr>
<tr>
<td>T1-T2/T1-M1</td>
<td>0.9±0.2</td>
<td>0.9±0.1</td>
</tr>
<tr>
<td>T1-T2/T2-M2</td>
<td>0.8±0.1</td>
<td>0.9±0.1</td>
</tr>
<tr>
<td>T1-T2/P1-P2</td>
<td>0.8±0.1</td>
<td>0.9±0.1</td>
</tr>
<tr>
<td>Posterior functional unit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M1-M2/P1-P2</td>
<td>0.9±0.1</td>
<td>0.7±0.1</td>
</tr>
<tr>
<td>P1-P2/P1-M1</td>
<td>1.3±0.2</td>
<td>1.2±0.2</td>
</tr>
<tr>
<td>P1-P2/P2-M2</td>
<td>1.2±0.2</td>
<td>1.1±0.2</td>
</tr>
</tbody>
</table>

3-2-3: Annulo-papillary Muscle Apparatus of the Tricuspid Valve

The distances between each papillary muscle and the crystals of the tricuspid annulus and the percentage of change through the cardiac cycle were analyzed and reported in Table 9. No significant change were observed by repeated measures ANOVA (p<0.05) for the segment lengths between each papillary muscle and its corresponding commissural crystals of the tricuspid annulus. Bonferroni/Dunn test was used to evaluate significant differences found by ANOVA resulting in significant differences for IVC versus IVR and IVR versus MD with p<0.01.
Table 9: Distance between the three papillary muscles of the tricuspid valve and the tricuspid annulus.

* = p<0.05 (repeated-measures ANOVA).

IVC: Isovolumic contraction, Ej: Ejection, IVR: Isovolumic relaxation, midD & D: Diastole, Spm, Apm & Ppm: Papillary muscles of the RV, S, AS, PS: Septal, anteroseptal and posteroseptal tricuspid annulus, AP, P and PS: Anteroposterior, posterior and posteroseptal annulus

<table>
<thead>
<tr>
<th></th>
<th>Spm-S</th>
<th>Spm-AS</th>
<th>Spm-A</th>
<th>Spm-AP</th>
<th>Spm-P</th>
<th>Spm-PS</th>
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</thead>
<tbody>
<tr>
<td>IVC</td>
<td>26.58±1.68</td>
<td>20.62±5.08</td>
<td>26.11±7.22</td>
<td>28.48±5.43</td>
<td>36.24±2.54</td>
<td>38.05±4.06</td>
</tr>
<tr>
<td>Ej</td>
<td>25.88±2.83</td>
<td>20.99±5.47</td>
<td>28.26±6.13</td>
<td>29.84±3.81</td>
<td>35.74±1.38</td>
<td>37.28±3.38</td>
</tr>
<tr>
<td>IVR</td>
<td>25.76±2.79</td>
<td>20.65±4.55</td>
<td>28.47±5.42</td>
<td>31.53±2.74</td>
<td>39.34±1.94</td>
<td>39.85±2.89</td>
</tr>
<tr>
<td>D</td>
<td>26.41±2.06</td>
<td>19.90±4.16</td>
<td>27.04±4.83</td>
<td>30.11±4.37</td>
<td>39.18±2.65</td>
<td>40.43±4.21</td>
</tr>
<tr>
<td>midD</td>
<td>26.76±1.53</td>
<td>19.73±4.25</td>
<td>24.63±7.39</td>
<td>27.51±5.17</td>
<td>36.78±2.51</td>
<td>38.45±3.77</td>
</tr>
<tr>
<td>% change</td>
<td>7.00±4.70</td>
<td>5.00±3.80</td>
<td>14.00±8.20*</td>
<td>16.00±9.30*</td>
<td>14.90±4.00*</td>
<td>10.10±1.40*</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Apm-S</th>
<th>Apm-AS</th>
<th>Apm-A</th>
<th>Apm-AP</th>
<th>Apm-P</th>
<th>Apm-PS</th>
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</thead>
<tbody>
<tr>
<td>Ej</td>
<td>37.06±4.88</td>
<td>34.75±6.68</td>
<td>28.52±2.91</td>
<td>21.31±3.85</td>
<td>27.70±6.02</td>
<td>38.76±4.59</td>
</tr>
<tr>
<td>IVR</td>
<td>33.67±4.92</td>
<td>33.04±6.28</td>
<td>28.00±3.29</td>
<td>21.32±3.64</td>
<td>27.27±6.66</td>
<td>36.69±4.96</td>
</tr>
<tr>
<td>D</td>
<td>36.08±3.31</td>
<td>34.04±5.51</td>
<td>27.86±3.00</td>
<td>21.40±3.62</td>
<td>27.73±7.52</td>
<td>38.33±4.74</td>
</tr>
<tr>
<td>% change</td>
<td>11.70±7.90*</td>
<td>8.60±2.40*</td>
<td>4.50±2.10</td>
<td>3.90±1.60</td>
<td>5.10±1.90</td>
<td>8.20±2.40*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Ppm-S</th>
<th>Ppm-AS</th>
<th>Ppm-A</th>
<th>Ppm-AP</th>
<th>Ppm-P</th>
<th>Ppm-PS</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVC</td>
<td>28.41±2.75</td>
<td>41.61±5.41</td>
<td>43.26±5.78</td>
<td>33.32±5.99</td>
<td>25.31±2.42</td>
<td>19.82±2.95</td>
</tr>
<tr>
<td>Ej</td>
<td>28.04±2.53</td>
<td>41.08±3.79</td>
<td>43.47±4.54</td>
<td>33.89±4.56</td>
<td>26.48±3.93</td>
<td>19.99±4.08</td>
</tr>
<tr>
<td>IVR</td>
<td>27.60±2.59</td>
<td>41.34±4.16</td>
<td>44.30±4.47</td>
<td>34.89±3.93</td>
<td>27.08±4.16</td>
<td>19.90±3.82</td>
</tr>
<tr>
<td>D</td>
<td>28.00±2.32</td>
<td>41.19±4.96</td>
<td>44.29±5.24</td>
<td>34.88±4.77</td>
<td>26.44±2.70</td>
<td>19.64±2.85</td>
</tr>
<tr>
<td>midD</td>
<td>28.66±3.03</td>
<td>40.50±6.04</td>
<td>42.11±6.52</td>
<td>32.68±6.16</td>
<td>24.18±2.23</td>
<td>19.75±2.89</td>
</tr>
<tr>
<td>% change</td>
<td>7.9±2.7</td>
<td>7.9±2.6</td>
<td>9±5.1*</td>
<td>11±6.9*</td>
<td>13.5±7.2*</td>
<td>5.4±3</td>
</tr>
</tbody>
</table>
The distances between the septal papillary muscle and the crystals of the annulus expanded between Ej and D and decreased during the end of diastole and the end of isovolumic contraction. The distance between the anterior papillary muscle and the crystals of the annulus increased between IVR and the end of diastole and decreased between IVC and the end of ejection. The distance between the posterior papillary muscle and the annulus followed the same trend as the one of the anterior papillary muscle.

Figure 21 shows the variations through the cardiac cycle of the distances between each papillary muscle tips and their corresponding commissural crystal, of Spm-P, Apm-S and Ppm-A.
Figure 21: In one sheep, variations through the phases of the cardiac cycle of the
distances between the PM tips and the corresponding commissures.

The flat line between the PM tips and their corresponding commissure showed no
variations during the cardiac cycle.

IVC: Isovolumic contraction, Ej: Ejection, IVR: Isovolumic relaxation, midD & D:
Diastole, Spm, Apm & Ppm: Papillary muscles of the RV
S, AS, PS: Septal, anteroseptal and posteroseptal tricuspid annulus
AP, P and PS: Anteroposterior, posterior and posteroseptal annulus
PA & RV: Pulmonary artery and right ventricle

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3-2-4: Annulo-papillary Units of the Tricuspid Valve

During the different times of the cardiac cycle, the relationships of the lengths of the quadrilateral AS-AP-A-S that defined the anterior annulo-papillary apparatus, the quadrilateral AS-PS-P-S that defined the septal annulo-papillary apparatus and the quadrilateral AP-PS-P-A that defined the posterior annulo-papillary muscle apparatus are in Table 10. Each ratio remained consistent during the cardiac cycle. The ratios that involved a segment of the annulus and the segment between the two corresponding papillary muscles and the ratios that involved two distances between the tip of a papillary muscle and its corresponding commissural crystal were close to one. However, they were different from one when the ratios involved a segment of the annulus and the distance between the tip of the papillary muscle and the corresponding crystal for the septal and posterior annulo-papillary apparatus. These last ratios were also close to one for the anterior annulo-papillary unit. These consistent ratios showed the same percentage of change of lengths for the different segments during the cardiac cycle. Therefore, as for the mitral papillary unit, these three quadrilaterals stayed trapezoid-like during the cardiac cycle.
Table 10: Relationships between the segments of the septal, anterior and posterior papillary muscle apparatus.

IVC: Isovolumic contraction, Ej: Ejection, IVR: Isovolumic relaxation, midD & D: Diastole, Spm, Apm & Ppm: Papillary muscles of the RV
S, AS, PS: Septal, anteroseptal and posteroseptal tricuspid annulus
AP, P and PS: Anteroposterior, posterior and posteroseptal annulus

<table>
<thead>
<tr>
<th>Anterior Annulo-Papillary apparatus</th>
<th>AS-PA/Spm-Apm</th>
<th>AS-PA/Spm-AS</th>
<th>AS-PA/Apm-PA</th>
<th>AS-Spm/PA-Apm</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVC</td>
<td>0.901</td>
<td>0.992</td>
<td>0.974</td>
<td>1.019</td>
</tr>
<tr>
<td>Ej</td>
<td>0.887</td>
<td>0.969</td>
<td>0.945</td>
<td>1.026</td>
</tr>
<tr>
<td>IVR</td>
<td>0.853</td>
<td>1.030</td>
<td>0.979</td>
<td>1.052</td>
</tr>
<tr>
<td>D</td>
<td>0.842</td>
<td>1.054</td>
<td>0.963</td>
<td>1.095</td>
</tr>
<tr>
<td>midD</td>
<td>0.878</td>
<td>0.997</td>
<td>0.940</td>
<td>1.062</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Septal Annulo-Papillary apparatus</th>
<th>AS-PS/Spm-Ppm</th>
<th>AS-PS/AS-Spm</th>
<th>AS-PS/PS-Apm</th>
<th>AS-Spm/PS-Apm</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVC</td>
<td>0.940</td>
<td>1.652</td>
<td>1.764</td>
<td>0.937</td>
</tr>
<tr>
<td>Ej</td>
<td>0.961</td>
<td>1.648</td>
<td>1.711</td>
<td>0.963</td>
</tr>
<tr>
<td>IVR</td>
<td>0.947</td>
<td>1.748</td>
<td>1.815</td>
<td>0.963</td>
</tr>
<tr>
<td>D</td>
<td>0.949</td>
<td>1.829</td>
<td>1.864</td>
<td>0.981</td>
</tr>
<tr>
<td>midD</td>
<td>0.928</td>
<td>1.706</td>
<td>1.747</td>
<td>0.976</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Posterior Annulo-Papillary apparatus</th>
<th>PA-PS/Apm-Ppm</th>
<th>PA-PS/PA-Apm</th>
<th>PA-PS/PS-Spm</th>
<th>PA-Apm/PS-Spm</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVC</td>
<td>0.930</td>
<td>1.418</td>
<td>1.542</td>
<td>0.919</td>
</tr>
<tr>
<td>Ej</td>
<td>0.973</td>
<td>1.432</td>
<td>1.526</td>
<td>0.939</td>
</tr>
<tr>
<td>IVR</td>
<td>0.993</td>
<td>1.438</td>
<td>1.571</td>
<td>0.915</td>
</tr>
<tr>
<td>D</td>
<td>0.977</td>
<td>1.463</td>
<td>1.632</td>
<td>0.896</td>
</tr>
<tr>
<td>midD</td>
<td>0.946</td>
<td>1.429</td>
<td>1.553</td>
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</table>
3-3: Geometrical Changes in the Mitral and Tricuspid Papillary Muscle Planes

3-3-1: Papillary Muscles of the Mitral Valve

M1-M2-ALV presented a 72% decrease from $2.15 \pm 0.45 \text{ cm}^2$ to $1.24 \pm 0.34 \text{ cm}^2$ between IVC and IVR. The inter-papillary muscle distance M1-M2 decreased from 29.8 ± 6 mm to 23.8 ± 7 mm (-32%) between IVC and IVR. Distance between M1 and ALV decreased from 23.14±5.4-mm to 15.7 ± 3.5 mm (-47%) between IVC and IVR. Distance between M2 and ALV decreased from 19.1 ± 4.2 mm to 15.5 ± 3.6 mm between IVC and IVR. Table 11 shows the significant variations of the mitral papillary muscle plane during the cardiac cycle. Figure 22 shows the variation through the cardiac cycle of the distances of the papillary muscle plane of the mitral valve.

Table 11: Variations of distances through the cardiac cycle for the papillary muscle plane of the mitral valve.

* = p<0.05 (repeated-measures ANOVA). Significant Bonferroni/Dunn test with p<0.05, ** = IVC versus IVR, ***= IVC versus D, †= ejection versus IVR, ‡= ejection versus D, †= Isovolumic relaxation versus midD, IVC: Isovolumic contraction, Ej: Ejection, IVR: Isovolumic relaxation, midD & D: Diastole, M1-M2: Papillary muscles of the LV, ALV: Crystal on the LV wall at the level of the papillary muscles.

<table>
<thead>
<tr>
<th></th>
<th>M1-M2 (mm)</th>
<th>M1-ALV (mm)</th>
<th>M2-ALV (mm)</th>
<th>M1-M2-ALV Area (cm²)</th>
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</thead>
<tbody>
<tr>
<td>IVC</td>
<td>29.8±6.1*</td>
<td>21.65±4.79*</td>
<td>21.39±4.95*</td>
<td>2.1±0.45*</td>
</tr>
<tr>
<td>Ej</td>
<td>28.7±5.52†</td>
<td>20.77±4.43†</td>
<td>21.08±4.73†</td>
<td>2.02±0.41†</td>
</tr>
<tr>
<td>IVR</td>
<td>23.8±7</td>
<td>15.80±2.8†</td>
<td>17.28±4.27</td>
<td>1.24±0.34†</td>
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<tr>
<td>D</td>
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<td>18.41±4.63</td>
<td>18.52±4.84</td>
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<td>MD</td>
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<td>21.22±4.62</td>
<td>20.19±5.41</td>
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</table>
Figure 22: Variations of M1-M2, M1-ALV, M2-ALV through the phases of the cardiac cycle.

Contraction of the ventricle during the systole is shown by the diminution of the interpapillary muscle plane distance.

IVC: Isovolumic contraction, Ej: Ejection, IVR: Isovolumic relaxation, midD & D: Diastole, M1-M2: Papillary muscles of the LV, ALV: Crystal on the LV wall at the level of the papillary muscles. Ao & LV: Aorta and Left ventricle

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3-3-2: Papillary Muscles of the Tricuspid Valve

The area defined by the septal (Spm), anterior (Apm) and posterior (Ppm) papillary muscles crystals contracted by 22 ± 14% (p>0.05, ANOVA) between IVC and IVR. The three segment motions (Spm-Apm, Spm-Ppm and Ppm-Apm) were homogeneous during the cardiac cycle in distance and time. Spm-Apm presented a total change of 15 ± 12% (p>0.05, ANOVA), Apm-Ppm of 12 ± 5% (p>0.05, ANOVA) and Spm-Ppm of 10 ± 4% (p>0.05, ANOVA). These lengths decreased during the systole and increased during the diastole (Table 12 & Figure 23).

Table 12: Variation of the inter-papillary muscle distance of the tricuspid valve during the phases of the cardiac cycle.
Spm, Apm & Ppm: Septal, anterior and posterior papillary muscles of the RV
IVC: Isovolumic contraction, Ej: Ejection, IVR: Isovolumic relaxation, midD & D: Diastole,

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<th>Apm-Ppm (mm)</th>
<th>Spm-Ppm (mm)</th>
<th>Area (cm²)</th>
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<tr>
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<td>Ej</td>
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<td>IVR</td>
<td>29.55±4.20</td>
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<tr>
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<td>37.68±3.71</td>
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<td>32.02±4.53</td>
<td>36.72±4.65</td>
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</table>
Figure 23: Variations of the interpapillary muscle distances of the tricuspid valve during the phases of the cardiac cycle.

Spm, Apm & Ppm: Septal, anterior and posterior papillary muscles of the RV  
IVC: Isovolumic contraction, Ej: Ejection, IVR: Isovolumic relaxation, midD & D:  
Diastole, PA & RV: Pulmonary artery and right ventricle

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3-3-3: Motion of Both Papillary Muscle Planes:

Both papillary muscle plane areas decreased during systole from IVC to IVR (see table 11 & 12 for values). During the contraction of the ventricles, M1-M2, Spm-Apm and Apm-Ppm decreased by 32%, 15 ± 12% and 12 ± 5% respectively; however, Spm-Ppm (10 ± 4% of change) decreased during the IVC, but increased during the ejection period. Figure 24 represents the variation of the interpapillary muscle distance at the 5 time points of the cardiac cycle.
Figure 24: Variations of the papillary-muscles distances of both atrioventricular valves during the cardiac cycle.

M1 & M2: papillary muscle of the LV
Spm, Apm & Ppm: Septal, anterior and posterior papillary muscles of the RV
IVC: Isovolumic contraction, Ej: Ejection, IVR: Isovolumic relaxation, midD & D: Diastole,
4: Discussion

4-1: Mitral and Tricuspid Valve Annuli

It was first shown in dogs that the shape and size of the mitral and tricuspid annuli varied continuously during the cardiac cycle [99,123]. Other studies with sonomicrometry array localization, marker angiography and three dimensional echocardiography confirmed their dynamic changes during the cardiac cycle [26,41,101,102]. Our findings on the diameter and segment length changes of both annuli resulting in shape and area variations during the cardiac cycle are consistent with previous reports of different groups who studied separately the mitral and the tricuspid annuli. In our experiment, the mitral annulus decreased in size during systole with the contraction of the antero-posterior (AM-PM: 15.9 ± 10 %) and commissure-commissural (P1-P2: 13.1 ± 4%) diameters of the annulus. These results are different from the findings of Gorman et al. who studied the mitral valve in sheep using sonomicrometry and reported an expansion of the AM-PM diameter of the annulus during the IVC and an expansion of P1-P2 during ejection [119], while we found in our experiment a contraction (figure 14). These differences may depend on the variability between sheep breeds. The time variations of AM-PM and P1-P2 diameters were associated with the motion of the posterior (PM-P1: 12.6 ± 4% and PM-P2: 14.3 ± 3.5%) and lateral segments (T1-P1: 10.7 ± 6.5% and T2-P2: 16 ± 6%) of the annulus during the cardiac cycle. The intertrigonal distance that was previously considered to remain constant or to change minimally during the cardiac cycle was actually found to expand during ejection along with the expansion of the aortic root. The expansion of the intertrigonal distance (T1-T2 10.5 ± 5%, p>0.05) during the cardiac cycle was associated with significant
expansion of the anterior segment lengths of the annulus (T1-AM: 11 ± 7%, AM-T2: 12 ± 7%) and corresponded to the dynamic balance of the aorto-mitral junction [121]. Mitral annulus deformation is closely related to aortic root dynamics. The intertrigonal distance expands during systole and allows for aortic root expansion, probably to maximize ejection.

For the tricuspid valve, the long axis diameter centered on the posterior part of the annulus (P-S: 21.6 ± 8.4 %, P-AS: 15.8 ± 5 % and P-A: 13.4 ± 7%) and the short axis diameters, septum-anteroposterior free wall (S-A: 11 ± 5 % and S-AP: 11.1 ± 4.5%) presented the major changes during the cardiac cycle. The minimal change corresponded to the segment A-PS (7 ± 3%). Thus the deformation of the annulus was associated with the motion of the posterior part of the annulus. According to Hiro et al. [41], the posteroseptal commissure in Targhee sheep corresponds to the tail of the pear shape of the tricuspid annulus. In humans, the change of the tricuspid valve depends on the expansion of the free wall segment of the narrow tail of the pear shaped annulus or the anteroseptal portion of the annulus [108]. We found the same relative distance changes (15-20%) between each segment defined by the six crystals of the annulus as Hiro et al [41] showed an homogenous change (12%) of the insertion of the three leaflets. The motion of these annulus segments was, however, not homogeneous in time. The anteroposterior segments (A-AP and P-AP) of the free wall and the postero-septal (S-PS) part of the annulus were increasing during ejection while the anteroseptal annulus (S-AS and A-AS) and the posterior segment P-PS were contracting. Thus the lateral annulus motion was following the septal annulus motion and the anterior annulus motion was following the posterior free wall motion.
According to our knowledge, there was no previous report describing the motion of both annuli during the cardiac cycle. We described the variations of the annulus shapes by their diameter changes. The mitral annulus had more significant changes in the axis parallel to the septum than in the free wall-septum axis AM-PM: 15.9 ± 10 % versus P1-P2: 13.1 ± 4%, which correspond to the findings of Gorman et al. [119]. The long axis (P-AS) of the tricuspid annulus expanded during ejection while the short axis (S-AP) was decreasing, following the motion of the diameters of the mitral valve. The tricuspid annulus long axis increase was associated with an early expansion of the tricuspid area during ejection. The contraction of the LV and the mitral annulus may create a dynamic balance of the atrioventricular valve septal junction. Our finding about the area of the mitral valve corresponds to that of Ormiston et al. who showed that the mitral annular area was increasing gradually during diastole and then was decreasing until midsystole. The mitral area started to increase again during late systole [100]. By using the Heron formula, we added triangles that were not on the same plane and respected the saddle shape of the annulus.

4-2: Annulo-papillary Muscle Apparatus of Mitral and Tricuspid Valves

In cadaveric hearts, the distances between the tip of both papillary muscles and the trigones or the point between the middle and lateral or septal scallops of the posterior leaflets were measured and found to be equal [124]. It has been shown in dogs that the orthogonal distance between the plane of the mitral annulus and the tips of both papillary muscles had a variation of less than 1-mm during the cardiac cycle with different heart rates and preload conditions [120]. In our study, the distance between each papillary muscle...
muscle tip and the hemicorresponding mitral annulus remained the same during the entire cardiac cycle. We studied the Apex-PM tip changes during the cardiac cycle and observed an elongation followed by a shortening of the distance during systole. Komeda et al. reported the same variations of the papillary muscle lengths [120]. We compared Apex-PM tip changes to the variations of the averaged distances between the apex and the mitral annulus crystals, which correspond to an average of the LV wall variations. We found a significant difference for the average change during the cardiac cycle (Apex-M1 versus Apex-annulus, p=0.002 and Apex-M2 versus Apex-annulus, p=0.017) and for the relative expansion during the IVC (Apex-M1 versus Apex annulus, p=0.01 and Apex-M2 versus Apex-annulus, p=0.01). The constant distance between the papillary muscles tips and the differences in changes between the apex-annulus distance and apex-papillary muscle distances suggest that both papillary muscles do not present the same changes as the ventricular wall during the phases of the cardiac cycle. The papillary muscles seem to be functionally different from the LV wall. The papillary muscles have variable morphology [104,105] and were always described as part of the LV in the conventional anatomy textbooks. Their muscular fibers of the body and base were understood to be in direct continuity with the myocardium of the LV. However, using x-ray multidetector CT with interactive 3-D images, Axel [125] has shown that the base of the human papillary muscles is not in continuity with the LV wall but through trabeculae carneae with clear demarcation spaces between both structures. These facts suggest that anatomically, the papillary muscles are different from the LV wall. Therefore, the papillary muscles, functionally and anatomically different from the LV wall, may function as independent structures that serve to maintain a constant distance between the annulus and their tip.
This continuity between the mitral valve annulus and the papillary muscles has to be preserved for LV performance and prevents postoperative mid-ventricle rupture in cases of mitral valve replacement [126].

For the tricuspid valve, our results also showed a remarkable geometry of the annulus-papillary muscle complex. According to our knowledge, no one previously reported that each papillary muscle of the tricuspid valve remained at a constant distance from its corresponding commissure. Traditionally, in the RV, three tricuspid papillary muscles are described and identified as anterior, posterior and septal. In their anatomical work on 100 human hearts, Viktor et al. found that the papillary muscles of the tricuspid valve exhibited endless variations in number, size, shape, fusion and location [93]. We showed that a single anterior muscle constituted the tricuspid valve predominantly and that numerous muscles among the septum constituted the posteroseptal and anteroseptal groups (Experiment 1). Among these muscles, a posteroseptal one at the corner between the septum and the free wall and the conus PM were always present at the level of the anteroseptal and posteroseptal commissures. These muscles corresponded to the placement of the crystals during the experiment. L. Axel also studied the RV papillary muscles and found also that they were not attached to the RV wall but to the trabeculae tendinae [125] and that they were anatomically distinct from the RV wall. In the case of the RV, the papillary muscles may also correspond to independent structures that maintain a constant distance between their tips and the annulus.

We studied the relationships between the segments of the quadrilateral constituted by two PM tips and the intertigonal or the latero-lateral mitral annulus and by two papillary muscles and their corresponding commissures in the tricuspid valve. For the
mitral valve our results showed a constant ratio close to one in each calculation. For the tricuspid valve, our results showed a constant ratio (different from one) during the phases of the cardiac cycle between interpapillary muscle and commissure-commissure distances and equal to one between papillary muscle-commissure distances. The mitral and tricuspid valves may be described as two (for the mitral) and three (for the tricuspid) annulo-papillary units constituted by two papillary muscles and the corresponding annulus with the leaflet base in between.

4-3: Papillary Muscle Motion

During systole, with the contraction of the ventricles, the interpapillary muscle distance in the LV decreased by 32% while the Apm-Ppm and Apm-Spm decreased by 15% and 12%, respectively. However, the distance between Spm and Ppm, the papillary muscles inserted on the septum in the RV increased by 10%. We demonstrated that the long axis diameter (P-AS) and the septal segment (S-PS) of the tricuspid annulus expanded during ejection (figure 16 & 17) while the septum was contracting. At the level of the papillary muscles, the PM tip motion seemed to reproduce the motion of the tricuspid annulus. The contraction of the LV may create, not only at the annulus level but also at the papillary muscle level, a dynamic balance with the sub-valvular apparatus of the tricuspid valve during ejection. One explanation for this finding may be a physiologic bulging of the septum during contraction. According to the anatomical concept of Torrent-Guasp, a muscular band wrapped upon itself forms the heart [36]. The RV is formed by horizontal fibers wrapped on the helical bands that constitute the LV. During systole the successive contractions of the basal and apical loop of the LV band make the
muscle thicker and more spherical [127]. The septum may become prominent inside the RV during ejection, while the pressure inside the LV is increased, and thus the distance between the Spm and Ppm increases.

In conclusion, we studied the motion of both atrioventricular valves in vivo in normal ovine hearts with sonomicrometry array localization. Both annuli were linked by the septum and presented the same directional change during the cardiac cycle. The subvalvular apparatus of both valves was associated with the annuli in a remarkable geometrical pattern. The LV papillary muscles remained at a constant distance from the hemi-corresponding mitral valve and the RV papillary muscles remained at constant distance from their corresponding commissures. The papillary muscles within each ventricle were associated with the each other to form with the trigones and the lateral mitral annulus or with their corresponding commissure areas on the tricuspid annulus a trapezoid-like quadrilateral. These quadrilateral sides presented constant ratios during the cardiac cycle.

Although sonomicrometry has an outstanding spatial and temporal resolution [119], differences in surgical placement and anatomic variations of the animals may explain deviations in measurements between research groups or even within the same group. To minimize this problem, the same surgeon performed all surgeries. This experiment was also conducted as an acute, open chest, open pericardium study on an anesthetized animal. This associated with the deleterious effect of cardio-pulmonary bypass and ischemia could have resulted in abnormal valve behavior.
Experiment 3: Comparison of Sonomicrometry and TTE Data in Sheep

1: Trans-thoracic echocardiography in sheep

The traditional long axis (aortic root to apex), short axis (perpendicular to long axis) and four chambers (traversing both ventricles and atria through mitral and tricuspid valves) views [128] of trans-thoracic echocardiography in sheep are obviously different from human patients. The TTE in human patients has been described as iterative and dependent on anatomical characteristics of the patient and manipulation of the operator [129]. The three dimensional structures are sliced in two-dimensional images and allow the measurements of intracardiac structures. To recognize precisely the structures that are measured by TTE in sheep where no references are available, comparisons between TTE data and sonomicrometry data were undertaken.

2: Materials and Methods

2-1: Echo data acquisition

Targhee sheep (n=6) were anesthetized and a trans-thoracic echocardiography was performed (HP, 5500) by the same operator. The sheep was positioned on its right side on the operating table. A short-axis view was acquired through the 5th left intercostal space about 4 cm above the sternum. A long-axis view was acquired through the 4th left intercostal space 4 cm above the sternum. The short axis view allowed seeing the LV and its papillary muscles but did not allow any measurements on the RV. Only the long axis view allowed the analysis of both annuli and both ventricles because of the anatomical conformation of the sheep.
Left ventricular and RV measurements were performed. For the LV: on short-axis view, maximum and minimum inter-papillary muscle distances (M1-M2) at the level of the head of papillary muscles were measured. On long-axis view, maximum and minimum M2-posterior annulus dimension and M2-anterior annulus (tethering distance) were measured. On the same view, maximum and minimum antero-posterior mitral annulus diameters were measured. Only the long-axis view was used for the measurements in the RV. The annulus diameter, the distance between the apex and the septal part of the annulus and the distance between the apex and the free wall part of the annulus were measured in systole and diastole. The distance between the tip of the free wall papillary muscle and the free wall annulus and the distance between the free wall PM and the septal annulus were acquired in systole and diastole. The distance between the septal papillary muscles and the septal annulus and the distance between the septal papillary muscle and the free wall annulus were also measured in systole and diastole.

2-2: Surgical experiment

Sonomicrometry data from crystals implanted in the sheep described above in the LV, RV, mitral and tricuspid annuli were used in this experiment. Three beats were selected from the filtered sono data, using Sonometric’s software (SonoSOFT Version 3.1.4, Sonometrics Corp, London, Ontario, Canada). Maximum and minimum distances between the following crystals were found for each beat and were used for comparisons. For the LV dimension: The inter-papillary muscle distances measured on TTE were compared with the inter-papillary muscle distance using the crystals implanted on the head of both papillary muscles (M1-M2).
For the mitral and tricuspid annuli: The TTE a mitral annulus diameter was compared with the antero-posterior distances of the mitral annulus defined by the following crystals: AM-PM, T1-P1, T2-P2, T1-PM, T2-PM, AM-P1 and AM-P2. The TTE tricuspid annulus diameter was compared with the distance defining the septo-lateral diameter (S-A, S-AP or S-P).

For the LV: M2-posterior annulus distances from TTE were compared with the distance between the head of the postero-medial papillary muscle and the posterior annulus M2-P1, M2-PM and M2-P2. The tethering distance (M2-anterior annulus) from TTE was compared with the distance between M2-AM, M2-T1 and M2-T2.

For the RV: The distance between free wall PM and the septal annulus from TTE were compared with Apm-PS, Apm-S and Apm-AS. The distance between free wall PM and the free wall annulus from TTE were compared with the Apm-A, Apm-AP and Apm-P.

2-3: Statistical analysis:

Samples of sheep were drawn randomly from a population of Targhee sheep with an assumed normal distribution. Because of the small number of our samples and to allow the use of parametric tests, normal distribution of our numbers was checked by a normal-probability-plot. Statistical analysis was performed using the unpaired t-test with p<0.05 significance.

3: Results

3-1: Data Comparison

The validity of the comparison of the sono and TTE groups was determined by matching the weight and hemodynamic characteristics of the two groups. The weight of
the TTE group was not significantly different from the weight of the sono group (57.6 ± 6 kg versus 72 ± 21 kg respectively, p = 0.2). Comparison of cardiac output (CO) and aortic systolic pressure were not significant (3.3 ± 0.4 L/min versus 2.9 ± 0.4 L/min, p = 0.24 and 72 ± 5 mmHg versus 67 ± 2 mmHg, p = 0.26, respectively).

In short axis view, the interpapillary muscle distance (M1-M2) measured in TTE was 27.7 ± 6.1 mm in ED and 26.4 ± 5.8 in ES. These measurements were not different from 29.8 ± 6.1 mm in ED (unpaired t-test, p = 0.24) and from 23.8 ± 7 mm in ES (unpaired t-test, p = 0.12).

In long axis view, the TTE measurements for the mitral and tricuspid annulus diameters are reported in Table 13. The TTE diameter of the mitral annulus was not significantly different from AM-PM and from AM-P1 in ED and ES and from T1-PM in ED. The TTE measurement of the tricuspid annulus was not significantly different from S-AP, S-P and AP-AS in ED and ES. Altogether, the 2-D picture acquired by TTE corresponded to a slice of the heart through the mitral annulus at the level of the segments AM-T1 and PM-P2 and through the tricuspid annulus at the level of the segments S-AS and AP-P (Figure 25).
Table 13: Comparison of TTE long axis view and sonomicrometry measurements (in mm) of the two groups for the LV and the mitral valve.

ED: end diastole. ES: end systole.
AM & PM: anterior & posterior mitral annulus
T1 & T2: trigones
P1 & P2: Lateral mitral annulus
S, AS & PS: septal, anteroseptal & posteroseptal tricuspid annulus

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For the mitral valve, the TTE tethering distance (ED: 39.54 ± 5.24; ES: 40 ± 5.27) was not significantly different from the sono measurements of M2-AM (ED: 38.22 ± 4.57, p=0.76; ES: 38.62 ± 5.2, p=0.99) and of M2-T1 (42.54 ± 4.97, p=0.14; ES: 41.62 ± 5.12, p=0.17) but was significantly different from M2-T2 (ED: 30.17 ± 4.5, p<0.005; ES: 30.6 ± 4.6, p<0.005). M2-posterior mitral annulus (ED: 25 ± 3.23 and ES: 25.7 ± 2.83) was not significantly different from M2-P1 (ED: 41.01 ± 8.26, p=0.59; ES: 39.51 ± 7.47, p=0.6) M2-PM (ED: 32.34 ± 6.41, p=0.25; ES: 34.4 ± 5.7, p=0.54) but was significantly different from M2-P2 (ED: 26.7 ± 4.9, p<0.005; ES: 28.24 ± 4.8, p<0.005). These results are consistent with the previous results on the mitral annulus.

For the tricuspid valve, the TTE distance papillary muscle free wall-septal annulus (ED: 31.48 ± 6.04; ES: 36.08 ± 2.87, p=0.12) was not significantly different from the sono measurement of Apm-S (ED: 38.25 ± 2.86, p=0.14; ES: 36.08 ± 2.87, p=0.12) and of Apm-AS (ED: 35.85 ± 5.5, p=0.53; ES: 34.04 ± 4.8, p=0.38) but was significantly different from Apm-PS (ED: 39.33 ± 3.83, p=0.03; 38.33 ± 4.1, p=0.04). For papillary muscle free wall-free wall annulus, the TTE measurement was not significantly different from Apm-P (ED: 28.1 ± 5.92, p=0.97; ES: 27.73 ± 6.51, p=0.32), from Apm-AP (AD: 21.22 ± 3.44, p=0.1; ES: 21.4 ± 3.14, p=0.05) Apm-A (ED: 28.14 ± 2.36, p=0.98; ES: 27.86 ± 2.6, p=0.22). These results were consistent with the previous results if we consider that Apm-P and Apm-A present the same dimension.
3-2: Correlation of Trans-thoracic Echocardiography and Sonomicrometry

Measurements:

Altogether, the 2-D picture acquired by the TTE corresponded to a longitudinal slice of the heart (Figure 25):

At the level of the LV:
- through the mitral annulus at the level of the segments AM-T1 and PM-P2,
- through the postero medial papillary muscle M2.

At the level of the RV:
- through the tricuspid annulus at the level of the segments S-AS and AP-P,
- through the septum between Spm and Ppm,
- through Apm
Figure 25: Correlation in sheep between a slice of the sound waves at the level of the annuli (left) and the TTE picture (long axis view acquired through the 4th intercostal space).

AM: Anterior mitral annulus
PM: Posterior mitral annulus
T1 and T2: Left and right trigone
P1 and P2: Left and right lateral mitral annulus
S, AS and PS: Septal, anteroseptal and posteroseptal tricuspid annulus
A, AP and P: Anterior, anteroposterior and posterior tricuspid annulus.
LV and RV: Left and right ventricle
Apm: Anterior papillary muscle of the right ventricle.
4: Discussion

In experimental studies with sheep, different methods may be used to measure the geometrical variations of the heart. Sonomicrometry crystals were developed by Smith et al. [122]. Using sound waves and piezoelectric crystals, records of distances between crystals can be obtained 200 times per second. The sonomicrometry technology was used to study the mitral valve physiology [119], the importance of the stay chords [66] and the aortic valve during the cardiac cycle [130]. Another technology to measure the geometry of the heart is to implant radiopaque markers to silhouette the LV, annulus and papillary muscles. Using a biplane videofluoroscopic machine, two-dimensional images from the two X-ray views are acquired and analyzed through a computer at 60 Hz. With that technology in the research laboratory of cardiovascular sciences at Stanford University, geometrical changes of the LV after MI or chordal cutting [65] were studied. These two technologies measure at high frequency and with great precision the variations of the distances between two crystals or between two radiopaque markers. However, a cardiopulmonary bypass is needed to implant crystals and radiopaque markers. The advantage of the sonomicrometry is the 200 Hz frequency of data acquisitions. However, sonomicrometric crystals have to be connected to the sono-computer to record the data. In case of a survivor model, the chest of the animal has to be closed with the crystal wires under the skin and additional measurements must be performed by opening the skin again. The use of radiopaque markers allows researchers to study the sheep while anesthetized only. Thus, in a survivor model, two sets of measurements with these technologies can be “safely” recorded: After implantation, and at sacrifice. The surgeon places the crystals or the radiopaque markers under direct view. All the distances
measured are between anatomical places chosen and known by the surgeon. Because of
the non-invasiveness of TTE image acquisition, the measures can be made repetitively
and allows the follow-up of geometrical changes of the ventricle and the mitral valve in a
survivor model. The TTE allows the measurement on 2-D pictures of distances between
structures that have an assumed precise position on a 3-D heart. Further the 2-D
echocardiography can be done with the sheep not anesthetized, restrained in a sling for
example, and therefore, avoids the surgical implantation of sonomicrometry crystals or
radiopaque markers thus reducing the morbidity and mortality associated with a survivor
model. TTE technique in sheep is different from humans and no reference on TTE
technique exists for the sheep. Due to the anatomy and the position of the transducer,
traditional TTE views are not possible to acquire in sheep. As in humans, the heart is
closer to the ribs in the left side of the chest in sheep. The acquisition of an image
through the right part of the chest was not possible because of the presence of the right
lung. The air in the lung does not transmit the sound waves of the crystal vibration and
does not allow one to acquire reliable pictures. Therefore, the view of the heart was taken
on the left side of the chest. A four to five centimeter space between the inferior and
superior part of the lung, at the level of the sternum and the 4th and 5th intercostal space
was used (Personal observation on 50 sheep). Through this space it was possible to
acquire a long- and short-axis view of the heart. The short-axis view allows the
acquisition of an image at the level of the papillary muscles of the mitral valve, but the
view through the annulus was not possible. The measurements of the interpapillary
muscle distance were comparable to the M1-M2 distance as measured by
sonomicrometry. To analyze both atrioventricular valves, only a long-axis view through
the 4th intercostal space was available. We compared TTE distances available to sonomicrometry measurements. The comparison of our data showed that the long-axis view acquired by TTE in sheep corresponded to the analysis of the antero lateral part of the mitral annulus (around AM-P1) and to the short-axis diameters (S-AP or S-P) of the tricuspid valve (Figure 25). The presence on the TTE image of a papillary muscle head in the LV close to the septum corresponded to the postero-medial papillary muscle, M2. The presence of a PM in the RV close to the free wall corresponded to the anterior papillary muscle Apm that we described as well isolated and constant (Experiment 1).

The TTE examination in sheep is not standardized and as in humans is iterative and largely determined by the anatomic characteristics of the sheep and manual manipulation of the transducer by the operator. The presence of a good “window” that enables the transmission of the ultrasound signal to the heart is not predictable. The same operator performed all the echocardiograms and the measurements to minimize any differences.

Conscious of the complexity and invasiveness of sonomicrometry, we undertook a study comparing sonomicrometry versus TTE measurements. In an attempt to study the geometry of both atrioventricular valves in sheep we compared sonomicrometry findings with TTE measurements obtained on anesthetized sheep and found that TTE measurements were comparable to sonomicrometry measurements. The long axis view acquired through the 4th left intercostal space in sheep provided the distance between the postero-lateral and anteromedial mitral annulus and between the septal and lateral part of the tricuspid annulus. This view allowed the analysis of both leaflets and postero-medial PM of the mitral valve and of the Apm of the tricuspid valve. These findings confirmed the possibility of performing chronic animal studies with TTE follow-up.
Experiment 4: Percutaneous induction of a postero-lateral infarct by injection of 100% ethanol and analysis of LV and RV geometrical changes.

1: Review of Existing Animal Models of Heart Failure:

The objectives of the animal model are to mimic the condition of human ischemic MR, which corresponds with a LV dysfunction associated with functional MR. The chronic experimental models have been directed towards inducing either global cardiomyopathy or a localized infarction. Ischemic heart failure can be studied with a large animal model to evaluate the effects and benefit-to risk ratio of new medical and surgical therapies.

1-1: Heart Failure Induced by Toxin:

Toxin administration to animals can mimic heart failure. The most commonly used toxin is doxorubicin, an anti-neoplastic drug, well known for its cardiac toxicity. Eighty-nine cases of heart transplantation in patients with doxorubicin –induced cardiomyopathy were reported by United Network for Organ Sharing between 1990 and 1996. In animals, many experiments demonstrate a decrease in cardiac function after doxorubicin administration.

Tessier et al [131] associated the use of low dose doxorubicin (0.5-1 mg/kg) systemic infusion with the surgical creation of an arterio-venous (carotid-jugular vein) fistula. Evaluation of the heart dysfunction was done with 2-D echocardiography measuring the LVEDD, LVESD and LV shortening fraction. They showed in goats that 0.5 mg/kg of doxorubicin injected weekly during 13 weeks created an increase in LVESD.
of 48 ± 12% and in LVEDD of 24 ± 4%. Also, shortening fraction decreased concomitantly by 32 ± 1%. When 1 mg/kg was injected, similar but more pronounced results were observed with a 38 ± 23% increase in LVESD and 10.3 ± 5.6% in LVEDD as well as a 41.7 ± 3.2% decrease in shortening fraction. All these results remain stable during three months after the 13th injection. The respective role of the doxorubicin infusion and the arterio-venous fistula could not be determined because of the absence of a control group. Chekanov et al. [132] used the same protocol with a daily injection of doxorubicin resulting in a creation of acute stable heart failure.

To avoid systemic toxicity, intracoronary injection of doxorubicin was reported by Shah et al. [133]. In 20 dogs, 1mg/kg of doxorubicin was administrated through a 2nd diagonal catheter resulting in accelerated heart failure in 12 weeks. Results were assessed by 2-D echocardiography measurements. All measurements were significantly different through the follow-up (LVEDD: +17.5%, p<0.0001; EF: −38.3% p<0.0001).

Histological changes in both ventricles included myocyte hypertrophy, loss of myofibrillar material and vacuolization, which are comparable to findings in clinical human heart failure. However 60% survival rate were reported at 12 weeks with nine sudden cardiac deaths.

Reviewing the intracoronary doxorubicin administration as a chronic heart failure model in dogs, shows that this drug can be used effectively to produce heart failure without systemic complications [134-136]. However, LV depression and mortality rates are dose-dependent and vary from one study to the other (mortality reported from 0% to 40-50%[135]). This high mortality rate is likely unacceptable for most heart failure studies and result variations question the reproducibility of this method in dogs.
LV intramyocardial injection of snake cardiotoxin induced a well-delineated transmural lesion. Rajnoch et al [137] reported the snake cardiotoxin *Naja Mossambica Mossambica* injected directly in the LV wall exposed through a LV thoracotomy in nine sheep. This method created a scar and was followed by remodeling of the LV. This original method was used to study LV wall motion after the implantation of satellite cells into the myocardium. All these techniques can create heart failure with LV dilatation. However, the presence of mitral or tricuspid insufficiency was not reported in these studies.

1-2: Heart Failure Induced by Rapid Pacing

Rapid ventricular pacing or tachycardia induced heart failure is the most common heart failure animal model associated with MR. Dogs were most commonly used [138] and to a lesser degree swine and sheep[139]. At 240 bpm during 3-4 weeks, dogs develop heart failure with many features of human end-stage heart failure, but rapid reversal of signs with profuse diuresis occurs at termination of pacing [140]. Byrne et al [139] created a pacing protocol which allowed a slower heart failure development. The pacing rate was modified through the study according to cardiac output and the clinical status of the animal. Therefore the authors were able to measure and study the distinct levels of heart failure. Patel showed also that increasing the duration of ventricular pacing to ten weeks created a long-term model of heart failure [141]. Results were assessed by 2-D echocardiography measuring a 155% increase in LV volume at 12 weeks.

Timek et al [142] studied the mechanism of the MR associated with an ovine model of tachycardia-induced cardiomyopathy. Timek showed that the mechanism of the
MR was not associated with a tethering effect. LVES and LVED volumes, mitral annulus area (36 ± 14% and 46 ± 13% in end diastole systole respectively), interpapillary muscles distance (from 29.9 ± 4.2 to 35 ± 4.1 mm in end diastole and from 23.9 ± 3 mm to 30.4 ± 3.7 mm in end systole) increasing significantly. However, impaired mitral leaflet coaptation was not due to a displacement toward the ventricle of the coaptation point of the anterior and posterior leaflet. Mitral leaflets were at the same position relative to the annulus in systole. Therefore, incomplete mitral coaptation and resulting MR were due to mitral annulus dilatation.

In this ovine model of tachycardia-induced cardiomyopathy, the annulus dilated more in the septo-lateral (+25±12 %) than in the commissure-commissure dimension (+9±5%). The saddle shape of the annulus was preserved but flattened [143]. Tachycardia-induced heart failure is effective for a stable LV dilatation and depression of EF if associated with a long and slow pacing protocol. Associated MR is mainly due to a dilatation of the mitral annulus in the septo-lateral dimension.

1-3: Heart Failure Induced by Ischemia

Several animal models reproduced ischemic events in the animal heart to mimic the development of ischemic heart failure in humans. Gelatin sponge intracoronary embolization created LV failure in pigs [144]. In a controlled study, pigs underwent catheterization of the LAD using a 2.5Fr catheter, with the tip of the catheter placed between the first and the second diagonal branches. Small gelatin sponge particles homogenized and mixed with heparanized saline were then infused in the distal part of the LAD. Recanalization of the LAD occurred in all animals with poor run-off at 1 week.
LVEDD and LVESD increased significantly at 4 and 8 weeks. Plasma BNP levels, reported to increase with the severity of congestive heart failure [145,146], were significantly higher in infarcted animals than in the control group.

Microembolization of microsphere was also used in dogs to create a model of heart failure [147]. The technique that used cardiac catheterization of the left coronary ostium followed by injections of microspheres was performed and discontinued when EF measured by 2-D echocardiography was between 30 and 40%. LVED and LVES volumes increased significantly at three months from 62 ± 4 to 78 ± 4 ml (p=0.001) and from 38 ± 3 to 47 ± 3 ml (p=0.001), respectively.

Llaneras et al [35] described a model of coronary ligature in sheep. Ligation of the 1st and 2nd marginal branches (group 1) infarcted 23 ± 3% of LV mass, did not infarct the posterior papillary muscle and increased significantly LV cavity size. Ligation of OM2 and OM3 (group 2) infarcted 21.4 ± 4% of the LV and completely infarcted the PPM. Ligation of OM2, OM3 and PDA (group 3) infarcted 40.4 ± 7.2% of the LV mass and included PPM and the posterior one third of the ventricular septum. Group 1 and 2 sheep were followed during 8 weeks and MR was measured by echocardiography. MR developed in group 2 (average MR: +2.7) and not in group 1. Severe MR developed immediately in group 3 and 83% of sheep died within 3 hours. Ventricular dilatation was assessed by angiography measuring LVED and LVES areas. LVED and LVES dilatation were significant in Group 2 at 8 weeks (p<0.01) and in group 3 one hour after the infarction (p<0.01).

Gorman et al [148] and Moainie et al [149] observed that ligation of the first two diagonal branches of the left descending artery in sheep produced massive dilatation of
the LV within 8 weeks. Both groups reported that around 24% of the LV mass with 82% of the anterior PM mass were infarcted. LVED and LVES volumes increased significantly (+73.2% and +115.3% respectively) at 8 weeks after the procedure and the EF significantly decreased \((p<0.05)\). Gorman et al also reported the acute ligation of the 1\textsuperscript{st} and 2\textsuperscript{nd} diagonal and OM1 which infarcted 33% of the LV and the entire APM. LVESD and area increased by 12.5% and 34.2%, respectively. However no MR was reported when coronaries which supplied the anterior wall were ligated. Therefore according to Gorman et al [148], infarct size and location determined the development of MR in the ovine model.

2: Materials and Methods

2-1: Induction of the Myocardial Infarction

The sheep were heparinized with 10,000 Units i.v. plus an additional 5,000 Units/hour. A catheter was introduced (6F Pinnacle, Terumo, Inc., Somerset, NJ, USA) via the left carotid by percutaneous puncture or cut down. An angiogram was performed using a C-arm (OEC 9400, GE Medical Systems, Inc., Waukesha, WI, USA) via a guiding catheter (LA6HS1, multipurpose hockey stick 1, Medtronic, Inc., Minneapolis, MN, USA) to identify the second (OM2) and third (OM3) obtuse marginal branches of the circumflex artery. A balloon catheter (D3S3020, Over-the-Wire Balloon Dilatation Catheter, Medtronic, Inc., Minneapolis, MN, USA) was positioned in the origin of OM2 and inflated beyond the first branch (4 bar). Therefore, the distal part of OM2 was isolated from the coronary circulation. Without deflating the balloon, 1 to 3 ml of 100% ethanol (EtOH) was injected into the distal part of OM2. After 5 minutes, the balloon was
deflated and the heart was allowed to recover for 30 minutes. The guide wire and the balloon catheter were then advanced into OM3, inflated again distal to its origin (4 bar), and 2 to 3 ml of 100% EtOH was injected. We empirically decided to limit the total volume of EtOH injected to 5 ml. After 5 minutes, the balloon catheter was deflated. Aortic pressure was recorded through the angiocatheter. Cardiac output and pulmonary artery pressure were measured through the Swan-Ganz catheter before and after the procedure. During the entire procedure, amiodarone (40 mg/h) and lidocaine (4 mg/min) were infused to prevent arrhythmia. Before each EtOH injection, magnesium (1g i.v.), lidocaine (60 mg i.v.) and bretylium (25 mg i.v.) were injected.

2.2: Data Acquisition and Follow-up

Transthoracic echocardiography was performed before the procedure (baseline), after the procedure, at weekly intervals, and at sacrifice. The sheep was positioned upright in a fenestrated sling for the TTEs. Left ventricular end-systolic (LVESD) and end-diastolic (LVEDD) dimensions and the interpapillary muscle tips distance at end-systole (M1-M2 ES) and end-diastole (M1-M2 ED) were recorded through a short axis view. The mitral valve annulus diameter was measured and the presence of IMR and/or ITR was evaluated in the long axis view. The severity of IMR and of ITR was determined by the length of the jet (1+ present; 2+ half the distance to the atrial walls; 3+ reaching the atrial wall, and 4+ for reverse pulmonary vein flow or if the jet reached the inferior vena cava) [150]. Displacement of the papillary muscles was calculated as the distance between their tips and to the contralateral mitral annulus ("tethering distance"). Valve coaptation height or "tenting distance" was measured as the distance between the leaflet’s...
coaptation point and the mitral annulus plane at end-diastole. The ejection fraction (EF) was calculated using the Teichhoz formula for the end-diastolic and systolic volumes [151]. The following RV dimensions were measured at baseline and at sacrifice in ES and ED on the long axis view: the tricuspid annulus diameter, Apm-free wall annulus, Apm-septal annulus and RV diameter at the level of the Apm.

After 6 to 10 weeks, the animals were again anesthetized. The chest was opened through the fourth intercostal space. An epicardial echocardiogram was performed to analyze LV and RV function and the degree of IMR and ITR. A Swan-Ganz catheter was placed via the left jugular vein to record pulmonary artery and RV pressures and cardiac output. After heparinization with 10,000 Units, the animal was euthanized with an injection of 60 mEq of KCl in the ascending aorta and the heart was excised.

2-3: Macroscopic and Histology Analysis

The hearts were cut into eight or nine 1-cm thick transverse slices. The slices were incubated in 2% triphenyl tetrazolium chloride (Sigma Chemical, St. Louis, MO, USA) for 30 minutes at room temperature [152]. The individual slices were weighed and photographed (Canon Powershot A70). The left ventricular and infarcted areas were quantified using planimetry software (AutoCAD Autodesk, San Rafael, CA) to determine the percentage of LV area infarcted, which was used to determine the LV mass infarcted. The slices were fixed in 10% formalin for histologic analysis. The fixed samples were dehydrated and embedded in PolyFin wax (Polysciences, Inc, Warrington, PA, USA), sectioned at 5 μm, and collected on Superfrost® plus slides (VWR Scientific, West
Representative sections were stained with hematoxylin and eosin (H&E) for general tissue and cellular morphology.

2-4: Statistical Analysis

Data were evaluated with repeated measures analysis of variance (ANOVA) explored by 3 paired student t-test comparing each time point (baseline versus immediate post-infarction, post-infarction versus sacrifice, and baseline versus sacrifice). A p-value < 0.016 was defined as statistically significant (Bonferroni corrected).

3: Results

3-1: Percutaneous Induction of Myocardial Infarction

The EtOH ablation procedure was performed in six sheep with an average weight of 53.6 Kg (range 64.5 to 39.5 Kg). The left carotid introducer was placed percutaneously in 4 and with a cut-down in 2 sheep. The baseline coronary angiograms showed that the LV apex was supplied by the left anterior descending artery in five cases (type A) and by the circumflex branches in one case (type B). These findings were similar to those reported by Llaneras et al. [35]. In five cases, it was easy to visualize OM1 and OM2 by videofluoroscopy. In four sheep, the coronary anatomy was similar. The origins of OM2 and OM3 were well-individualized branches off the circumflex artery. In three of these cases, each branch had two EtOH injections (Figure 26). In one case, OM2 and OM3 were very small, and OM4 was injected followed by irreversible ventricular fibrillation that terminated the procedure. In two other cases (one type A and one type B), a large branch of the circumflex artery divided distally into OM2 and OM3. In these cases, a
single bolus of EtOH was injected in the large branch (4 and 5 ml respectively). The mean volume of 100 % EtOH injected was 1.5 ml in OM2 and 2.6 ml in OM3 (for a total of 4 ml/animal). The hemodynamic and echocardiographic data at baseline and immediately after the procedure are shown in Table 14. In all cases, frequent multiform ventricular nonsustained tachycardia occurred at the time of injection, which stopped spontaneously, when the injection was terminated. In all animals the EKG, showed severe ST segment elevation after the EtOH injection (Figure 27).
Figure 26: Coronary angiogram of the left coronary before (left) and after (right) 100% EtOH injection.

The distal part of the artery is no more apparent after the injection of contrast product in the coronaries. EtOH: ethanol, OM2 & OM3: Obtuse marginal arteries 2 and 3.

Figure 27: EKG at the end of the procedure showing S-T segment elevation.
3-2: Development of Ischemic Cardiomyopathy

Five animals were followed for an average of 8 ± 1.3 weeks. The two animals with a single large OM that received only one injection of EtOH developed early clinical heart failure at 2 weeks post-procedure, requiring the administration of furosemide (20 mg twice/week) followed by daily doses (20 mg) during the last week before sacrifice at 6 and 7 weeks. The other three animals showed signs of clinical heart failure after the fourth week. They were sacrificed at weeks 8 and 9. At the time of sacrifice, all animals were in heart failure. They were breathing rapidly, had pale noses, stretched their hind legs, increased their water intake and retained fluid. The average weight increased by 16% (p=0.043), from 56.4 ± 7.6 kg (range 44 to 64.5 kg) to 65.3 ± 3.9 kg (range 63.1 to 71.2 kg). The hemodynamic and echocardiogram data at the end of the study are shown in Table 14. At sacrifice, the mean grade of IMR was 2.8 ± 0.45+ and ITR was 2.1 ± 0.5+. The LVEDD increased from 43.9 ± 3.51 mm to 58.4 ± 5.94 mm (33.7 ± 17%, p=0.009) and the LVESD increased from 31.7 ± 3.4 to 50.6 ± 8.47 mm (62 ± 40%, p=0.015). The diastolic distance between papillary muscles increased from 28.2 ± 6.8 to 36.4 ± 5.4 mm (32 ± 24%, p=0.01). The systolic distance between papillary muscles increased from 21.3 ± 3.4 to 29.6 ± 6.2 mm (40.7 ± 32%). The tethering and tenting distances increased by 32.7 ± 28% and 108 ± 65% (p=0.036), respectively. The mitral annulus diameter had enlarged by 18.5 ± 15.3% in diastole and by 29.7 ± 41.3% in systole.
Table 14: Hemodynamic and echo data, before and after EtOH injection and at sacrifice.

M1: antero-medial left papillary muscle  
M2: postero-lateral left papillary muscle  
LVEDD & LVESD: Left Ventricular End-Diastolic/Systolic Dimensions in short axis view  
MR&TR: Mitral and Tricuspid regurgitation, PAP; Pulmonary artery pressure  
OM2 & OM3: Obtuse marginal arteries 2 & 3  
*: Repeated measure analysis of variance, p<0.05 repeated measures ANOVA significant  
**: Comparison between baseline and sacrifice, p<0.016 (Bonferroni corrected)  
***: Comparison between after injection and sacrifice, p<0.016 (Bonferroni corrected).  
The differences were not significant for the paired t-test between baseline and after injection. Significant dilatation of the left ventricle occurred during the follow-up after the EtoH injection.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>After injection OM2-OM3</th>
<th>Sacrifice</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAP mm Hg</td>
<td>25/18</td>
<td>28.6/22</td>
<td>28/13</td>
</tr>
<tr>
<td>Cardiac output L/min</td>
<td>3.18</td>
<td>2.87</td>
<td>3.92</td>
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<td>Aortic pressure mmHg</td>
<td>87/66</td>
<td>86/67</td>
<td>72/55</td>
</tr>
<tr>
<td>EKG</td>
<td>normal</td>
<td>ST-segment elevation</td>
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<tr>
<td>EF%*</td>
<td>56.4±7</td>
<td>48±9</td>
<td>29.6±14******</td>
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<tr>
<td>M1-M2 ED mm*</td>
<td>28.2±6.8</td>
<td>32.4±4.64</td>
<td>36.4±5.4*** ***</td>
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<td>M1-M2 ES mm</td>
<td>21.3±3.36</td>
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<td>MR*</td>
<td>0</td>
<td>0.2</td>
<td>2.8±0.45*** ***</td>
</tr>
<tr>
<td>LVEDD short axis, mm*</td>
<td>43.9±3.51</td>
<td>48.8±5.08</td>
<td>58.4±5.94**</td>
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<tr>
<td>LVESD short axis, mm*</td>
<td>31.7±3.4</td>
<td>37.5±3.15</td>
<td>50.6±8.47**</td>
</tr>
<tr>
<td>TR*</td>
<td>0.2</td>
<td>0.5</td>
<td>2.1±0.5** ***</td>
</tr>
<tr>
<td>Tethering distance</td>
<td>37.1±6</td>
<td>37.5 ± 6.59</td>
<td>47.2±3.76</td>
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<tr>
<td>Tenting distance mm*</td>
<td>4.7±1.3</td>
<td>6.6 ± 1.6</td>
<td>10.25±0.5**</td>
</tr>
<tr>
<td>Mitral annulus ES</td>
<td>29.6±5.6</td>
<td>31 ± 2.4</td>
<td>36.3±2</td>
</tr>
<tr>
<td>Mitral annulus ED</td>
<td>31.5±5.4</td>
<td>33.2 ± 4.7</td>
<td>37.3±2.9</td>
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We also observed the development of a TR during the development of the MR. At sacrifice we observed a directional jet toward the atrial septum on epicardial echocardiography (figure 28). The dimension of the tricuspid annulus increased by $11 \pm 7\%$ ($p=0.03$) in ED, and by $8 \pm 6\%$ ($p=0.04$) in ES. The distance between the papillary muscle on the free wall (Apm) and the annulus at the level of the free wall increased by $5 \pm 3\%$ ($p=0.05$) in ED, and by $3 \pm 7\%$ ($p=0.48$) in ES. The distance between Apm and the annulus at the level of the septum increased by $8 \pm 5\%$ ($p=0.03$) in ED, and by $16 \pm 24\%$ ($p=0.26$) in ES. The distance between the septum wall and the free wall increased by $21 \pm 17\%$ ($p=0.04$) in ED, and by $0.35 \pm 23\%$ ($p=0.01$) in ES. The dimension of the RV and the tricuspid valve are reported in table 15. Figure 29 shows the progressive TTE changes during the follow-up.

Table 15: Variations of RV dimensions at baseline and at sacrifice.

FW: Free wall, Apm: Anterior papillary muscle of the RV, ED: End diastole, ES: End systole

<table>
<thead>
<tr>
<th></th>
<th>Pre EtOH</th>
<th>Sacrifice</th>
<th>Paired t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tricuspid annulus</td>
<td></td>
<td></td>
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<tr>
<td>ED</td>
<td>21.1±1.2</td>
<td>23.6±2.2</td>
<td>0.03</td>
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<tr>
<td>ES</td>
<td>19.5±1.8</td>
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<td>0.04</td>
</tr>
<tr>
<td>Apm-FW annulus</td>
<td></td>
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<tr>
<td>ED</td>
<td>29±5.6</td>
<td>30.9±6.9</td>
<td>0.05</td>
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<tr>
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<td>Apm-septal annulus</td>
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<tr>
<td>ED</td>
<td>28.2±1.2</td>
<td>30.6±1.3</td>
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<tr>
<td>ES</td>
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<td>RV diameter at level</td>
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<td>Apm ED</td>
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<td>ES</td>
<td>9.6±1.9</td>
<td>12.7±2</td>
<td>0.01</td>
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</tbody>
</table>
Figure 28: Directional jet of the ITR at sacrifice.

The directional jet toward the atrial septum corresponded to a tethering of the septal leaflet.
RA: Right atrium,
RV: Right ventricle
LV: Left ventricle

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Figure 29: Mean variations of all sheep in MR, TR, LVEDD, EF and M1-M2 ED (during the follow-up).

MR & TR: Mitral and Tricuspid regurgitation
EF: Ejection fraction
M1-M2 ED: Interpapillary muscle distance in end diastole
LVEDD: Left ventricle end diastolic dimension
3-3: Histology Analysis

The infarct area was 22 ± 3.5% (range 19.1% to 24%) of the LV area and had a mean calculated weight of 48.2 ± 6.1g. Macro and microscopically, the posterior papillary muscle was not infarcted. Histology of the infarcted area showed marked thinning of the ventricular wall with no inflammatory response (Figures 30A and 32B). Small bundles of cardiomyocytes were present in the subendocardial area (Figure 30C). The remainder of the wall consisted of bands of fibroblasts alternating with adipocytes within a finely fibrillar background. Ghost cells without residual nuclear material and with a waxy eosinophilic cytoplasm were occasionally observed (Figure 30D). Small vessels surrounded by occasional normal looking lymphocytes could be seen near the periphery of the infarct. No foreign body giant cell response was identified. These changes abruptly gave way to a normal appearing myocardium at the periphery of the induced lesion. No epicardial abnormalities were identified.
Figure 30: Histologic sections of the infarcted ventricular wall (H & E stained)

A: x4, Border zone. Normal cardiomyocytes: White arrow.
B: x10, small bundles of cardiac myocytes present in a subendocardial location
C: x40, Border zone, bands of fibroblasts in a finely fibrillar background with bundles of cardiomyocytes
D: x40, ghost cell (white arrow), collagen and fibroblasts
4: Discussion

The incidence of functional IMR after acute myocardial infarction has been reported to be between 13% [18] and 39% [19]. Its presence doubles the long-term (5 years) mortality [3], even in cases with mild or moderate regurgitation [22]. Although, IMR is known to be related to the location and extension of the infarct [23], successful early reperfusion does not reduce mortality [33]. Due to the high level of interest in this subject, it is obvious that far more questions than answers remain regarding the pathophysiology of IMR and, indeed, of the mechanisms involved in the remodeling of the well-perfused remote areas from the infarct [153]. In fact, it has been recently questioned whether IMR is the main cause for the poor prognosis of these patients or only a reflection of poor LV function secondary to remodeling [154]. These questions highlight the need for reproducible chronic experimental models. Furthermore, reduction annuloplasty, considered the standard surgical treatment for IMR, has been shown to have a 30% failure rate and no improvement in 5-year survival [59]. These unsatisfactory results have spurred interest in new surgical alternatives that need to be tested in large animals. Current chronic experimental models have been directed toward inducing either global cardiomyopathy or a localized infarction. Systemic or intracoronary injection of doxorubicin hydrochloride in dogs and goats [131,135], local transmural injection of snake cardiotoxin [137], and coronary micro-embolization [144,147] result in a variable degree of heart failure, but with absent or unmentioned IMR. The rapid pacing model of cardiomyopathy requires several weeks to create chronic heart failure but usually achieves only mild or moderate MR that is reversible when pacing is discontinued [139,143]. The mechanism responsible for the MR might be different according to the
experimental method used. In the rapid pacing ovine model, Timek and associates [142] have described significant mitral annulus dilatation, particularly in the septolateral direction, without papillary muscle displacement and leaflet tethering.

In a seminal anatomic study of the coronary artery distribution in sheep, Llaneras et al. [35] demonstrated the absence of collaterals and, the very precise and localized effects of selective coronary ligature. This systematic approach revealed that IMR did not follow the selective ligature of OM1 and OM2 while occlusion of OM2 and OM3 induced a posterolateral infarct with development of significant IMR at about 8 weeks. When ligature of OM4 was added, severe IMR appeared followed by death within hours. These models, extensively applied by Gorman and associates [148,155], have the disadvantage of needing a thoracotomy to access the selected arteries. Although a thoracotomy is often required to fully instrument the animal when radiopaque or ultrasound crystals are used, a previous or second thoracotomy is needed to either induce the infarction or to test a particular surgical procedure.

A previous review of the use of percutaneous alcohol injections in the interventricular septum for the treatment of hypertrophic cardiomyopathy suggested to us the possibility of applying it to induce IMR by infarcting the myocardium served by OM2 and OM3 [156-159]. Our results confirm the findings of Llaneras and associates [35]. In both cases, ligature or EtOH injection of OM2 and OM3 resulted in a localized and well-defined posterolateral infarct and significant IMR. In the current study, a central IMR appeared in all five survivors between 2 and 4 weeks after the procedure and evolved to 2.8+ at 8 ± 3.1 weeks. The development of significant functional tricuspid regurgitation was seen in all cases. Because of the difficult imaging of the tricuspid valve in sheep, we
could not evaluate the RV function when the animal was standing in a sling. Therefore we analyzed the geometry of the RV at baseline and sacrifice. A significant ITR appeared in all survivors between three and four weeks and its development followed the progression of the MR. This finding has not been previously reported in animals subjected to occlusion of the circumflex branches. It confirms its similar high incidence found in human cases with IMR [44].

The histology of the EtOH-induced infarct was similar to that described after alcohol septal ablation [160], i.e. an appearance typical of an old, well-defined infarct with signs of inflammation absent. Interestingly, contrary to Llaneras et al. [35], who reported the presence of an infarcted posterior papillary muscle in all cases, we found an intact posterior papillary muscle in all of ours independently of whether the LV apex was supplied by the left anterior or by the circumflex artery (type A or B heart). The infarct area and degree of IMR were similar in both the Llaneras report (21.4% and 3.18+ respectively) and in our model (22% and 2.8+). These similar end results in spite of the different status of the papillary muscle might be explained by the LV remodeling that displaces a perfused posterior papillary muscle. Also, the reports of Messas and associates in sheep [28] and of Khankirawatana et al. in humans [161] described paradoxical decrease in IMR severity in the presence of an ischemic papillary muscle. Recently, in a pig model of alcohol injection into the circumflex arteries, Li et al. [162] showed that the size of the infarct is related to the volume injected and not to the velocity of delivery [163]. In our study, the maximum volume was arbitrarily limited to 5 ml and the velocity of injection was controlled by hand and according to the arrhythmias that occurred during the injection.
Transthoracic echocardiography allowed us to follow each animal weekly avoiding a thoracotomy before sacrifice. The echocardiographic findings in our series confirm those reported in patients [23] and in sheep [27,28]. The mitral annulus dilated by 32% in systole and by 13% in diastole and the interpapillary muscle distance increased by 32% in diastole and by 40% in systole, resulting in an increase in leaflet tethering (32%+) and coaptation (108%+) distances. The 3-D data at 8 weeks post infarction reported by Otsuji et al [27] are similar to our 2-D data (Table 14). However, while Messas et al. [28] and Otsuji and associates have reported significant 3-D differences between pre and immediate post infarction, these differences obtained with 2-D echography did not reach statistical significance [148].

In the RV, between the baseline and sacrifice in our study, the annulus measurement increased by 11% in diastole and 8% in systole and the distance between the free wall and the septum increased by 21% in diastole and 35% in systole. As in our sheep, ITR has been associated with annulus dilatation [47,49] and RV enlargement [50]. Right ventricle enlargement may result in leaflet tethering [51,53]. Also, the distance between Apm and the septal annulus increased significantly in diastole by 8% in our sheep. However, the 16 ± 24% increase in systole was not significant. No significant increase was found for the distance between the Apm tip and the annulus at the level of the free wall (+5% in diastole and + 3% in systole).

According to experiment 3, the measured dilatation of the mitral annulus occurred in an antero-posterior axis and the measured dilatation of the tricuspid annulus occurred in the short axis. The dilatation of the mitral annulus in case of IMR has been described as homogeneous in the antero-posterior and septo-lateral dimensions [30]. The increase
of the short-axis diameter of the tricuspid annulus on our sheep corresponds to the segmental free wall dilatation observed by Deloche et al. in human valves [49].

Following the EtOH infarction, we observed the development of IMR and ITR associated with annulus, LV and RV enlargement. These enlargements resulted in tethering of the anterior mitral leaflet (the tethering distance increased by 32.7 ± 28% between baseline and sacrifice in systole) and an increase of the Apm-annulus distance at the level of the septum (+16 ± 24% in systole) in the RV. All these variations resulted in geometrical distortion of the remarkable and very precise geometry of the annuli and their corresponding subvalvular apparatus. The increased Apm-annulus distance at the level of the septum did not result in eventual tethering of the septal leaflet because no chord linked the Apm to the septal leaflet [93]. Tethering of the septal leaflet may have occurred by displacement of the Ppm and Spm that are linked by chords to the septal leaflets. The displacement of the papillary muscles inserted on the septum may be associated with LV enlargement. We showed in experiment 2 that both annuli and ventricles at the level of the papillary muscle were linked by the septum. According to the Laplace law, with the increasing dimensions of the LV, the pressure on the LV wall increased and resulted in significant bulging inside the RV. The bulging of the septum inside the RV had been described as associated with functional TR [164]. The increased bulging of the septum may have displaced the Spm and Apm enough to distort the geometry of the septo-anterior papillary muscle unit of the tricuspid valve. By minimal distortion of all the annulo-papillary units of the tricuspid valve, the moderate annulus and RV enlargement may have also facilitated the development of the ITR. The eccentric
jet described in human cases of septal bulging with ischemic LV dysfunction [164] was also observed at sacrifice on epicardial echocardiography in our sheep model (figure 28).

The small number of animals is obviously one major limitation of the studies. However, in the development of the animal model the fact that all but one reached the intended objective of inducing a significant IMR confirms the feasibility, simplicity and reproducibility of the method. Also, the results in sheep can not be applied uncritically to the human where, for instance, there is a diffuse coronary vascular inflammation, which has been associated with acute coronary syndrome. Whether our animal model of IMR following a postero-lateral infarct is similar to the clinical situation of an IMR secondary to an inferior infarct remains to be answered [23].

In conclusion, to study the functional tricuspid regurgitation from ischemic origin, we developed an original animal model of heart failure. The percutaneous selective injection of 100% EtOH in the second and third marginal coronary arteries of sheep heart resulted in a transmural LV infarction with no PM damage. During the eight weeks follow-up, remodeling of both ventricles was at the origin of ischemic mitral and tricuspid regurgitation. The non-invasiveness of this technique opens the field of chronic experimental ischemic cardiomyopathy to study the remodeling of the heart.
Experiment 5: Change in eNOS and nNOS expression by distance from the infarct in an ovine ischemic heart failure model

1: Nitric Oxide Synthase

1-1: Background

Nitric oxide (NO) is an endothelium derived relaxing factor synthesized from L-arginine by activation of NO synthase (NOS). Three isoforms of NOS are known: constitutive forms, discovered in neurons (NOS1 = nNOS) and endothelial cells (NOS3 = eNOS), and the inducible isoform (NOS2 = iNOS) discovered in macrophages. The three NOS isoforms share 50 to 60% homology of their amino acid sequence and are encoded by three different genes (respectively NOS1, NOS2 and NOS3). All three isoforms combine two functionally complementary portions, a carboxyl-terminal reductase domain homologous to cytochrome P450 reductase and an amino-terminal oxygenase domain containing binding sites for heme, L-arginine, and tetrahydrobiopterin (THB4). A calmodulin-binding domain in the middle connects the two portions. The three isoforms function presumably as homodimers (Figure 31). Within each monomer, the chemical reaction is the following:

\[
\begin{align*}
\text{O}_2 & \quad \text{O}_2 \\
\text{L-arginine} & \quad \text{N-hydroxy-L-arginine} & \quad \text{L-citrulline + NO} \\
\text{NADPH} & \quad 1/2\text{NADPH}
\end{align*}
\]
Figure 31: Nitric Oxide Synthase principles.

Within each monomer, electrons provided by NADPH are transferred from the flavins (FAD or FMN) in the carboxyl-terminal portion of the molecule to the heme iron (Black arrows). This is activated to bind O\(_2\) and in presence of L-arginine, to catalyze the synthesis of NO and L-citrulline (Grey arrow). Calmodulin seems to be required to allow the transfer of the electron. THB4 appears to stabilize the homodimeric conformation of the enzyme (modified from Xu et al., Nitric Oxide Website, www.wxumac.demon.co.uk).
Nitric Oxide Synthase and the Heart

NO is a highly diffusible gas that permeates cell membranes and cannot be stored. Its theoretical diffusion is limited within the cardiac tissue by the abundance of superoxide anions produced by actively contracting myocytes, which combine with NO to produce nitrogen derivatives, and by the abundance of cytoplasmic myoglobin, a heme-protein with a high affinity for NO. The autocrine effect is therefore predominant and may explain the multiple NOS synthases within the same tissue. The source of NO may be a predictor of its functional impact and thus the signaling specificity of NO is likely controlled at the level of its synthesis [94]. Both eNOS and nNOS are constitutively expressed in cardiomyocytes [94]. These isoforms are localized in key organelles that regulate cell function [165]. Endothelial NOS is localized in cell-membrane caveolae, which are organelles that link extracellular hormones with appropriate intracellular signaling effectors (eNOS is mostly associated with caveolin-3, the cardiomyocyte-specific structural protein of caveolae) [166]). It has been shown in endothelial cells that multiple protein interactions in caveolae regulate eNOS activity and its coupling to extracellular stimuli [167]. Endothelial NOS interacts with the major protein of caveolae, caveolin, which inhibits eNOS. In presence of Ca^{2+}, calmodulin causes dissociation of eNOS from caveolin [168]. This is modified by heat shock protein 90 (Hsp90), which binds to eNOS and facilitates the displacement of caveolin by calmodulin. Hsp90 belongs to a family of proteins that are synthetized by the cell in response to stress such as elevated temperature [167]. Among the NOS isoforms, nNOS is unique in containing a PDZ domain, a specific peptide sequence that allows it to recognize and bind to other proteins [169]. This PDZ domain may play a major role in
targeting the nNOS to specific cellular membranes [167]. In cardiomyocytes, nNOS localizes to the sarcoplasmic reticulum, the organelle responsible for the intracellular calcium cycle that drives excitation-contraction coupling [170]. Endothelial NOS is also expressed in endothelial and endocardial cells and nNOS is present in adrenergic and cholinergic fibers.

2-3-1: Endothelial NOS, nNOS and Cardiac Function in the Normal Heart

Endothelial NOS is localized in sarcolemmal caveolae with β-adrenergic receptors and L-type Ca^{2+} channels and nNOS is localized in the SR [170]. In the unstimulated heart, eNOS is not expressed at significant levels and has little impact on basal contractile [171] and relaxation [172] functions. In vivo studies showed an increase of contractility in nNOS -/- mice compared to wild type (WT) controls and on isolated cardiomyocytes, an increase of L-type calcium current and amplitude of contractile shortening were found [173]. When stimulating hearts or isolated LV myocytes from nNOS -/- mice, an attenuated positive force-frequency response in conjunction with impaired increase in SR calcium load in comparison to WT mice were found [174]. These findings showed a role for nNOS in reducing calcium entry, facilitating SR load and, thus, promoting relaxation. A promoting effect of nNOS on SERCA activity remains under question [94]. Nitric oxide synthase isoforms affect the contractile response to β-adrenergic stimulation. The β-adrenergic effect of isoproterenol was enhanced by L-NG-monomethyl-arginine (L-NMMA, a competitive inhibitor of all NOS isoforms) treatment in isolated rat cardiomyocytes. An in vivo study of eNOS -/- mice has shown a role for eNOS in limiting the inotropic effect of catecholamines [172]. However, no change was seen in normal subjects who received an intravenous dobutamine infusion and a
concurrent intracoronary infusion of L-NMMA [175]. The significant impact of eNOS on
the contractile response to β-adrenergic stimulation is due to the stimulation of a β3-
adrenoreceptor isoform on cardiac myocytes. This receptor signals through G-protein
coupling to eNOS activation and cGMP increases. Cyclic GMP was demonstrated to
antagonize the effect of beta-adrenergic signaling in cardiomyocytes through degradation
of cAMP, phosphorylation of calcium channel or ryanodine receptor or Troponin I
decreases myofilaments Ca\(^{2+}\) sensibility) (Figure 32) [94]. Neuronal NOS was also
shown to decrease the inotropic response to β-adrenergic stimulation in isolated
cardiomyocytes. In isolated LV myocytes from nNOS -/- mice, increased inotropic
response was found after exposure to a low concentration of isoproterenol [176]. The
limiting effect of nNOS-derived NO on L-type Ca\(^{2+}\) current may be implicated in the
positive response to β-adrenergic stimulation. However, in nNOS -/- animals in vivo, this
positive response was not seen and could even be reversed [170]. The profound alteration
of Ca\(^{2+}\) cycling in the stressed heart may be a major factor influencing the increase in
sarcolemma Ca\(^{2+}\) influx. Thus, nNOS may have a more important role in promoting
SERCA function and SR calcium load to promote diastole in response to catecholamines
in vivo. Endothelial NOS may act as a balance to prevent β-adrenergic overstimulation
[94]. Spatial localization of eNOS and nNOS in proximity with effector signaling
pathways allows NO to exert complex and precise autocrine regulation of cardiac
contraction, relaxation and rate [172]. Endothelial NOS was also shown to facilitate
excitation/contraction coupling in response to sarcomere stretch. Petrof et al. showed that
SR Ca\(^{2+}\) release was proportional to length change of the sarcomere and was abrogated in
cardiomyocytes from eNOS -/- mice. The mechanism appeared to involve an activation
of the ryanodine receptor facilitated by the localization of both proteins. Endothelial NOS-derived NO may serve as a local messenger that plays a role in the Anrep effect [177].

Figure 32: Endothelial and neuronal Nitric Oxide Synthase in the normal heart.

In the normal heart, eNOS promotes calcium increases in response to stretch (double black arrow) and protects the myocardium against excess catecholamine stimulation through the cGMP activation, that degrades cAMP, phosphorylates the calcium channel, ryanodon receptor and Troponin I (bold black arrow). Neuronal NOS limits the beta-adrenergic increase in calcium transient (bold grey arrows). This function may be less relevant in vivo compared with its activation of SERCA activity to sustain SR calcium load in response to inotropic interventions and promote cytosolic calcium removal and diastolic relaxation. These two isoforms would then cooperatively sustain excitation/contraction coupling and cardiac contraction. (Modified from Pelat et al. [94])

![Diagram of endothelial and neuronal Nitric Oxide Synthase in the normal heart.](image-url)
2-3-2: Endothelial NOS, nNOS and Cardiac Function in the Diseased Heart

Nitric oxide synthase isoforms appear to have a role in cardiac remodeling. Indeed, in a mouse model of an anterior myocardial infarct, the infarct size was unchanged in eNOS-/− mice in comparison with WT. However, the remodeling of the eNOS-/− mouse hearts presented decreased capillary density and greater myocyte width, as well as diastolic and systolic dysfunction in comparison with the WT at both day two and 28 after the MI [178].

Intracoronary L-NMMA infusion in moderate heart failure patients with an etiology of dilated cardiomyopathy had no significant effect on basal function and the pacing of these patients was not altered either. Thus, NO may have little influence on cardiac basal function and force-frequency relationships in moderate heart failure patients [179]. However, Hare et al showed that NO inhibited the positive inotropic response to β-adrenergic stimulation in LV dysfunction patients using the same kind of protocol with dobutamine infusion [180]. This study supported the role of NO in modulation of cardiac function in heart failure patients.

In heart failure patients with idiopathic dilated cardiomyopathy, eNOS has been reported to be down-regulated [181,182]. Damy et al reported up-regulation of nNOS in these patients with a translocation of the enzyme from the SR to the sarcolemma membrane [182]. The decreased nNOS activity in the SR associated with the increased nNOS activity in the sarcolemma membrane may be a central pathological event that enhances the importance of the spatial confinement of NOS isoforms within the cardiomyocyte [169]. The role of nNOS as an adaptative mechanism to preserve diastolic Ca^{2+} levels and SR calcium load in the failing cardiomyocyte is not yet established.
The failing dilated heart is limited in the amount it can increase stroke volume as any increase in diastolic volume greatly increases the filling pressure of the ventricle. The sarcomeres in the wall are at their maximal length and cannot adapt [83]. The eNOS down-regulation and nNOS up-regulation and translocation may participate in the altered Frank-Starling adaptation of the failing heart through defective stretch-dependent contractile reserve and altered relaxation [171].

Dynamic changes in expression of the enzymes, substrates and cofactors as well as change in repartition of NOSs in the different structures of the cardiomyocyte are probably associated with the heart failure [94].

2: Materials and Methods

The hearts from the animals who underwent a percutaneous injection of 100% EtOH in the OM2 and OM3 (experiment 4) and five control hearts from age-matched animals obtained from a local abattoir (Lolo Locker, Lolo, MT, USA) were analyzed.

2-1: Preparation of Tissue

The right and left ventricles were cut into 8 to 9 transverse slices. On the 5th slices from the apex, samples, about 1cm wide, of myocardium from seven different areas were removed, snap frozen in liquid nitrogen and stored at -70°C until used. The areas were sampled from the LV anterior wall (A1), LV posterior wall (A2) RV posterior wall (A3), inferior septum (B1), RV free lateral wall (B2), superior septum (C1) and RV anterior wall (C2). The distances between the sampled areas and the MI were also measured.
2-2: Protein isolation, SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and Western Blot Analysis

For protein isolation, the snap-frozen heart tissue was allowed to thaw on ice and then was weighed. The samples were homogenized using a Tissuemizer in 4 vol/wet weight of sample in Triton lysis Buffer [20mM Tris.HCl (pH 7.6), 0.5% Triton X-100, and 20% glycerol] supplemented with protease inhibitors (Leupeptin, Aprotinine and PMSF). Extracts were then clarified by centrifugation (14,000 X g for 20 min) and the supernatant was removed for protein determination, SDS-PAGE and Western blot analysis. Final protein concentration of the myocardial extracts was determined with a standardized colorimetric assay (Bio Rad Protein Microassay from Bio-Rad Laboratories, Hercules, California, USA) according to the package insert. Protein concentration was adjusted to the appropriate concentration with PBS-T followed by the addition of Laemmli denaturing buffer at the ratio of one volume/volume. The samples were then heated at 100°C for 20 minutes. The Laemmli denaturing buffer contains sodium dodecylsulfate (SDS) that denatures the proteins by wrapping around the polypeptide backbone. SDS also confers a negative charge to the polypeptides in proportion to their lengths, leaving the proteins with a constant charge-to-mass ratio. The protein extracts isolated from ovine heart samples were separated on a 6% denaturing polyacrylamide gel and electrophoretically transferred to Hybond-PVDF membrane (Amersham, Arlington Heights, IL, USA). The membranes were incubated with a blocking solution of 5% non-fat dry milk in tris-buffered saline containing 0.1% Tween (TBS-T) to block all the non-specific binding sites of the proteins. After blocking, the membranes were incubated for one hour at room temperature with a 1:250 dilution of the primary antibody either eNOS
or nNOS antiserum (Transduction Laboratories, Lexington KY, USA). The primary antibody was followed by the secondary antibody at a 1:1000 dilution (goat anti-mouse horseradish peroxidase conjugated antibody, Pierce Inc, Rockford, IL, USA). All dilutions were made in 5% non-fat dry milk in TBS-T. The membranes were washed three times between each step with TBS-T. Immunoreactive bands were visualized by incubation with 5 ml of the luminol enhancer solution and 5ml of the stable peroxidase buffer provided in the kit for 1 minute (SuperSignal West Femto Maximum Sensitivity Substrate, Pierce Inc, Rockford, IL, USA). Bands were quantified with a computer-based imaging system (1D Image Analysis Software, Kodak Digital Science, Scientific Imaging System, Eastman Kodak Company, Rochester, NY, USA). To compare the optical density of the bands between the different gels, we used the lowest optical density for the area corresponding to a distance of 3 cm from the infarct as a reference value on each gel. The optical densities of the other bands were normalized according to the reference band density within the same gel.

2-3: Immunohistochemistry

The snap frozen heart tissues were used for immunohistochemistry. Representative slices from each area were placed in optimum cutting temperature compound, frozen on dry ice (-70°C) and cut in sections (6 μm) with a Cryotome (Thermo Electron Corporation, Milford, MA, USA) and transferred to aminoalkylsilane-treated slides (Superfrost Plus; Fisher Scientific, Santa Clara, CA). Cryostat sections were post fixed with 100% ethanol and stored at -80°C until used. The tissue sections were first incubated in PBS-T and 1% goat serum for 30 minutes, to eliminate non-specific binding of the primary antiserum to
tissue sections and to block endogenous peroxidase activity. Then, the sections were incubated with the eNOS antiserum or nNOS antiserum (Transduction Laboratories, Lexington KY, USA) at a 1:250 dilution for 1 hour at room temperature. After washing with PBS-T, the sections were incubated with appropriate fluorescent secondary antibodies (Alexa Fluor 546, Molecular Probes™, Invitrogen Inc, Carlsbad, CA, USA) at a dilution of 1:1000 for one hour at room temperature in the dark. Sections were washed again with PBS-T and mounted. An Olympus IX51 microscope (Olympus America Inc., Melville, NY, USA) equipped with epifluorescence optics was used to visualize the immunofluorescence. Immunostaining intensity was quantified at a magnification of 200X for ten different layers per area using an image program analysis (Image Pro Plus, version 5.0.1, Media Cybernetics Inc., Silver Spring, MD, USA).

2-4: Statistical Analysis

Data are expressed as mean ± standard deviation. Statistical significance was determined by using the unpaired student-t test in case of comparison of two groups. ANOVA was used in case of comparison of more than two groups. p<0.05 was considered significant.

3: Results

Four hearts of the animals (53.3 ± 6.4 Kg body weight) that underwent the EtOH infarction procedure were available for this experiment.
3-1: Development of Ischemic Cardiomyopathy

The left carotid introducer was placed percutaneously in three and with a cut-down in one sheep. The total volume of 100% EtOH injected was 2.5 ± 1.5 ml/animal. These four animals were followed for an average of 7.5 ± 1.5 weeks and they all developed clinical heart failure after the fourth week. Their weight presented a 24% increase from 54.3 ± 5 kg to 67.4 ± 3, p=0.04. As reported previously in experiment 4, the hemodynamic characteristics were not significantly changed (cardiac output varied from 3.3 ± 0.4 to 3.9 ± 1, p=0.2 and aortic systolic pressure did not change). The echocardiographic data showed, as previously described an increase in distance between baseline and before the sacrifice (Table 16). The infarct area was 21 ± 2% of the LV area and had a mean calculated weight of 44.6 ± 3.6 g.

Table 16: Geometric variations between baseline and sacrifice measured with Echocardiography in four sheep

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3-2: Expression of eNOS and nNOS

The expression of eNOS was barely detectable in normal hearts and was expressed in all areas of the diseased heart as shown by western blot analysis (Figure 33). Among the ICM group, no significant difference was found for eNOS expression in the LV, RV and septum according to the area sampled (ANOVA: p=0.712). However, relative expression of eNOS presented significant variations when samples where regrouped according to the distance from the MI (ANOVA, p=0.018). The relative optical density of eNOS expression appeared to be inversely correlated with the distance from the MI with an R-value of 0.723 (Figure 34). The immunohistochemical analysis (Figure 35) confirmed that eNOS was expressed in cardiomyocytes and that the intensity of eNOS staining was greater in ICM tissue. The measure of optical density intensity was increased ten fold in ICM hearts compared to controls (grades: 1 ± 4.5 versus 0.11 ± 0.32, p=0.002). The eNOS-staining pattern appeared to be peripheral. The staining did not appear to encompass the entire periphery of the majority of stained cells.

Neuronal NOS was not detected on Western blot analysis even with increased concentration of proteins loaded (25, 50 and 100 µg/lane) using a 1:250 dilution of nNOS antibody. Immunoreactivity for nNOS was also not seen on stained tissue sections (1:250 dilution).

Immunoreactive bands at the level of 70Kda were seen by Western blot analysis for eNOS and nNOS. These bands may correspond to degradation products from eNOS. Cross-reaction with nNOS antibodies may explain their appearance on nNOS Western blots (Personnal communication from the technical service representative of Transduction Laboratories, BD Biosciences, Lexington, KY, USA)
Figure 33: eNOS expression in sheep heart eight weeks after infarction.

A: eNOS band in ICM, expression in all areas (50 µg per lane);
B: eNOS bands in control hearts, expression detectable in A1, A2 and A3 areas and undetectable in other areas (50 µg per lane);
C: eNOS band in A2 area of the nine sheep, expression barely detectable in control hearts (20 µg per lane).
Figure 34: Plot showing a significant correlation between relative expression of eNOS in the diseased heart samples and the distance from the MI.

ROD: Relative optical density
Figure 35: Representative immunofluorescence micrographs of ovine heart sections of failing heart and controls, labelled with eNOS (200X magnification).
Endothelial NOS was up-regulated in ischemic heart and seemed to be more expressed closer to the infarct.
4: Discussion

Our finding of weak eNOS expression in normal cardiomyocytes of sheep origin corresponds to previous studies in human hearts [182,183]. This insignificant level of eNOS expression in the cardiomyocyte may be due to the minor effect of eNOS-derived NO on the basal contractile function of the heart [94]. Our study demonstrated increased expression of eNOS in ovine heart failure eight weeks after a postero lateral infarction. This result is consistent with a report by Baker et al. [184] who showed that chronic hypoxia increased eNOS expression in the immature rabbit heart. Fukuchi et al. [183] studied 28 failing human hearts with various etiologies and reported increased expression of eNOS irrespective of the underlying pathogenesis of heart failure. In dogs, Yamamoto et al [185] reported increased eNOS activity in isolated LV cardiomyocytes from tachycardia-induced heart failure. In our sheep model of early heart failure, the peripheral eNOS-staining pattern suggested the presence of eNOS in the sarcolemma. This result is consistent with previous studies that reported eNOS localizing to membranae caveolae where it can regulate the β-adrenergic stimulation [169]. With heart failure, the decrease in cardiac output stimulates the sympathetic nervous system with an increase in circulating level of catecholamines [90,186]. Endothelial NOS has been reported to limit the inotropic effect of the catecholamines [172] and thus may be up-regulated in our sheep to counteract a β-adrenergic overstimulation of the heart. This finding is different from other studies where it was reported that eNOS is down regulated in human late heart failure [94,182]. However, the studies that reported eNOS down-regulation in heart failure were done on end-stage heart failure patients whose etiologies were mostly due to idiopathic dilated cardiomyopathy. In these studies, samples of patient hearts were
obtained after death or during cardiac transplantation and the duration of end-stage heart failure of these patients was not reported [94,182]. In end-stage heart failure, a desensitization of the heart to catecholamines caused by a decrease in β-receptor density has been shown [187]. The mechanism that accounts for myocardial β-adrenergic-receptor down-regulation is presumably related to exposure to increased levels of catecholamines [90]. Taken together, these results suggested in our sheep a temporal “adaptative” up-regulation of eNOS to mitigate the excessive β-adrenergic stimulation of the heart in early heart failure. This might have been followed by a down-regulation of eNOS associated with the decrease in the β-adrenergic-receptor density. The alteration of eNOS expression may be dynamic during the course of development of a specific disease thus participating in the altered Frank-Starling adaptation of the terminally failing heart [94].

The up-regulation of eNOS was observed in cardiomyocytes from sheep hearts eight weeks after an MI. Heart failure symptoms and evidence of geometrical remodeling of the hearts, such as a significant increased LV diameter and the presence of functional MR and TR, were observed in all sheep after the fourth week. Endothelial NOS was up-regulated in all areas of the heart and we found that the level of eNOS expression was correlated with the distance from the MI. This correlation has not been previously reported in the literature. Fukuchi et al. reported increased eNOS expression mostly in the subendocardial area in failing human myocardium irrespective of the site of infarction in ischemic cardiomyopathy [183]. The mechanism responsible for our finding may correspond to stretch since the quantitative loss of cardiomyocytes following an MI results in work overload. The reduced stroke volume is compensated by an increased end-
diastolic volume. Thus the filling pressure increases on cardiomyocytes that are already lengthened. During the progression toward heart failure, the cardiomyocyte stretch tends to remain at a high level with persistent reduced EF [90]. According to Wilson et al. [89], the remodeling of the heart results in differences in the level of stretch of the myocardium: the further from the MI, less stretch is applied. The stretch of the sarcomere has a facilitatory effect on the Ca$$^{2+}$$ release process resulting in an increased contraction [85]. In vitro, Petroff et al [177] showed that eNOS-derived NO enhanced the release of Ca$$^{2+}$$ in response to sarcomere stretch through a direct activation of the ryanodin receptor independently from cGMP [177]. Altogether, this suggested that in early heart failure the expression of eNOS might be up-regulated to potentiate the SR calcium release according to the amount of stretch exerted on the cardiomyocytes. Direct activation of signaling molecules or release of paracrine or autocrine signaling factors appears to be among the different pathways to adapt the cardiomyocyte to stretch [188]. The exact mechanisms underlying the production of eNOS-derived NO that modulates the excitation/contraction coupling, as observed by Petroff et al. [177] in the case of increased stretch, remain unknown and eNOS may be associated with or may act as a “stretch sensor”.

Endothelial NOS was assumed to be the only constitutive isoform expressed in the cardiomyocyte until the report of Xu et al in 1999, who showed that nNOS localized to the murine and human SR [189]. Damy et al [182] reported a weak expression of nNOS in the SR of normal human heart. Using the same antibody, we were unable to detect nNOS in our samples by western blot analysis or immunohistochemistry. After a review of the literature, no previous report was found on the detection of the different NOS isoforms in the sheep cardiomyocyte. A lack of nNOS expression in the sheep
cardiomyocytes may explain the absence of detectable levels of nNOS in our samples. However, the presence of nNOS in many mammalian cardiomyocytes such as humans [182,189], mice [173,176,189] and rabbits [189] has been reported. The sheep cardiomyocytes may express a different isoform of nNOS, therefore the difference in species may be the reason for a potential lower cross reactivity between the nNOS antibody (mouse anti-rat antibody) that we used and the nNOS isoform in sheep myocardium.

It has been reported that nNOS derived NO regulates myocardial contraction by controlling Ca\(^{2+}\) handling in cardiomyocytes [173]. In late human heart failure, eNOS expression that localizes to the sarcolemma is down-regulated [94,182] while greater expression and activity of nNOS and its translocation from the SR to the sarcolemma has been reported [182]. The defect of eNOS-derived NO in the sarcolemma might be compensated by the translocation of nNOS from the SR to the cell membrane. This implicates an altered regulation of the role of nNOS in the autocrine control of cardiac contractility. The loss of NO in the SR and thus, the regulation on the Ca\(^{2+}\) load of this organelle, may be a central pathophysiological event [169]. In our failing heart, there was up-regulation of eNOS that appeared to be localized to the sarcolemma. The increase of NO in the sarcolemma may be related with an adaptative response to the increased systemic catecholamines in heart failure. The absence of detectable nNOS in our model may be due not only to the reasons cited above (absence of expression or difference in species), but also its dynamic expression. The NOS isoforms may present a dynamic pattern of expression in the development of heart failure as postulated by Pelat et al. [94].
Limitations of the study

We used a sheep model to study the expression of eNOS and nNOS in early ischemic heart failure. We did not find any report on the expression of eNOS and nNOS in sheep cardiomyocytes in the literature. The physiology of eNOS and nNOS expression in sheep cardiomyocytes might be different from the other species studied.

The controlled hearts in our study were obtained from a local abattoir from age-matched sheep. These sheep were assumed to be healthy and to present a normal expression of eNOS and nNOS.

The induction of an MI in our sheep model of heart failure was done by the percutaneous injection of 100% EtOH that created a MI of 21 ± 2% of the LV. The histology showed a well-defined MI surrounded by a fibrillar web. In humans, Raute-Kreinsen [160] studied the myocardial tissue from patients who underwent EtOH ablation of the septum in the case of hypertrophic cardiomyopathy. The myocardial tissue from patients who died minutes to two years following the EtOH ablation showed a preserved fixed necrotic area that was not infiltrated by leukocytes. After one month, a web of collagen was produced by the fibroblasts around the fixed myofibrils (ghost cells). The border zone of the damaged myocardium was, however, transformed to scar tissue by leukocytes. The pathophysiology of the transformation and stabilization of the 100% EtOH ablated area is not known and may have its own signaling characteristics that may have influenced the eNOS and nNOS expression level. In our model, the MI was also induced acutely in a sheep model that did not present any evidence of coronary disease. In humans, the progressive evolution of atherosclerotic lesions and inflammation that
plays a key role in coronary disease [2] may influence the NOS expression in the infarcted heart.

Furthermore, the pathophysiology of the different diseases that lead to heart failure may also be different. The heart failure syndrome can be defined as a constellation of signs and symptoms caused by inadequate performance of the heart, but the subject of heart failure requires a number of definitions to denote hemodynamic alterations, clinical symptoms and pathology. More precise analysis and classification might be based on differences in pathology and biochemistry [90].

**Mechanisms of Functional Ischemic Tricuspid Regurgitation**

1: Heart Remodeling and Stretch

The analysis of the normal geometry of the tricuspid valve in isolated pig hearts (Experiment 1) and the study of the normal geometrical variations of the mitral and tricuspid valves during the cardiac cycle (Experiment 2) allowed us to understand the remarkable geometry of both valves that are linked by the septum. In Experiment 4, we demonstrated that, following an MI, both mitral and tricuspid valve regurgitations were due to ventricular enlargement that altered the geometry of the mitral and tricuspid valves. More precisely, these valve distortions occurred at the level of the annulo-papillary muscle apparatus. These geometrical alterations were consistent with the ventricular remodeling observed in human cases of heart failure [2]. According to the Laplace Law, the ventricular enlargement increases the mechanical stress exerted on the
ventricular wall, thus the stretch on the cardiomyocytes [90]. The macroscopic remodeling of the myocardium is associated with alterations of the cardiomyocytes and the extra-cellular matrix [90]. During the remodeling process, the stretched cardiomyocytes change their phenotype with marked enlargement in size associated with deterioration in contractile function [1]. In heart-failure patients with an apparently normal mitral valve, it has been shown that the mitral valve leaflets had significant alterations in both cellularity and extracellular matrix [190]. These findings suggest that not only the myocardium, but also the mitral leaflets, thus, the entire heart, remodel as an adaptative response to overload [191]. The cardiomyocyte hypertrophy with the laying down of new sarcomeres in parallel is accomplished with biochemical alterations in both the contractile proteins and activating membrane systems [1]. It has been shown that the stretch of cultured cardiomyocytes results in gene transcription and protein synthesis that mimics the load-induced hypertrophic response in vivo [192]. The activation of a large number of cellular signal transduction pathways follows an ischemic event [188]. These signals may compensate for the increased wall stress on the surviving myocardium. Within the myocardium, the biochemical alterations include dysregulation of myosin gene expression [91], loss of myofilament and alterations of cytoskeletal proteins [193], alterations in excitation/contraction coupling [90,194] and desensitization of β-adrenergic signaling [187]. The dynamic equilibrium of the extracellular matrix, regulated by the metalloproteinases (MMPs) and their inhibitors (tissue inhibitors of metalloproteinases or TIMPs) changes also in the myocardium of failing hearts [195,196] and the proliferation and increased secretory activity of fibroblasts increase also fibrosis and collagen remodeling of the myocardium [197]. We demonstrated in our study, that the ventricular
remodeling was associated with an up-regulation of eNOS that was correlated with the
distance from the infarct (Experiment 5). According to Wilson et al., the further from the
MI, the less stretch is applied to the cardiomyocytes [89]. Thus, the up-regulation of
eNOS expression associated with the geometrical alterations of the ventricle appears to
be correlated with the degree of stretch. This finding corresponds to a previous study on
regional changes of MMPs and TIMPs that were also correlated with the distance from an
anterior MI [89]. This correlation between protein expression and distance from the MI
suggests that the direct hemodynamic forces may be the drivers of protein expression and
extracellular matrix changes. The initial stimulus that starts the remodeling process is
almost invariably stretching of the myocardium [1]. The remodeling process seems to not
be unidirectional and, according to some authors, might undergo a “reverse remodeling”
process [198,199]. It has been observed in some end-stage heart failure patients, that
unloading the left ventricle with a left ventricular assist device induces reverse
remodeling. Reduction of the mechanical stress on the myocytes through the reduction in
intraventricular volume appears to play an important role in reverse remodeling [200].
However, unloading on its own seems to be inadequate [1]. The explanation for the
cellular and extracellular changes observed in heart failure is probably more complex.
Indeed, heart failure involves many compensatory systems to adjust to the reduced
cardiac output [2,90]. These compensatory mechanisms have been shown to have
potential harmful effects on the myocytes, fibroblasts and endothelial cells and may also
participate in the development of contractile dysfunction [2,91,92]. For example, it has
been reported that the increased circulating level of catecholamines following an MI [90]
may produce apoptosis of cardiomyocytes [1]. In our early heart failure model, the up-
regulation of eNOS may be an “adaptative process” to counteract the excess of β-adrenergic stimulation and protect the cardiomyocytes. Heart failure may be viewed as a “neurohormonal model”, in which heart failure progresses as a result of the overexpression of active molecules exerting deleterious effects on the heart and circulation [2]. However, the neurohormonal model fails to explain the disease progression in heart failure [91]. During the remodeling process, not only the changes that occur in the biology of the failing myocardium but also the geometric alterations of the LV contribute to the development of the LV dilatation and dysfunction [1,2]. Therefore, the remodeling process appears to be the consequence of both stretching and activated compensatory mechanisms. The interrelationship between contractile dysfunction and cardiac remodeling is responsible for disease progression in heart failure [91]. The progressive cardiomyocyte hypertrophy considered as a physiological adaptative response to increased load may eventually become maladapative resulting in a self perpetuating pathological process of continuous remodeling leading to irreversible heart failure [1].

2: Clinical Application

Surgical therapies that directly affect cardiac remodeling should improve cardiac function and the natural history of heart failure. Restoration of the LV and RV geometry aims at decreasing myocardial stress and may initiate a reverse remodeling process. For this geometric restoration, mitral annuloplasty is being performed in nearly 80% of patients with ischemic mitral regurgitation [56]. An undersized mitral ring is the standard technique that by reducing the mitral orifice increases leaflet coaptation and consequently
reduces the IMR [54]. However, mitral annuloplasty is sometimes inadequate, following annuloplasty for ischemic MR a rate of 30% for recurrent MR has been reported [58, 59]. Papillary muscle displacement as well as LV dysfunction and enlargement are responsible for the recurrent MR [60]. Chordal cutting was described as a new surgical approach for the treatment of ischemic MR [201]. However, experimentation on sheep showed the importance of these chords as essential for the valvular-LV geometry. Transection of these chords did not prevent acute ischemic mitral regurgitation and resulted in LV systolic dysfunction in sheep [64, 65] and also narrowed the aorto-mitral angle and increased the papillary muscles-fibrous trigones distances [66]. To relocate the postero-lateral papillary muscle, Kron et al. added a suture between the left trigone and the papillary muscle in patients with previous inferior myocardial infarction and ischemic MR [56]. In case of papillary muscle displacement, to reestablish constant distances between the tip of the papillary muscles and the corresponding hemi-mitral annulus could lead to better outcomes due to the restoration of the geometry of the annulo-papillary muscle unit. The importance of the annulo-papillary muscle continuity and therefore of the annulo-papillary unit geometry for global LV function in prosthetic valve replacement has also been widely recognized. The preservation of papillary muscles-annulus continuity is either restored by conservation of chords [126] or by use of polytetrafluoroethylene to create new chords [124]. In cases of mitral homograft implantation, conservation of the recipient’s papillary muscles and suturing of the allograft to their tips preserves the constant distance between the tip of the papillary muscles and the mitral annulus plane [120].

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For the restoration of the RV, the annuloplasty in the case of ITR has poor results with a high incidence of recurrence [79] that may be predicted by tricuspid valve tethering after annuloplasty [202]. The mechanism of functional TR is not only the result of tricuspid annulus dilatation, but also the result of a bulging septum [164]. The annulus dilatation, the RV enlargement and the presence of the septum bulging in the RV resulted in annulo-papillary muscle apparatus distortion. This finding in a sheep model of heart failure may explain the recurrence and the persistence of the apical displacement of the tricuspid leaflets [51]. It is hoped that a better knowledge of the dynamic relationship between the septum and the RV will promote new surgical techniques.

As previously seen, stretch of the myocardium appears to not be the only factor involved in the remodeling process. Both eNOS and nNOS appear to regulate cardiomyocyte functions [169]. The up-regulation of eNOS expression in early heart failure may be an important step in the pathophysiology of ischemic heart failure. The correlation of eNOS expression with the distance from the infarct suggested a correlation with the degree of stretch. A better understanding of the signaling pathway of the NOS isoforms in the heart may lead to new pharmacologic therapy.

3: Limitations of the Study

Given the many aspects of the present work, a discussion has followed each experiment. The following limitations are of a general nature that applies to the overall characteristics of all experiments. To study the normal geometry of the atrioventricular valves, we based our study on animal hearts, which may present different anatomical characteristics compared to human hearts. The remarkable geometry of the mitral and
tricuspid valves of the sheep may not be exactly the same in the human. However, our findings about the mitral geometry are consistent with the anatomical finding of Sakai et al. [124] on human hearts. The small number of animals also involved in all our experiments is obviously one major limitation. Our findings about the geometrical changes of the LV and RV that lead to IMR and ITR corresponded to the remodeling of a sheep heart following a postero-lateral infarction. In sheep, the localization of the infarct may play a role in the remodeling process. Our original method to induce the infarction with the percutaneous injection of 100% ethanol may have also its own signaling characteristics [160] that may have influenced the microscopic and macroscopic remodeling. We also compared the expression of eNOS in our infarcted sheep’s heart to the expression in normal sheep hearts obtained from an abattoir. These age-matched sheep were assumed to be healthy without any pathology that may have influenced our results.
Conclusion

Patients in heart failure from ischemic origin may present mitral and tricuspid functional regurgitation. Ischemic mitral regurgitation doubles the mortality at five years [21] and when tricuspid functional regurgitation is associated, the one-year mortality doubles [42]. Remodeling of the whole heart results in geometrical distortion of both atrioventricular valves, creating valve incompetences. We studied the geometrical variations of the ventricles in an original sheep model of heart failure. For the mitral valve, annulus dilatation, LV enlargement and a tethering effect on the anterior mitral leaflet were at the origin of a central regurgitation by lack of coaptation of the leaflets. For the tricuspid valve, annulus dilatation, chamber enlargement and lateral bulging of the septum toward the RV were associated with the functional TR. Alteration of the geometry of the annulo-papillary muscle apparatus were at the origin of the regurgitation of both valves. These findings may lead to new surgical approaches.

The geometrical distortions are associated with molecular changes in the heart wall. Seven areas that represented the whole heart - the anterior, LV, RV and septum, the lateral RV, the middle of the septum, the posterior RV and LV wall were studied eight weeks following a EtOH-induced lateral MI in four sheep and compared to five control hearts from a local abattoir. In the ischemic heart eNOS was increased ten-fold compared to the controls. The expression of eNOS was correlated with the distance from the MI and thus the amount of stress. Because eNOS is known to play a role in stretch adaptation, the up-regulation of eNOS may be correlated with an adaptative process of the stretched cardiomyocyte. However, we did not find evidence of the presence of nNOS in the
samples. These, according to our knowledge, previously unreported findings, eight weeks after a MI resulting in an ovine model of heart failure, may enhance the dynamic response of eNOS to the resulting stretch.

Whether these findings are applicable to the ischemic human heart is unknown and further studies are warranted.

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