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ORIGINAL ARTICLE

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Aortic stenting in the growing sheep causes aortic endothelial dysfunction but not hypertension: Clinical implications for coarctation repair

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Abstract

Background: Stent implantation is the treatment of choice for adolescents and adults with aortic coarctation (CoAo). Despite excellent short-term results, 20%–40% of the patients develop arterial hypertension later in life, which was attributed to inappropriate response of the aortic baroreceptors to increased stiffness of the ascending aorta (ASAO), either congenital or induced by CoAo repair. In particular, it has been hypothesized that stent itself may cause or sustain hypertension. Therefore, we aimed to study the hemodynamic and structural impact following stent implantation in the normal aorta of a growing animal.

Methods: Eight female sheep completed the study and a stent was implanted in four. Every 3 mo we measured blood pressure of the anterior and posterior limbs and left ventricular function by echocardiography. Twelve months later invasive pressure was measured under baseline and simulated stress conditions. Expression of genes indicating oxidative stress (OS), endothelial dysfunction (ED) and stiffness, as well as pathological examination were performed in ascending (ASAO) and descending aorta (DSAO).

Results: SOD1 and MMP9 gene expression were higher in ASAO of the stented animals, compared to DSAO and controls, while NOS3 was decreased. No differences were found in blood pressure and echocardiographic parameters. No histological differences were found in the aorta of the two groups of animals.

Conclusions: Stent does not affect central and peripheral hemodynamics, cardiac structure and function even in the long term. However, the finding of markers of OS and increased stiffness of ASAO, proximal to the stent, points to molecular mechanisms for increased cardiovascular risk of patients with stented CoAo.

KEYWORDS
coarctation, endothelial dysfunction, hypertension, oxidative stress, stress genes, stent implantation

1 | INTRODUCTION

Aortic coarctation (CoAo) accounts for 5%-7% of all congenital heart disease[1] and it is the sixth most common cardiovascular malformation, with an incidence of 1:3000 live births.[2]

Life expectancy of these patients is reduced despite CoAo repair because of late complications, such as recoarctation, late aneurysm formation, aortic dissection and rupture, premature coronary and cerebrovascular disease, many of which can be accounted for by arterial hypertension.

Even after a complete neonatal correction, patients with CoAo show increased stiffness of the ascending aorta (ASAO) compared to the descending aorta (DSAO).[3–8] The increase of stiffness, which finds a histological explanation in the increased amount of collagen and reduced content of elastic fibers and of smooth muscle cells (SMC) in ASAO,[9] has a linear correlation with severity of hypertension.[10]

CoAo repair by stent implantation has become the treatment of choice for adolescents and adults,[11] but concerns have been raised regarding the possible negative impact of the stent itself on the aortic compliance becoming responsible for worsening of hypertension.[12–16] The alterations of the laminar flow at the level of the interface between the native aorta and the stent[13] and the consequent induction of oxidative stress (OS) could in fact worsen hypertension by altering aortic distensibility.[17]

The study of the impact of a stent on the aortic wall in patients born with CoAo is challenging since these patients show a congenitally altered aortic wall compliance since birth.[3–8] Therefore, we designed a longitudinal proof of concept study to assess the impact of stenting on central and peripheral hemodynamics, cardiac and aortic structure and function by implanting a stent in the normal aorta of a growing sheep and following it up to adult age.

2 | METHODS

2.1 | Animals

For the study we employed twelve female Bergamasca breed sheep between 3 to 5 mo of age, provided by a sheep farm (Magonara Maurizio, Villa Estense, Padua, Italy).

All animal experiments were conformed to the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes and the study project was communicated to the Italian Ministry of Health (project registration number 90/08 C21).

2.2 | Study protocol

The animals were randomly assigned to the Stent (STENT) or Sham group (SHAM) in a 2:1 ratio.

The study lasted 12 mo. Every 3 mo we measured invasive blood pressure (BP) of the auricular artery, noninvasive pressure of the anterior and posterior limbs and left ventricular function by echocardiography. Invasive pressure was also measured under baseline and simulated stress conditions at the end of follow-up before sacrifice.

2.3 | Anesthesia protocol

Two different anesthesia protocols were employed. General anesthesia with oro-tracheal intubation and mechanical ventilation was adopted for invasive studies. Unconscious sedation was employed for periodic echocardiographic follow-up.

The general anesthesia protocol was the following: premedication with midazolam 0.3 mg/kg and methadone 0.2 mg/kg, induction with propofol 4-6 mg/kg (dose effect), maintenance with inhalation of Isoflurane (dose effect).

Periodic sedation protocol was: premedication with midazolam 0.2 mg/kg, and maintenance of sedation with Propofol 0.5 mg/kg followed by constant infusion at 0.1 mg/kg/min.

2.4 | Cardiac catheterization

Two different catheterization protocols were employed:

2.4.1 | First catheterization

Under general anesthesia, the animal was positioned in right lateral recumbence. After surgical scrub of the neck, arterial access was obtained by surgical exposure of the right common carotid artery and by modified Seldinger technique placing a 12 French introducer (fr) into the artery (Check-Flo Performer Introducer 12 fr, Cook Medical).

Once arterial access was achieved under fluoroscopic guidance (Radius System S9, Multi Image, Italy), a 5 fr Gensini Catheter (Balt Extrusion, Montmorency, France) connected to a multiparameter monitoring system (Cardiocap II, Dathex-Ohmeda – GE Healthcare) by a pressure transducer (TruWave Disposal Pressure Transducer, Edward Lifescience, Switzerland) was employed to collect invasive pressure data in the left ventricle, ASAO and DSAO.

Once baseline pressure data were recorded, aortogram was performed. At this time, in the animals randomized to the stent group (STENT), a 39 mm long CP Stent (NuMed Inc, Cornwall, Canada) mounted over a 23 mm balloon angioplasty catheter (Crystal Balloon 23x40 mm, Balt Extrusion, Montmorency, France) was implanted in the DSAO distally to the take-off of the left subclavian artery. To avoid the stented region to become obstructive during the animal growth we intentionally dilate the stent to the diameter of DSAO of an adult sheep. A second contrast injection was performed to evaluate the correct stent position. In the stent group a new subset of invasive pressure data was obtained before ending the procedure.

At the end of the procedure, the 12 fr introducer was removed, the right common carotid artery surgically reconstructed, and the animal awakened.

2.4.2 | Second catheterization

Under general anesthesia, in right lateral recumbence, arterial access was gained by percutaneous puncture of the right common femoral artery

TABLE 1 Somatic growth

	SHAM (n=4)	STENT (n=4)	*p*
Day 0	30.6 ± 2.1	29.3 ± 2.1	ns
Day 90	38.5 ± 2.8	37.9 ± 4.8	ns
Day 180	48.2 ± 3.9	48.7 ± 4.6	ns
Day 270	56.0 ± 5	59.5 ± 3.4	ns
Day 360	57.8 ± 4.7	62.2 ± 4.1	ns

Values are expressed in kg and as mean ± standard deviation (ns: no statistical significance). n is the number of animals in each group.

with a modified Seldinger technique placing an 8 fr introducer into the arterial lumen (Check-Flo Performed Introducer 8 fr, Cook Medical).

As previously described, firstly invasive data were obtained under baseline conditions in both groups of animals. A simulated exercise stress test was then performed by continuous iv infusion of Dobutamine titrated up to the maximum dose of 10 mcg/kg/min with incremental steps of 2,5 mcg/kg/min. For each step of the stress test, when the steady state was achieved, invasive pressure recording was obtained in the left ventricle, ASAO and DSAO.

2.5 | Animal sacrifice

At the end of the second catheterization the animals, still under general anesthesia, were sacrificed by administering intravenously a solution of embutramide, mebenzonium iodide and tetracaine hydrochloride (Tanax, Intervet, Milan, Italy).

2.6 | Blood pressure measurement

BP was measured invasively and noninvasively every 90 days in both STENT and SHAM animals. To obtain awake invasive BP measurement, a catheter in the auricular artery on the abaural surface of the ear was connected to a monitor (Cardiocap II, Dathex-Ohmeda, GE Healthcare). Care was taken in handling the animal to reduce stress conditions. All animals were allowed to remain quietly in the measurement room for 5-10 min before attempting BP measurement to minimize white coat effect that could alter pressure values. Noninvasive BP measurement was monitored under unconscious sedation: an inflatable cuff (Critikon, GE Healthcare) was employed to obtain noninvasive oscillometric limb pulse pressure (Dathex-Ohmeda, GE Healthcare). The cuff was placed to the anterior and posterior limbs at the same height of the heart.

2.7 | Echocardiography

In both groups of animals, every 90 days a transthoracic echocardiographic evaluation of the left ventricle was obtained. After sedation the animal was positioned in the right lateral recumbence. The echocardiographic examination was performed according to the previously described protocol.[56]

2.8 | Blood tests

Every 90 days blood samples were obtained for determination of complete blood count with differentials, and plasma total proteins, albumin, globulin, BUN, creatinine, bilirubin, AST, ALT, GGT, LDH, CK, ALP, Ca, P, Mg, Na, K, Cl, glucose.

2.9 | Histological analysis

After sacrifice, from each animal the aorta was harvested for histological analysis.

After formalin fixation, the aorta was opened longitudinally and digital images were acquired. For histopathological analysis, 3 tissue samples were cut from each aorta: a sample 1 cm proximal and distal to the stent and one sample from the area in which the stent was inserted. Aortas from untreated control animals were sampled at corresponding levels.

After sampling, the aortic tissue was immediately embedded in paraffin and subsequently cut and stained in hematoxylin-eosin (H&E) and elastic Weigert van Gieson. Medial thickness was quantified by obtaining digital images with a light microscope (Zeiss Axioplan2IE and Zeiss AxioCam MRc cameras; Carl Zeiss MicroImaging GmbH, Jena, Germany) and measuring the distance between the internal and external elastic laminas in elastic Weigert van Gieson–stained segments. The mean thickness value was determined. Intimal proliferation at the level of stent implantation was also assessed.

2.10 | Gene expression

For a detailed description of the methods relative to aortic gene expression refer to Supporting Information methods (Appendix S1). Specimens from DSAO and ASAO, respectively 1 cm proximal and distal to the stent, were isolated and preserved in RNAlater solution (Qiagen, Hilden, Germany). After removal of RNAlater, the tissue sample was immersed in liquid nitrogen and conserved at −80°C until use. After RNA extraction, the expression of the following genes, markers

TABLE 2 Direct auricular blood pressure in awake SHAM and STENT sheep at baseline and follow up

	Systolic blood pressure		Diastolic blood pressure		Pulse pressure		
	SHAM (n=4)	STENT (n=4)	SHAM (n=4)	STENT (n=4)	SHAM (n=4)	STENT (n=4)	*P* value
Day 0	107 ± 10	121 ± 8	74 ± 11	96 ± 7	33 ± 10	25 ± 2	ns
Day 90	112 ± 10	102 ± 11	76 ± 4	73 ± 14	35 ± 8	29 ± 11	ns
Day 180	119 ± 8	119 ± 8	85 ± 7	76 ± 10	34 ± 10	43 ± 6	ns
Day 270	111 ± 12	115 ± 15	80 ± 7	82 ± 9	31 ± 8	31 ± 11	ns
Day 360	108 ± 14	112 ± 8	75 ± 9	82 ± 6	34 ± 6	30 ± 5	ns

Values are expressed in mm Hg as mean ± SD (ns: no statistical significance). n is the number of animals in each group.

TABLE 3 Noninvasive pressure of anterior and posterior limbs in sedated SHAM and STENT sheep

	Systolic blood pressure anterior limbs		Systolic blood pressure posterior limbs		Diastolic blood pressure anterior limbs		Diastolic blood pressure posterior limbs		
	SHAM (n=4)	STENT (n=4)	SHAM (n=4)	STENT (n=4)	SHAM (n=4)	STENT (n=4)	SHAM (n=4)	STENT (n=4)	*P* value
Day 90	134 ± 12	122 ± 24	129 ± 8	116 ± 15	82 ± 11	84 ± 20	78 ± 8	68 ± 15	ns
Day 180	129 ± 14	117 ± 18	116 ± 7	117 ± 11	84 ± 13	79 ± 4	66 ± 12	77 ± 7	ns
Day 270	121 ± 17	134 ± 4	116 ± 11	134 ± 2	68 ± 4	97 ± 21	65 ± 12	87 ± 4	ns
Day 360	118 ± 12	125 ± 16	123 ± 8	124 ± 5	72 ± 8	71 ± 14	69 ± 10	67 ± 16	ns

Values are expressed in mm Hg as mean ± SD (ns: no statistical significance). n is the number of animals in each group.

of OS, ED, matrix remodeling and stiffness were tested by means of Real-Time PCR: SOD1, NOS3, ICAM-1, E Selectin, Caspase 3, and MMP9.

Validation of specificity of qPCR assay was performed by melt-curve analysis and by agarose gel analysis. β-actin was used as housekeeping gene. Data analyses were performed with the iQTM Optical System Software (Bio-Rad, Hercules, CA). The comparative cycle threshold method (ΔΔCt), which compares the difference in cycle threshold values between groups, was used to obtain the relative fold change of gene expression.

2.11 | Statistical analysis

All data are expressed as mean ± SD. Nonparametric paired and unpaired tests were used for the statistical analysis. One-way ANOVA was employed to analyze time dependent differences. Statistical significance was assumed at $P < .05$. Statistical analysis was performed with SPSS 15 software package (SPSS, Chicago, IL).

3 | RESULTS

3.1 | Outcome

Between July 2008 and March 2011, 12 animals were enrolled in the study and a stent was implanted in the aortic isthmus in eight of them.

In the group of stented animals, three died. The first one died during the procedure because of aortic dissection, two animals died because of sepsis, 2 and 3 days after the procedure. One animal within the stent group was excluded from the study because of the development of severe aortic regurgitation following bacterial endocarditis. Four animals in the STENT group and four animals in the SHAM group completed the 12-mo follow-up and their data were compared.

3.2 | Somatic growth

STENT and SHAM animals showed a similar somatic growth as presented in Table 1. In particular, at the time of the first catheterization STENT and SHAM animals weighted respectively 29.3 ± 2.1 and 30.6 ± 2.1 kg and reached a similar weight at the end of the follow up weighting respectively 62.2 ± 4.1 and 57.8 ± 4.7 kg.

3.3 | Blood pressure

There were no differences in term of direct auricular, indirect oscillometric and direct aortic pressure neither at the first catheterization nor at the following examinations up to the end of the 12 mo of follow-up between the two groups of animals.

Tables 2 and 3 report the direct auricular BP in awake SHAM and STENT sheep and noninvasive limb BP in sedated SHAM and STENT groups. Table 4 reports the hemodynamic data obtained at the time of the first and second cardiac catheterization.

3.4 | Dobutamine stress test

The results of the dobutamine stress test are depicted in Figures 1 and 2. In both group of animals, heart rate (Figure 1) and BP in the ASAO and DSAO (Figure 2) did not differ significantly.

3.5 | Echocardiography

Supporting Information Table S1 summarizes the echocardiographic data obtained at the time of the first cardiac catheterization and every 90 days. None of the variables showed a statistical difference during follow-up in the two groups of animals.

TABLE 4 Invasive pressure in ascending and DSAO at the time of the first cardiac catheterization (day 0) before and after stent implantation and at the time of the second cardiac catheterization (day 360) in SHAM and STENT groups

	Systolic pressure ascending aorta		Systolic pressure descending aorta		Diastolic pressure ascending aorta		Diastolic pressure descending aorta		
	SHAM (n=4)	STENT (n=4)	SHAM (n=4)	STENT (n=4)	SHAM (n=4)	STENT (n=4)	SHAM (n=4)	STENT (n=4)	*P* value
Prestent day 0	88 ± 19	94 ± 19	88 ± 19	95 ± 18	77 ± 23	74 ± 21	74 ± 18	73 ± 19	ns
Poststent day 0	/	100 ± 20	/	101 ± 27	/	78 ± 21	/	74 ± 26	ns
Day 360	99 ± 9	99 ± 9	99 ± 10	98 ± 8	83 ± 8	84 ± 8	83 ± 9	82 ± 8	ns

Values are expressed in mm Hg as mean ± SD (ns: no statistical significance). N is the number of animals analyzed in each group.

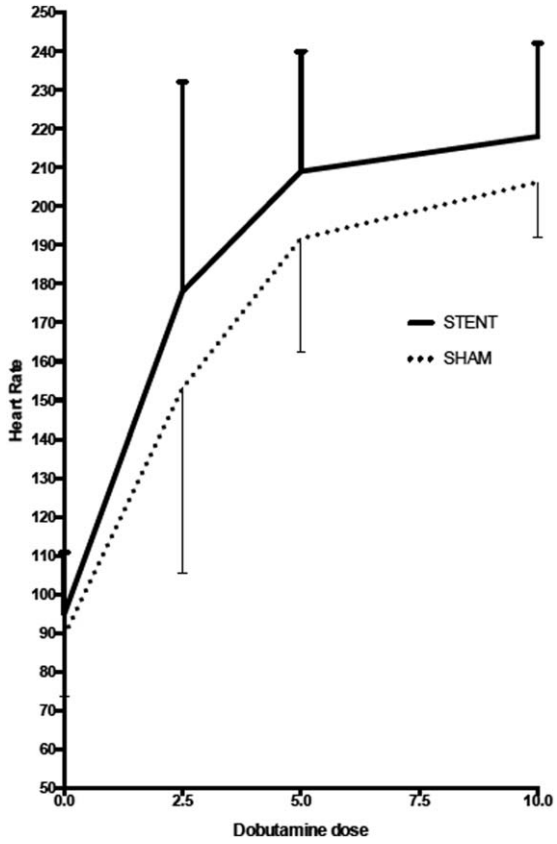

FIGURE 1 Heart rate at different dobutamine doses in stent and sham sheep. Dobutamine doses are expressed in mcg/kg/min. Heart rate values are expressed in beats per minute

3.6 | Blood tests

None of the biochemical parameters was consistently different among groups during time (data not shown).

3.7 | Histological analysis

Histology of the aorta in control animals showed that the aortic wall consists of 3 layers like in humans. However, the media is composed by smooth muscle cells (SMC) and layers of elastic and collagen fibers with a disarrayed spatial arrangement. In STENT, the findings in the nonstented aortic segments were the same as in controls, reflecting a species-related feature rather than a stent-related structural change. In the control aortas, the mean thickness of the tunica media was 2369 $\pm$50 μm.

Substantial differences were seen when comparing medial thickness of nonstented segments with stented aortic wall specimens. Significant medial thinning was observed in all stent-covered regions. At this level, the elastic and collagen fibers were stretched. In particular, the mean aortic wall thickness was 2951 $\pm$ 570 μm proximal to the stent, 2496 $\pm$ 94 distally and 1524 $\pm$ 80 within the covered aortic segment ($P < .001$). No inflammatory infiltrate was seen either proximal or distal to the stent.

In all stent-covered regions, the metal struts left indentations in the aortic wall. Mild neointimal proliferation, partially covering stent mesh and never determining stenosis of the lumen, was observed in treated animals. Stent struts were not always uniformly distended and neointima covering was incomplete in some areas. Despite neointima proliferation, aortic wall thickness at stent level was reduced in comparison with segments proximal and distal to the device (Figures 3 and 4).

3.8 | Gene expression

NOS3 expression was significantly lower in ASAO of stented animals compared to ASAO of controls (1.04 $\pm$ 0.17 vs. 1.34 $\pm$ 0.36), while SOD1 expression was higher in ASAO of stented animals (1.42 $\pm$ 0.39) compared to ASAO of controls (0.95 $\pm$ 0.16) and to DSAO of both stented and controls animals (0.68 $\pm$ 0.11 and 0.77 $\pm$ 0.07, respectively). Similarly, MMP9 expression was higher in ASAO of stented animals (1.68 $\pm$ 0.45) compared to ASAO of controls (0.96 $\pm$ 0.07) and to DSAO of both stented and controls animals (1.06 $\pm$ 0.25 and 1.22 $\pm$ 0.46, respectively). The relative fold change of gene expression of SOD1, NOS3, ICAM-1, E Selectin, Caspase 3, and MMP9 in STENT is shown in Figure 5 and the relevant data are reported in Supporting Information Table S2. These data indicate increased focal aortic OS associated with compensatory SOD1 overexpression, and accelerated vascular remodeling, that may herald increased wall stiffness in ASAO of stented aortas.

4 | DISCUSSION

Animal models are essential tools for cardiovascular research to understand pathophysiological processes and to develop new therapies.[18] Among large animal models, sheep are most suitable because of their docility, slow growth and easy housing in long-term follow up studies. Furthermore, the sheep is a good animal model for cardiovascular studies because its heart is similar in dimensional and functional parameters to the human heart and its vascular anatomy bears close resemblance to the human vasculature.[19,20] The present study in grown up sheep shows that aortic stent implantation does not cause hypertension and cardiovascular remodeling but it increases the expression of markers of OS, ED and aortic stiffness. Thus, this proof of concept study rules out a direct effect of stent in inducing hypertension in the normal aorta of a growing animal, but highlights molecular mechanisms that may have a role in increasing the cardiovascular risk of patients with stented CoAo.

The long-term prognosis of patients after CoAo repair is jeopardized by development or persistence of arterial hypertension, which affects up to 40% of patients at follow-up.[21–26] Its pathogenesis is poorly understood. Many different mechanisms have been involved: hyperactivity of the renin-angiotensin-aldosterone system,[27] impaired aortic compliance,[4,5,15,28] altered sensibility of the baroreceptors,[3,29,30] peculiar aortic shape,[22] ASAO relative hypoplasia,[31] generalized arterial and aortic dysfunction,[4,7,31] and ED,[3] that could not be corrected by either surgical or endovascular repair. It has also been hypothesized that the turbulent flow across the repaired segment promotes medial thickening and predisposes these patients to the development of hypertension by increasing the parietal stress in ASAO, even in the

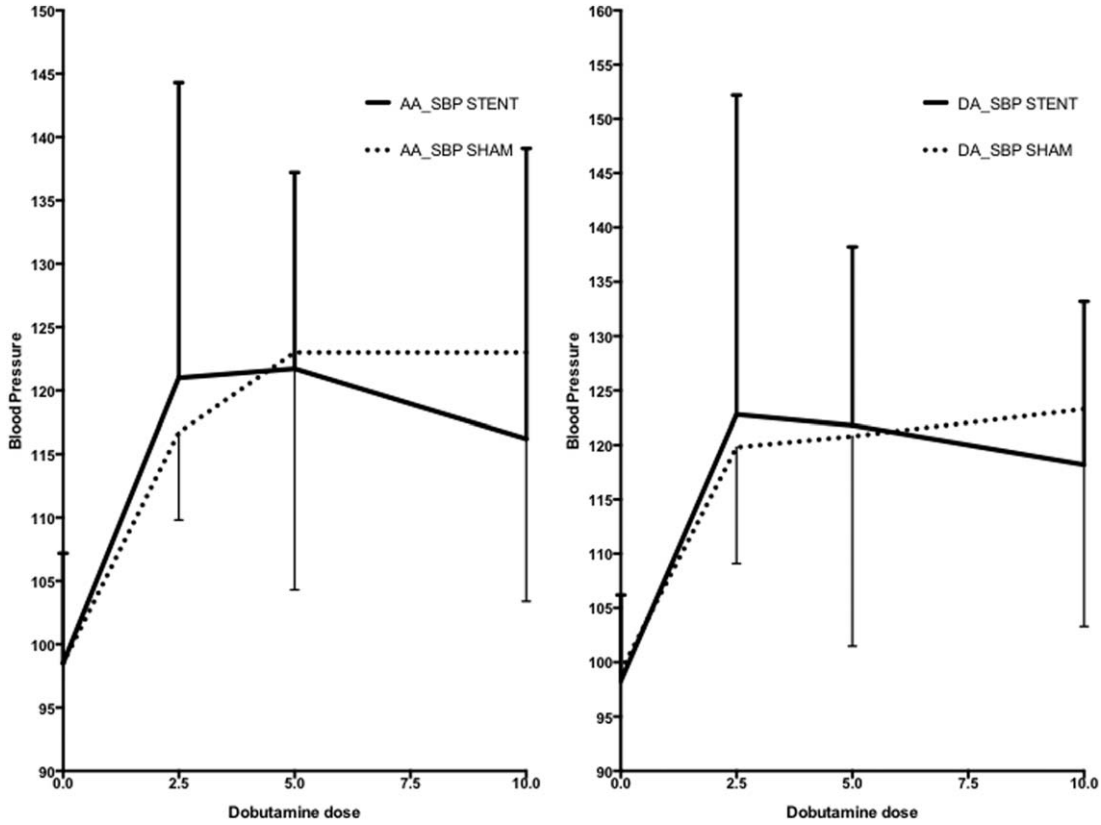

FIGURE 2 Mean systolic pressure in ascending and DSAO at different dobutamine doses in stent and sham sheep. In the left panel, systolic blood pressure in the ASAO, in the right panel, systolic blood pressure in the DSAO. Dobutamine doses are expressed in mcg/kg/min. Blood pressure values are expressed in mm Hg

absence of significant recoarctation.[6,32–34] The altered aortic compliance, which is a feature of all the patients with CoAo even before correction,[5,10,29,30] may lead to the development of high BP by impairing baroreceptor reflexes.[9,30]

Stent implantation is the treatment of choice for adolescents and adults with CoAo.[11] Despite excellent immediate results, few long-term data are available and no data are available about the possible long-term negative effects of the stent on the development or maintenance of hypertension. Some authors argued that the stent could further reduce aortic compliance by causing loss of pulsatile energy, increased impedance to blood flow with reduction of distal perfusion, increased reflection of pressure waves and increased mechanical stress at the interface between stent and native aorta.[12–14,16,35] Others demonstrated that ASAO of patients born with CoAo is stiffer than ASAO of the normal population and this is not corrected either by surgery or stent implantation, but they did not confirm alterations of aortic compliance by stenting.[36–38]

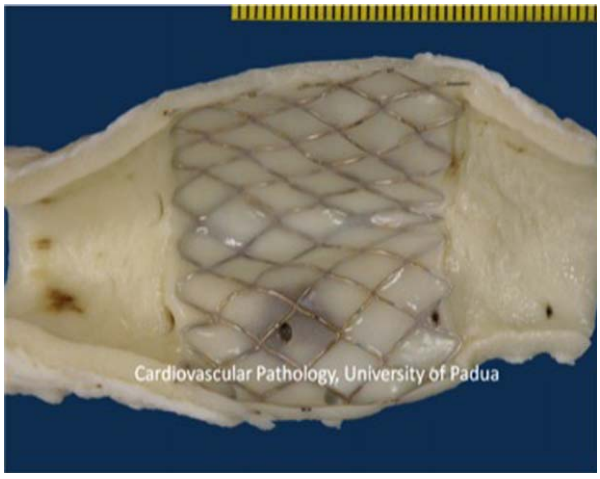

FIGURE 3 Formalin-fixed aortic stented segment after section of the wall. Wall thickness at stent level is reduced from segments above and below the device

Our long-term study demonstrates that the stent does not alter the overall hemodynamics of the aorta in agreement with what it was reported by Pihkala et al. in a short-term experiment in pigs.[39] In fact, after 12-mo follow-up in full grown up sheep, the aortic pressure behavior was similar in the two groups of animals both at rest and under simulated exercise stress and there were no differences in left ventricular dimension and function. However, alterations of the normal laminar blood flow at the level of the interface between the stent and the native artery induced OS, ED and increased protein turn over in the ASAO above the stent.

The role of the ED is gaining more and more interest in the pathogenesis of hypertension. Alteration of endothelium-mediated vasodilatation is present in patients with hypertension, dyslipidemia, diabetes

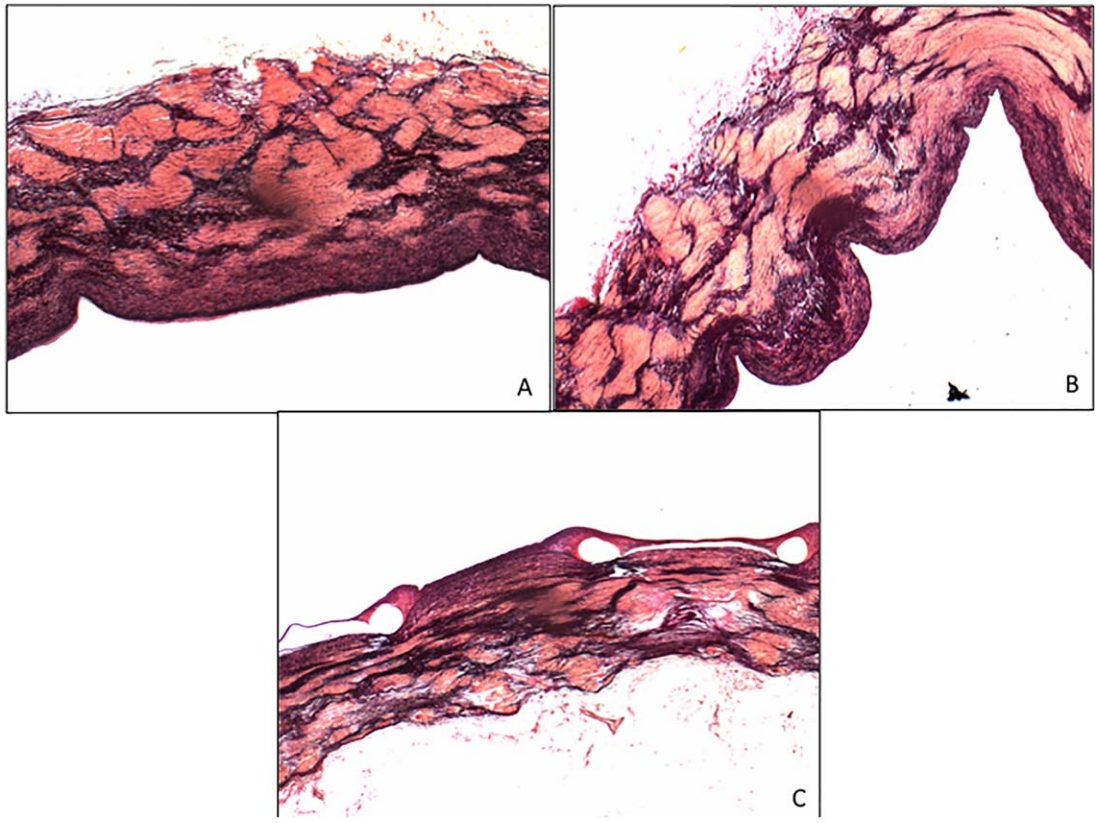

FIGURE 4 Histological sections of aortic wall. ASAO in a STENT (A) and a SHAM (B) sheep. Stented aortic segment (C) with mild neointimal proliferation around the mesh

and coronary artery disease.[40–42] Endothelial damage plays a critical role in restenosis after stenting,[43] but nothing is known on endothelial function in the presence of a stent in the isthmic region. In our experimental model, in stented animals we found decreased expression of NOS3 and increased gene expression of SOD1 and MMP9 in ASAO. The decreased NO production by NOS3, the increased OS, suggested by high compensatory SOD1 expression, and the increased collagen turnover, expressed by elevated expression of the MMP9 limited to ASAO proximal to the stent and not in DSAO, underlie ED, possibly caused by backward reflection of the pressure waves determined by the stent.

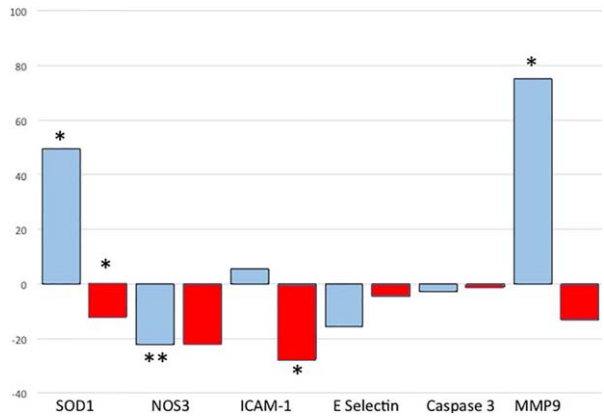

FIGURE 5 Relative percent change of gene expression in the ascending (blue bars) and descending (red bars) aorta of STENT. The data are expressed as percent change of gene expression in the same aortic segment of SHAM, taken as reference value. (* $P < .05$, ** $P < .01$ vs SHAM)

The activation of proteolytic and apoptotic processes contributes to structural changes of the extracellular matrix and SMC layers of the arterial vessels. MMP family plays a critical role in the degradation of the proteins of the extracellular matrix and in the physiologic remodeling of the vessels.[44,45] The overexpression of MMP proteins shown in our experimental model can lead to inappropriate vascular remodeling favoring aneurysm formation, restenosis and accelerated arteriosclerosis.[44,46–48] MMP9 is, in fact, an important member of the MMP family and increased levels of this protein were found in patients with coronary artery disease, type 2 diabetes and early arteriosclerosis.[46,48–50] Increased levels of this protein are associated with increased stiffness of the aortic wall and increased mortality for coronary artery diseases.[51,52] Production of collagen fibers at a faster rate than the elastic ones makes the arterial wall stiffer.[52] All these structural changes in ASAO may predispose to aneurysm formation and dissection, well-known complications of CoAo repair.[1]

The correlation between higher aortic wall stiffness and higher risk of hypertension has been well demonstrated.[53] In patients with

a stiff aortic wall there is an improper matching between aortic diameter and flow that cause an increase in forward arterial pressure wave amplitude, elevated pulse-wave velocity and premature wave reflection. The resulting increase in pulsatile hemodynamic load increases cardiac afterload, reduces diastolic coronary flow, and damages microcirculation, particularly in high-flow organs such as the kidneys and brain.[54,55] The changes of SOD1, NOS3, and MMP9 gene expression, in our long term experiment in the ovine animal model were not accompanied by anatomically and functionally evident cardiac and vascular abnormalities. Our data, therefore, suggest that the development of hypertension in patients with repaired CoAo needs a preexisting aortopathy to accelerate the increase of aortic wall stiffness.[3–6,28]

The main study limitation is the small number of animals employed. Its strength is the remarkably long follow-up with animals followed up from infancy to maturity, mimicking the human development from adolescence to adult age.

Having implanted a stent in the aorta of otherwise normal animals, we conclude the stent induces OS, ED, and increased stiffness in ASAO but not hypertension. Even if we did not find any vascular remodeling of the aorta in this animal model, OS, ED, and aortic matrix changes following stent implantation is worrisome. At present we cannot predict what clinical relevance they may have on the aorta of patients who have a congenital abnormality of the aortic wall since birth as in CoAo. They may lead to vascular changes with development of aneurism, a well-known complication of coarctation repair. Therefore, we suggest periodic noninvasive examination of the proximal aorta in patient with stented COAO for early recognition of long term complications, such as aneurysm formation, even in the absence of hypertension following repair.

CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest with the contents of this article.

AUTHOR CONTRIBUTIONS

Andrea Semplicini, Ornella Milanesi and Daniele Bernardini: designed the study and reviewed the paper

Nicola Maschietto, Luca Semplicini: wrote the paper

Nicola Maschietto, Ilaria Jacopetti and Ornella Milanesi: Performed the invasive procedures and wrote the relative sections

Luca Semplicini and Helen Poser: performed the periodic echocardiographic study and wrote the relative sections

Giulia Maria De Benedictis and Tommaso Pilla: performed the anesthesia and wrote the relative section

Luca Aresu: Performed the post-mortem examination and wrote the relative section

Cristina Basso e Stefania Rizzo: performed the histological examination of the aorta and wrote the relative section

Giulio Ceolotto and Arianna Cattelan: were in charge of the molecular biology examinations and wrote the relative section

Gabriele Gerardi: was in charge of the health of the animals and the periodic morphometric evaluations of the animals

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