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Faculdade de Medicina da Universidade do Porto

Fisiopatologia e tratamento da hipertensão pulmonar: desenvolvimento de modelos experimentais, modulação farmacológica e nutricional

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À Ana. Aos meus Pais. À minha Irmã Patrícia.

Science does not rest upon solid bedrock. The bold structure of its theories rises, as it were, above a swamp. It is like a building erected on piles. The piles are driven down from above into the swamp, but not down to any natural or 'given' base; and when we cease our attempts to drive our piles into a deeper layer, it is not because we have reached firm ground. We simply stop when we are satisfied that they are firm enough to carry the structure, at least for the time being.

The Logic of Scientific Discovery (1959)

Some scientists find, or so it seems, that they get their best ideas when smoking; others by drinking coffee or whisky. Thus there is no reason why I should not admit that some may get their ideas by observing, or by repeating observations.

Realism and the Aim of Science (1983)

Karl Popper

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Resumo

A hipertensão pulmonar, síndrome de etiologia diversificada definida hemodinamicamente pela elevação das pressões da artéria pulmonar, associa-se a importante morbi-mortalidade e tem merecido atenção crescente por parte da comunidade médico-científica. Apesar dos inúmeros desenvolvimentos terapêuticos recentes, como, por exemplo, os antagonistas dos receptores da endotelina-1, as resistências vasculares pulmonares elevam-se inexoravelmente e o ventrículo direito acaba por ser incapaz de tolerar a elevação de pós-carga. A falência ventricular, ou insuficiência cardíaca, direita é uma importante causa de mortalidade e um determinante fundamental do prognóstico, sendo também prioritária a pesquisa nesta área. No entanto, as acções miocárdicas dos vasodilatadores arteriolares pulmonares, em uso corrente, não estão bem caracterizadas. Na hipertensão pulmonar severa, ocorre igualmente disfunção ventricular esquerda, cujos mecanismos também não estão completamente esclarecidos. Embora a maioria das evidências aponte para a interacção ventricular como aspecto fundamental, algumas sugerem a ocorrência de disfunção intrínseca. Com a progressão da doença e activação inflamatória é igualmente frequente o desenvolvimento de perturbações do metabolismo miocárdico e caquexia. A caquexia cardíaca complica um número importante de casos de insuficiência cardíaca, agravando o seu prognóstico. De facto, os insuficientes cardíacos com índices de massa corporal elevado têm paradoxalmente melhor sobrevida. Analogamente, as dietas do tipo Ocidental, hipercalóricas, sendo factores de risco cardiovascular bem estabelecidos, poderão ter efeitos inteiramente distintos na insuficiência cardíaca e caquexia. Foram objectivos da presente dissertação: (i) esclarecer se o ventrículo esquerdo desenvolve disfunção intrínseca na hipertensão pulmonar, e quais os mecanismos responsáveis, (ii) avaliar o teste hemodinâmico de tolerância diastólica à elevação aguda de pós-carga como índice funcional, comparando miocárdio saudável com miocárdio insuficiente, (iii) averiguar quais os efeitos de um regime alimentar do tipo ocidental na progressão da hipertensão pulmonar, insuficiência cardíaca e caquexia cardíaca, e, finalmente, (iv) caracterizar os efeitos miocárdicos e vasculares pulmonares funcionais e moleculares dos antagonistas da endotelina-1. Para o efeito, levámos a cabo múltiplos estudos num modelo experimental de hipertensão pulmonar induzida pela monocrotalina no rato, complementados com um estudo no perioperatório de cirurgia cardíaca. Demonstrámos (i) disfunção miocárdica ventricular esquerda intrínseca na hipertensão pulmonar e identificámos potenciais mecanismos patogénicos, nomeadamente a activação neuroendócrina e inflamatória, (ii) que a tolerância diastólica à elevação aguda de pós-carga discrimina disfunção miocárdica e reforça o acoplamento íntimo entre contracção e relaxamento, (iii) que regimes alimentares do tipo ocidental atenuam a hipertensão pulmonar e melhoram a função miocárdica e caquexia cardíaca, provavelmente por supressão da resposta inflamatória, segundo a hipótese lipoproteína-endotoxina, e por maior aporte energético e optimização do metabolismo miocárdico, e, por último, (iv) que os antagonistas da endotelina-1 não só atenuam a hipertensão pulmonar, como também preservam a função miocárdica, em parte por actividade anti-inflamatória e modulação de reguladores agudos do tono vascular pulmonar e da função miocárdica. Numa perspectiva translacional, estes trabalhos contribuíram para uma compreensão mais ampla da disfunção ventricular esquerda na hipertensão pulmonar e dos mecanismos de acção dos antagonistas da endotelina-1,

sugerindo que antagonistas com efeitos agudos, utilizados por via endovenosa, possam ser alternativas à terapêutica crónica, por via oral, por exemplo no período perioperatório e em cuidados intensivos. Forneceram também evidência experimental *in vivo* para o "paradoxo da obesidade" e hipótese lipoproteína-endotoxina, apoiando a realização de trabalhos clínicos que testem possíveis benefícios na caquexia cardíaca. Finalmente, demonstrámos a íntima correlação entre a função sistólica e diastólica e demonstrámos claramente que mesmo as pressões arteriais usuais podem contribuir para a disfunção diastólica na insuficiência cardíaca.

Abstract

Pulmonary hypertension is a syndrome of diverse etiology that can be defined on haemodynamic grounds by elevated pulmonary artery pressures. It portends high morbidity and mortality and has deserved increased recognition by the medical and scientific communities. Despite the latest therapeutic developments, such as endothelin-1 receptor antagonists, pulmonary vascular resistance still evolves and the right ventricle is unable to bare progressive increases in afterload. Right ventricular failure is an important cause of mortality and a fundamental prognosis determinant and therefore has been considered a research priority. Still, the myocardial actions of currently used lung vessel vasodilators are not well defined. In severe pulmonary hypertension patients also develop left ventricular dysfunction, but the underlying mechanisms are also incompletely understood. Though the majority of evidence points to a fundamental role of ventricular interaction, some reports suggest intrinsic left ventricular myocardial dysfunction. Moreover, with disease progression and inflammatory activation disturbances of myocardial metabolism and cardiac cachexia further aggravate the clinical condition. Cardiac cachexia complicates an important part of heart failure cases and substantially worsens its prognosis. In point of fact, heart failure patients with high body mass indices paradoxically show improved survival. Analogously, it would not be surprising that well established cardiovascular risk factors such as Western-type diet regimens could actually have entirely distinct effects in heart failure and cardiac cachexia. With the works carried out we aimed at (i) establishing whether the left ventricular myocardium is intrinsically dysfunctioning in pulmonary hypertension and what could be the responsible mechanisms, (ii) assessing an haemodynamic test based on evaluation of end-diastolic pressure elevation induced by acute afterload elevations as a tool to discriminate failing from healthy myocardium, (iii) evaluating the effects of a Western-type diet in pulmonary hypertension, heart failure and cardiac cachexia, and, finally, (iv) characterizing the myocardial and lung vessel actions of endothelin-1 antagonists. For these purposes we conducted studies in the rat model of monocrotaline-induced pulmonary hypertension, and a perioperative evaluation of patients undergoing coronary artery bypass grafting. We have documented (i) intrinsic left ventricular myocardial dysfunction and potential causal mechanisms, namely neuroendocrine and inflammatory activation, in pulmonary hypertension, (ii) the accuracy of diastolic tolerance to acute afterload elevation in discriminating myocardial dysfunction, and the close relationship between contraction and relaxation, (iii) attenuation of pulmonary hypertension, and improvements in myocardial function and cardiac cachexia with a Western-type diet, partly due to anti-inflammatory actions, according to the lipoprotein-endotoxin hypothesis, and partly to the increased energy intake and preservation of myocardial metabolism, and, lastly, (iv) that endothelin-1 antagonists not only attenuate pulmonary hypertension but also preserve myocardial function, due at least in part to an anti-inflammatory action and modulation of acute regulators of lung vessel tone and myocardial function. Our findings have contributed to a better understanding of left ventricular dysfunction in pulmonary hypertension and of the pharmacological actions of endothelin-1 antagonists, suggesting that intravenous acute blockade may be an effective replacement therapy in patients who cannot use the oral route, such as those undergoing surgery or admitted to intensive care. We have also provided *in vivo* experimental support for the “obesity paradox” and the lipoprotein-endotoxin

hypothesis, sanctioning clinical trials in cardiac cachexia patients. Finally we have demonstrated the close relationship between contractility and relaxation and clearly demonstrated that even baseline systemic arterial pressures can contribute to diastolic dysfunction in heart failure.

Índice

Introdução

A hipertensão pulmonar é um síndrome de etiologia diversificada que se define em termos hemodinâmicos pela elevação da pressão média da artéria pulmonar para valores superiores a 25 mmHg (Fishman 2004). Ao longo das duas últimas décadas, este síndrome tem sido alvo de interesse crescente por parte da comunidade médica e científica, particularmente no que concerne a uma das formas etiológicas mais raras, a hipertensão arterial pulmonar. Estas formas de hipertensão pulmonar, para além dos critérios diagnósticos usuais, apresentam um critério hemodinâmico adicional, a normalidade das pressões de preenchimento ventriculares esquerdas, assim como ausência de outras causas prováveis. Apesar da sua incidência e prevalência serem extremamente baixas (Humbert et al. 2006), a elevada morbilidade e mortalidade, com sobrevida estimada de cerca de 50 % aos 2 anos, de acordo com registos da década de 80 (McLaughlin e Suissa 2010), motivaram uma intensa investigação no campo da fisiopatologia e terapêutica que levaram à aprovação, por parte da *European Medicines Agency* e da *Food and Drug Administration* dos Estados Unidos da América, de vários fármacos vasodilatadores pulmonares arteriolares que fazem já parte da prática médica corrente, muitas vezes em combinação (Rabinovitch 2008). Estes avanços resultaram numa melhoria global da sobrevida e qualidade de vida destes doentes (Galie et al. 2009). No entanto, as resistências vasculares pulmonares elevam-se inexoravelmente e o ventrículo direito, que inicialmente tolera o aumento progressivo de pós-carga, por vários mecanismos compensatórios, como hipertrofia, dilatação e alteração da sua geometria, acaba por se tornar incapaz de tolerar a elevação sustentada de pós-carga. A falência ventricular, ou insuficiência cardíaca, direita é uma importante causa de mortalidade (Humbert 2009) e um determinante fundamental do prognóstico (D'Alonzo et al. 1991). Apesar do seu papel fulcral, apenas em 2006 foi reconhecida a importância da função miocárdica ventricular direita na qualidade de vida e sobrevida destes doentes, sendo considerada a investigação nesta área uma prioridade nas ciências cardiovasculares (Voelkel et al. 2006). Desde então, vários trabalhos têm contribuído para uma melhor compreensão da fisiopatologia da disfunção ventricular direita; no entanto, esta área dá ainda os seus primeiros passos, encontrando-se ainda por esclarecer, por exemplo, quais as acções miocárdicas dos fármacos recentemente aprovados para terapêutica crónica da hipertensão pulmonar (Bogaard et al. 2009a). Na hipertensão pulmonar crónica severa com atingimento da função ventricular direita, outros mecanismos emergiram como potenciais alvos terapêuticos, nomeadamente o reconhecimento do compromisso concomitante da função ventricular esquerda e da actividade inflamatória e neuroendócrina, sistémica e local, como

mecanismos que contribuem decisivamente para a deterioração funcional. Adicionalmente, o envolvimento de alterações de metabolismo sistémicas, pulmonares e miocárdicas, condicionadas pela menor perfusão tecidular, congestionamento vascular mesentérico, sobrecarga miocárdica e mediadores neuroendócrinos e inflamatórios , e o desenvolvimento de caquexia cardíaca (Carr et al. 1989; le Roux et al. 2005; von Haehling et al. 2009) também têm sido progressivamente integradas numa visão mais alargada deste síndrome.

Como complemento a esta introdução, reproduzimos na íntegra um artigo de revisão que sumaria os principais desenvolvimentos na compreensão fisiopatológica e na abordagem clínica da hipertensão pulmonar. Em seguida, abordamos em maior detalhe outros aspectos que têm vindo a ser apontados como tendo maior relevância actual no esclarecimento da fisiopatologia da hipertensão pulmonar crónica e disfunção ventricular direita. Com os trabalhos desenvolvidos, e incluídos na presente dissertação, pretendemos contribuir para um melhor esclarecimento de alguns destes tópicos.

Conceitos de fisiopatologia e abordagem clínica actual da hipertensão pulmonar

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Review

Current pathophysiological concepts and management of pulmonary hypertension

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ABSTRACT

Pulmonary hypertension (PH), increasingly recognized as a major health burden, remains underdiagnosed due mainly to the unspecic symptoms. Pulmonary arterial hypertension (PAH) has been extensively investigated. Pathophysiological knowledge derives mostly from experimental models. Paradoxically, common non-PAH PH forms remain largely unexplored. Drugs targeting lung vascular tonus became available during the last two decades, notwithstanding the disease progresses in many patients. The aim of this review is to summarize recent advances in epidemiology, pathophysiology and management with particular focus on associated myocardial and systemic compromise and experimental therapeutic possibilities. PAH, currently viewed as a panvasculopathy, is due to a crosstalk between endothelial and smooth muscle cells, inammatory activation and altered subcellular pathways. Cardiac cachexia and right ventricular compromise are fundamental determinants of PH prognosis. Combined vasodilator therapy is already mainstay for refractory cases, but drugs directed at these new pathophysiological pathways may constitute a signicant advance.

1. Introduction

Pulmonary hypertension (PH), is dened by mean pulmonary arterial (PA) pressure (mPAP) elevation above 25 mm Hg at rest[1] . In most cases, PH accompanies cardio-respiratory conditions and does not involve the pulmonary vasculature. However, more rarely it may present itself as pulmonary arterial hypertension (PAH), dened additionally by normal left ventricular (LV)lling pressure[2]. PAH is viewed as a vasoproliferative disease with characteristic pathological abnormalities, such as arteriolar plexiform lesions, as found in most of cases. Initial symptoms, mainly fatigue and dyspnea, are usually vague and insidious, thus most cases are diagnosed when cardiac output (CO) is already low[3]. Right ventricular (RV) failure due to PH is an important cause of death [4] whose complex pathophysiological

Abbreviations:5-HT, 5-Hydroxytryptamin, serotonin; 5-HT $_{2A}$, serotonin type 2A receptor; 6MWT, 6-minute walk test; AC, adenylate cyclase; AS, atrial septostomy; BMP, bone morphogenetic protein; BMPR1, bone morphogenetic protein receptor 1; BMPR2, bone morphogenetic protein receptor 2; BNP, type B natriuretic peptide; CaL, L-type Ca^{2+}-channel; CC, cardiac cachexia; CCB, Ca^{2+}-channel blocker; CCR2, chemokine receptor 2; CCR5, chemokine receptor 5; CDK, cyclin-dependent kinase; cGMP, cyclic guanosine monophosphate; CHD, congenital heart disease; CO, cardiac output; CO–A/R, co-repressors or activators; COPD, chronic obstructive pulmonary disease; CT, computerized tomography; CTD, connective tissue disease; CTEPH, chronic thromboembolic pulmonary hypertension; CVC, central venous catheter; CX3CR1, chemokine receptor 1; CXCR4,-chemokine receptor; DCA, dichloroacetate; DL_{CO}, carbon monoxide diffusion; e^-, electron; ECM, extracellular matrix; EF, ejection fraction; EGFR, epidermal growth factor receptor; EPC, endothelial progenitor cells; ERA, endothelin-1 receptor antagonists; ET-1, endothelin-1; ET_A, endothelin-1 type A receptor; ETC, electron transport chain; FDA, Food and Drug Administration; fPAH, familial pulmonary arterial hypertension; GC, guanylate cyclase; G_q, protein G_q; HF, heart failure; HIF-1, hypoxia-inducible factor-1; HIV, human immunodeciency virus; HLT, heart-lung transplantation; Id, inhibitor of DNA binding proteins; IL-6, Interleukin-6; IP, prostaglandin receptor; IP_3, inositol 3-phosphate; iPAH, idiopathic pulmonary arterial hypertension;*iv*, intravenous; Kv1.5, O $_2$-sensitive K^+-channels; LHD, left-heart disease; LV, left ventricular; LT, lung transplantation; MCP-1, monocyte chemotactic protein-1; MLC, myosin light-chain; MLCK (−P), myosin light-chain kinase, and respective phosphorylated form; MMP, matrix metalloproteinases; mPAP, mean pulmonary artery pressure; NFAT, nuclear factor of activated T lymphocytes; NIH, National Institutes of Health; NO, nitric oxide; NRCT, non-randomized clinical trial; O_2^-, superoxide anion; PA, pulmonary arterial; PAP, pulmonary artery pressure; PAH, pulmonary arterial hypertension; PASMC, pulmonary artery smooth muscle cell; PCH, pulmonary capillary haemangiomatosis; PCWP, pulmonary capillary wedge pressure; PEA, pulmonary endarterectomy; PDE, phosphodiesterases; PDE_5, type 5 phosphodiesterase; PDEi, phosphodiesterase inhibitors; PDGF, platelet derived growth factor; PDGFR, platelet derived growth factor receptor; PDH (−P), pyruvate dehydrogenase, and respective phosphorylated form; PDK, pyruvate dehydrogenase kinase; PGI_2, prostacyclin; PH, pulmonary hypertension; PKA, protein-kinase A; PKG, protein-kinase G; PPH, portopulmonary hypertension; PTE, pulmonary thromboembolism; PVOD, pulmonary veno-occlusive disease; PVR, pulmonary vascular resistance; QOL, quality of life; RANTES, regulated upon activation, normal T expressed and secreted; RCT, randomized clinical trials; RHC, right-heart catheterisation; ROS, reactive oxygen species; RV, right ventricular; RVAD, right ventricular assist device;*sc*, subcutaneous; SDF-1, stromal cell-derived factor-1; SLE, systemic lupus erythematosus; SOD, superoxyde dismutase; SPAP, systolic pulmonary artery pressure; SR, sarcoendoplasmic reticulum; TGF-, transforming growth factor -; TGF-R, transforming growth factor -receptor; TP, ThromboxaneA $_2$ receptor; TnC, tenascin C; TNF-, tumor necrosis factor-; trp, transient receptor potential; TTCW, time to clinical worsening; TxA_2, thromboxane A_2; VEGF, vascular endothelial growth factor; VEGR, vascular endothelial growth factor receptor; WHO, World Health Organization.

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Table 1
New classication for pulmonary hypertension (PH) from the 4th World Symposium on PH (Dana Point, 2008).

Pulmonary arterial hypertension (PAH)	Non-PAH pulmonary hypertension (PH)	
	Well dened cause	Unclear or multifactorial
PAH (1)	*Left-heart disease (2)*	*Unclear/multifactorial mechanisms (5)*
Idiopathic	Systolic dysfunction	Haematologic disorders
Hereditary	Diastolic dysfunction	*Myeloproliferative disorders,etc.*
Drug/toxin induced	Valvular disease	Systemic disorders
Disease associated	*Lung diseases/hypoxia (3)*	*Vasculitis, sarcoidosis, neurobromatosis, etc.*
CTD	COPD	Metabolic disorders
HIV infection	Interstitial lung disease	*Glycogen storage disease,thyroid disorders,etc.*
Portal hypertension	Sleep-disordered breathing	Congenital heart disease
Systemic-pulmonary shunts	Chronic exposure to high altitude	(Other than systemic-pulmonary shunt)
Schistosomiasis	Broncho pulmonary dysplasia	Other
Chronic haemolytic anaemia	Developmental abnormalities	*Fibrosing mediastinitis,chronic renal failure on dialysis,etc.*
Subclass of PAH (1′)	*CTEPH (4)*	
PVOD and PCH		

Classes are presented between parentheses. CTD, connective tissue disease; HIV, human immunodeciency virus; PVOD, pulmonary veno-occlusive disease; PCH, pulmonary capillary angiomatosis; COPD, chronic obstructive pulmonary disease; CTEPH, chronic thromboembolic PH.

mechanisms are just beginning to be understood. The last decades have been prolic in experimental and clinical studies in both PAH and PH. Several new drugs have become available[3]. Nevertheless, the prognosis remains poor, and many patients require transplantation [5]. The present review aims to summarize the most recent concepts on the epidemiology, pathophysiology, diagnosis and management of PH[6,7].

2. Aetiology and classication

Several conferences on PH have been fostered by the World Health Organization (WHO). A classication was proposed in 1973 and then modied at Evian in 1988 to better reproduce pathophysiology and clinical presentation. At Venice in 2003, the term primary PH was substituted for idiopathic PAH (iPAH) and pulmonary veno-occlusive disease (PVOD) and pulmonary capillary haemangiomatosis (PCH) were grouped under a single PAH subcategory. In 2008, the 4th World Symposium held in Dana Point (Table 1) endorsed the expression "non-PAH PH" to address categories other than PAH. Additionally, left-heart disease PH was subdivided in systolic heart failure (HF), diastolic HF and valvular heart disease, and schistosomiasis was included as a new class of disease-associated PAH.

3. Diagnosis

During the 4th Conference (Table 2) exercise values were excluded as a criteria for diagnosis since the increase in mPAP during exercise frequently exceeds 30 mm Hg among the elderly [8]. Additionally, non-invasive echocardiographic criteria of systolic tricuspid regurgitant velocity were contemplated[9]. Nevertheless, transpulmonary ow and pulmonary venous pressure are not reliably measured by echocardiography thus right-heart catheterisation (RHC) remains the gold standard while echocardiography is usually a screening exam. RHC is mandatory in every patient, allowing the selection of patients that may benet from Ca^{2+}-channel blockers (CCB), the positive responders during vasoreactivity test, those in whom mPAP drops more than 10 mm Hg or to values bellow 40 mm Hg with normal or increased CO, after administration of a short-acting vasodilator, such as nitric oxide (NO)[10], epoprostenol or adenosine[7].

Table 2
New diagnostic criteria for pulmonary hypertension (PH) from the 4th World Symposium on PH (Dana Point, 2008).

Method	Normal	Borderline	Clear
mPAP (mm Hg)	<21	21–25	>25
systolic tricuspid regurgitation ($m.s^{-1}$)	<2.5	2.5–2.8	>2.8

mPAP, mean pulmonary artery pressure.

4. Epidemiology

The incidence and prevalence of PAH were estimated to be 2.4–7.6 cases/million/year and 15–26 cases/million, respectively, in large population studies[11,12]. Worldwide prevalence is hard to appraise, but it is surely underdiagnosed[13]and its onus is likely greater than recognized, given the newly revealed associations with haemodialysis [14], the metabolic syndrome [15], and developing world diseases, such as human immunodeciency virus (HIV) infection, schistosomiasis, and sickle cell disease[16]. Apart from iPAH no precise estimates of incidence or prevalence are available. Nevertheless, non-PAH PH is increasingly recognized as a major health burden. HF is the most common cause of pulmonary hypertension (PH). Not only up to 60% of patients with severe LV systolic dysfunction but also 70% of those with HF and normal ejection fraction (EF)[17]develop PH[18,19]. Moreover, PH aficts 70% of patients with rheumatic heart disease [20]. Many patients develop chronic thromboembolic PH (CTEPH) after pulmonary thromboembolism (PTE)[20]or PH during the progression of chronic obstructive pulmonary disease (COPD). Prevalence ranges from 35 to 90% according to stage[21,22]. Systolic PAP (SPAP) is mostly limited to values ranging from 25 to 35 mm Hg, and severe PH is uncommon in advanced COPD[23]. Nevertheless, some patients develop disproportionate PH. These warrant particular attention[21], but even modest PH has a strong impact on quality of life (QOL) and survival[22]. Right HF, its most severe complication, is responsible for 10–30% of admissions due to decompensated HF[24]. Presently COPD is already responsible for 84% of*cor pulmonale*cases and, due to smoking, will be the 3 rd cause of death by 2020 [23]. Portopulmonary hypertension (PPH) is a pulmonary-hepatic vascular disorder that aficts approximately 5–6% of patients referred for liver transplantation due to advanced liver disease. It is an underrecognized complication that adversely affects survival, after liver transplantation but presumably also in the early stages of liver disease[32,33].

5. Clinical presentation and workup

Severe disease may present with chest pain, palpitation, oedema, ascites, and syncope[9]but earlier treatment, at reversible stages, is fundamental. Diagnosis is challenging, a delay of 2 to 3 years is common and a high suspicion level is needed[13]. The clinician maynd RV hypertrophy on the electrocardiogram and hilar PA prominence on the chest X-ray. Echocardiography, generally undertaken after a suspicion, may show increased SPAP, estimated by the velocity of tricuspid

regurgitation jet, and/or increased RV outow tract acceleration time. It is fundamental to evaluate valve or primary myocardial disease, as well as the degree of RV hypertrophy and dysfunction[9] . Comprehensive echocardiographic evaluations of RV function have been proposed as useful approaches to risk stratication in PAH[25], although magnetic resonance imaging techniques have also been used [26]. Regarding differential diagnosis, patients with suspicion of PTE should undergo the highly sensitive ventilation-perfusion (V-Q) scan. Staging and operability also relies on chest computerized tomography (CT) and angiography. High-resolution CT is useful to assess PVOD or PCH and to diagnose interstitial lung or connective tissue disease (CTD)[7,9]. Finally antinuclear antibodies, autoimmune disease markers, HIV and viral hepatitis screening, coagulation disorder markers (eg, protein S and C, lupus anticoagulants, von Willebrand factor) and type B natriuretic peptide (BNP) may be carried out for differential diagnosis [7,9]. The key feature differentiating PH resulting from left-heart disease (LHD) is elevated pulmonary capillary wedge pressure (PCWP), which is absent in PAH [27]. To establish the diagnosis of PPH patients must present with portal hypertension and not only haemodynamic criteria for PH, in the absence of other causes, but also increased pulmonary vascular resistance (PVR)[28]. Functional respiratory evaluation relies on spirometry and carbon monoxide diffusion (DL_{CO}). Spirometry may be markedly altered in lung disease, whereas minor changes are found in iPAH. DL_{CO} impairment correlates with lung vascular surface area and PAH severity [29]. The 6-minute walk test (6MWT), a common clinical trial end-point that evaluates moderate to severe heart or lung disease, is an easily performable and reproducible test originally developed as a surrogate of peak O_2 consumption (Table 3). It correlates well with CO, PVR, O_2 consumption, QOL, and predicts mortality in PAH [30].

Table 3
The 6-minute walk test (6MWT) in pulmonary hypertension (PH).

Features	
	Submaximal exercise test
	Correlates well with activities of daily living (useful for moderately severe functional impairment)
	Non-specic (evaluates the response of all systems)
	Well tolerated (nevertheless, appropriate response to an emergency should be available)
Measurements	
	Dyspnoea and fatigue self-rating at the beginning and end (according to the Borg scale, see legend)
	Distance walked
Demographic and anthropometric determinants	
	Gender, age and ethnicity
	Height and weight
Advantages	
	Practical and inexpensive to perform (no equipment or specially trained technicians needed)
	Reproducible (estimated coefcient of variability of 8%)
	Ongoing monitoring of cardiopulmonary disease progression
	Evaluation of response to therapy
Limitations	
	Merely a rough estimate of the general functional status (does not discard specic assessment tools)
	Lack of validation for connective tissue disease associated PAH (musculoskeletal involvement)
	"Ceiling effect"for patients with better baseline capacity
	Biases: disturbed cognition, motivational factors, test repetition, musculoskeletal limitations, etc.

A comprehensive perspective on the 6MWT including indications, contraindications, safety precautions, technical aspects, biases, can be found in the guidelines from the American Thoracic Society[134]. The 6MWT measures the distance that a patient can walk on aat surface in a period of 6 min, patients are allowed to stop and rest. The normal walked distance for healthy 60 year-old men and women of average constitution is approximately 630 and 550 m, respectively[135], whereas idiopathic PAH patients on World Health Organization functional class IV usually walk less than 200 m[30]. A clinically important improvement in walking distance for the PAH patient is generally 44–76 m[103].
Borg scale: (0) nothing at all, (0.5) just noticeable, (1) very slight, (2) slight, (3) moderate, (4) somewhat severe, (5) severe, (7) very severe, and,nally, (10) maximal[136].

Nevertheless, since it depends on many individual variables, it is not a reliable marker of disease progression[7] . Additionally, its validity has been questioned for CTD [31]. Cardiopulmonary exercise testing, regarded by most as gold-standard in exercise capacity evaluation and still a cornerstone in PAH functional evaluation, also assesses PH prognosis[32], but requires an experienced laboratory[33,34].

6. Pathophysiology

Although no animal model completely recapitulates human PAH, combining multiple insults, according to the multiple-hit hypothesis, yielded severe phenotypes that closely mimic it[35]. Pathophysiological knowledge, derived mostly from these animal studies, once viewed PH as an imbalance between pulmonary vasoconstrictors and vasodilators [36]. While prostacyclin (PGI_2) and NO normally govern vascular tone, endothelin-1 (ET-1), thromboxane A_2 (TxA_2) and serotonin (5-HT) take over in PH. Not surprisingly, lung arteries vasodilators have been the mainstay of therapy (Fig. 1)[3]. Nevertheless, recent research showed this view to be highly incomplete.

6.1. PAH as panvasculopathy

PAH is currently viewed as a panvasculopathy, accompanied by histological features as intimal hyperplasia, medial hypertrophy, and arteriolar occlusion by thrombosis, inltration by inammatory cells or angioproliferative plexiform lesions (Fig. 1)[7]. Apoptosis may generate apoptosis-resistant endothelial cell phenotypes that cross-talk with PA smooth muscle cell (PASMC) through growth factors such as transforming growth factor-(TGF-), that are involved in endothelial cell and broblast transdifferentiation and PASMC proliferation[37]. Metalloproteinase activation leads to the disruption of the basement membrane enabling inammatory cell recruitment and further generation of mitogenic peptides[38]. The main mechanisms involved in inammation, endothelial progenitor cell (EPC) recruitment, growth factor activity and extracellular matrix remodeling are summarized inFig. 1. PAH shares a mitochondrial-metabolic abnormality with cancer, the "Warburg phenotype", a shift from oxidative phosphorylation to glycolysis (despite adequate O_2 supply) that enhances proliferation and prevents apoptosis (Fig. 2). Hyperpolarization of the mitochondrial membrane, reduced production of reactive oxygen species (ROS), normoxic-activation of hypoxia inducible factor-1, overexpression of pyruvate dehydrogenase kinase (PDK) and decreased expression of O_2-sensitive K^+ channels (Kv1.5) have been postulated to underlie changes in mitochondrial O_2 sensing [39]. PDK activation suppresses aerobic glucose metabolism and decreased Kv1.5 conductance depolarizes the membrane. Dichloroacetate (DCA), a mitochondrial PDK inhibitor and Kv1.5 channel opener, improved PAH[39]both by activating pyruvate dehydrogenase (PDH) and aerobic metabolism and by restoring membrane potential and ROS production[40].

6.2. Genetics of PAH

Mutations in bone morphogenetic protein (BMP) receptor-2 (BMPR2), a constitutively active receptor responsive to TGF-superfamily (including BMP), are seen in more than 80% of familial PAH (fPAH) cases, leading to loss of smad signalling (Fig. 3) and therefore to increased proliferation and decreased differentiation of PASMC[41,42]. Still, penetrance is low and the mutation is seen only in 10 to 20% of non-fPAH[43]. Other genetic mechanisms predispose to PAH, namely single-nucleotide polymorphisms of Kv1.5[18], transient receptor potential (trp) channels [13], and serotonin transporters [44]. Trp channels regulate contractility and cell proliferation by intracellular Ca^{2+}[45]. Elevated 5-HT levels and 5-HT transport have been implicated in PAH pathogenesis[44].

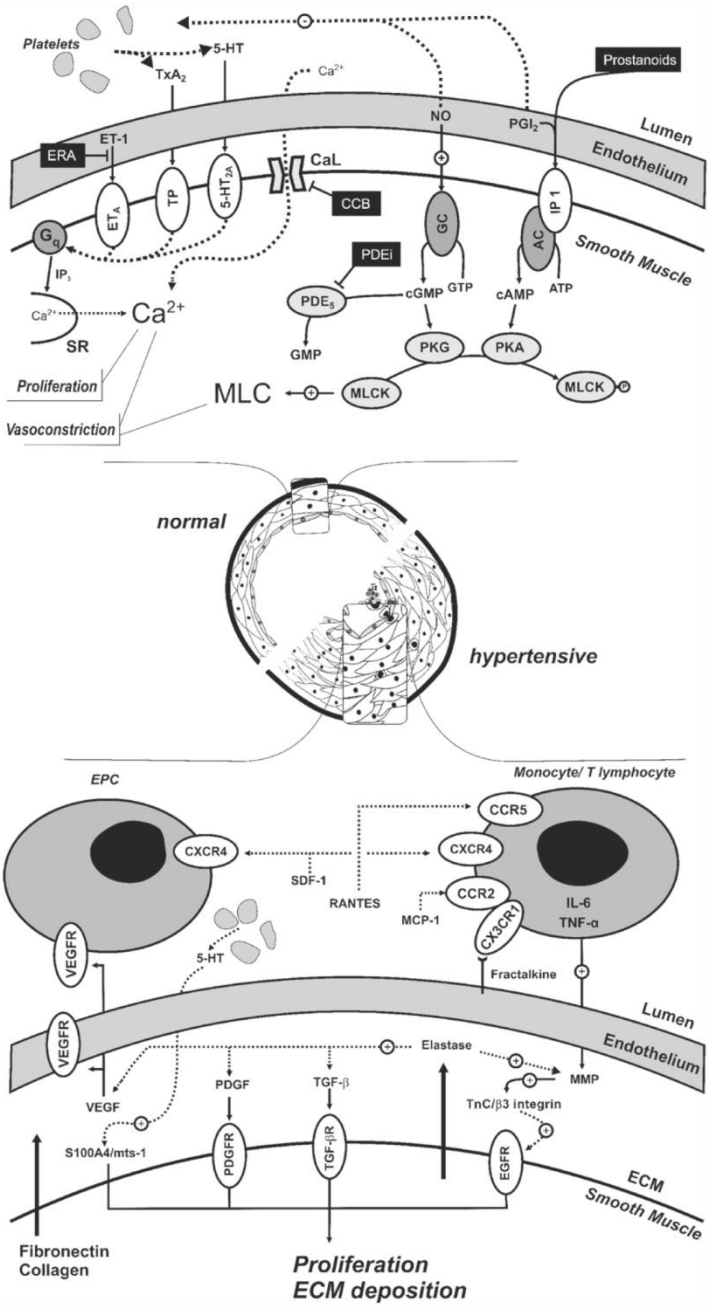

Fig. 1. Pulmonary artery smooth muscle cell (PASMC) constriction and proliferation mechanisms. The major sites of action of lung vasodilator drug classes are shown in the upper panel, namely Ca^{2+}-channel blockers (CCB), endothelin-1 (ET-1) receptor antagonists (ERA), phosphodiesterase inhibitors (PDEi) and prostanoids. Myosin light-chain (MLC) kinase (MLCK) is inactivated upon phosphorylation (MLCK-P). Other mechanisms are presented in the lower panel. Several cytokines, beyond interleukin-6 (IL-6) and tumor necrosis factor- (TNF-), mostly produced bybroblasts, such as stromal cell-derived factor-1 (SDF-1), monocyte chemotactic protein-1 (MCP-1), fractalkine, RANTES (regulated upon activati on, normal T expressed and secreted), and vascular endothelial growth factor (VEGF) are upregulated and induce PASMC proliferation and monocyte recruitment, while monocytes upregulate the -chemokine receptor (CXCR4) and chemokine receptors 1, 2 and 5 (CX3CR1, CCR2 and CCR5, respectively)[46]. Elastase, early activated in PH, triggers growth factors release from the extracellular matrix (ECM) and induces tenascin C (TnC) through the activation of matrix metalloproteinases (MMP). When TnC binds surface integrins on PASMCs cell-survival signals are generated and growth factor receptors are further activated. Serotonin (5-HT) induces proliferation of PASMCs by stimulation of S100A4/Mts1, a S100 Ca^{2+}-binding protein family member with metastasis-inducing ability[3]. Endothelial progenitor cells (EPC) may participate in vessel repair, but on the other hand also take part in plexiform lesions[146]. Other abbreviations: TxA_2, thromboxane A_2; G_q, protein G_q; IP_3, inositol 3-phosphate; SR, sarcoendoplasmic reticulum; ET_A, ET-1 type A receptor; TP, TxA_2 receptor; $5\text{-}HT_{2A}$, 5-HT type 2A receptor; CaL, Type L Ca^{2+}-channel; GC, guanylate cyclase; AC, adenylate cyclase; IP, prostacyclin receptor; PDE_5, phosphodiesterase type 5; PKG, protein-kinase G; PKA, protein-kinase A; VEGFR, VEGF receptor; PDGF, platelet derived growth factor; TGF-, transforming growth factor-; PDGFR, PDGF receptor; TGFR, TGF-receptor; EGFR, epidermal growth factor receptor.

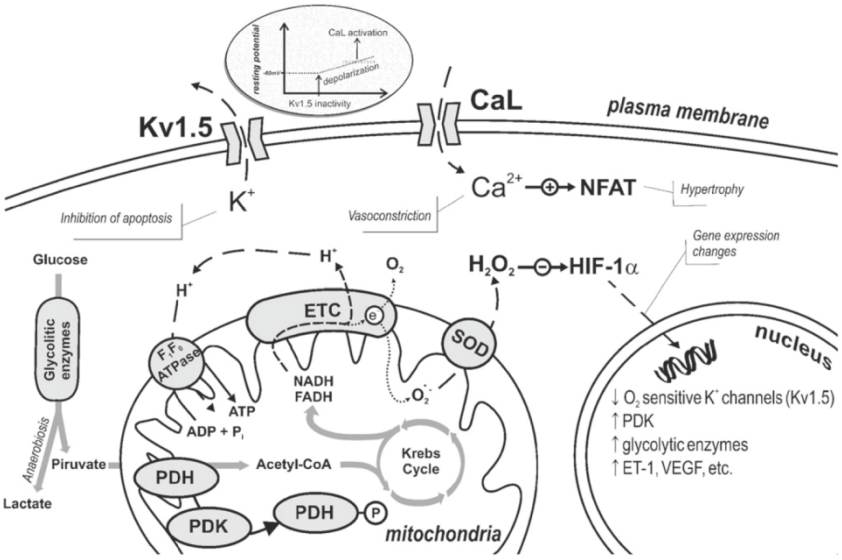

Fig. 2. Reactive oxygen species (ROS), disturbed O_2 sensing, and mitochondrial dysfunction in pulmonary arterial hypertension (PAH). During oxidative phosphorylation, electrons (e^-) are conveyed by the electron transport chain (ETC) from donors (NADH and FAH) to O_2, but minor side reactions also generate by-products, as superoxide anion (O_2^-) that must be detoxied to H_2O_2 by superoxyde dismutase (SOD)[147]. Under normoxia H_2O_2 constitutively opens plasma membrane O_2-sensitive K^+-channels (Kv1.5) and inhibits hypoxia-inducible factor-1(HIF-1) activity, whereas during hypoxic vasoconstriction, ROS and H_2O_2 production are decreased, Kv1.5 channels close, the plasma membrane depolarizes, Ca^{2+} enters the cell and myocytes contract. In PAH, mitochondrial abnormalities, most notably pyruvate dehydrogenase kinase (PDK) activation, shift metabolism toward anaerobic glycolysis and impair the ETC. Reduced ROS production, nuclear translocation of HIF-1, and decreased expression of Kv1.5 ultimately lead to sustained membrane depolarization, L-type Ca^{2+}-channel (CaL) activation and hypertrophy by Ca^{2+}-calcineurin-dependent activation of the nuclear factor of activated T lymphocytes (NFAT)[146] . PDH, pyruvate dehydrogenase, and respective phosphorylated form (−P); ET-1, endothelin-1; VEGF, vascular endothelial growth factor.

6.3. Inammation

The inammatory state of the vessel wall has recently gained interest as primary event, rather than mere consequence of the disease [46]. Autoantibodies and in ltration by in ammatory cells are common in PAH associated with CTD but are also seen in iPAH[46]. Increased levels of cytokines and their receptors have been demonstrated, particularly in iPAH patients[47], who also present heightened expression of inammatory cell-associated nuclear factor of activated T lymphocytes (NFAT)[48]. Cytokines involved in the pathogenesis of chronic inammatory diseases and cancer, such as tumor necrosis factor-(TNF-) and IL-6, may play a role in PAH vasculopathy[49]. Our group has tested an anti-inammatory approach in experimental models of PH with variable success[50,51] . Inammatory activation may also underlie systemic manifestations, for instance cardiac cachexia (CC). CC is characterized not only by neuroendocrine and inammatory activation but also by suppressed appetite and nutritional derangements[52]and poses a signicant prognostic burden on HF patients [53]. CC accompanies the progression of PH, indeed, patients with severe PH have exaggerated and early post-prandial satiety hormone response[54].

6.4. The RV in PH

The RV effectively serves as a thin, compliant reservoir for blood returning to the LV whose primary function of is to deliver deoxygenated blood to the lungs, while maintaining low-pressure perfusion [55]. It is thus best suited for volume work and unable to suddenly withstand high PAP. Sudden afterload decreases stroke volume and dilates the RV [56], whereas progressive overload allows gradual hypertrophy, remodeling and substantial increases in mPAP. Curiously, although RV response partly determines the outcome [26,57], despite the fact that the RV was shown to be an independent therapeutic target in experimental PH [58], and even though RV remodeling is potentially reversible, as seen after lung transplantation (LT)[59] , little is known about the mechanisms underlying RV dysfunction [55]. Many have shown neuroendocrine activation can contribute to RV hypertrophy [60,61], brosis and apoptosis, as well as to oxidative stress, and activation of in ammatory cytokines and growth factors [55,62]. A state of myocardial hibernation has been proposed based on systolic ow impediment to coronary arteries which is proportional to RV pressure and mass [63]. In contrast to the normal exible metabolism, in RV hypertrophy the myocardium relies solely on anaerobic glucose metabolism partly due to PDK activation [64] and possibly impaired mitochondrial energy-producing ability [65]. Moreover, changes in cardiomyocyte redox state can underlie electrophysiological instability and remodeling, by mechanisms similar to those already described for pulmonary vessels [66]. Experimental ndings and clinical observations suggest that elevated mPAP cannot be the single driver for RV failure [62], therefore, targeting the RV may be a promising approach [6]. As for the LV myocardium, echocardiography shows compromised LV function in various aetiologies of PH [67], mainly due to ventricular interdependence and impaired lling [68]. Nevertheless, myocardial abnormalities partly underlie LV dysfunction. Indeed, despite immediate restoration of LV geometry and RV function, LV lling is only normalized 1 year after single-LT in severe PH [69], and combined heart-lung transplantation (HLT) is favored if LV function is impaired because the LV may not recover after LT alone [70]. We have con rmed intrinsic LV myocardial dysfunction and neuroendocrine activation experimentally [61,71].

6.5. Pathophysiology of non-PAH PH

Contrarily to PAH, and paradoxically, few data are available on the pathophysiology of the far more common non-PAH PH. Regarding chronic pulmonary disease, several mechanisms are potentially responsible. Hypoxia, such as is found at high altitude, is known to

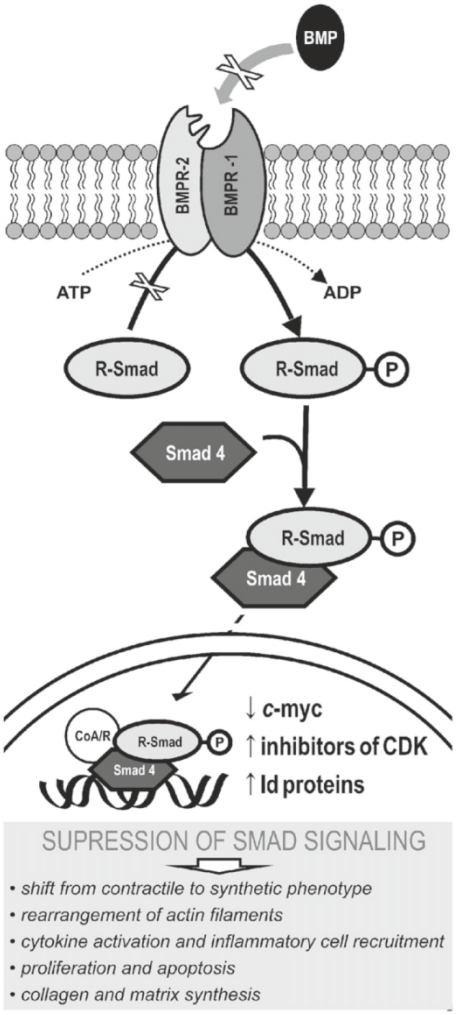

Fig. 3. Growth-promoting pathways in bone morphogenetic protein (BMP) receptor type 2 (BMPR-2) mutations. Bone morphogenetic protein (BMP) receptor type 1 (BMPR-1) and -2 dimerize upon activation by BMP and initiate a cytosolic receptor-activated Smad protein-signaling cascade. Smads (homology with drosophila's mothers against decapentaplegic–MAD–and Caenorhabditis elegans' small phenotype–sma–proteins) ultimately complex with common partner Smad4 and translocate to the nucleus. The weak Smad–DNA interaction requires co-repressors or activators (Co–A/R) [148]. Signal disrupting mutations in BMPRII can be found in the ligand-binding domain, in the kinase domain or in the cytoplasmic tail. Suppression of Smad signalling partly underlies the hypertrophic and proliferative phenotype of pulmonary artery smooth muscle cells (PASMC) [149]. In normal PASMC, BMP stimulates transcriptional activation of cyclin-dependent kinase (CDK) inhibitors and repression of c-*myc*. CDK inhibitor prevents progression in cell cycle, while c-*myc* encodes a transcriptional activator responsible for growth and proliferation [150]. Inhibitor of DNA (Id) binding proteins, a family deprived of DNA-binding domain that acts by inhibition of transcription factors, are also major targets. Failure to induce Id genes makes PASMC unresponsive to the growth suppressive effects of BMPs [151]. Prostanoids, by cyclic adenosine monophosphate and a direct effect on the Id promoter, drive the expression of Id proteins[148].

induce PH, but low arterial O_2 is not an independent predictor of mPAP, therefore after the Evian Conference COPD-associated PH was no longer classied as'associated with hypoxemia'[29]. Pulmonary vessels in COPD consistently develop intimalbro-elastic thickening and overall muscularisation but this does also not provide a consistent explanation [72]. Endothelial dysfunction and inammation, are currently viewed as the key to vascular remodeling [73]. Findings strongly suggest an involvement of vasoactive mediators and cytokines [72]. Plasma IL-6 correlates with mPAP and certain IL-6 genotypes are associated with PH development in COPD [74]. Indeed, vascular remodelling and endothelial dysfunction can be observed in mild COPD without hypoxaemia and in ordinary smokers[75] . Symptomatic CTEPH affects 3.8% of patients within 2 years of initial PTE[76] , but up to 5.1% of patients may develop denite CTEPH[77] . Unlike PAH, CTEPH is mainly associated with obstructions in larger vessels. Its pathophysiology remains obscure, while most argue that it results from recurrent pulmonary embolism, it has also been suggested that endothelial dysfunction could lead to thrombus formation*in situ*, and, in fact many patients do not have a clear history of embolism[78]. Variable degrees of small vessel disease, a PAH-like vasculopathy, accompany CTEPH and the mechanisms that underlie them are probably common to PAH, namely endothelial dysfunction [79]. As for LHD, two major mechanisms underlie PH, an hydrostatic and a vasoreactive. Increased lling pressures are transmitted to the pulmonary circulation and generate, initially, pulmonary venous hypertension, but, later on, also PVR increase. SPAP correlates tightly and is roughly twice the PCWP[80]. When the compensatory mechanisms of the highly distensible pulmonary vasculature are surpassed PA pressure increases rst on exertion and later on also at rest. Endothelial dysfunction, sympathetic-adrenergic stimulation and disturbances of 5-HT, TxA_2 and angiotensin-II production further aggravate PH [81], contributing to structural changes at the capillary level, namely swelling of the endothelial cells, thickening of the basal lamina, and proliferation of reticular and elastic brils. These changes participate in increasing PVR, decreasing permeability of the vascular bed, and lower the possibility of developing pulmonary edema, but ultimately lead to increased likelihood of right ventricular failure [82]. These changes are initially reversible if cardiac lling pressures are reduced, but on the long term become irreversible and pose a relative contraindication to cardiac transplantation[83].

6.6. Prognosis

Although PAH has been most extensively studied, its rarity, diverse etiology and changing therapeutics preclude an estimation of yearly mortality rates. An early registry followed 194 patients with iPAH from 1981 to 1985 and estimated a median survival of 2.8 years, with 1-, 3-, and 5-year survival rates of 68, 48, and 34%, respectively[84]. Present-day registries, however, reveal a better prognosis, with 1 year survival ranging 83 to 88% and 3 year survival 58 to 72%[85]. A risk-prediction equation could be derived from multivariate analysis, including gender, 6MWT, and CO at diagnosis as covariates [86]. Four variables were associated with increased 1-year survival: WHO functional class I, 6MWT$\geq$440 m, BNP<50 pg/mL, and $DL_{CO}\geq$80% of predicted [86]. Recently, echocardiographic evaluation of RV function has also been successfully used for risk stratication in PAH[25]. The progression in non-PAH PH is generally slower and the overall prognosis is better. Still, there is a substantial impact on QOL and survival[22,27]. The level of PAP is a good indicator of prognosis in COPD and a 50% 5-year survival rate has been reported with PH[87]. Regarding CTEPH, survival changed dramatically. Before the advent of pulmonary endarterectomy (PEA) patients who had mPAP higher than 30 mm Hg steadily progressed to PH and 2 year-survival was lower than 20% after it reached 50 mm Hg [88]. Currently, in experienced centres and carefully selected patients, PEA provides remarkable haemodynamic and clinical improvement with low procedural mortality rate[5]. In severe HF, the EF of the RV is the most important determinant of short-term prognosis among hemodynamic variables[89]. Although increased mPAP is frequently coupled with reduced RV function, exceptions must be taken in account during prognostic stratication[19].

Table 4
Summary of randomized clinical trials (RCT) on pulmonary arterial hypertension (PAH).

Class	Drug	Year	Author	Study type	Sample	Patients	Follow-up	Positive outcomes
Prostanoid	Epoprostenol	1996	Barst[96]	RCT (not blind)	iPAH (WHO III–IV)	81	12 weeks	Haemodynamics, QOL, WHO class, survival
	Treprostinil (sc)	2002	Simmoneau[98]	RCT	iPAH, CTD and CHD (WHO II-IV)	470	12 weeks	Haemodynamics, 6MWT, clinical evaluation
	Iloprost (inh)	2002	Olschewski[99] (AIR)	RCT	iPAH, CTD and CTEPH (WHO III-IV)	203	12 weeks	Haemodynamics, 6MWT, QOL, WHO class
	Beraprost	2002	Galiè[137] (ALPHABET)	RCT	iPAH, CTD, CHD, portal hypertension and HIV (WHO II-III)	130	12 weeks	Exercise tolerance, 6MWT, clinical evaluation
		2003	Barst[138]	RCT	iPAH, CTD and CHD (WHO II-III)	116	1 year	Exercise tolerance, 6 MWT, TTCW,
ERA	Bosentan	2002	Rubin[103] (BREATHE1)	RCT	iPAH, CTD or SLE (WHO III-IV)	213	16–28 weeks	Exercise tolerance, 6MWT, WHO class, TTCW;
		2005	McLaughlin[139]	RCT (not blind)	iPAH (WHO III-IV)	169	3 years	Survival (NIH prediction)
		2006	Galiè[140] (BREATHE5)	RCT	CHD (WHO III)	54	16 weeks	Haemodynamics, 6 MWT
		2008	Galiè[106] (EARLY)	RCT	iPAH, CTD, CHD, HIV (WHO II)	185	6 months	Haemodynamics, NT-pro-BNP and TTCW
		2008	Jais[123] (BENEFIT)	RCT	CTEPH (WHO II–IV)	157	16 weeks	Haemodynamics
	Ambrisentan	2005	Galiè[141]	RCT (dose ranging)	iPAH, CTD, HIV and anorexigen (WHO II–III)	64	12 + 12 weeks (not-blind)	Haemodynamics, 6 MWT, clinical evaluation
		2008	Galiè[142] (ARIES 1 and 2)	RCT	iPAH, CTD, HIV and anorexigen	202 + 192 (parts 1 and 2)	12 weeks	6MWT, WHO class, QOL, TTCW, NT-pro-BNP
		2009	Oudiz[108] (ARIES 1, 2 and E)	RCT	iPAH, CTD, HIV and anorexigen	383	2 years	6MWT, TTCW and survival (combined outcome)
	Sitaxsentan	2004	Barst[143] (STRIDE1)	RCT	iPAH, CTD and CHD (WHO II–IV)	178	12 weeks	Haemodynamics, 6MWT, WHO class
		2006	Barst[144] (STRIDE2)	RCT	iPAH, CTD and CHD (WHO II–IV)	245	18 weeks	6MWT, WHO class
PDEi	Sildenal	2005	Galiè[110] (SUPER1)	RCT	iPAH, CTD and CHD (WHO II–IV)	278	12 weeks	Haemodynamics, 6MWT, WHO class
	Tadalal	2009	Gàlie[145] (PHIRST)	RCT (dose ranging)	iPAH, CTD, CHD, HIV and anorexigen	405	16 weeks	Haemodynamics, WHO class, 6MWT, TTCW, QOL
Combined	Bosentan + Iloprost (inh)	2006	McLaughlin[114] (STEP)	RCT	iPAH, APAH (WHO III)	67	12 weeks	Haemodynamics, WHO class, TTCW
	Epoprostenol + sildenal	2008	Simmoneau [115](PACES)	RCT	iPAH and CTD	267	16 weeks	Haemodynamics, exercise tolerance, QOL, TTCW
	Bosentan or sildena l + treprostinil (inh)	2010	McLaughlin[100] (TRIUMPH I)	RCT	iPAH, CTD, HIV and anorexigen (WHO III–IV)	255	12 weeks	QOL, NT-pro-BNP

RCT on PAH therapeutics are summarized according to drug class, drug type and publication date. Study acronyms are presented when applicable. Studies enrolling less than 50 patients as well as studies involving specic PAH groups, namely HIV-related and portal hypertension-related were excluded. iPAH, idiopathic pulmonary arterial hypertension; WHO, World Health Organization; QOL, quality of life; 6MWT, 6-minute walk test; NIH, National Institute of Health; APAH, disease associated pulmonary arterial hypertension; CTD, connective tissue disease APAH; CHD, congenital heart disease APAH; CTEPH, chronic thromboembolic pulmonary hypertension; HIV, human immunodeciency virus APAH; TTCW, time to clinical worsening; SLE, systemic lupus erythematosus APAH; NT-pro-BNP, N terminal fragment of pro-type B natriuretic peptide. Study acronyms stand for: AIR, Aerosolized Iloprost Randomized; ALPHABET, Arterial Pulmonary Hypertension and Beraprost European Study Group; BREATHE, Bosentan Randomized trial of Endothelin Antagonist THErapy Study Group; EARLY, Endothelin Antagonist tRial in miLdlY symptomatic PAH patients; BENEFiT, Bosentan Effects in iNopErable Forms of chronic Thromboembolic pulmonary hypertension; ARIES, Ambrisentan in pulmonary hypertension, randomized, double blinded, placebo controlled, multicenter, efcacy studies); STRIDE, Sitaxsentan To Relieve ImpaireD Exercise; SUPER, Sildenal Use in Pulmonary Arterial Hypertension Study Group; PHIRST, Pulmonary Arterial Hypertension and Response to Tadalal; STEP, Safety and pilot efcacy Trial in combination with bosentan for evaluation in pulmonary arterial hypertension; PACES, pulmonary Arterial hypertension Combination study of epoprostenol and sildenal; TRIUMPH I, efcacy and tolerability of inhaled Treprostinil sodium in patients with severe pulmonary arterial hypertension.

7. Therapeutics

Treatment of PAH has evolved considerably over the past decade, many treatment algorithms have been proposed, mainly based on studies conducted in patients with iPAH and PAH associated with CTDs. InTable 4we summarize the major therapeutic studies on PAH.

Several general measures can be recommended. Regarding exercise practice, patients may practice low level aerobic exercise, such as walking, whereas exertion that may lead to breathlessness, dizziness or chest pain should be avoided. Some patients may not tolerate high altitudes, for instance during airplane ights, and therefore require in-ight O_2 administration. It is currently recommended for patients either in WHO classes III and IV or whose arterial O_2 pressure is below 60 mm Hg. Dietary sodium restriction can be advised particularly in RV failure ($\leq$2.4 g.d^{-1}), but current European Society of Cardiology guidelines do not recommend it. Immunization against common respiratory pathogens is recommended[7]. PAH is a contraindication to pregnancy due to the high mortality rate[90].

Despite the lack of randomized controlled trials (RCT), the initial therapeutic, and largely supportive, approaches to the treatment of PAH were anticoagulation, diuretics, O_2 therapy and digoxin. Observational studies suggested improved survival after anticoagulation in patients with iPAH, therefore most experts recommend anticoagulation in iPAH, heritable PAH, and PAH due to use of anorexigens (titrated to international normalized ratio of 2.0–3.0). As for non-iPAH, anticoagulation may be advised for severe cases[7]. Diuretics are used to manage HF symptoms. O_2 therapy in hypoxemia is employed strictly to avoid vasoconstriction. Based on a short-term effect study, digoxin may be used in patients with low CO, but its use is clearly only recommended in patients with supraventricular tachyarrhythmias

[91]. During the last two decades substantial RCT and pharmacological research have yielded several new and more effective alternatives to treat PH. The main pharmacological classes will be briey presented. Most of the studies are small scaled and short-termed, not suitable for survival analysis, but a recent meta-analysis found an overall bene t in mortality[92]. Nevertheless, most therapies reduce mPAP by only 10–20%, with the exception of strong responders to CCB. Despite all the advancement, many patients still remain symptomatic, with a suboptimal QOL and warrant combined therapy or even invasive or surgical procedures.

7.1. Ca^{2+} channel blockers

A marked improvement in survival rates was shown with long-term high-dose CCB therapy for patients with iPAH and a positive vasoreactivity test[93]. Long acting nifedipine, diltiazem, or amlodipine are more commonly used. If there is no recovery to functional classes I or II patients are deemed as non-responders and should discontinue CCB. True responders are rare in non-iPAH[94] . Indiscriminate use is not recommended, due to systemic vasodilation and negative inotropic effects[95].

7.2. Prostanoids

There are presently several commercially available prostanoid formulations. Intravenous (*iv*) epoprostenol was the rst shown to improve functional class, hemodynamics and survival in a 12-week follow-up period in patients with iPAH of classes III and IV [96]. These bene cial effects were reproduced in long-term observational comparisons with historical controls [97]. Moreover, epoprostenol was also evaluated in CTD associated PAH and other forms of non iPAH with favourable outcomes. Presently, because of the complex administration and cumbersome follow-up, epoprostenol use is mainly con ned to highly experienced centres. Patients must keep a central venous catheter (CVC) and handle drug preparation and infusion. Dosing must be carefully titrated. Most patients do well with an initial in-hospital dose of 2 $ng.kg^{-1}.min^{-1}$ and a dose range between 25 and 40 $ng.kg^{-1}.min^{-1}$. Unfortunately, substantial side-effects have been reported, namely ushing, headache, and sudden death after abrupt discontinuation, as well as risk of infection related to CVC [7]. Treprostinil, a longer half-life prostanoid, amenable to administration by subcutaneous (*sc*) route, circumventing the need for CVC, showed minor benecial effects in patients with functional classes II–IV of idiopathic, CTD and (CHD) associated PAH [98]. The Food and Drug Administration (FDA) approved it for functional classes II–IV also by *iv* route, when the *sc* route is not tolerated due to pain or erythema. It is currently not approved by the European Medicines Agency (EMA). On another attempt to facilitate administration, iloprost was developed for inhalation by aerosol device. After a 12-week administration, iloprost improved the 6MWT and functional class in a multicentre RCT enrolling patients with PAH of different aetiologies [99]. Treprostinil is now also available by inhalation [100], and trials of oral formulations have been iniciated (FREEDOM, Trial of Oral Treprostinil in Pulmonary Arterial Hypertension).

7.3. Endothelin receptor antagonists

We have previously reviewed the role of ET-1 and its antagonists (ERA) in cardiovascular pathophysiology [101]. Briey, after a small magnitude RCT had shown improvement in the 6MWT, mPAP and CO with the non-selective ERA bosentan [102], a larger scale study conducted in patients with idiopathic or CTD associated PAH, reproduced these ndings and reported improvement in time to clinical worsening (TTCW), a secondary endpoint dened as a composite of mortality, LT, hospitalization, discontinuation due to lack of recovery or need for epoprostenol or atrial septostomy (AS)[103]. As an important side-effect, bosentan dose-dependently altered hepatic function. Anemia can also occur and the FDA therefore recommends liver function test and haematocrit surveillance[7]. Long-term evaluation as arstline drug in functional class III patients also revealed good results, although many patients demanded prostanoids[104]. In fact, improved survival was only demonstrated by comparison with historical data from epoprostenol treated iPAH WHO class III patients, and unfortunately the two cohorts were not comparable[105] . By now, bosentan has also been tested in CHD, HIV-associated PAH and CTEPH with favourable results. Moreover, it has been successfully used in a large sample of mildly symptomatic, class II, multiple cause-PAH patients improving haemodynamics and TTCW [106]. Sitaxentan a selective ET_A ERA initially was shown to have comparable effects to bosentan in iPAH and PAH associated with CTD or CHD, but has been withdrawn from market due to two fatal cases of liver failure [107]. Ambrisentan, another selective ET_A ERA, also improved the 6MWT and TTCW, which was reproducible in long-term studies [108]. It is approved by the FDA since 2007 and it has also been approved by the EMA for PAH patients in functional classes II and III. Indeed, it is the only ERA approved for WHO class II.

7.4. Phosphodiesterase inhibitors

Phosphodiesterases (PDE) degrade cyclic guanosine monophosphate (cGMP) therefore PDE inhibitors (PDEi) potentiate the effects of cGMP generated by NO activation of guanylate cyclase. NO and NO donors have been extensively used as a rescue therapy to mitigate mPAP in the perioperative period and in the critically ill patient, particularly in children[109]. Sildena l, the rst used PDEi, was shown to improve 6MWT, WHO functional class and mPAP in idiopathic, CTD or CHD associated PAH, but there were no differences in TTCW[110] . The FDA approved sildenal in low doses for patients with PAH although there is some debate as to whether higher doses might confer additional benets[111]. Other PDEi are currently under study. Tadala l, recently approved by both FDA and the EMA, has a longer half-life than sildenal and is amenable to once-daily dosing. Nevertheless, unlike sildena l, due to its hepatic metabolism and renal clearance, dose adjustments are recommended for patients with renal or hepatic function impairment [112].

7.5. Combination therapy

The possibility to combine distinct drug classes that target different molecular pathways in order to improve clinical efcacy and minimize side-effects is an attractive perspective. After an initial attempt to combine bosentan and epoprostenol in a small scale and underpowered trial conducted on patients with either iPAH or PAH associated to CTD that proved unsuccessful [113], another trial that combined inhaled iloprost with bosentan in patients who remained symptomatic showed improvement in functional class, TTCW and haemodynamics [114]. More recently, the addition of sildenal to PAH patients who remained symptomatic on a stable dose ofivepoprostenol improved the 6MWT, as well as mPAP, CO, and TTCW [115], while the addition of inhaled treprostinil to WHO III and IV PAH patients undergoing either bosentan or sildenal chronic therapy showed only clinical benets in QOL[100], and the introduction of oral treprostinil failed to achieve statistical signicance in 6MWT (FREEDOM, unpublished results).

7.6. Invasive and surgical strategies in PAH

These include AS and LT or HLT. Other possibilities, such as the RV mechanical assist devices (RVAD) are still poorly investigated. AS creates a right-to-left shunt that unloads the RV, decreases mPAP, and improves LV lling. The increase in CO offsets the shunting of deoxygenated blood and ameliorates O_2 delivery. Increased CO allows bridging to transplantation in up to 40% of patients [116]. Nevertheless, it is merely palliative and procedural mortality is still high therefore it is just a last resort for patients on maximal medical therapy and inotropic support. Improved

techniques are being currently explored to reduce procedural risk [5]. Currently, PAH is responsible for approximately 4% of LT and HLT, and although there is a substantial procedural related mortality, the long-term outcome is better than with medical therapy alone, with a 47% survival after 5 years [117]. The type of transplant is still a matter of debate and highly related to the experience of each centre. Generally HLT is preferred either when patients have intractable HF or are dependent on inotropic support or if PH is secondary to CHD or LHD [70].

7.7. Therapeutic algorithm

Management must be tailored to each patient according to disease severity, comorbid conditions, drug side-effects and each centre's experience. CCB are reserved for iPAH patients with a positive vasoreactivity test and stable hemodynamics, otherwise first line therapy should consist of ERA or PDEi, unless the oral route is not available, patients are in functional class IV or present overt RV failure. In these cases, the first choice is an *iv* prostanoid. Moreover, combination therapy should always be kept in mind, particularly when side-effects arise or patients are not responding. Enrolment in clinical trials with newer pharmacological agents may be an option but AS and transplantation should be considered before systemic deterioration. Early referral for transplantation is crucial particularly for refractory cases [7]. A simplified therapeutic algorithm is suggested in Fig. 4.

7.8. Non-PAH PH

Patients will benefit from medical optimization of their primary disease, but significant PH may persist. Some patients actually present disproportionate PH not easily attributable to the underlying condition.

In left-heart disease prostanoids, with the exception of inhaled route, are usually contraindicated due to systemic vasodilation [118]. ERA trials have been interrupted prematurely due mainly to side-effects and absence of clinical benefit, even with reduced dose [119], but selected cases may benefit from short trials as a bridge to transplantation [120]. As

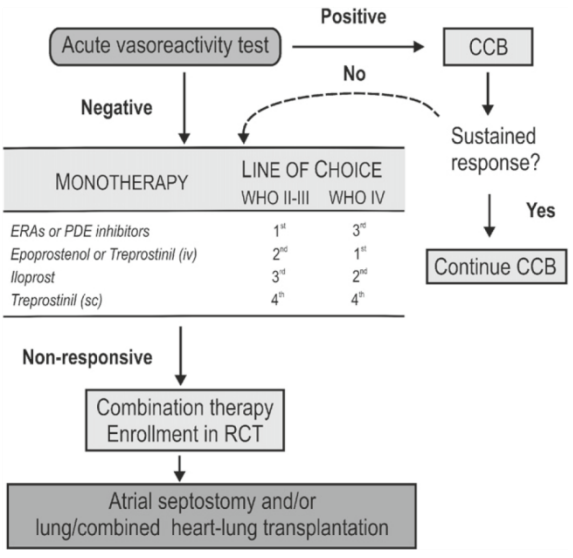

Fig. 4. Algorithm for pulmonary arterial hypertension (PAH) management. CCB, calcium channel blockers; WHO, World Health Organization; ERA, endothelin-1 receptor antagonists; PDEi, phosphodiesterase inhibitors; RCT, randomized clinical trials; *iv*, intravenous; *sc*, subcutaneous. *, among the ERAs only ambrisentan is approved for WHO class II patients.

for PDEi short-term hemodynamic benefits, as well as long-term improvements have been documented [121].

Mild levels of PH are amenable to optimization of medical therapy in COPD. If PH is disproportionate, and other PH causes have been ruled out, many centres are routinely employing vasodilators despite V-Q mismatch [122]. CTEPH is potentially curable with PEA [5]. Yet, many patients are not candidates so they remain anticoagulated and on diuretics. Many centres are promptly using new PAH drugs off-label if there is associated vasculopathy [123].

7.9. Recent progresses and future targets in PH

Based upon the most recent experimental findings, clinical trials targeting altered metabolic and signalling pathways are warranted. DCA and Kv1.5 channel gene transfer have been successful in experimental studies [39], as well as trp channel inhibitors, growth factor receptor inhibitors and intracellular kinase inhibitors [3,124]. Inammatory response modulation has also been a major research topic. After several animal studies demonstrating beneficial effects of statins [124], possibly due to pleiotropic effects, a human study disappointingly showed no long-lasting improvement [125]. Other immunomodulatory agents have been successful in animal experiments [50,126], but benefical effects are mainly confined to CTD associated PAH [127]. We have also reported disturbances in endogenous endocrine and paracrine systems [128,129] that may be targeted. Another tempting possibility is the recruitment or infusion of EPC. The number and function of EPCs predicts prognosis, and most currently used drugs increase circulating EPC numbers [130]. Circulating EPCs home to sites of endothelial injury, promote revascularization and improve vascular homeostasis [131], endothelial dysfunction may be related to the lack of EPCs [130]. Finally, we must bear in mind that RV failure is the final and most severe complication of PH. Agents such as levosimendan that vasodilate lung vessels but are also positive inotropes are predictably good therapeutic tools. Still, the clinical efficacy of these drugs has only just started to be evaluated [132,133].

Conict of interest statement

None declared.

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Fisiologia do ventrículo direito e circulação pulmonar

A embriologia, anatomia e fisiologia do ventrículo direito e circulação pulmonar diferem substancialmente das do ventrículo esquerdo e circulação sistémica, sendo, portanto, incorrecto extrapolar de conceitos bem estabelecidos da fisiologia cardiovascular quando procuramos compreender a dinâmica do ventrículo direito.

A septação ventricular completa e o desenvolvimento de dois sistemas circulatórios em série foi fundamental à adaptação à vida terrestre e à endotermia (Koshiba-Takeuchi et al. 2009). O primórdio cardíaco é uma estrutura tubular única, mas disparidades na expressão génica de factores de transcrição e moléculas de sinalização intracelular condicionam um padrão de assimetria direito-esquerdo que determina uma curvatura das estruturas e a formação de um órgão maduro marcadamente assimétrico (Chen et al. 2010), em que o ventrículo direito é o último componente a desenvolver-se (Moorman e Christoffels 2003). O desenvolvimento embrionário, a partir da curvatura de uma estrutura tubular única, repercute-se na complexidade geométrica da cavidade ventricular direita do adulto, que assume uma forma em crescente, em secção transversa, e uma forma triangular, no plano longitudinal, envolvendo a face antero-medial do ventrículo esquerdo. Durante o desenvolvimento embrionário, os pulmões não participam nas trocas respiratórias, o leito vascular pulmonar apresenta resistências elevadas e as cavidades ventriculares equalização de pressões e idêntica espessura parietal mas, logo após o parto, com o início da ventilação, e participação pulmonar nas trocas respiratórias, há uma rápida transição para o padrão circulatório adulto (Rudolph 2010). O leito vascular pulmonar do adulto é constituído por uma rede capilar extensa e altamente distensível, com baixa resistência e impedância, que lhe permite acomodar com facilidade todo o volume de ejecção, mesmo quando o débito cardíaco aumenta, como acontece durante o exercício físico, mantendo pressões baixas na artéria pulmonar, comparativamente com a circulação sistémica (Sarnoff e Berglund 1954). As baixas pressões da circulação pulmonar repercutem-se em menor trabalho de ejecção e pressão intraventricular direitas (Sarnoff e Berglund 1954), bem como em massa miocárdica e espessura parietal inferiores (Foale et al. 1986; Lorenz et al. 1999). O ventrículo direito, cujo volume tele-diastólico é superior ao do ventrículo esquerdo (Sheehan e Redington 2008), adapta-se facilmente a alterações abruptas de pré-carga e retorno venoso, por exemplo, durante as flutuações inerentes à ventilação (Sheehan e Redington 2008), uma vez que é mais complacente que o ventrículo esquerdo (Joyce et al. 2000). Está, portanto, bem adaptado para encurtar e ejectar facilmente sangue contra as resistências reduzidas do leito vascular pulmonar. No entanto, contrariamente ao que sucede com o ventrículo esquerdo, a espessura parietal reduzida e o menor trabalho de ejecção que é capaz de desenvolver tornam-no

relativamente incapaz de desenvolver pressões elevadas e, por conseguinte, de ultrapassar elevações agudas de pós-carga. De facto, elevações agudas da pressão na artéria pulmonar superiores a 40-50 mmHg desencadeiam diminuição da fracção de ejecção, dilatação ventricular direita e falência cardíaca direita (Guyton et al. 1954).

Fisiopatologia da disfunção ventricular direita na hipertensão pulmonar

A insuficiência cardíaca direita pode definir-se, numa analogia com a insuficiência cardíaca esquerda, como um síndrome complexo, de etiologia diversificada, que surge como consequência de qualquer perturbação anatómica ou funcional que limita a capacidade de preenchimento ou de ejecção do ventrículo direito, cursando com retenção de fluidos, redução do débito cardíaco e maior risco de arritmias (Haddad et al. 2008; Voelkel et al. 2006).

Contrariamente ao que sucede nas elevações agudas da pressão da artéria pulmonar, o ventrículo direito consegue adaptar-se a elevações progressivas de carga. De qualquer modo, a sobrecarga crónica de volume é melhor tolerada (Marino et al. 1985), ao passo que a sobrecarga de pressão normalmente progride rapidamente para insuficiência cardíaca direita, com excepção dos casos particulares em que esta é exercida desde o nascimento, sobre o miocárdio ventricular direito do recém-nascido, que apresenta maior massa relativa e está adaptado às pressões circulatórias pulmonares elevadas do período fetal, como acontece em muitas cardiopatias congénitas (Hopkins 2005).

O miocárdio ventricular direito adapta-se cronicamente à sobrecarga de pressão através de hipertrofia, da adopção de uma geometria mais elipsóide e da dilatação da sua cavidade, que resultam numa redução da tensão parietal e recrutamento da reserva de pré-carga (Boxt et al. 1992; Horan et al. 1981). O miocárdio ventricular direito hipertrofiado aumenta o seu trabalho de ejecção e a capacidade de desenvolvimento de pressão por forma a ultrapassar as resistências vasculares pulmonares, preservando, assim, o débito cardíaco. No entanto, apesar das alterações compensatórias iniciais o miocárdio ventricular direito acaba por entrar num círculo vicioso de descompensação com dilatação ventricular e redução do débito cardíaco. A nível do cardiomiócito, os mecanismos moleculares subjacentes a esta progressão terão semelhanças com os mecanismos de adaptação do ventrículo esquerdo à sobrecarga de pressão, como sucede, por exemplo, na hipertensão arterial sistémica, e posterior descompensação, mas relativamente poucos estudos documentam quais as modificações efectivamente envolvidas (Bogaard et al. 2009a).

Para além da sobrecarga, mecanismos adicionais devem estar implicados, uma vez que a constrição da artéria pulmonar raramente desencadeia falência ventricular direita (Bogaard et

al. 2009b). Foi claramente demonstrada a contribuição da isquemia em doentes com hipertensão arterial pulmonar (Gomez et al. 2001). Realmente, enquanto o ventrículo direito é usualmente perfundido tanto durante a sístole como durante a diástole, uma vez que a pressão aórtica é sempre superior à pressão ventricular direita, na hipertensão pulmonar pode não suceder o mesmo (Urabe et al. 1985), adicionalmente, o consumo energético do miocárdio sobrecarregado aumenta contribuindo para o desequilíbrio entre consumo e aporte de oxigénio (Cross 1962). A progressão de hipertrofia compensatória para disfunção não é uniforme na população de doentes com hipertensão pulmonar, poderão contribuir para esta heterogeneidade diferenças de predisposição genética, relacionadas, por exemplo, com polimorfismos no gene da enzima de conversão da angiotensina (Abraham et al. 1995), e graus diversos de activação neuroendócrina (Nootens et al. 1995), e inflamatória (Sharma et al. 2003).

Como mecanismo adicional de agravamento da doença, a dilatação ventricular direita e o desenvolvimento de pressões progressivamente mais elevadas, com elevação da tensão parietal (Quaife et al. 2006), condicionam regurgitação tricúspide por dilatação do anel e incompetência do aparelho valvular. Esta contribui para uma sobrecarga adicional de volume e para um círculo vicioso de dilatação e disfunção ventricular e congestionamento sistémico (Voelkel et al. 2006).

Disfunção ventricular esquerda na hipertensão pulmonar

O fenómeno de interacção ventricular corresponde ao comprometimento da função, ou débito, de um ventrículo secundária à hipertrofia, distensão, elevação de pressões ou disfunção do ventrículo contralateral. Compreende dois mecanismos distintos, um de interacção em série, pelo qual a queda do débito de um ventrículo condiciona uma redução da pré-carga do outro, e outro de interacção em paralelo, mediado pelo septo interventricular, pericárdio e fibras musculares partilhadas pelos dois ventrículos, que também é designado por interdependência ventricular (Morris-Thurgood e Frenneaux 2000). Originalmente proposta por *Bernheim*, no início do Século XX, como potencial mecanismo explicativo para o congestionamento venoso sistémico na insuficiência cardíaca, o ventrículo esquerdo dilatado limitaria a complacência ventricular direita e o retorno venoso (Santamore e Dell'Italia 1998). O efeito complementar, pelo qual a distensão e hipertensão ventricular direitas comprometem a função ventricular esquerda recebe, ainda hoje, frequentemente, a designação de efeito de *Bernheim* reverso. A dilatação e elevação sustentada da tensão parietal ventricular direita comprometem a função ventricular esquerda. Diversos mecanismos têm sido apontados como responsáveis. Classicamente, o desvio do septo interventricular para a esquerda, que se traduz em rectilinização ou deslocamento paradoxal, em conjunto com a restrição pericárdica, uma vez que

o ventrículo direito dilatado partilha o mesmo espaço físico com o ventrículo esquerdo, contribuem para a redução efectiva da complacência e a perturbação do preenchimento ventricular esquerdo. O mecanismo fundamental parece ser a persistência de pressão intracavitária e elevação do volume ventricular direito durante o relaxamento isovolumétrico e preenchimento precoce do ventrículo esquerdo, como consequência do prolongamento do tempo de ejecção e contracção do ventrículo direito face à maior resistência vascular e impedância do leito vascular pulmonar (Triffon et al. 1988). Durante o preenchimento inicial, o volume e geometria do ventrículo esquerdo são, então, perturbados, sendo claramente evidente o atraso na abertura da válvula mitral e início do preenchimento precoce (Stojnic et al. 1992). No entanto, com a progressão da doença, também o débito do ventrículo direito diminui, condicionando uma redução da pré-carga ventricular esquerda (Gurudevan et al. 2007), e das próprias pressões da artéria pulmonar, sendo talvez por este facto que os melhores preditores de prognóstico não são os níveis de pressão arterial pulmonar mas sim o débito cardíaco e os índices de função ventricular direita (Sandoval et al. 1994). O envolvimento ventricular esquerdo assume particular importância uma vez que a hipoperfusão coronária secundária à hipotensão pode contribuir para o agravamento da disfunção ventricular direita, num círculo vicioso que frequentemente precipita eventos fatais (Zamanian et al. 2007).

Para além dos mecanismos mecânicos e hemodinâmicos, várias evidências sugerem que factores adicionais possam contribuir para a disfunção ventricular esquerda. Assim, na hipertensão pulmonar crónica severa, mesmo depois da reversão das alterações de geometria ventricular, após transplantação pulmonar, a disfunção ventricular esquerda persiste frequentemente (Xie et al. 1998), o que motiva a prática de transplantação cardiopulmonar conjunta na maior parte dos casos de hipertensão pulmonar severa com disfunção ventricular esquerda concomitante (Pielsticker et al. 2001). Estes dados poderão ser interpretados admitindo que o miocárdio ventricular esquerdo sofre um processo de remodelagem no contexto da hipertensão pulmonar. Algumas evidências experimentais e anatomopatológicas sugerem isso mesmo (Laks et al. 1969). A redução sustentada de pré-carga, ou *unloading*, poderá originar esta remodelagem (Lisy et al. 2000). Por outro lado, não seria surpreendente que mecanismos neurohumorais pudessem mediar remodelagem ventricular esquerda. Gomez *et al*. propuseram que o ventrículo direito, no contexto de sobrecarga, libertasse uma substância que actuasse no ventrículo esquerdo (Gomez et al. 1993). No entanto, estas hipóteses não foram devidamente testadas ou fundamentadas experimentalmente.

A avaliação funcional do miocárdio ventricular esquerdo no contexto de hipertensão pulmonar e hipertrofia ou falência ventricular direita é uma tarefa extremamente complexa, será difícil

destrinçar qual o seu estado funcional intrínseco uma vez que este se encontra sujeito a mediação neuroendócrina sistémica e local e interacção ventricular. Estas limitações podem contornar-se por metodologias de avaliação *in vitro*, nomeadamente em preparações de coração isolado (Lamberts et al. 2007) e de feixes musculares isolados (Kogler et al. 2003), e *in vivo*, utilizando índices funcionais independentes da carga (Faber et al. 2006). Actualmente, a melhor avaliação funcional ao nível de feixes musculares isolados é a relação força-frequência (Endoh 2004). A elevação da tensão desenvolvida pelos feixes musculares para frequências de estimulação crescentes é uma resposta fisiológica e um mecanismo importante de regulação da contractilidade que reflecte a reserva contráctil, de forma independente da carga, constituindo uma metodologia adequada para aferir a gravidade da disfunção contráctil do miocárdio: o miocárdio insuficiente apresenta, caracteristicamente, relações força-frequência negativas (Endoh 2004). Contudo, a obtenção de relações força-frequência positivas no miocárdio saudável depende de uma técnica experimental extremamente cuidadosa em que a manutenção da temperatura e oxigenação permanente dos feixes musculares, a utilização de baixas concentrações de cálcio e a dissecção de feixes extremamente finos de cardiomiócitos (evitando a hipóxia das regiões centrais) são fulcrais (Layland e Kentish 1999). Quanto à utilização de índices funcionais independentes da carga convencionais, derivados das relações pressão-volume tele-sistólica e tele-diastólica, que são o padrão na avaliação hemodinâmica invasiva da função intrínseca dos ventrículos (Burkhoff et al. 2005). Estes índices são teoricamente independentes da carga, uma vez que são obtidos com reduções gradativas da pré-carga, durante oclusões agudas da veia cava inferior, no entanto, em grupos experimentais com níveis basais de pré-carga distintos, as variações suscitadas pelas oclusões podem não abranger o mesmo âmbito de pressões e, deste modo, os grupos dificilmente serão comparáveis (Burkhoff et al. 2005).

Tolerância diastólica à pós-carga

Leite-Moreira *et al*. desenvolveram um índice funcional que avalia a reserva funcional diastólica por elevação abrupta de pós-carga, forçando a contracção isovolumétrica em ciclos cardíacos isolados, designado por tolerância diastólica à pós-carga. Este índice, de obtenção simples, embora nunca tenha sido aplicado na patologia, ou no ser humano, é teoricamente um índice funcional independente quer da pós-carga quer da pré-carga, que poderá ultrapassar esta limitação (Leite-Moreira e Gillebert 1994; Leite-Moreira et al. 1999). Adicionalmente, correlaciona-se tanto com a função diastólica como com a função sistólica (Gillebert et al. 1997).

O relaxamento miocárdico é um processo dinâmico que se segue à contracção mas se inicia ainda durante a ejecção ventricular. O miocárdio saudável responde compensatoriamente a elevações ligeiras de pós-carga (Leite-Moreira e Gillebert 1994), mas nas elevações mais acentuadas de pós-carga o relaxamento lentifica-se, estando esta lentificação relacionada com a função sistólica (Eichhorn et al. 1992). A transição entre aceleração e lentificação do relaxamento ocorre a um nível de carga relativa (que pode definir-se pelo cociente entre pressão máxima desenvolvida num ciclo pós-carregado e pressão máxima desenvolvida numa contracção isovolumétrica), que reflecte a reserva de pós-carga (Gillebert et al. 1997). No miocárdio saudável o relaxamento está completo antes do final da diástole (Leite-Moreira e Gillebert 1994; Weisfeldt et al. 1978), portanto o principal mecanismo subjacente à subida das pressões tele-diastólicas na disfunção diastólica é a maior rigidez miocárdica (Gaasch e Zile 2004; Leite-Moreira 2006). Durante décadas advogou-se que por maior que fosse a perturbação do relaxamento, simples alterações do relaxamento não poderiam ser responsáveis pela elevação das pressões tele-diastólicas (Gilbert e Glantz 1989), no entanto, evidências mais recentes, obtidas experimentalmente, demonstram que, nos extremos de carga, mesmo o miocárdio saudável pode desenvolver elevação das pressões tele-diastólicas como consequência da lentificação do relaxamento (Leite-Moreira e Correia-Pinto 2001). No entanto, estes resultados experimentais ainda não foram reproduzidos em modelos experimentais de insuficiência cardíaca ou no ser humano.

Activação neuroendócrina na hipertensão pulmonar

De forma análoga à insuficiência cardíaca, no curso da hipertensão pulmonar crónica são activados vários sistemas neuroendócrinos que contribuem para a fisiopatologia deste síndrome. Os mediadores neuroendócrinos que se associam à evolução da disfunção cardiomiocitária são produzidos tanto localmente, pelo miocárdio sobrecarregado, como em outros tecidos, como resposta à hipoperfusão sistémica (Baker et al. 1990; Ferrari et al. 1996). Efectivamente, os mediadores neuroendócrinos são activados precocemente na sequência de eventos que medeia entre a sobrecarga e a hipertrofia ventricular (Roncon-Albuquerque et al. 2006), e têm sido extensivamente investigados na insuficiência cardíaca. Destacam-se a angiotensina II e a endotelina-1, ambas factores parácrinos de crescimento libertados agudamente após sobrecarga miocitária (Ito et al. 1993; Sadoshima et al. 1993). Estes mediadores desempenham um papel primordial não só na resposta hipertrófica mas também na remodelagem miocárdica adversa e disfunção miocárdica (Baker et al. 1990; Rothermund et al. 2000). Com o conhecimento crescente nesta área o papel destes mediadores neuroendócrinos a nível local e tecidular assume cada vez maior relevância, em detrimento das suas acções

sistémicas (Takemoto et al. 1997). E várias vias e mecanismos subcelulares e moleculares envolvidos nas acções destes mediadores encontram-se já bem estabelecidos (Dorn e Force 2005). O envolvimento de mediadores neuroendócrinos na progressão da hipertensão pulmonar e falência ventricular direita não foi alvo de avaliação tão detalhada, mas é também reconhecido, tanto a nível experimental (Miyauchi et al. 1993) como a nível clínico (Nootens et al. 1995).

Papel da endotelina-1 e efeitos farmacológicos dos seus antagonistas

Os conhecimentos iniciais de patogenia levaram à construção de uma hipótese fisiopatológica para a hipertensão pulmonar sustentada no desequilíbrio entre agentes vasodilatadores e vasoconstritores (Christman et al. 1992). Nomeadamente, uma redução da actividade da prostaciclina e no óxido nítrico, que preponderam no controlo do tono vascular pulmonar em condições fisiológicas, e uma elevação da endotelina-1 e tromboxano A_2. Entre os mediadores neuroendócrinos envolvidos na fisiopatologia da hipertensão pulmonar com acção vasoconstritora pulmonar destaca-se a endotelina-1, que para além da acção vasoconstritora sistémica e pulmonar também tem acções inotrópicas positivas no miocárdio saudável (Brunner et al. 2006). A activação sustentada do sistema da endotelina-1, quer nos vasos pulmonares, quer no miocárdio, é um elemento importante na fisiopatologia da maior parte das formas de hipertensão pulmonar, resultando, para além da vasoconstrição, em remodelagem deletéria (Brunner et al. 2006). Adicionalmente, na activação sustentada o seu efeito inotrópico positivo torna-se irrelevante (Mollmann et al. 2007).

O sucesso do antagonismo de sistemas neuroendócrinos na terapêutica da insuficiência cardíaca e dos síndromes coronários agudos (Rehsia e Dhalla 2010), que também foi reproduzido para o antagonismo da endotelina-1 em modelos experimentais (Mulder et al. 1997), não teve igual impacto na aplicação clínica a pacientes com insuficiência cardíaca (Kalra et al. 2002; O'Connor e Colaboradores 2003). Na hipertensão pulmonar, no entanto, o antagonismo crónico da endotelina-1 obteve grande sucesso terapêutico, tendo sido talvez a principal evolução terapêutica pela possibilidade de administração cómoda, por via oral, com benefícios claramente demonstrados na capacidade funcional e hemodinâmica (Channick et al. 2001), e no adiamento da deterioração clínica (Rubin et al. 2002). Efectivamente, os antagonistas da endotelina-1 tiveram bons resultados como fármacos de primeira linha em doentes em classe funcional III modificada da Organização Mundial de Saúde (Provencher et al. 2006). e também em classes funcionais inferiores, e, portanto, com doença menos grave (Galie et al. 2008), e possivelmente melhorarão a sobrevida, de acordo com comparações efectuadas com coortes

históricas (Sitbon et al. 2005). No entanto, muitos dos seus mecanismos de acção a nível subcelular e molecular encontram-se ainda por esclarecer, particularmente no que concerne às suas acções miocárdicas (Bogaard et al. 2009a).

Apesar da disponibilidade oral ser uma vantagem fundamental na terapêutica crónica da hipertensão pulmonar, que esteve, em boa parte, na base da rápida inclusão do *Bosentan*, um antagonista dual, ou não selectivo, da endotelina-1, na prática médica, esta deixa de o ser na terapêutica aguda, por exemplo durante intervenções cirúrgicas e no ambiente de cuidados intensivos. No entanto, a hipertensão pulmonar é um síndrome muito frequente e com consequências marcantes precisamente nestes contextos em que a via oral muitas vezes não está disponível e em que são desejáveis fármacos com tempos de latência e duração de acção curtos, preferencialmente administráveis por via endovenosa e em perfusão, sendo a velocidade de infusão titulada para obter um efeito hemodinâmico desejado (Blaise et al. 2003; Kerbaul et al. 2005; Zamanian et al. 2007). De facto, a hipertensão pulmonar constitui um risco importante nos doentes críticos (McNeil et al. 2003) e cirúrgicos, tanto na cirurgia cardíaca(Reich et al. 1999) como na cirurgia não cardíaca (Ramakrishna et al. 2005), e a doença crítica e intervenções cirúrgicas constituem factores de risco para agudização da hipertensão pulmonar (Kerbaul et al. 2005), no entanto a investigação nesta área limita-se a pequenos estudos em que se testaram a dobutamina, o levosimendan, a milrinona, os inibidores da fosfodiestérase, os prostanóides e o óxido nítrico. Os antagonistas da endotelina-1 também já foram testados, principalmente no contexto da hipertensão pulmonar aguda. O *Tezosentan*, um antagonista não selectivo da endotelina-1, desenvolvido para administração por perfusão endovenosa, de curta latência e efeito rápido (Clozel 1999), cuja segurança e tolerabilidade já foi demonstrada em doentes com insuficiência cardíaca (O'Connor e Colaboradores 2003), foi empregue com sucesso em modelos experimentais de hipertensão pulmonar aguda em animais endotoxémicos (Konrad 2007, 2004), com lesão pulmonar aguda induzida pelo ácido oleico (Wang et al. 2004), com hipertensão pulmonar aguda induzida pelo tromboxano (Fitzgerald et al. 2004) e por aspiração de mecónio (Geiger 2008, 2006). Foi também eficaz na redução das pressões da artéria pulmonar num modelo experimental de hipertensão pulmonar sustentada por hiperfluxo em cordeiros recém-nascidos (Fitzgerald et al. 2004), mas os seus efeitos na hipertensão pulmonar crónica, quer no contexto experimental quer no âmbito clínico, por exemplo como fármaco de substituição do *Bosentan* no período perioperatório ou durante o internamento em cuidados intensivos ainda não foram investigados satisfatoriamente. De facto, apenas dois trabalhos abordam esta questão, um ensaio clínico de fase III, levado a cabo em doentes submetidos a cirurgia cardíaca com hipertensão pulmonar pré-operatória secundária a doença cardíaca esquerda, aos quais foi

administrado *Tezosentan* nas primeiras 24h pós-operatórias (AC-051-350), que foi interrompido por ausência de diferenças, e um ensaio com *Sitaxsentan*, um antagonista selectivo dos receptores ET_A em que se obtiveram melhorias hemodinâmicas na separação da circulação extracorporal durante a cirurgia de revascularização miocárdica (Joffs et al. 2001; Toole et al. 2010).

Os mecanismos farmacológicos de acção vascular pulmonar e miocárdica dos antagonistas da endotelina-1 não estão bem caracterizados a nível funcional e molecular. A maior parte dos vasodilatadores pulmonares é igualmente inotrópica negativa, seja por acção directa no miocárdio, seja por acção indirecta induzindo hipotensão sistémica e menor perfusão coronária (Zamanian et al. 2007), ou simplesmente pela redução da pós-carga (Rex et al. 2008). A vasodilatação na hipertensão pulmonar também agrava frequentemente o acoplamento ventilação-perfusão e favorece o desenvolvimento de hipóxia (Blanco et al. 2010). No caso dos antagonistas da endotelina-1 estes efeitos farmacológicos encontram-se pouco explorados. Sabe-se que o *Bosentan* parece preservar a contractilidade e o acoplamento ventrículo-vascular (Rondelet et al. 2003) e que parte do seu efeito vasoactivo pulmonar se deve à maior actividade pulmonar da síntase do óxido nítrico constitutiva (Girgis et al. 2005). No entanto, outros mecanismos moleculares, particularmente as acções miocárdicas ainda não são conhecidas. Sabe-se, no entanto, que a endotelina-1 é um importante activador inflamatório (Juergens et al. 2008) e que em modelos experimentais o antagonismo agudo da endotelina-1 teve importantes efeitos anti-inflamatórios (Gamze et al. 2007).

Activação inflamatória na hipertensão pulmonar

Apesar da perspectiva fisiopatológica da hipertensão pulmonar como resultante do desequilíbrio entre vasoconstritores e vasodilatadores pulmonares ter contribuído de forma decisiva para o advento da maior parte dos fármacos que são correntemente utilizados na terapêutica da hipertensão pulmonar crónica, nomeadamente prostanóides, antagonistas da endotelina-1 e inibidores das fosfodiesterases, esta é francamente incompleta. De facto, as evidências mais recentes apontam para a interpretação fisiopatológica deste síndrome, principalmente na forma de hipertensão pulmonar arterial, como vasculopatia, em que ocorre uma extensa remodelagem das arteríolas pulmonares, infiltração por células inflamatórias e activação inflamatória local, e se desenvolvem lesões angioproliferativas (McLaughlin et al. 2009). O desenvolvimento de linhas celulares endoteliais resistentes à apoptose, a interacção entre as células endoteliais e as células musculares lisas vasculares através da acção de factores de crescimento e a transdiferenciação, quer de células endoteliais quer de fibroblastos em células musculares lisas, contribuem para a

remodelagem no sentido da hipertrofia e hiperplasia da camada muscular, que contribuem para um aumento progressivo das resistências vasculares independentemente da mediação vasoactiva (Sakao et al. 2009). Adicionalmente, as células vasculares pulmonares adquirem um fenótipo metabólico semelhante ao descrito para as neoplasias, o fenótipo de *Warburg*, no qual domina a glicólise anaeróbia. Este fenótipo altera vias de sinalização intracelular e favorece a resistência à apoptose, a proliferação e a vasoconstrição (Bonnet et al. 2006).

A activação inflamatória local, por outro lado, deixou de ser encarada como uma mera consequência da patologia, e passou a ser entendida como um factor fundamental na sua progressão (Dorfmuller et al. 2003). Várias citocinas envolvidas na patogenia das doenças inflamatórias crónicas e das doenças neoplásicas, como, por exemplo, o factor de necrose tumoral-α e a interleucina-6, desempenham um importante papel no desenvolvimento e progressão não só das alterações vasculares pulmonares, como possivelmente também da remodelagem e disfunção miocárdica e das manifestações sistémicas da hipertensão pulmonar (Sharma et al. 2003), de forma análoga ao que ocorre na insuficiência cardíaca esquerda (Dunlay et al. 2008; von Haehling et al. 2004). O papel das citocinas inflamatórias, dos infiltrados inflamatórios perivasculares e de mecanismos auto-imunitários foi inicialmente demonstrado na hipertensão arterial pulmonar associada a doenças do tecido conjuntivo, mas, posteriormente, também noutras formas de hipertensão arterial pulmonar e mesmo nas formas não arteriais (Dorfmuller et al. 2003), tendo um papel particularmente importante na progressão das lesões vasculares pulmonares da doença pulmonar obstrutiva crónica (Joppa et al. 2006; Sin e Man 2006).

Metabolismo no miocárdio insuficiente

Na progressão para a insuficiência cardíaca os cardiomiócitos sofrem um conjunto de alterações que conduzem a uma perda progressiva de função. Entre estas destacam-se a maior expressão das isoformas β das cadeias pesadas de miosina, a perda progressiva de miofilamentos contrácteis, as alterações nas proteínas do citosqueleto, as alterações das proteínas reguladores do acoplamento excitação-contracção e cinética do cálcio e a dessensibilização das vias de sinalização do sistema simpático-adrenérgico (Dhalla et al. 2009; Lehnart et al. 2009). No entanto, mais recentemente, para além destas alterações clássicas de remodelagem miocárdica, reconhece-se um papel fundamental de perturbações metabólicas. Nos estádios mais avançados da insuficiência cardíaca o miocárdio apresenta uma redução do seu conteúdo de trifosfato de adenosina e converte de forma ineficiente energia em trabalho mecânico, em boa parte pela perturbação do metabolismo oxidativo (Ingwall 2009; Ormerod et al. 2008) e pelo desvio

metabólico que favorece a glicólise em detrimento da oxidação de ácidos gordos (Davila-Roman et al. 2002). Embora esta questão seja controversa, é opinião quase consensual que a disfunção metabólica está envolvida causalmente na disfunção e remodelagem miocitárias (Stanley et al. 2005). Assim sendo, novas armas terapêuticas que tenham como alvo a regulação do metabolismo poderão ter um impacto considerável no prognóstico da insuficiência cardíaca, uma vez que actuarão por intermédio de vias moleculares distintas das dos fármacos actualmente em uso, sem efeito inotrópico negativo (Bristow 2000). Como mera curiosidade, relatos históricos descreveram uma melhoria sintomática na insuficiência cardíaca após ingestão de açúcar de cana (Goulston 1911). Estes mecanismos têm sido fundamentalmente investigados na insuficiência cardíaca esquerda, mas tudo leva a crer que sejam também relevantes na insuficiência cardíaca direita e hipertensão pulmonar.

No miocárdio saudável, o aporte constante do oxigénio é fundamental para o metabolismo energético, mais precisamente para a fosforilação oxidativa, que é responsável por cerca de 90% da produção de trifosfato de adenosina (Ventura-Clapier et al. 2004). Vários substratos energéticos, como ácidos gordos, glícidos e lactato podem ser metabolizados pelo ciclo do ácido tricarboxílico miocárdico gerando equivalentes redutores (Bing et al. 1954). A produção de trifosfato de adenosina adapta-se ao consumo e às necessidades, de acordo com o trabalho desenvolvido pelo miocárdio, podendo a actividade mitocondrial aumentar cerca de 90% (Mootha et al. 1997). Usualmente, no miocárdio adulto, em condições de repouso a β-oxidação de ácidos gordos é responsável por até 60 a 90% da produção de trifosfato de adenosina, enquanto que a oxidação do piruvato e lactato oriundos da glicólise são responsáveis pelos restantes 10 a 40% (Wisneski et al. 1985), no entanto, os corações do feto e recém-nascido, hipóxicos, dependem mais da glicólise anaeróbia, uma vez que os sistemas enzimáticos responsáveis pela oxidação de ácidos gordos têm maturação tardia (Ostadal et al. 1999). Normalmente, na presença de ácidos gordos é inibida a glicólise no miocárdio adulto, de acordo com a concepção original de *Randle* (Garland et al. 1963). De facto, a oxidação de ácidos gordos aumenta as razões acetil-CoA/CoA livre e NADH/NAD+, o que activa a cínase da desidrogénase do piruvato, que, por sua vez, fosforila e , desta forma, inibe a desidrogénase do piruvato. Durante o metabolismo glicolítico, a glicose-6-fosfato produz duas moléculas de trifosfato de adenosina e é convertida em piruvato. O complexo enzimático da desidrogénase do piruvato é responsável pelo passo limitante da glicólise aeróbia, a descarboxilação oxidativa irreversível do piruvato em acetil-CoA e a internalização desta na mitocôndria (Stanley et al. 2005). Caso não haja internalização na mitocôndria, o piruvato é convertido em lactato no citoplasma (glicólise não-oxidativa ou anaeróbia). O miocárdio saudável é capaz de adaptar-se rapidamente não só às

exigências metabólicas mas também à disponibilidade de substratos, o que depende de mecanismos de regulação enzimática alostéricos, acção de metabolitos reguladores e uma reciprocidade efectiva entre os substratos. Por exemplo, sempre que os níveis plasmáticos de corpos cetónicos se elevam, como sucede na insuficiência cardíaca, estes são extraídos e oxidados, em detrimento de outros substratos (Lommi et al. 1998).

No entanto, com a progressão da patologia cardiovascular ocorrem alterações sustentadas na expressão e actividade enzimáticas, sob a coordenação de factores de transcrição como o factor de transcrição indutível pela hipoxia, que acentua o metabolismo glicolítico (Semenza 2000), e o receptor dos peroxissomas activado por estímulos proliferativos, que estimula a fosforilação oxidativa de ácidos gordos e inibe a glicólise (Finck 2007; Gulick et al. 1994). Na progressão da insuficiência cardíaca está inequivocamente demonstrada uma menor actividade do receptor dos peroxissomas activado por estímulos proliferativos e um desvio metabólico correspondente, favorecendo a glicólise em detrimento da oxidação de ácidos gordos (Barger et al. 2000; Garnier et al. 2003). Todavia, o significado biológico desta adaptação ainda não é consensual. Se por um lado, a glicólise é mais eficiente em termos de produção de trifosfato de adenosina em função do consumo de oxigénio, o que satisfaz as necessidades energéticas acrescidas do miocárdio hipertrofiado e insuficiente (Burkhoff et al. 1991; Stanley et al. 2005), sendo a eficiência menor na presença de concentrações elevadas de ácidos gordos uma vez que estes induzem algum grau de desacoplamento da fosforilação oxidativa, por intermédio de proteínas desacopladoras (Schrauwen et al. 2003), por outro lado o metabolismo de ácidos gordos gera maior número de moléculas de trifosfato de adenosina (van Bilsen et al. 2009) e o desenvolvimento de trabalho mecânico é extremamente dependente dos ácidos gordos em doentes com insuficiência cardíaca (Tuunanen et al. 2006). Também quanto ao papel do receptor dos peroxissomas activado por estímulos proliferativos, há evidências contraditórias, a nível experimental a sua activação tanto agravou a disfunção contráctil e a hipertrofia (Young et al. 2001) como as inibiu (Ogata et al. 2002), o que poderá dever-se a diferenças nos modelos experimentais e espécies ou estirpes de animais estudadas.

Outra alteração metabólica típica na progressão da insuficiência cardíaca é o desenvolvimento de insulino-resistência (Mamas et al. 2010). Esta constitui um factor preditor independente de mau prognóstico (Doehner et al. 2005). Embora não sejam claros os mecanismos que ligam a insulino-resistência à insuficiência cardíaca, acredita-se que o tecido adiposo e o vasto leque de citocinas e mediadores bioactivos que este produz possam constituir o elo fundamental (Berg e Scherer 2005). Destacando-se o papel de adipocinas e citocinas inflamatórias (Wisniacki et al. 2005).

Caquexia cardíaca

A designação caquexia, da etimologia Grega "kakós" e "hexis", cuja tradução para língua portuguesa é "má condição", foi pela primeira vez aplicada por Hipócrates a casos clínicos graves, provavelmente de insuficiência cardíaca ou hepática (Doehner e Anker 2002). Uma declaração de consenso recente define-a como perda ponderal de pelo menos 5% num intervalo de tempo inferior a 12 meses ou, alternativamente, a redução do índice de massa corporal para valores inferiores a 20 $Kg.m^{-2}$, em conjunto com pelo menos 3 de 5 critérios (diminuição da força muscular, fadiga, anorexia, índice de massa magra baixo, ou alterações analíticas como inflamação, anemia ou albumina sérica reduzida), em doentes que apresentam doenças crónicas (Evans et al. 2008). Várias doenças crónicas podem associar-se a caquexia para além da insuficiência cardíaca, nomeadamente patologia oncológica, doença pulmonar obstrutiva crónica, doenças infecciosas e inflamatórias crónicas. O elemento comum a todas é uma extensa activação inflamatória (Deswal et al. 2001). Efectivamente, tanto a insuficiência cardíaca como a hipertensão pulmonar são síndromes multi-sistémicos que afectam os sistemas musculo-esquelético, imunitário, neuroendócrino e renal (Dorfmuller et al. 2003; Francis 2001). Nos casos mais graves de insuficiência cardíaca, estima-se que cerca de metade dos doentes desenvolvam caquexia (Filippatos et al. 2000), e numa população geral de doentes com insuficiência cardíaca este valor rondará os 15% (Anker e Coats 1999). A caquexia cardíaca é um preditor independente e importante de mau prognóstico, elevando a mortalidade de 17% para 50% aos 18 meses (Anker et al. 1997).

A caquexia é então uma complicação de doenças crónicas e um síndrome com mecanismos fisiopatológicos complexos e ainda incompletamente compreendidos, entre os quais a anorexia, a má-absorção e perturbações gastrointestinais, as alterações do metabolismo e a activação inflamatória e neuroendócrina (von Haehling et al. 2007). A activação inflamatória e as perturbações neuroendócrinas e imunológicas correlacionam-se fortemente com a perda de massa corporal (Anker et al. 2003). O factor de necrose tumoral-α, em particular, poderá constituir a via final comum subjacente à caquexia em todas as doenças crónicas. Inicialmente isolado a partir de murganhos infectados com soro do bacilo de *Calmette-Guérin* após tratamento com lipopolissacarídeo, foi designado "caquexina" porque mimetizava a acção de necrose tumoral do LPS e inibia a lípase das lipoproteínas, pressumindo-se que desempenharia um papel na caquexia cardíaca (Beutler et al. 1985). Sabe-se hoje que os seus efeitos integram as principais alterações que acompanham a progressão da insuficiência cardíaca, tais como a disfunção ventricular, a remodelagem ventricular, a apoptose dos cardiomiócitos, o desenvolvimento de anorexia e caquexia, a redução do fluxo sanguíneo muscular, a disfunção

endotelial e a insulino-resistência (Meldrum 1998). A anorexia é também um elemento comum a todas as formas de caquexia. Na caquexia cardíaca os pacientes apresentam redução de apetite e da ingestão alimentar, o que poderá dever-se parcialmente ao aumento da actividade da melanocortina e à sensação de saciedade induzidas por citocinas pró-inflamatórias (Inui 1999). No caso da caquexia cardíaca, o tracto gastrointestinal parece desempenhar um papel fulcral. O edema e hipoperfusão gastrointestinal contribuem para a má-absorção (Witte e Clark 2002) e perda de proteínas, particularmente nos casos de falência direita. Nas condições mais extremas, os pacientes apresentam enteropatia com perda de proteínas e hipoproteinemia (Thorne et al. 1998). Por outro lado, o edema da parede visceral (Niebauer et al. 1999), a maior permeabilidade resultante quer da hipoperfusão e isquemia da mucosa (Krack et al. 2005),e também da acção de citocinas (Rauchhaus et al. 2000a) facilitam a translocação bacteriana. A translocação bacteriana, de acordo com a visão prevalecente, será a principal responsável pela activação inflamatória na insuficiência cardíaca severa (Rauchhaus et al. 2000a; Wagner et al. 1998), embora alguns autores proponham mecanismos alternativos, designadamente a hipoperfusão e hipóxia tecidular (Tsutamoto et al. 1998).

Quanto ao factor de necrose tumoral-α, o principal factor de transcrição subjacente quer à sua actividade celular quer à indução da sua síntese é o factor de transcrição nuclear-κB, ou factor de transcrição das cadeias leves κ de células B activadas. A activação deste factor de transcrição tem sido associada a vários processos patológicos que acompanham a progressão da insuficiência cardíaca, incluindo a libertação de citocinas pelos cardiomiócitos, o stress oxidativo, a hipertrofia e a remodelagem do miocárdio (Valen et al. 2001). Esta família de factores de transcrição é responsável pela regulação de mais de 150 genes alvo. Na maioria das células, os complexos de factor de transcrição nuclear-κB estão presentes no citoplasma sob a forma inactiva, ligada ao inibidor-κB. Vários estímulos, entre os quais o lipopolissacarídeo e citocinas, resultam na fosforilação, ubiquitinação e degradação subsequente do I-κB, libertando um conjunto de aminoácidos no NF-κB que promove a translocação nuclear e a ligação a elementos de resposta específicos dos genes alvo nos cardiomiócitos (Valen et al. 2001).

A prevalência e o papel da caquexia cardíaca em doentes com hipertensão pulmonar estão ainda pouco explorados, no entanto, a sua consequência terminal, a insuficiência cardíaca direita, associa-se, como todas as formas de insuficiência cardíaca, a caquexia cardíaca, ainda mais porque na insuficiência cardíaca direita predomina o quadro de congestionamento sistémico e da mucosa gastrointestinal (Ajayi et al. 1999; Thorne et al. 1998). Apesar de tudo, estudos clínicos de pequena magnitude têm sugerido uma prevalência elevada da caquexia nestas populações de doentes (Habedank et al. 2009; le Roux et al. 2005).

Nutrição na insuficiência cardíaca e caquexia

O papel da nutrição como factor de risco cardiovascular está bem estabelecido. A *American Heart Association* recomenda a redução do total de calorias ingeridas, a redução da ingestão de gorduras, especialmente saturadas e colesterol, bem como o aumento da ingestão de legumes, frutas e produtos lácteos com baixo teor em gordura (Azhar e Wei 2006; Krauss et al. 2000). A avaliação clínica de períodos prolongados de restrição calórica em pacientes não-obesos corrobora estas recomendações, uma vez que se observaram melhorias na função cardiovascular (Meyer et al. 2006), metabolismo, função de diversos órgãos (Chou et al. 2010) e prevenção de aterosclerose (Fontana et al. 2004). No entanto, estas recomendações poderão não ser aplicáveis à insuficiência cardíaca grave, associada a perturbações do metabolismo cardiomiocitário e caquexia. De facto, enquanto a obesidade é reconhecidamente um factor de risco para desenvolvimento de insuficiência cardíaca (Hubert et al. 1983), particularmente insuficiência cardíaca com fracção de ejecção preservada (Powell et al. 2006), na insuficiência cardíaca estabelecida, paradoxalmente, a obesidade torna-se um factor de prognóstico favorável (Abel et al. 2008), que reduz a mortalidade (Oreopoulos et al. 2008). A este achado de estudos epidemiológicos dá-se vulgarmente a designação de "paradoxo da obesidade". Para além dos índices de massa corporal elevados, também níveis elevados de colesterol se associam paradoxalmente a melhor sobrevida (Curtis et al. 2005; Rauchhaus et al. 2003). Como hipótese explicativa, a nível fisiopatológico, para além das diferenças óbvias atribuíveis aos contextos clínicos, ou seja, a obesidade pode constituir um factor de risco no ser humano saudável mas tornar-se um factor protector no caso de doença (Kalantar-Zadeh et al. 2004), tem sido apontada a hipótese da lipoproteína-endotoxina. Segundo esta hipótese, as lipoproteínas ligam-se e neutralizam os lipopolissacarídeos circulantes, protegendo o organismo da resposta inflamatória (Rauchhaus et al. 2000b). As diversas classes de lipoproteínas ligam-se aos lipopolissacarídeos proporcionalmente ao seu conteúdo de colesterol (Flegel et al. 1993) e inactivá-lo-ão por intermédio da formação de micelas (Wurfel et al. 1994). A menor disponibilidade de lipopolissacarídeos diminui a libertação de citocinas inflamatórias quer por células mononucleares, mediada pelo ligação ao receptor *toll-like* 4 e ao CD14 (Beutler 2000), quer pelos cardiomiócitos (Wagner et al. 1998). Estes mecanismos paradoxais sugerem que dietas hipercalóricas, do tipo Ocidental, ricas em lípidos saturados e glícidos simples, que constituem factores de risco para obesidade, síndrome metabólico e insuficiência cardíaca, possam ter efeitos distintos na insuficiência cardíaca estabelecida, particularmente quando complicada por caquexia. Deveras, sabemos que o suporte nutricional pode contrariar a anorexia das doenças crónicas e prevenir parcialmente a caquexia (Laviano et al. 2005), sendo a

subnutrição proteico-energética um aspecto crescentemente investigado em doenças crónicas e na caquexia cardíaca (Akner e Cederholm 2001; Azhar e Wei 2006). O papel terapêutico de estimulantes do apetite e de suplementação entérica tem sido investigado, com resultados favoráveis (von Haehling et al. 2009), particularmente na doença crítica (Stapleton et al. 2007).

Para além dos efeitos na caquexia, a dieta pode influenciar de forma marcada a progressão da insuficiência cardíaca, tanto no aspecto funcional metabólico como em termos de remodelagem miocárdica. Vários trabalhos experimentais têm investigado o papel de nutrientes particulares e, em muitos destes, surpreendentemente, nutrientes que têm sido conotados com doença cardiovascular, não apresentam efeitos deletérios (Sharma et al. 2007). Assim, uma dieta rica em lípidos aumentou a fosforilação oxidativa mitocondrial, desviando os ácidos gordos de vias citotóxicas, sem agravar a progressão da insuficiência cardíaca num modelo experimental de enfarte do miocárdio (Rennison et al. 2007), e dietas ricas em gorduras atenuaram a disfunção ventricular e a remodelagem induzidas pela sobrecarga de pressão, comparativamente com dietas ricas em glícidos (Duda et al. 2008), e a hipertrofia e remodelagem num modelo experimental de hipertensão arterial sistémica, comparativamente com dietas pobres em gorduras (Okere et al. 2005). O papel dos nutrientes não parece estar apenas limitado à alteração de vias metabólicas e modulação da bioenergética miocárdica mas sim ser mais abrangente, modulando vias de sinalização intracelular. Assim, o enriquecimento alimentar em ácidos gordos de cadeia intermédia preserva a função miocárdica e a fosforilação oxidativa, provavelmente por indução do receptor dos peroxissomas activado por estímulos proliferativos (Iemitsu et al. 2008). De facto, este factor de transcrição é activado pelos lípidos nas dietas hipercalóricas (Clarke et al. 1999; Wu et al. 2001).

Modelos experimentais de hipertensão pulmonar

Embora nenhum modelo animal recapitule completamente todas as características da hipertensão pulmonar arterial humana, o estudo da fisiopatologia da hipertensão pulmonar tem sido levado a cabo essencialmente em modelos animais de hipertensão pulmonar arterial (Ryan et al. 2011). A combinação de diferentes agressões, de acordo com a hipótese das agressões múltiplas, permitiu desenvolver fenótipos mais severos que mimetizam melhor as características da hipertensão pulmonar arterial humana (Robbins 2004).

O modelo experimental mais frequentemente utilizado, pela sua reprodutibilidade e facilidade de execução, é o modelo de hipertensão pulmonar induzida pela monocrotalina no rato (Brown et al. 1998; Jasmin et al. 2001; Lalich e Merkow 1961; Werchan et al. 1989). A monocrotalina é um alcalóide pirrolizidínico, isolado a partir da *Crotalaria spectabilis*, que, após desidrogenação

pelo citocromo P450 hepático num pirrol reactivo, adquire acção tóxica no endotélio, provocando uma endarterite obliterativa das arteríolas pulmonares. As lesões histológicas pulmonares são semelhantes às da hipertensão pulmonar arterial (Kay et al. 1967; Ryan et al. 2011), sendo a monocrotalina desprovida de acção tóxica directa no miocárdio (Chen et al. 1998). Este modelo experimental contribuiu de forma muito relevante para a evolução terapêutica recente na hipertensão pulmonar arterial. Praticamente todos os fármacos que tiveram sucesso na hipertensão pulmonar arterial humana também foram eficazes neste modelo, com a excepção do *Beraprost* (Stenmark et al. 2009). Adicionalmente, neste modelo, a activação neuroendócrina assemelha-se à da insuficiência cardíaca (Brunner 1999; Leineweber et al. 2002), pelo que tem sido empregue também no estudo da evolução da resposta hipertrófica do miocárdio da fase compensada para insuficiência cardíaca (Buermans et al. 2005; Werchan et al. 1989) e na comparação entre as alterações funcionais e moleculares dos dois ventrículos, uma vez que o ventrículo esquerdo é influenciado apenas pela activação neuroendócrina enquanto o ventrículo direito é simultaneamente sobrecarregado (Kogler et al. 2003; Leineweber et al. 2002; Schott et al. 2005; Seyfarth et al. 2000). Como característica adicional, este modelo animal evolui rapidamente para insuficiência cardíaca descompensada e fatal, com activação inflamatória, anorexia e caquexia acentuadas e perda de massa músculo-esquelética (Dalla Libera et al. 2004; Steffen et al. 2008).

Objectivos

1. Caracterizar o fenótipo contráctil do ventrículo esquerdo na hipertensão pulmonar experimental, procurando estabelecer:
    a. O estado funcional intrínseco do miocárdio *in vitro*;
    b. Evolução da disfunção e mecanismos moleculares;
    c. O papel de mecanismos neuroendócrinos na disfunção ventricular esquerda;
    d. Se a elevação aguda de pós-carga desencadeia intolerância diastólica à pós-carga e permite detectar disfunção *in vivo*.
2. Estudar o efeito de um regime alimentar hipercalórico, do tipo Ocidental, na caquexia que acompanha a hipertensão pulmonar experimental, avaliando concretamente:
    a. As repercussões na hipertensão pulmonar e função ventricular;
    b. Modificações na composição corporal e ganho ponderal;
    c. As alterações suscitadas no metabolismo e na activação inflamatória e neuroendócrina;
    d. Mecanismos subcelulares subjacentes, nomeadamente a modulação de factores de transcrição.
3. Avaliar os efeitos hemodinâmicos e neuroendócrinos, na hipertensão pulmonar crónica experimental, do antagonismo não-selectivo, agudo, da endotelina-1 com o *Tezosentan*, no intuito de analisar:
    a. Se os efeitos do antagonismo agudo são sobreponíveis aos do antagonismo crónico, com *Bosentan*, e mais concretamente:
        i. Se o antagonismo agudo apresenta benefícios hemodinâmicos;
        ii. Se o *Tezosentan* por via endovenosa pode ser usado como um substituto do *Bosentan* quando a via oral é inconveniente.
    b. Qual a acção miocárdica da endotelina-1 neste modelo e as consequências do seu antagonismo;
    c. Quais os mecanismos vasoactivos, reguladores agudos da função contráctil e anti-inflamatórios envolvidos nos efeitos miocárdicos e vasculares pulmonares da endotelina-1.
4. Reproduzir em pacientes submetidos a revascularização miocárdica os achados experimentais, animais, de intolerância diastólica à elevação abrupta de pós-carga e correlacionar a disfunção diastólica com a função contráctil.

Resultados

Apresentados sob a forma de artigos publicados ou no prelo.

1. *Disfunção miocárdica ventricular esquerda intrínseca e activação neuroendócrina na hipertensão pulmonar experimental*

   Lourenço, A. P., R. Roncon-Albuquerque Jr, C. Brás-Silva , B. Faria , J. Wieland, T. Henriques-Coelho, J. Correia-Pinto e A. F. Leite-Moreira. 2006. "Myocardial dysfunction and neurohumoral activation without remodeling in left ventricle of monocrotaline-induced pulmonary hypertensive rats.". Am J Physiol Heart Circ Physiol 291(4):H1587-1594.

2. *Mecanismos de progressão da disfunção ventricular esquerda e avaliação da tolerância diastólica à pós-carga na hipertensão pulmonar experimental*

   Correia-Pinto, J. C., T. Henriques-Coelho, R. Roncon-Albuquerque Jr, A. P.Lourenço, G. Melo-Rocha, F. Vásques-Nóvoa, T. C. Gillebert e A. F. Leite-Moreira. 2009. "Time course and mechanisms of left ventricular systolic and diastolic dysfunction in monocrotaline-induced pulmonary hypertension.". Basic Res Cardiol 104(5):535-545.

3. *Efeitos de um regime alimentar do tipo Ocidental na progressão da hipertensão pulmonar, insuficiência cardíaca e caquexia cardíaca na hipertensão pulmonar experimental*

   Lourenço, A. P., F. Vásques-Nóvoa, D. Fontoura, C. Brás-Silva, R. Roncon-Albuquerque Jr, e A. F. Leite-Moreira. 2011. "A Western-type diet attenuates pulmonary hypertension with heart failure and cardiac cachexia in rats.". J Nutr 141(11):1954-60.

4. *Efeitos hemodinâmicos e neuroendócrinos do antagonismo agudo e crónico da endotelina-1 na hipertensão pulmonar experimental*

   Lourenço, A. P., F. Vásques-Nóvoa, J. Oliveira-Pinto, D. Fontoura, R. Roncon-Albuquerque Jr, e A. F. Leite-Moreira. 2011. "Haemodynamic and neuroendocrine effects of tezosentan in chronic experimental pulmonary hypertension.". Int Care Med (no prelo).

5. Avaliação da tolerância diastólica à pós-carga em pacientes submetidos a cirurgia de revascularização miocárdica com graus variáveis de disfunção sistólica

   Leite-Moreira, A. F., A. P. Lourenço, R. Roncon-Albuquerque Jr, T. Henriques-Coelho, M. J. Amorim, J. Almeida, P. Pinho, e T. C. Gillebert. 2011. "Diastolic tolerance to systolic pressures closely reflects systolic performance in patients with coronary heart disease.". Basic Res Cardiol (no prelo).

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Myocardial dysfunction and neurohumoral activation without remodeling in left ventricle of monocrotaline-induced pulmonary hypertensive rats

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Lourenço, André P., Roberto Roncon-Albuquerque, Jr., Carmen Brás-Silva, Bernardo Faria, Joris Wieland, Tiago Henriques-Coelho, Jorge Correia-Pinto, and Adelino F. Leite-Moreira. Myocardial dysfunction and neurohumoral activation without remodeling in left ventricle of monocrotaline-induced pulmonary hypertensive rats. *Am J Physiol Heart Circ Physiol* 291: H1587–H1594, 2006. First published May 5, 2006; doi:10.1152/ajpheart.01004.2005.—In monocrotaline (MCT)-induced pulmonary hypertension (PH), only the right ventricle (RV) endures overload, but both ventricles are exposed to enhanced neuroendocrine stimulation. To assess whether in long-standing PH the left ventricular (LV) myocardium molecular/contractile phenotype can be disturbed, we evaluated myocardial function, histology, and gene expression of autocrine/paracrine systems in rats with severe PH 6 wk after subcutaneous injection of 60 mg/kg MCT. The overloaded RV underwent myocardial hypertrophy ($P < 0.001$) and fibrosis ($P = 0.014$) as well as increased expression of angiotensin-converting enzyme (ACE) (8-fold; $P < 0.001$), endothelin-1 (ET-1) (6-fold; $P < 0.001$), and type B natriuretic peptide (BNP) (15-fold; $P < 0.001$). Despite the similar upregulation of ET-1 (8-fold; $P < 0.001$) and overexpression of ACE (4-fold; $P < 0.001$) without BNP elevation, the nonoverloaded LV myocardium was neither hypertrophic nor fibrotic. LV indexes of contractility ($P < 0.001$) and relaxation ($P = 0.03$) were abnormal, however, and LV muscle strips from MCT-treated compared with sham rats presented negative ($P = 0.003$) force-frequency relationships (FFR). Despite higher ET-1 production, BQ-123 (ET_A antagonist) did not alter LV MCT-treated muscle strip contractility distinctly ($P = 0.005$) from the negative inotropic effect exerted on shams. Chronic daily therapy with 250 mg/kg bosentan (dual endothelin receptor antagonist) after MCT injection not only attenuated RV hypertrophy and local neuroendocrine activation but also completely reverted FFR of LV muscle strips to positive values. In conclusion, the LV myocardium is altered in advanced MCT-induced PH, undergoing neuroendocrine activation and contractile dysfunction in the absence of hypertrophy or fibrosis. Neuroendocrine mediators, particularly ET-1, may participate in this functional deterioration.

gene expression; myocardial contractility; neuroendocrine stimulation; endothelin-1; heart

IN CARDIAC HYPERTROPHY AND FAILURE, the myocardium is subjected to a combination of two key factors: increased biomechanical load and enhanced neuroendocrine stimulation. The understanding of the relative contribution of these two kinds of stimuli as triggers underlying alterations of myocardial phenotype is only now starting to emerge. Models of experimental pulmonary hypertension (PH) are particularly valuable in this respect because although both ventricles are exposed to neuroendocrine stimulation, only the right ventricle (RV) experiences increased biomechanical load (18). The role of overload as a cause of myocardial hypertrophy, molecular remodeling, and dysfunction in the progression to heart failure (HF) has been substantially underscored in these PH experimental models (16, 18, 30, 32), since most of the changes are restricted to the hypertrophied RV. Yet, some modifications also have been identified in the left ventricular (LV) genome and proteome of rats with hypoxia-induced (32) and monocrotaline (MCT)-induced PH (30). Studying the normally loaded LV of PH rats may be very helpful to ascertain the actions of neuroendocrine systems per se. Curiously, neuroendocrine actions have been considerably highlighted in models of experimental PH. Particularly, endothelin-1 (ET-1) contributes to the progression of cardiopulmonary alterations in MCT-induced PH (24), and chronic therapy with either a selective ET_A or a nonselective $ET_{A/B}$ receptor antagonist improves the survival and ameliorates pulmonary blood flow and RV hemodynamics but, interestingly, also reverses dysfunctional LV contractility and relaxation in MCT-treated rats (14).

The present study was undertaken to characterize the molecular and contractile phenotype of the LV myocardium of MCT-treated rats with advanced PH. After hemodynamic evaluation and morphometric characterization of PH rats 6 wk after MCT injection, we assessed in both ventricles histological features such as myocardial fibrosis and myocyte diameters, and we quantified mRNA levels of genes previously implicated in autocrine/paracrine activation during HF progression such as ET-1, angiotensin-converting enzyme (ACE), type B natriuretic peptide (BNP), angiotensinogen, and aldosterone synthase. We further evaluated the contractile phenotype of the LV myocardium of MCT rats in vitro, performing force-frequency relationships (FFR) in LV muscle strips. Because a considerable increase in myocardial ET-1 mRNA levels was observed, we assessed to greater extent both ET-1 myocardial production by immunostaining and its myocardial actions by acutely blocking ET_A receptors with BQ-123 in LV muscle strips and chronically treating MCT-injected rats with the dual endothelin receptor antagonist bosentan.

METHODS

Animal model. Seven-week-old male Wistar rats (Charles-River, Barcelona, Spain) were housed in groups of five per cage with a controlled environment under a 12:12-h light-dark cycle at a room temperature of 22°C. Rats randomly received either a subcutaneous injection of MCT (60 mg/kg; Sigma Chemical, St. Louis, MO) or an

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equal volume of vehicle and were studied 36–40 days (6 wk) after injection. Previously, we (8) observed that rats develop PH and RV hypertrophy, without overt HF, 22–25 days after subcutaneous injection of 60 mg/kg MCT. Knowing that the time elapsed from injection of MCT to the development of severe hypertrophy and HF is strongly dependent on the dose in relation to the age of the animals studied (35), we decided to study rats 6 wk after injection. Because MCT-treated rats consume significantly less food, the amount of chow fed to sham rats was restricted to the quantity consumed by MCT-treated rats during the previous day, to avoid noticeable differences in nutritional state (18). A group of randomly selected MCT-treated rats underwent chronic daily therapy with 250 mg/kg bosentan (kindly provided by Actelion Pharmaceuticals) administered by gavage (25 mg/ml in 5% gum arabic) starting 2 days after MCT injection and ending 48 h before experimentation. Experiments were subjected to the Portuguese law on animal welfare and conform to the *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 85-23, revised 1996), having been performed at the Faculty of Medicine of the University of Porto (Porto, Portugal), which is a governmental Institution granted to perform animal research.

Hemodynamic assessment. As previously reported (8), animals (MCT treated and sham, $n = 7$ each) were anesthetized with an intraperitoneal injection of pentobarbital sodium (6 mg/100 mg), mechanically ventilated (Harvard Rodent Ventilator model 683), and compensated for per operative fluid losses. The pericardium was widely opened after a median sternotomy was performed. A silk thread was passed around the ascending aorta to transiently occlude it during the experimental protocol. RV and LV pressures were measured with high-fidelity micromanometers (Millar Instruments SPR-407) inserted into the ventricular cavities. LV septal-lateral diameter was assessed with a sonomicrometer amplifier (Triton Electronics, San Diego, CA) after the implantation of ultrasonic crystals in the interventricular septum and epicardial surface of the LV free wall as previously described (3). After stabilization for 15 min, basal RV and LV peak rate of pressure rise (dP/dt_{max}), end-diastolic pressure (EDP), peak systolic pressure (SP), LV end-diastolic dimension (LVEDD), and peak systolic LV isovolumetric pressure (LVP_{iso}) were recorded. Relaxation rate was estimated with the time constant τ by fitting the isovolumetric pressure fall to a monoexponential function, as previously described (20). Parameters were sampled with a frequency of 1,000 Hz. Recordings were made with respiration suspended at end expiration. An ECG lead (II) was recorded throughout. RV and septum plus LV free wall wet weights were determined at the end of the experimental protocol after anesthetic overdose. Transmural LV and RV free wall samples were snap frozen in liquid nitrogen and stored at −80°C for subsequent molecular analyses.

Intact muscle strip preparations. Briefly, rats were anesthetized with intraperitoneal ketamine-xylazine (50 mg/kg and 8 mg/kg, respectively), a left thoracotomy was performed, and beating hearts were quickly excised and immersed in modified Krebs-Ringer (KR) solution containing (in mM) 93 NaCl, 5 KCl, 1 $MgSO_4$, 1.2 KH_2PO_4, 10 glucose, 1.25 $CaCl_2$, 20 $NaHCO_3$, 1 NaH_2PO_4, 20 $NaC_2H_3O_2$, and 5 U/l insulin with cardioplegic 2,3-butanedione monoxime (BDM; 2.5%) equilibrated with 95% O_2-5% CO_2. Thin muscle strips (cross-sectional diameter < 400 μm) were carefully isolated from LV papillary muscles under a dissecting microscope (Leica Wilde M651), mounted vertically in a 10-ml Plexiglas organ bath, and attached to an electromagnetic length-tension transducer (University of Antwerp, Antwerp, Belgium). Twenty minutes later, bathing solutions were replaced by modified KR solution without BDM. Preload was estimated according to muscle dimensions, and electrical stimulation was set 10% above threshold and at a frequency of 0.5 Hz. Muscles were allowed to stabilize, and the experimental protocol was begun once three comparable basal isotonic and isometric contractions were separately recorded with an interval of at least 10 min. Temperature was kept at 35°C and pH at 7.4 throughout the experimental protocol. In one set of LV muscle strips from sham ($n = 9$), MCT ($n = 9$) and MCT + bosentan rats ($n = 8$), isometric FFR were obtained after an initial period of contraction at 0.5 Hz by stepping up the frequency of stimulation at 3-min intervals and sequentially recording five contractions at 1, 2, 3, 4, and 5 Hz. In another set of LV sham ($n = 7$), MCT ($n = 7$), and MCT + bosentan ($n = 6$) muscle strips, the effects of ET_A antagonism were evaluated by recording several isotonic and isometric contractions before and 20–30 min after a concentration of 1 μM of the selective ET_A antagonist BQ-123 (Sigma Chemical) was added to the bath. Developed tension (mN/mm^2) was computed, using software, from the weight and length of the muscle strip by assuming an ellipsoid shape. Muscle maximum cross-sectional diameters measured with a microscope (Leica DM4000B) were similar ($P = 0.15$) in sham (252 ± 24 μm), MCT (280 ± 27 μm), and MCT + bosentan muscle strips (305 ± 41 μm), excluding the possibility that differences in muscle strip dimensions had an impact on contractile function.

Histology and immunohistochemistry. Transverse 4-μm-thick sections of paraffin-embedded, formalin-fixed specimens encompassing the RV, interventricular septum, and LV free wall were stained and photographed with a digital camera (Leica DFC320) in a group of additional sham ($n = 4$) and MCT rats ($n = 4$). The shortest diameter of 50 transversally cut, randomly selected cardiomyocytes from the RV, septum, and LV free wall myocardium was measured with image analyzer software (Leica IM-1000) at the level of the nucleus in hematoxylin-eosin-stained sections. Two independent, blinded observers ranked histological sections stained with Masson's trichrome for collagen as having no fibrosis (*grade 0*); a localized small amount of fibrosis (*grade 1*); mild, patchy fibrosis (*grade 2*); moderate, diffuse fibrosis (*grade 3*); or severe, diffuse fibrosis (*grade 4*). Immunohistochemical staining of ET-1 was performed with a 1:800 dilution of rabbit ET-1 antiserum (T-4050; Peninsula Laboratories) at 4°C for 16 h after initial incubation with 0.3% H_2O_2 and blocking solution (TR-004-HD; Labvision) at room temperature. The sample was then sequentially exposed at room temperature to biotinylated goat anti-rabbit (TR-004-HD; Labvision) for 15 min, to streptavidin peroxidase (TR-004-HD; Labvision) for 15 min, and, finally, to 4% 3,3-diaminobenzidine (TR-004-HD; Labvision) for 10 min. Counterstaining was performed with hematoxylin.

Relative quantification of mRNA. Two-step real-time RT-PCR was performed as previously described (8). Briefly, after total mRNA extraction (no. 74124; Qiagen), standard curves were obtained for each gene correlating ($R \geq 0.98$) the mRNA quantities in graded dilutions of a rat cardiac tissue sample with the respective threshold cycles (second derivative maximum method). Equal amounts of mRNA from every sample underwent three separate two-step real-time RT-PCR experiments for each gene, using SYBR green as marker (no. 204143; Qiagen). GAPDH was used as internal control, because its mRNA levels were similar in the studied groups. Results are relative to the mean obtained for the sham group (set as arbitrary unit) and normalized for GAPDH. Specific PCR primer pairs for the studied genes are presented in Table 1.

Statistical analysis. Data are expressed as means ± SE. Statistical analysis used a two-way ANOVA to compare cardiomyocyte hypertrophy data; a repeated-measures two-way ANOVA followed by Holm-Sidak's method for multiple comparisons was applied to data from FFR in intact muscle strip preparations; and Fisher's exact test was used to evaluate myocardial fibrosis after categories were grouped. One-way ANOVA and Student's *t*-test were used to compare other results as indicated. Statistical significance was set at a two-tailed value of $P < 0.05$.

RESULTS

Rats were studied 6 wk after injection, at which point the mortality was null in sham rats but ~85% in MCT and 50% in MCT + bosentan rats. All MCT rats presented clinical signs of overt HF, namely, lethargy, labored breathing, cachexia, vein

Table 1. *Primers used in mRNA quantification*

Gene	Sequence 5′ → 3′
GAPDH	fw: TGG CCT TCC GTG TTC CTA CCC
	rev: CCG CCT GCT TCA CCA CCT TCT
ET-1	fw: CCA TGC AGA AAG GCG TAA AAG
	rev: CGG GGC TCT GTA GTC AAT GTG
ACE	fw: GCA GGC CAG CAG GGT CCA CTA CAC
	rev: GAC CTC GCC ATT CCG CTG ATT CT
BNP	fw: GGA CCA AGG CCC TAC AAA AGA
	rev: CAG AGC TGG GGA AAG AAG AG
Angiotensinogen	fw: CGG ACA GCA CCC TAT TTT TCA ACA
	rev: GAG GCG CAC TGG GGC TGG AT
Aldosterone synthase	fw: AAT GGC GCT TCA ACC GAC TG
	rev: GAG CAT TCT GAC GCA CCT TCT TTT
HIF-1α	fw: CTA ACA AGC CGG GGG AGG AC
	rev: TCA TAG GCG GTT TCT TGT AGC

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ET-1, endothelin-1; ACE, angiotensin-converting enzyme; BNP, type-B natriuretic peptide; HIF-1α, hypoxia-inducible factor-1α.

and liver engorgement, pleural effusion, and ascites. Although MCT + bosentan rats also presented these clinical signs, they distinguished themselves from MCT rats because they showed less lethargy on a qualitative evaluation. The body weight (BW) of sham rats was similar to the BW of MCT and MCT + bosentan rats (266 ± 16, 240 ± 13, and 250 ± 12 g, respectively; $P = 0.37$).

Cardiac hemodynamics, morphometry, and histology. The hemodynamic features of the experimental groups are summarized in Table 2. MCT rats showed a marked increase in RVSP compared with sham rats ($P < 0.001$). This was accompanied by other modifications in hemodynamic profile, such as increased values of RV dP/dt_{max} ($P = 0.014$), τ ($P < 0.001$), and RVEDP ($P = 0.003$), but also by morphometric (Fig. 1) and histological changes (Table 3 and Figs. 1*C* and 2), such as increased ratio of RV weight to BW ($P < 0.001$), increased RV cardiomyocyte diameters ($P < 0.001$), and significantly higher grades of myocardial fibrosis ($P = 0.014$). In contrast to the RV myocardium, the grade of myocardial fibrosis observed in the LV of MCT rats did not differ from that of sham rats (Table 3 and Fig. 2), and despite the tendency toward an increase in the ratio of LV and septum as a whole to BW (Fig. 1), only septal ($P < 0.001$) and not LV free wall cardiomyocytes presented increased diameters (Fig. 1*C*). Likewise, the LV filling pressures as estimated by LVEDP and LVEDD were unaltered. The maximum developed pressures (LVSP), however, were reduced ($P < 0.001$) as well as the indexes of contractility, dP/dt_{max} ($P < 0.001$) and LVP_{iso} ($P < 0.001$),

Table 2. *Hemodynamic data*

	Sham	MCT
n	7	7
	RV	
RVSP, mmHg	20.8±1.3	51.4±4.1*
RVEDP, mmHg	0.9±0.4	3.9±0.7*
dP/dt_{max}, mmHg/s	936±72	1,411±173*
τ, ms	7.8±0.7	37.8±2.3*
	LV	
LVSP, mmHg	95.1±2.5	60.2±3.5*
LVEDP, mmHg	6.7±0.6	7.8±0.9
LVEDD, mm	6.4±0.8	5.4±0.8
dP/dt_{max}, mmHg/s	4,749±323	2,205±272*
LVP_{iso}, mmHg	193.1±7.2	131.5±6.6*
τ, ms	21.5±1.7	26.9±2.2*

Values are means ± SE. MCT, monocrotaline-treated rats; RV, right ventricle; RVSP, right ventricular systolic pressure; RVEDP, right ventricular end-diastolic pressure; LV, left ventricle; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; LVEDD, left ventricular end-diastolic dimension; dP/dt_{max}, peak rate of ventricular pressure rise; LVP_{iso}, peak systolic isovolumetric pressure; τ, time constant of isovolumetric relaxation. Comparisons were performed using Student's *t*-test. *$P <$ 0.05 vs. sham.

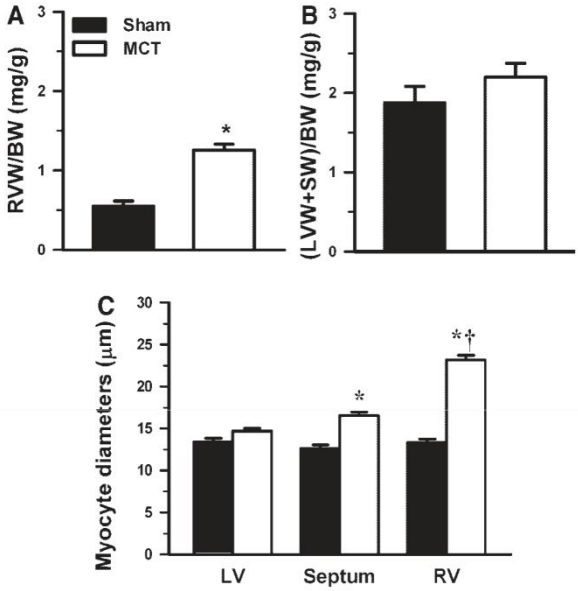

Fig. 1. Hypertrophic response to monocrotaline (MCT)-induced pulmonary hypertension. *A*: right ventricle weight (RVW)-to-body weight (BW) ratio. *B*: left ventricle free wall weight + interventricular septum weight (LVW+SW)-to-BW ratio. Student's *t*-test was used to compare sham ($n = 7$) and MCT-treated rats ($n = 7$). RVW/BW was significantly increased in MCT rats (*$P <$ 0.001). *C*: LV free wall, interventricular septum, and RV free wall cardiomyocyte diameters from sham ($n = 4$) and MCT-treated rats ($n = 4$). Two-way ANOVA and Holm-Sidak's method for multiple comparisons were used in the statistical analysis. Septal cardiomyocytes of MCT-treated rats were significantly larger than cardiomyocytes from sham rats and from the LV of MCT-treated rats (*$P < 0.001$), yet the largest cardiomyocytes were those from the RV of MCT-treated rats, which were significantly larger than the corresponding septal myocytes (†$P < 0.001$).

Table 3. *Myocardial fibrosis*

Fibrosis Grade	Sham RV	Sham LV	MCT RV	MCT LV
0	3]	2	0	1
1	1]	2	0	3
2	0	0	3]*	0
3	0	0	1]*	0
4	0	0	0	0

Fibrosis grades: 0, no fibrosis; 1, localized small amount of fibrosis; 2, mild patchy fibrosis; 3, moderate, diffuse fibrosis; and 4, severe, diffuse fibrosis. *P* value was calculated using Fisher's exact test after grouping categories (*grades 0* and *1* in sham rat RV vs. *grades 2* and *3* in MCT-treated rat RV). *$P = 0.014$ vs. sham RV ($n = 4$ for sham and MCT).

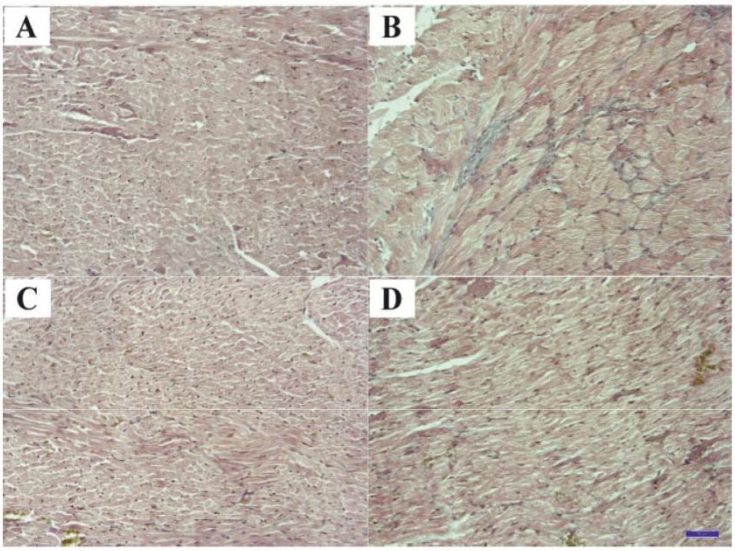

Fig. 2. Representative photomicrographs (×200) of Masson's trichrome-stained sections from sham and MTC-treated rats for evaluation of myocardial fibrosis. *A*: sham rat RV. *B*: MCT-treated rat RV. *C*: sham rat LV. *D*: MCT-treated rat LV. Note the increased fibrosis in the RV of MCT-treated rats.

whereas the relaxation time constant τ was increased ($P = 0.03$) in the LV of MCT rats. The heart rate of MCT rats was significantly decreased as well (sham = 272 ± 14 beats/min vs. MCT = 200 ± 17 beats/min; $P = 0.008$). Chronic therapy with bosentan after MCT injection attenuated RV hypertrophy ($P < 0.001$) as assessed by a reduction in the ratio of RV free wall to LV free wall plus interventricular septum weight (Fig. 3).

Autocrine/paracrine activation. Changes in the expression of genes involved in autocrine/paracrine activation during the progression to heart failure in MCT-treated rats with and without subsequent chronic therapy with bosentan are summarized in Fig. 4. ACE and ET-1 mRNA levels were increased in both the RV and the LV free wall myocardium of MCT rats ($P < 0.01$), whereas BNP mRNA overexpression was restricted to the RV myocardium ($P < 0.001$). The RV myocardium of MCT rats also presented a more pronounced upregulation of the ACE gene ($P = 0.03$), but the increase in ET-1 mRNA levels was not different between ventricles ($P = 0.694$). The indiscriminate increase in ET-1 production was

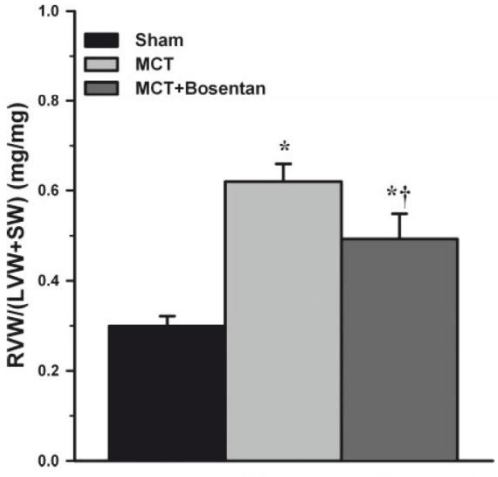

Fig. 3. RVW-to-LVW+SW ratios in sham ($n = 7$) and MCT-treated rats with (MCT+bosentan, $n = 7$) and without subsequent chronic daily therapy with 250 mg/kg bosentan by gavage (MCT, $n = 7$). As evaluated with one-way ANOVA, *$P < 0.001$ vs. sham and †$P < 0.001$ vs. MCT.

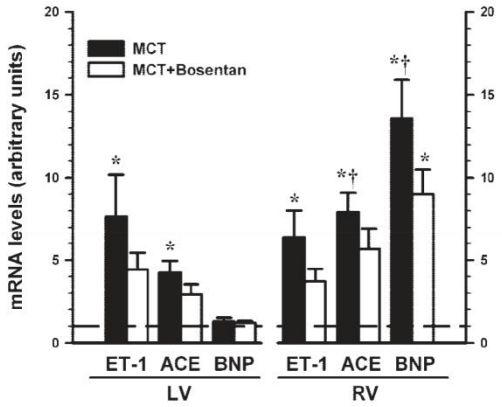

Fig. 4. LV (*left*) and RV (*right*) mRNA levels of angiotensin-converting enzyme (ACE), endothelin-1 (ET-1), and type-B natriuretic peptide (BNP) in rats with MTC-induced pulmonary hypertension with (MCT, $n = 7$) and without chronic daily therapy with 250 mg/kg bosentan by gavage (MCT+bosentan, $n = 7$). Results are expressed as arbitrary units after normalization for GAPDH. The arbitrary unit was set as the average value of the sham group and is presented as a reference line. ACE and ET-1 gene expression were increased in both ventricles of MCT compared with sham ($n = 7$) rats (*$P < 0.01$, as evaluated with one-way ANOVA), whereas MCT+bosentan showed no significant differences in mRNA levels from sham mRNA levels. In MCT and MCT+bosentan, BNP was only overexpressed in the RV (*$P < 0.001$, as evaluated with one-way ANOVA). ACE and BNP were significantly more upregulated in the RV than in the LV of MCT (†$P = 0.03$ and $P = 0.002$ as evaluated with Student's *t*-test, respectively).

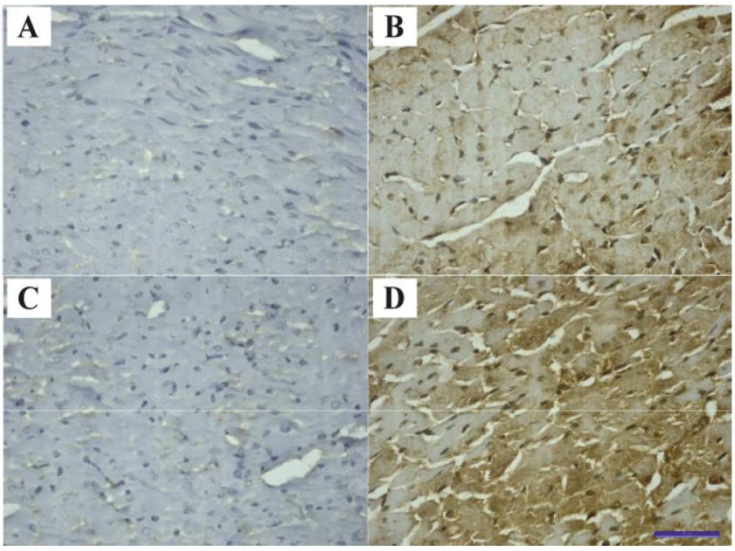

Fig. 5. Representative photomicrographs (×400) showing ET-1 immunohistochemical staining in sham and MCT-treated rat RV and LV free wall myocardium. *A*: sham rat RV. *B*: MCT-treated rat RV. *C*: sham rat LV. *D*: MCT-treated rat LV. ET-1 immunostaining was almost confined to the vascular endothelium in the RV (*A*) and LV (*C*) myocardium of sham rats, whereas in addition to endothelial marking there was also a striking cardiomyocyte staining in the RV (*B*) and LV (*D*) myocardium of MCT-treated rats. Notice as well the cardiomyocyte hypertrophy in the RV myocardium of MCT-treated rats (*B*).

confirmed by a striking immunostaining of cardiomyocytes from both the LV and RV myocardium of MCT rats, whereas the staining in sham rat myocardium was found almost exclusively in vascular endothelium (Fig. 5). The MCT + bosentan group showed no significant differences in the gene expression of ACE and ET-1 in both the RV and the LV compared with either the sham or MCT group, indicating an intermediate level of gene expression (Fig. 4). RV BNP mRNA levels were significantly increased compared with sham rats ($P < 0.001$), and although their increase was of lower magnitude, it did not differ from that of MCT rats.

No significant differences were observed in the RV and LV expression of aldosterone synthase (1.2 ± 0.7 vs. 1.0 ± 0.2, $P = 0.4$, and 1.7 ± 0.8 vs. 1.0 ± 0.2, $P = 0.22$, respectively) or angiotensinogen (1.2 ± 0.3 vs. 1.0 ± 0.1, $P = 0.29$, and 1.3 ± 0.4 vs. 1.0 ± 0.1, $P = 0.22$, respectively) in MCT-treated rats compared with sham rats. The gene expression of transcription factor HIF-1α was studied in the LV of MCT-treated rats to assess a possible role of hypoxia on neuroendocrine activation. No differences were observed (sham = 1.0 ± 0.1 arbitrary units vs. MCT = 1.3 ± 0.2 arbitrary units; $P = 0.22$).

Contractile properties of intact LV muscle strips. Isometrically contracting muscle strips from sham and MCT + bosentan LV showed a steady increase in developed tension between 1 and 5 Hz, indicating positive FFR, whereas MCT LV muscle strips responded in the opposite way ($P < 0.001$), presenting overall negative FFR (Fig. 6). Baseline force development was not different ($P = 0.16$) among the three groups and averaged 2.2 ± 0.4 mN/mm^2. Adding the selective ET_A antagonist BQ-123 to in vitro contracting LV intact muscle strips induced different responses ($P = 0.005$) in sham compared with MCT and MCT + bosentan muscle strips: a negative inotropic effect was elicited in sham muscle strips ($P < 0.032$), whereas the tension developed by MCT and MCT + bosentan muscle strips remained unaltered (Fig. 7).

DISCUSSION

We have demonstrated that the LV myocardium of MCT-treated rats with long-standing PH presents autocrine/paracrine system activation in the absence of a direct hemodynamic stress, and although myocardial hypertrophy and fibrosis are not observed, its contractile phenotype is disturbed.

MCT-treated rats developed PH, RV myocardial hypertrophy, and fibrosis. A higher expression of genes that take part in the processes of molecular remodeling was observed in the chronically overloaded RV myocardium but also in the LV myocardium. Despite the pronounced upregulation of ACE and

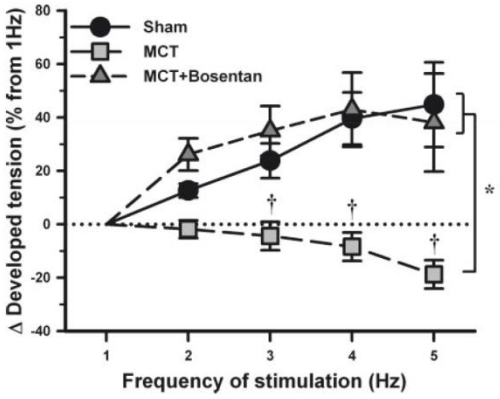

Fig. 6. Force-frequency relationships (FFR) obtained in intact isolated muscle strips from the LV myocardium of sham ($n = 9$), MCT ($n = 9$), and MCT+bosentan rats ($n = 8$). Results are presented as percent variation from baseline (Δ) of developed tension at 1 Hz. Sham and MCT+bosentan LV muscle strips show positive FFR, whereas FFR are negative in MCT LV muscle strips. *$P < 0.001$ for interaction, as evaluated with two-way repeated-measures ANOVA. †$P < 0.001$ vs. sham at an equivalent frequency of stimulation, as evaluated with Holm-Sidak's method for multiple group comparisons.

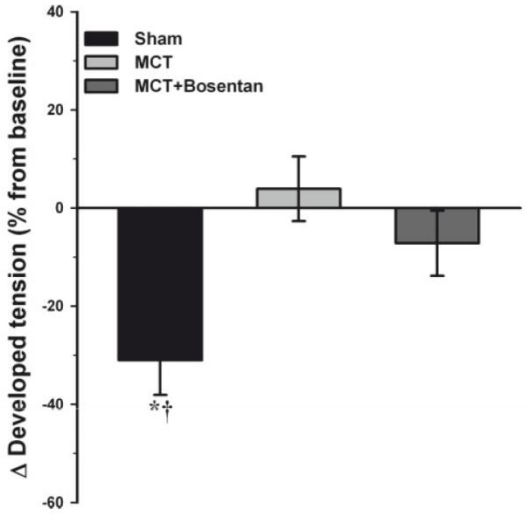

Fig. 7. Effects of the selective ET_A antagonist BQ-123 on the LV intact muscle strips of sham (n = 7), MCT (n = 7), and MCT+bosentan rats (n = 6). Results are presented as percent Δdeveloped tension after BQ-123 treatment. Sham LV muscle strips showed a negative inotropic effect (P = 0.032, as evaluated with paired t-test), whereas the tension developed by MCT and MCT+bosentan muscle strips was unaffected (P = 0.005, as evaluated with one-way ANOVA).

ET-1 without counterregulatory activation of BNP gene expression in the LV of MCT-treated rats, neither myocardial hypertrophy nor fibrosis was observed, underscoring the primordial role of overload on their development (17).

Opposite to BNP and ACE, ET-1 gene expression and peptide production were similar in the RV and LV of MCT-treated rats. Several explanations can be advanced to substantiate the magnitude of LV production of ET-1. First, neurohumoral activation typically accompanies unloading (21), but because neither the LV filling pressures nor LVEDD of MCT-treated rats were lowered and their LV were atrophic, we believe unloading cannot fully explain ET-1 upregulation. Second, some degree of hypoxemia also was acknowledged in MCT-treated rats (23, 37); hence, we studied the mRNA levels of HIF-1α, a central marker of hypoxia-induced ET-1 expression, (15) having observed no differences among groups. Third, various circulating mediators (2, 24) and the sympathetic nervous system (31) are likely inducers of ET-1 gene expression in MCT-treated rats, although their influence would be expected to take place earlier in the development of PH (18). Finally, because ET-1 is an autocrine/paracrine factor in angiotensin II-induced hypertrophy (6, 12), we must point out the possibility that angiotensin II generated by the overactive ACE may contribute to ET-1 activation.

MCT animals presented significant reductions of both LVSP and heart rate. This has been interpreted previously as consequence of continuous severe sickness (24). Tachycardia would be expected as a reflex response to a lower blood pressure, but MCT-treated rats presented an impaired response to the sympathetic nervous system as manifested by reductions of β-adrenoceptor and norepinephrine neuronal transporter densities and of norepinephrine neuronal transporter and G protein-coupled receptor kinase activity (18).

Reduction of LVSP in MCT-treated rats was accompanied by disturbed hemodynamic contractility and relaxation indexes, as previously described (14). Albeit, LV fibrosis and atrophy were ruled out, and even though invasive hemodynamic indexes were obtained with a widely open pericardium, thus reducing ventricular interdependence (33), the majority of studies carried out in PH patients (22, 29) attribute LV dysfunction fundamentally to ventricular interdependence and impaired LV filling (22) or geometric modifications (4). This is supported by the development of septal hypertrophy in our group of MCT-treated rats but not by the evidence of unaltered LVEDP and LVEDD. Likewise, conserved LV volumes have been reported despite LV functional deterioration in PH (7). However, it should be stressed that because we evaluated LV dimensions through a simple septal-lateral diameter, which does not allow a thorough evaluation of LV volume, and therefore an accurate determination of LV preload, preload-dependent indexes such as dP/dt_{max} and peak isovolumetric pressures are insufficient to definitely establish impaired contractility.

To further elucidate the myocardial contractile phenotype of the LV in MCT-treated rats, safeguarding the possibility that our findings were not due to ventricular interdependence, we conducted experiments with LV myocardium on intact muscle strip preparations. The FFR is an important intrinsic regulatory mechanism of cardiac contractility; normal myocardium increases developed force with higher frequencies of stimulation over the frequency range close to the physiological heart rate, showing normal contractile reserve, but failing or dysfunctional myocardium loses this reserve, and therefore FFRs are excellent indicators for evaluating the severity of cardiac contractile dysfunction and cardiac reserve capacity as well as for the evaluation of the effectiveness of therapeutic agents in congestive HF (5). The normal myocardium from sham rat LV muscle strips presented positive FFR as expected (5, 16), whereas the muscle strips from the LV of MCT-treated rats showed overall negative FFR indicative of reduced contractile reserve (5), resembling the hypertrophied RVs of MCT-treated rats in earlier stages of PH (16, 34).

The strong local activation of ET-1 in the LV of MCT-treated rats could straightforwardly lead one to presume that this peptide might be partly underlying the disturbed contractile properties of MCT LV myocardium. Chronic effects of ET-1 have been previously studied. ET-1 administration augments basal force of contraction, prolongs relaxation, and significantly blunts responses to Ca^{2+} and isoprenaline (38). In the present study, negative FFRs were observed in the ET-1-overexpressing LV myocardium of MCT rats. Chronic ET-1 overactivity in heart disease has been associated with slower relaxation and impaired contractility through dysfunctional Ca^{2+} homeostasis and myosin heavy chain (MHC) isoform switch (10, 13, 26, 28). Curiously, the LV of rats with PH induced by either MCT (11) or hypoxia (32) undergoes MHC isoform switch, whereas ET_A receptor antagonists have noticeably been reported to partly prevent this switch in MCT RV myocardium (10). The acute effects of ET_A blockade were studied in vitro to ascertain whether there was a functional change in the acute myocardial effects of endogenous ET-1. We had previously reported a negative inotropic effect of the selective ET_A antagonist BQ-123, per se, on undissected rabbit RV papillary muscles with intact endothelium in vitro (19).

This also was observed in the present study in sham muscle strips. The contractility of LV muscle strips from MCT-treated rats, on the contrary, was unaffected by acute in vitro exposure to BQ-123, which might denote an abnormal functioning of the overactive endothelin system compared with the normal myocardium. Chronic therapy with bosentan did not restore the normal effects of BQ-123 on muscle strips of LV myocardium, but it restored the positivity of FFR compared with MCT-injected PH rats without subsequent treatment with bosentan. Indeed, although bosentan blunted MCT-induced PH as previously reported (9), reducing RV hypertrophy with concomitant attenuation of local neuroendocrine gene expression, the marked change in FFR in the LV of MCT-injected rat myocardium after chronic therapy with bosentan favors the hypothesis of a direct action of chronic ET-1 antagonism on the molecular and functional LV myocardial phenotype. Overall, these results suggest that the beneficial effects of chronic endothelin antagonism in MCT-treated rats (14) may not be dependent exclusively on attenuated PH and improved pulmonary blood flow but also on the myocardial effects of chronic ET-1 blockade.

Despite the similarities with human PH (25), given that MCT-induced PH has no human equivalent, any implication in human pathophysiology is limited. Nevertheless, we must point out that LV myocardial abnormalities may partly underlie LV dysfunction in patients with severe PH (36). Indeed, in patients with PH, normal LV filling is only restored 1 yr after single-lung transplantation even if LV geometry and RV function are immediately restored (36), and combined heart-lung transplantation is favored if severe impairment of LV function is also present because the LV may not recover after transplantation of the lungs alone (27). A patient with severely diminished LV function, however, tolerated double-lung transplantation and fully recovered cardiac function after therapy with bosentan. The authors wisely attributed this to a reduction in ventricular interaction but also admitted that bosentan might have acted directly on the myocardium (1).

In conclusion, MCT-treated rats with severe long-standing PH develop local autocrine/paracrine system activation that is not restricted to the overloaded ventricle. The LV myocardium expresses high levels of genes involved in autocrine/paracrine systems, but despite this, it does not undergo hypertrophy or fibrosis. The LV contractile phenotype, however, is altered: LV muscle strips from MCT-treated rats present negative FFR. Acute ET_A antagonism in vitro has distinct effects on contractile performance compared with sham myocardium, and chronic ET-1 blockade after MCT injection restores the positivity of LV myocardium FFR, suggesting a possible direct detrimental action of ET-1 overexpression in the LV myocardium of PH rats.

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ORIGINAL CONTRIBUTION

Time course and mechanisms of left ventricular systolic and diastolic dysfunction in monocrotaline-induced pulmonary hypertension

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Abstract Although pulmonary hypertension (PH) selectively overloads the right ventricle (RV), neuroendocrine activation and intrinsic myocardial dysfunction have been described in the left ventricle (LV). In order to establish the timing of LV dysfunction development in PH and to clarify underlying molecular changes, Wistar rats were studied 4 and 6 weeks after subcutaneous injection of monocrotaline (MCT) 60 mg/kg (MCT-4, $n = 11$; MCT-6, $n = 11$) or vehicle (Ctrl-4, $n = 11$; Ctrl-6, $n = 11$). Acute single beat stepwise increases of systolic pressure were performed from baseline to isovolumetric (LVPiso). This hemodynamic stress was used to detect early changes in LV performance. Neurohumoral activation was evaluated by measuring angiotensin-converting enzyme (ACE) and endothelin-1 (ET-1) LV mRNA levels. Cardiomyocyte apoptosis was evaluated by TUNEL assay. Extracellular matrix composition was evaluated by tenascin-C mRNA levels and interstitial collagen content. Myosin heavy chain (MHC) composition of the LV was studied by protein quantification. MCT treatment increased RV pressures and RV/LV weight ratio, without changing LV end-diastolic pressures or dimensions. Baseline LV dysfunction were present only in MCT-6 rats. Afterload elevations prolonged τ and upward-shifted end-diastolic pressure dimension relations in MCT-4 and even more in MCT-6. MHC-isoform switch, ACE upregulation and cardiomyocyte apoptosis were present in both MCT groups. Rats with severe PH develop LV dysfunction associated with ET-1 and tenascin-C overexpression. Diastolic dysfunction, however, could be elicited at earlier stages in response to hemodynamic stress, when only LV molecular changes, such as MHC isoform switch, ACE upregulation, and myocardial apoptosis were present.

Keywords Myosin heavy chain · Endothelin-1 · Angiotensin-converting enzyme · Apoptosis · Tenascin-C · Collagen

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Introduction

Although chronic pulmonary hypertension (PH) selectively overloads the right ventricle (RV), left ventricular (LV) dysfunction also manifests in the course of primary PH [32], chronic thromboembolism [13] and cor pulmonale [41]. Echocardiography-based studies carried out in PH patients suggested that one of the mechanisms contributing to LV dysfunction is ventricular interdependence and impaired LV filling [35]. Other studies, however, provide evidences that intrinsic LV myocardial abnormalities also contribute to LV dysfunction in severe PH [11, 12, 24, 33].

In fact, the LV of rats with severe longstanding PH induced by monocrotaline (MCT) presents local autocrine/paracrine system activation and muscle strips dissected from the LV myocardium show, in vitro, negative force–frequency relationships (FFR) [33]. Intrinsic LV myocardial abnormalities might help to explain late recovery of LV filling after single-lung transplantation in patients with PH, even if LV geometry and RV function are immediately restored [50]. They might explain as well, why combined heart–lung transplantation is favored for PH patients when severe impairment of LV function is present, since LV function may not sufficiently recover after transplantation of the lungs alone [39].

The timing of development and the underlying mechanisms of intrinsic LV myocardial dysfunction were incompletely investigated so far. In the present study, we analyzed biventricular hemodynamics, LV myosin heavy chain (MHC) protein isoforms, LV myocardial expression of genes involved in neurohumoral activation (angiotensin-converting enzyme and endothelin-1), extracellular matrix remodelling (tenascin-C gene expression and interstitial fibrosis) and apoptosis (TUNEL assay), 4 and 6 weeks after MCT injection. Hemodynamics were studied at baseline and in response to single-beat afterload elevations, which allow the detection of diastolic dysfunction that may not be evident during evaluation at rest, but is revealed during exercise or hemodynamic stress [9, 10, 19, 20, 26, 48].

Methods

Animal protocol

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and the experiments were performed according to the Portuguese law on animal welfare. Seven-weeks-old male Wistar rats ($n = 58$; Charles-River, Barcelona, Spain) were housed in groups of three per cage in a controlled environment under a 12:12 h light/dark cycle at a room temperature of 22°C. Rats randomly received, a subcutaneous injection of monocrotaline (MCT, 60 mg/kg; Sigma Chemical, St. Louis, MO, USA; $n = 36$) or an equal volume of vehicle (Ctrl, $n = 22$; 1 mL/kg). Animals had free supply of food and water. Hemodynamic studies and collection of samples for molecular and histological studies were carried out, for control and MCT-treated rats, at days 22, 24 or 26 (fourth week), Ctrl-4 ($n = 11$) and MCT-4 ($n = 11$), respectively, and at days 36, 38 or 40 (sixth week) after injection, Ctrl-6 ($n = 11$) and MCT-6 ($n = 11$), respectively.

Hemodynamic studies

Experimental preparation

Animals were anesthetized with an intraperitoneal injection of 60 mg/kg pentobarbital sodium and additional boluses of 20 mg/kg when needed, placed over a heating pad, and mechanically ventilated (Harvard Rodent Ventilator model 683) through a tracheostomy. The right jugular vein was cannulated and a pre-warmed 0.9% NaCl solution was administrated to compensate for perioperative fluid losses. The heart was exposed through a median sternotomy and the pericardium widely opened. The ascending aorta was dissected and surrounded by a silk thread in order to transiently occlude it during the experimental protocol. RV pressure was measured with a 2F high-fidelity micromanometer (SPR-324, Millar Instruments, Houston, TX, USA) inserted through the RV free wall into the RV cavity and LV pressure with a 3F high-fidelity micromanometer (SPR-407, Millar Instruments, Houston, TX, USA) inserted through an apical puncture into the LV cavity. LV septal to free wall diameter was recorded with ultrasonic crystals using a sonomicrometer amplifier (Triton Technology, San Diego, CA, USA). One crystal was placed into the left border of the interventricular septum and the other on the epicardial surface of the LV free wall, as previously described [3, 4]. A limb ECG (II) was recorded throughout. After the instrumentation period (typically 60 min), the animal was allowed to stabilize for 15 min before the beginning of the experimental protocol. Recordings were made with ventilation suspended at end-expiration. Parameters were converted on line to digital data with a sampling frequency of 1,000 Hz.

Data analysis

Peak rates of LV and RV pressure rise (dP/dt_{max}) and fall (dP/dt_{min}) were determined. RV and LV pressures were measured at end-diastole (EDRVP and EDLVP, respectively) and at peak-systole (RVP_{max} and LVP_{max}). Relaxation rate was estimated with the time constant τ, by fitting isovolumetric LV pressure fall to a monoexponential function. From baseline to isovolumetric, multiple graded LV pressure elevations were randomly performed by abruptly clamping the ascending aortic root starting in the diastole separating two heartbeats, as previously described [3, 4, 25, 31]. Beats preceding aortic obstruction were designated as baseline heartbeats and those immediately following it as test beats. Along with the baseline hemodynamics, four afterloaded test-beats with a peak systolic pressure of approximately 60, 70, 80, and 90 of the isovolumetric pressures, and the isovolumetric test-beat were selected for analysis. LV septum to free wall diameter was measured at end-diastole. Afterload-

induced shifts of the end-diastolic pressure–dimension relation (EDPDR) were assessed by measuring diastolic pressures at matched dimensions, close to end-diastole, as previously described [3, 4, 29, 30].

At the end of the hemodynamic study animals were euthanized with anesthetic overdose, the position of crystals and manometers was verified, the RV was carefully dissected from the interventricular septum and LV free wall, and transmural samples of LV free wall (excluding the septum) were collected for molecular studies. Two MCT-6 rats died during hemodynamic instrumentation, having been excluded from analysis.

Morphometric determination of cardiac fibrosis

Transverse-sections of paraffin-embedded, formalin-fixed specimens encompassing the LV free-wall were stained with Masson's Trichrome and photographed with a digital camera (Leica DFC320) in additional animals ($n = 5$ per group). A 330-point grid was superimposed on ten fields (400×) randomly selected on each section. Two independent blinded observers rated the images and the area-percent of blue staining, indicative of fibrosis, was calculated as follows: (total number of blue-positive points/total number of points) × 100.

Detection of apoptotic cardiomyocytes

To assess the extent of apoptosis the terminal deoxynucleotidyl-transferase-mediated dUTP nick end-labeling (TUNEL) assay was used (CardioTACS^{TM} in situ Apoptosis Detection Kit, R&D Systems, Minneapolis, MN, USA). Briefly, after deparaffinization tissue slides were immersed in phosphate-buffered saline (PBS) pH 7.4 and then permeabilized with proteinase K for 20 min at room temperature. Endogenous peroxidase activity was quenched using 5.0% hydrogen peroxide. Then, specimens were incubated in TdT labeling buffer for 5 min. After that, slides were incubated with a mix containing the terminal deoxynucleotidyl transferase (TdT), Mn^{2+} and biotinylated nucleotides for 1 h at 37°C, blocked with stop buffer, and incubated with streptavidin-HRP for 10 min at room temperature. After washing in PBS, the slides were finally developed using TACS Blue Label. Nuclear staining by Nucler Fast Red was performed as counterstaining. Positive control of the TUNEL assay was generated by staining by the treatment of the samples with TACS-Nuclease^{TM} prior to the labeling protocol. Negative control of the TUNEL assay was confirmed by staining of the heart tissue in the same manner without TdT. We used three sections obtained at a distance of 100 μm from each tissue block. TUNEL-positive cardiomyocytes were counted in at least 50 optical fields (400×) of each specimen. The apoptotic rate was expressed as a percentage of apoptotic cells of all cardiomyocytes per field.

Molecular studies

Relative quantification of mRNA

Two-step Real-time RT-PCR was performed as previously described [14, 15, 33, 40]. Briefly, after total mRNA extraction (no. 74124, QIAGEN), standard curves were obtained using graded dilutions of a rat cardiac tissue sample. The starting mRNA quantities were correlated ($R \geq 0.98$) with the respective threshold cycles, for each gene. Threshold cycles were automatically calculated by software (LightCycler, ROCHE) using the second derivative maximum method. An equal amount of mRNA from every sample underwent two separate two-step real time RT-PCR experiments for each gene, using SYBR green as marker (no. 204143, QIAGEN). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as internal control since its mRNA levels were similar in the studied groups. Results are normalized for GAPDH and presented in arbitrary units. The arbitrary unit was set as the mean obtained for the Ctrl-4 group. Specific PCR primer pairs for the studied genes were: endothelin-1 (ET-1), fw 5′-CCA TGC AGA AAG GCG TAA AAG-3′, rev 5′-CGG GGC TCT GTA GTC AAT GTG-3′; angiotensin-converting enzyme (ACE), fw 5′-GCA GGC CAG CAG GGT CCA CTA CAC-3′, rev 5′-GAC CTC GCC ATT CCG CTG ATT CT-3′; tenascin-C, fw 5′-TCT CCG GTG TAG CCC TCG TCA-3′, rev 5′-CAC CAT GGC CGC TGT CTC AA-3′; and GADPH, fw 5′-TGG CCT TCC GTG TTC CTA CCC-3′, rev 5′-CCG CCT GCT TCA CCA CCT TCT-3′.

Relative quantification of myosin heavy-chain isoforms

Total protein (15 μg) was separated by SDS-PAGE (3.0% stacking and 5.0% running polyacrylamide gels). Electrophoresis was carried out at constant voltage (60 V) for ~270 min (no. 165-3301, BIO-RAD), allowing the separation of myosin heavy-chain isoforms α and β isoforms in two distinct bands visible at ~200 KDa. Gels were silver-stained following the manufacturer's instructions (no. 161-0449, BIO-RAD) and the relative amount of the myosin heavy-chain isoforms in each sample was quantified by densitometry (Multilmage Light Cabinet; ALPHA INNOTECH CORPORATION).

Statistical analysis

Values were expressed as mean ± SEM. Differences in baseline hemodynamic parameters, morphology, and molecular studies were evaluated by two-way ANOVA.

Three-way ANOVA was employed to compare data from graded afterload elevations. Holm–Sidak's method was used for multiple comparisons when significant differences were detected. Statistical significance was assumed at a two-tailed value of $P < 0.05$.

Results

All MCT-6 animals presented clinical signs of congestive heart failure such as lethargic behavior, ruffled fur, cachexia, tachypnea and severe breathing effort, pleural effusion, and ascites. MCT-4 animals behaved similarly to control animals. Mortality was null in Ctrl-4 and Ctrl-6, 33% in MCT-4 and 70% in MCT-6. No significant differences were observed between Ctrl-4 and Ctrl-6 for morphometric, hemodynamic and molecular parameters.

Baseline hemodynamics and morphometry

MCT treatment resulted in progressively increasing RV peak-systolic pressures (Fig. 1a). Increased RV pressures were accompanied by selective RV hypertrophy, as denoted by the progressive increase of the RV to LV plus interventricular septum weight ratio (Fig. 1b). RV end-diastolic pressures were significantly increased in MCT-6 (4.9 ± 1.0 vs. 1.9 ± 0.5, 0.5 ± 0.3, and 1.1 ± 0.6 mmHg, in MCT-4, Ctrl-4, and Ctrl-6, respectively). Compared with the other groups, MCT-6 also presented lower body weight and heart rate (216 ± 9 vs. 287 ± 7, 265 ± 20, and 270 ± 20 beats/min, in MCT-4, Ctrl-4, and Ctrl-6, respectively).

Both systolic (Fig. 2) and diastolic parameters (Fig. 3) were preserved at baseline in the LV of MCT-4. MCT-6 animals, however, presented low peak LV systolic pressure and disturbed indexes of both LV contractility (Fig. 2), such as reduced peak isovolumetric pressures and dP/dt_{max}, and LV relaxation (Fig. 3), such as reduced dP/dt_{min} and prolonged time constant τ. Despite these hemodynamic disturbances neither the EDLVP nor the EDLVD were changed (Fig. 3).

Response of diastolic function to afterload elevations

Both MCT-4 and MCT-6 revealed an impaired response of diastolic function to afterload elevations (Fig. 4). This figure displays changes in the time constant τ (panel A) and shifts of the EDPDR (panel B) with increasing afterloads. Effects on τ are expressed by the ratio of test to baseline beats values. Time constant τ was differently affected by afterload in control and in MCT-treated rats. Control animals responded to afterload elevations with shortening of τ (acceleration of pressure fall) for almost the entire range of systolic LV pressures, apart from isovolumetric beats that showed a non-significant change in τ. On the contrary,

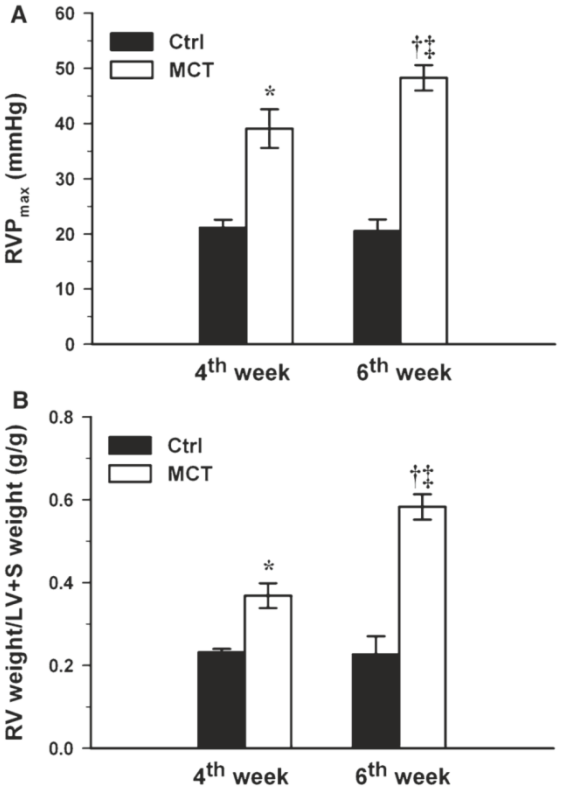

Fig. 1 Development of pulmonary hypertension and selective RV hypertrophy in response to monocrotaline (MCT) injection. **a** Peak systolic right ventricular pressures (RVP_{max}). **b** Right ventricular (RV) weight/left ventricular plus interventricular septum (LV + S) weight ratio. RVP_{max} progressively increased in MCT-4 and MCT-6; RV weight to LV weight ratio also increased in MCT-4 and MCT-6. $P < 0.05$: * vs. Ctrl-4; † vs. Ctrl-6; ‡ vs. MCT-4 ($n = 6$ in each group)

MCT-treated animals responded to afterload elevations with a prolongation of τ (slowing of pressure fall). This slowing was observed only with the two highest afterload levels in MCT-4, while in MCT-6 such slowing was more pronounced and present over the entire range of systolic LV pressures. With regard to the EDPDR, in response to afterload elevations, it remained unaffected in control animals, but was upward shifted in both MCT-4 and MCT-6 groups. This upward shift was of similar magnitude in MCT-4 and MCT-6 (Fig. 4b), but occurred at much lower systolic LV pressures in the later. Representative pressure–dimension loops are presented (Fig. 5).

Neurohumoral activation

The expression of genes involved in autocrine/paracrine systems was upregulated in the LV myocardium of MCT-treated rats compared with controls (Fig. 6). Angiotensin-converting enzyme mRNA levels were progressively

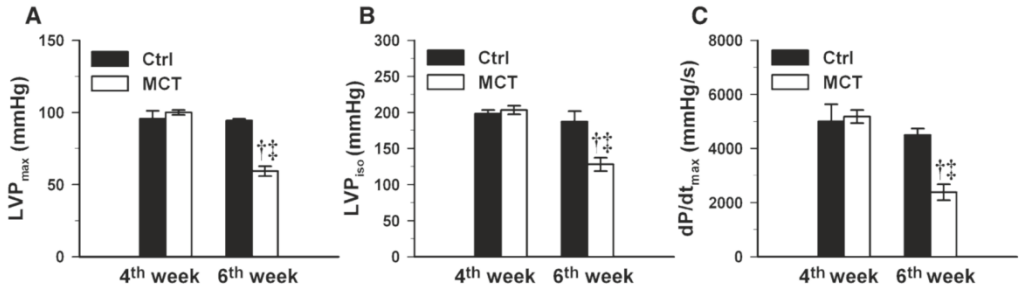

Fig. 2 Left ventricular (LV) systolic hemodynamic parameters. **a** Peak systolic LV pressure (LVP_{max}). **b** Peak isovolumetric LV pressure (LVP_{iso}). **c** Peak rate of LV pressure rise (dP/dt_{max}). $P < 0.05$: * vs. Ctrl-4; † vs. Ctrl-6; ‡ vs. MCT-4 ($n = 6$ in each group)

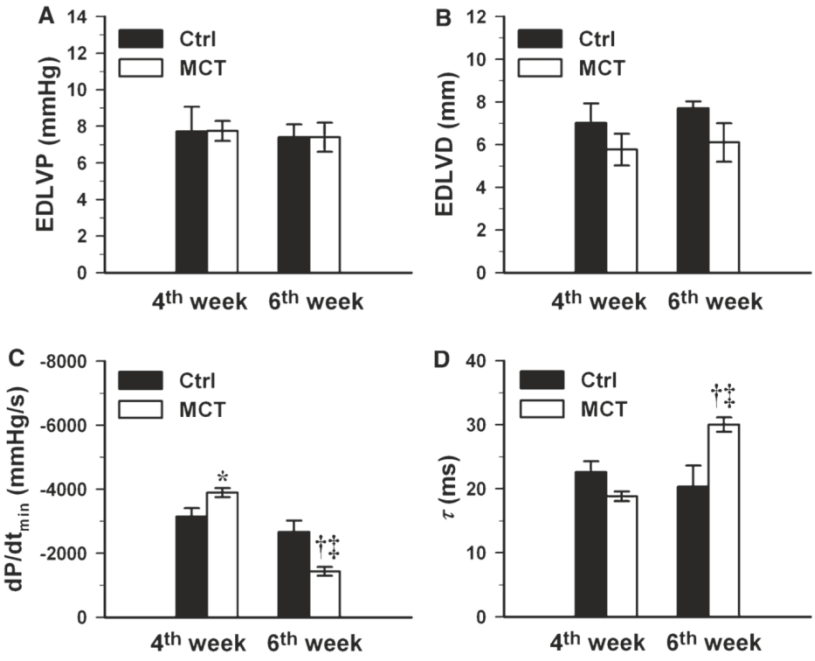

Fig. 3 Left ventricular (LV) diastolic hemodynamic parameters. **a** End diastolic left ventricular (LV) pressure (EDLVP). **b** End diastolic LV dimensions (EDLVD). **c** Peak rate of LV pressure fall (dP/dt_{min}). **d** LV time constant τ. Significant differences were found for dP/dt_{min} and τ, whereas no differences were observed for EDLVP and EDLVD. $P < 0.05$: * vs. Ctrl-4; † vs. Ctrl-6; ‡ vs. MCT-4 ($n = 6$ in each group)

increased in MCT-4 and MCT-6, whereas endothelin-1 expression was only elevated in MCT-6.

Apoptosis

Apoptosis was significantly augmented in LV myocardium of both MCT-4 and MCT-6 groups, as compared with controls (Fig. 7a). In the representative examples presented in Fig. 7b apoptotic cardiomyocytes can be easily identified in MCT groups.

Extracellular matrix composition

Regarding the extracellular matrix composition, we evaluated the gene expression of tenascin-C, an extracellular matrix glycoprotein as well as the LV interstitial collagen content. Both were significantly increased only in MCT-6 group (Fig. 8).

LV myofilaments

With regard to myofilament composition, a significant elevation of the relative expression of myosin heavy-chain β-isoform was observed in both MCT-4 and MCT-6 LV myocardium compared with Ctrl-4 and Ctrl-6, respectively (Fig. 9).

Discussion

The present study was undertaken to investigate the onset and progression of LV dysfunction in the course of PH and

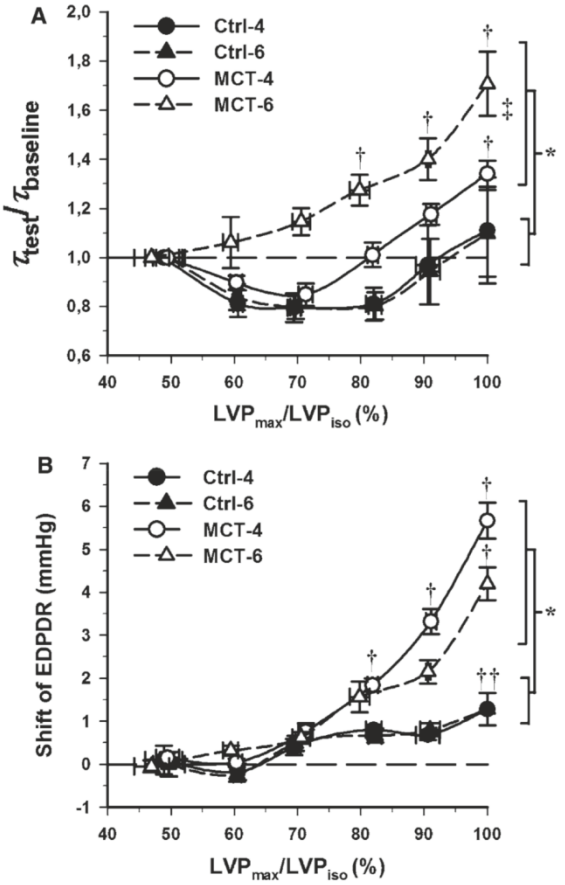

Fig. 4 Effects of acute left ventricular (LV) afterload elevations on LV diastolic function. **a** Fractional changes in time constant τ ($\tau_{test}/\tau_{baseline}$). **b** Upward shift in the end-diastolic pressure–dimension relation (EDPDR). Six beats with progressively higher peak systolic LV pressure (LVP_{max}) are represented: baseline, test-beats with a peak systolic LV pressure corresponding to 60, 70, 80, and 90% of the isovolumetric beat, and the isovolumetric test-beat (LVP_{iso}); $n = 6$ in each group. MCT-treated rats, distinctly from controls ($^*P = 0.002$), presented a prolongation of the time constant τ in test beats compared with baseline beat ($\dagger P < 0.01$), while controls presented an overall acceleration of relaxation and shortening of τ, with the exception of the isovolumetric test beats. In MCT-6 the changes in τ were more striking and observed for lower afterload levels than in MCT-4 ($\ddagger P = 0.007$). The upward shift of EDPDR in test-beats compared with baseline beat ($\dagger P < 0.01$) was more pronounced and observed for lower afterload levels in MCT-treated rats compared with controls ($^*P = 0.008$)

the underlying myocardial mechanisms, namely neurohumoral activation, extracellular matrix composition and apoptosis. A hemodynamic stress test with single-beat afterload elevations was used to detect early, load dependent, changes in LV diastolic function in animals with MCT induced PH.

MCT-treated rats progressively developed PH, as evaluated by RVP_{max}, selective RV myocardial hypertrophy, and increased EDRVP. As previously reported [33], MCT-6 rats showed clinical signs of congestive heart failure, disturbed indexes of LV contractility and relaxation, and increased expression of ACE and ET-1 in the LV myocardium. A more detailed discussion on the possible causes of neuroendocrine activation in the non-overloaded LV is provided elsewhere [33]. MCT-6 animals also presented reductions in body weight and LVP_{max}, previously interpreted as consequence of continuous severe sickness [36], as well as, diminished heart rate, suggesting an attenuation of the sympathetic nervous system response [25, 33]. In contrast, MCT-4 rats showed no baseline LV hemodynamic changes. Nonetheless, single afterloaded test-beats revealed diastolic function impairment in MCT-4. Systolic performance, as assessed by the contractility indexes LVP_{iso} and dP/dt_{max}, was unchanged, but diastolic dysfunction was elicited in response to increased afterload (afterload-induced diastolic dysfunction; [29, 30]). Diastole was evaluated with the time constant of isovolumetric relaxation τ and the end-diastolic pressure–diameter relation (EDPDR), the former evaluates active relaxation while the latter is an in vivo estimate of myocardial stiffness. The dynamic process of myocardial relaxation goes on from the ejection phase to the early filling period. The main hemodynamic manifestation of myocardial relaxation, however, is LV pressure fall and its analysis, through the time constant τ, allows a description of the course of myocardial relaxation [26]. Changes in afterload modify τ and the rate of LV pressure fall. While in healthy hearts small afterload elevations accelerate LV pressure fall and only marked afterload increases slow it, in failing hearts even slight increases in afterload may induce slower relaxation. At a similar systolic LV pressure, in failing hearts the relative load, defined as the ratio of systolic LV pressure to isovolumetric LV pressure, is higher, the afterload reserve is surpassed and afterload mismatch yields a pronounced slowing of LV pressure fall, compared with healthy hearts [10, 31].

Diastolic dysfunction is commonly defined, in hemodynamic terms, by an upward displacement of the EDPDR [26], which reflects a decrease in diastolic distensibility [13]. Classically, it was held that only chronic conditions that altered the compliance and distensibility of the LV could change the filling conditions to the point of modifying the EDPDR. However, more recent work demonstrated that the EDPDR could be acutely shifted by load [25, 31] and neurohumoral stimulation [27, 28, 37]. Afterload mismatch induces incomplete myocardial relaxation that can be large enough to increase LV pressure at end-diastole and cause diastolic dysfunction. A slower course of myocardial relaxation and the decrease in time available for the ventricle to relax are the major underlying mechanisms of afterload-induced diastolic dysfunction [29,

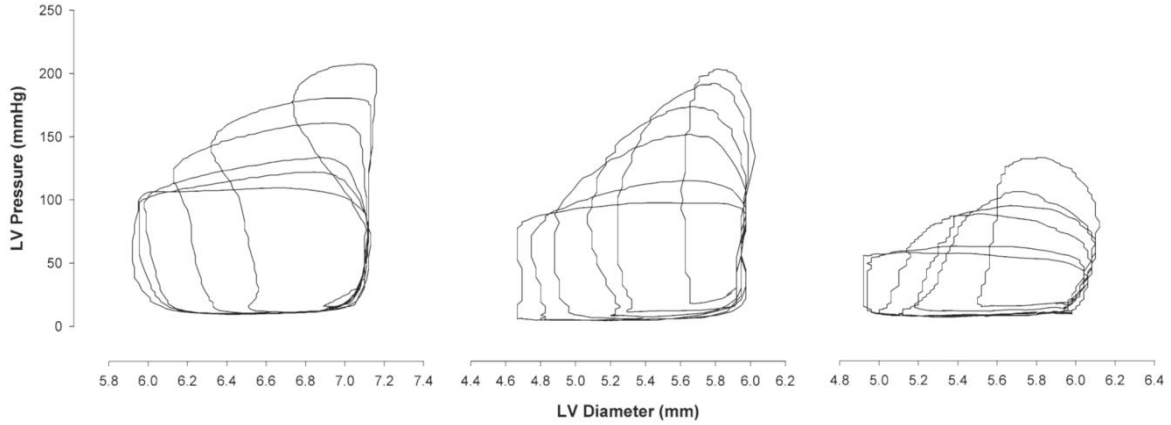

Fig. 5 Representative examples of left ventricular pressure-dimension (LVPD) loops of a Ctrl-4 *(left)*, a MCT-4 (*centre*) and a MCT-6 (*right*) animal at increasing afterload levels, from baseline up to isovolumetric. Peak systolic pressures are similar in Ctrl-4 and MCT-4 animals, but are clearly decreased in the MCT-6 animal. On the other side, diastolic pressures are nicely matched at all afterload levels in the control animal, but become progressively higher as afterload increases, both in MCT-4 and MCT-6 animals. As Ctrl-4 and Ctrl-6 animals were entirely similar only a representative example of Ctrl-4 is given

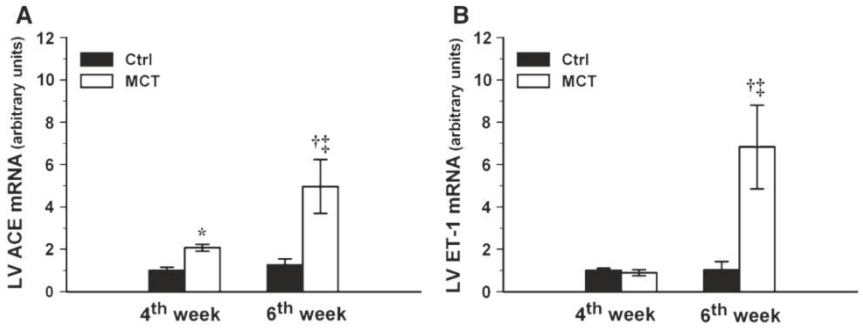

Fig. 6 Left ventricular (LV) mRNA levels of angiotensin-converting enzyme (ACE; **a**) and endothelin-1 (ET-1; **b**) in rats with monocrotaline (MTC)-induced pulmonary hypertension (MCT-4, $n = 6$; MCT-6, $n = 6$) and controls (Ctrl-4, $n = 6$; Ctrl-6, $n = 6$), 4 and 6 weeks after injection, respectively. Results are expressed as arbitrary units after normalization for GAPDH. The arbitrary unit was set as the average value of the Ctrl-4 group. ACE mRNA levels progressively increased in MCT-4 and MCT-6, whereas ET-1 mRNA levels were only increased 6 weeks after MCT injection $P < 0.05$: * vs. Ctrl-4; † vs. Ctrl-6; ‡ vs. MCT-4

30]. Compared with control rats, MCT-treated rats developed prolongation of time constant τ and a larger upward shift of the EDPDR indicating that afterload mismatch occurred at lower relative loads. Curiously, although MCT-6, unlike MCT-4, presented prolongation of τ at baseline that was aggravated with every step up in relative load, there were no differences between the two groups regarding the upward shift of the EDPDR induced by afterload. One possible reason is the difference in heart rates. Inappropriate increases in heart rate are known to upwardly displace the EDPDR even if relaxation rate is normal [49]. Conversely, the reduction in the heart rate of MCT-6 may have partly reverted afterload-dependent diastolic dysfunction by increasing the time available for relaxation and the extent of relaxation. Animals and patients with PH are known to respond badly to hemodynamic stress [19, 23, 45]. The present results suggest that increased systemic blood pressures may induce, or contribute to, hemodynamic deterioration in PH by leading to, or worsening of, LV diastolic dysfunction.

One of the most likely subcellular candidates for myocardial dysfunction is the altered myofilament composition. MCT-treated rats presented myosin heavy-chain isoform switch in the LV myocardium. The slower β-isoform was increased in the LV of both MCT-4 and MCT-6, as previously reported in different models of RV hypertrophy [17, 43, 46, 47]. Changes in the expression of enzymes involved in bioenergetics and cell metabolism such as pyruvate dehydrogenase kinase-4, acetyl-CoA dehydrogenase and mitochondrial ATP synthase [42, 43] as well as a reduced capillary proliferation [21] have also been described in the LV of PH rats. Contrastingly, calcium kinetics

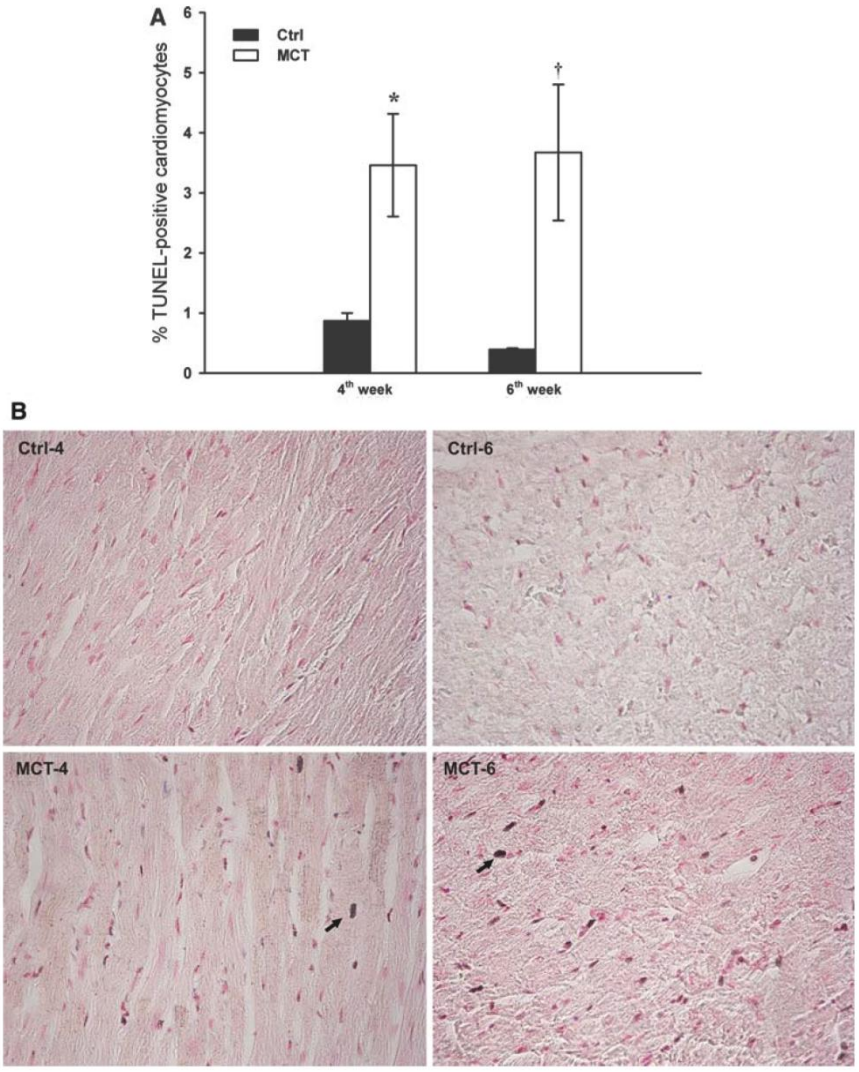

Fig. 7 Left ventricular (LV) apoptosis is expressed as TUNEL-positive cell percentage (**a**). There is a significant increase in apoptosis in both MCT-4 and MCT-6 groups ($P < 0.05$: * vs. Ctrl-4; † vs. Ctrl-6). Representative examples from rats with monocrotaline-induced pulmonary hypertension (MCT) and controls (Ctrl) 4 and 6 weeks after injection are presented in **b** (*arrows* apoptotic cells)

regulator proteins such as the Ca^{2+}-ATPase of the sarcoendoplasmic reticulum and phospholamban were consistently reported to be unaltered in the non-overloaded LV [21, 42, 43]. To further explore the underlying mechanisms of LV myocardial dysfunction, we investigated neurohumoral activation, extracellular matrix composition and apoptosis.

Regarding local autocrine/paracrine related gene expression, MCT-4 rats showed increased ACE mRNA levels in the LV myocardium. However, only MCT-6 rats presented markedly increased expression of ET-1, and ACE was further upregulated compared with MCT-4. The activation of autocrine/paracrine systems, particularly for ET-1, was concomitant with the development of LV systolic and diastolic dysfunction in the baseline hemodynamic evaluation. It has been suggested that cardiac ET-1 may play a critical role in the functional deterioration of LV during the transition to congestive heart failure [18]. Indeed, chronic administration of ET-1, for 5 days, to engineered heart tissues, derived from cells of neonatal rat hearts, decreased the contractile response to calcium and isoprenaline and changed myosin heavy chain isoform and sarcoendoplasmic reticulum Ca^{2+}-ATPase expression in a signaling pathway that involved protein kinase-C and the Na^{+}/H^{+} exchanger [51]. Lower heart rate, LVP_{max} and LV dysfunction under the stress of anesthesia and surgical manipulation could well be partly the consequence of refractoriness to adrenergic stimulation mentioned above. Further supporting the possibility that ET-1 might have an important role modulating LV function in PH we have previously demonstrated that chronic ET-1 blockade prevents functional deterioration of the LV

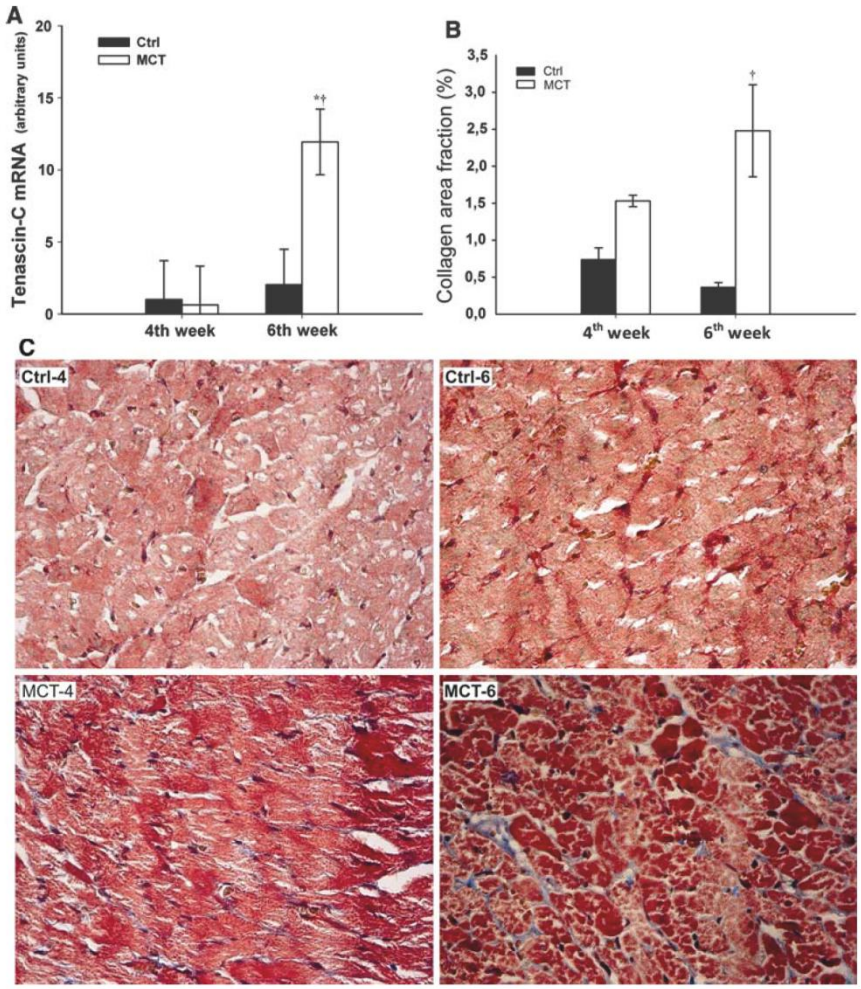

Fig. 8 Left ventricular (LV) mRNA levels of tenascin-C expressed as arbitrary units after normalization for GAPDH (**a**) was increased in MCT-6 group ($P < 0.05$: * vs. Ctrl-6; † vs. MCT-4). Collagen area fraction was increased in MCT-6 group ($P < 0.05$: * vs. Ctrl-6) (**b**). Representative examples of left ventricle myocardium stained with Masson's Trichrome of rats with monocrotaline-induced pulmonary hypertension (MCT) and controls (Ctrl) 4 and 6 weeks after injection, are presented in **c**

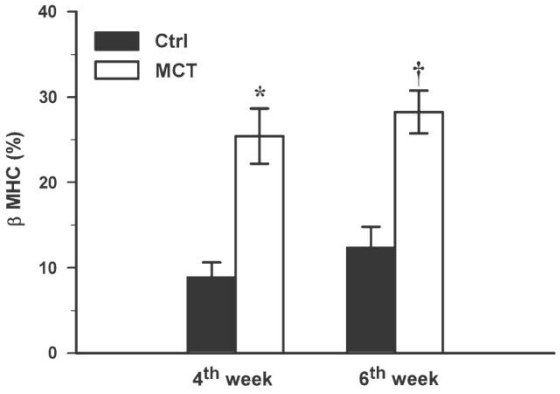

Fig. 9 Myosin heavy chain (MHC) isoform switch in monocrotaline-induced pulmonary hypertension, manifested as a significant increase of the β-MHC isoform that was similar in the MCT-4 and MCT-6 groups. $P < 0.05$: * vs. Ctrl-4; † vs. Ctrl-6 ($n = 6$ in each group)

myocardium in PH rats, restoring normal contractile response to increasing frequencies of stimulation [33].

A possible molecular mechanism involved in the left ventricular dysfunction that accompanied severe PH is altered extracellular matrix composition and fibrillar collagen content [6, 8, 38]. Tenascin-C is synthesized by fibroblasts, highly expressed in embryonic tissues, and upregulated by both mechanical stress [2] and neuroendocrine mediators [34] in adult tissues under pathological conditions such as myocardial infarction [16]. Additionally, several pro-inflammatory cytokines, growth factors and neurohumoral peptides that stimulate tenascin-C synthesis [8, 34, 42] also participate in myocardial dysfunction induced by MCT [6, 33]. Tenascin-C is an important regulator of extracellular matrix and induces several metalloproteinases which activation is implicated in cardiac remodeling [44]. In the present study, we could demonstrate

that there is a significant increase in myocardial interstitial collagen content and tenascin-C gene expression only 6 weeks after MCT injection, suggesting that changes in extracellular matrix composition may partly underlie the late deterioration of LV function in MCT-induced PH.

There are evidences suggesting that cell cycle and apoptosis pathways are co-activated in pressure-overload RV in MCT model [5]. In this sequence, we assessed the LV myocardial apoptosis, and found increased rates of apoptosis both in MCT-4 and MCT-6. Although there is some controversy about the role of apoptosis in chronic heart failure [1, 7, 22], this results suggest that it might be another possible mechanism involved in early events of LV dysfunction.

In conclusion, selectively RV overloaded MCT-treated rats develop, on hemodynamic assessment, LV dysfunction that is accompanied by myosin heavy-chain isoform switch and local LV autocrine/paracrine system activation. Despite the changes in myocardial molecular phenotype, such as MHC isoform switch, increased apoptosis and ACE upregulation, in the LV, 4 weeks after MCT injection, diastolic dysfunction can only be detected in MCT-4 by increasing afterload. Two weeks later, however, the LV myocardium of MCT-6 overexpresses ET-1 has increased collagen content and augmented gene expression of tenascin-C and presents baseline systolic and diastolic dysfunction. The transition to LV dysfunction and congestive heart failure in PH is a complex process that involves the activation of several subcellular pathways. Afterload-dependent diastolic disturbances of the LV may herald, or contribute to, hemodynamic deterioration in PH.

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A Western-Type Diet Attenuates Pulmonary Hypertension with Heart Failure and Cardiac Cachexia in Rats[1–3]

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Abstract

Western-type diets (WD) constitute risk factors for disease but may have distinct effects in heart failure (HF) with cardiac cachexia (CC). We evaluated hemodynamic, metabolic, and inflammatory effects of short-term WD intake in pulmonary hypertension (PH) with CC. Male Wistar rats randomly received 60 mg · kg^{-1} monocrotaline (M) or vehicle (C) and consumed either a 5.4-kcal · g^{-1} WD (35% animal fat, 35% simple carbohydrate, 20% protein, 0.4% Na^+) or a 2.9-kcal · g^{-1} (3% vegetable fat, 60% complex carbohydrate, 16% protein, 0.25% Na^+) normal diet (ND) for 5 wk. Mortality, energy intake, body weight (BW), metabolism, hemodynamics, histology, apoptosis, gene expression, transcription factors, and plasma cytokines were evaluated. Compared with the C-ND group, the M-ND group had PH, HF, and mortality that were significantly attenuated in M-WD. The extent of myocardial remodeling and apoptosis was higher in M-ND than in C-ND but lower in M-WD than in M-ND, while conversely, energy intake, BW, cholesterol, and TG plasma concentrations were lower in M-ND than in C-ND but higher in M-WD than in M-ND. M-ND had increased myocardial NF-κB transcription factor activity, endothelin-1, and cytokine overexpression and higher circulating cytokine concentrations than C-ND, which were lower in M-WD than in M-ND. PPARα activity, however, was lower in M-ND, but not in M-WD, compared with the respective C groups. WD attenuated PH and CC, ameliorating survival, myocardial function, metabolism, and inflammation, through transcription factor modulation, suggesting a beneficial role in CC. J. Nutr. 141: 1–8, 2011.

Introduction

CC[6], defined by a weight loss of >6% over 6 mo (1), accompanies HF in up to 50% of severe cases and independently determines a poor prognosis (2). As part of the complex mechanisms underlying CC are abnormalities of general metabolism as well as of the immune and neuroendocrine systems, with inflammatory activation playing a prominent role (3). Besides increased basal metabolism, reduced appetite, and gastrointestinal derangements that compromise energy intake (4), the failing heart undergoes extensive metabolic changes, namely a myocardial shift from FFA oxidation to glycolysis and reduced mitochondrial oxidative capacity that partly underlie the functional disturbances (5). Energy restriction has been shown to reduce cardiovascular risk and improve overall metabolism and organ function (6). AHA nutritional guidelines recommend avoiding simple carbohydrates and fat, particularly saturated, as main energy sources in a balanced diet (7). This may not apply, however, to severe HF with disturbed cardiomyocyte metabolism and CC. In fact, epidemiological studies strongly support that obesity and hypercholesterolemia paradoxically improve survival in HF (8), so it would not be surprising that a hypercaloric and cardiovascular risk-associated WD could have some benefits. Among the experimental models of CC, MCT-induced PH stands out with rapidly progressive HF and cachexia (9). Our goal was to test the effects of a WD rich in saturated animal fat and simple carbohydrates and with a higher salt content on survival, PH, myocardial function, remodeling, neuroendocrine and inflammatory activity, and cachexia in severe MCT-induced PH.

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[3] Supplemental Tables 1–4 and Figures 1–5 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at jn.nutrition.org.

[6] Abbreviations used: *Acadm*, medium-chain acyl CoA dehydrogenase; *Acsl1*, long-chain-fatty acid-CoA ligase 1; *Bax*, Bcl-2–associated X protein; *Bcl-2*, B-cell lymphoma 2; BP, blood pressure; BW, body weight; CC, cardiac cachexia; CI, cardiac index; CO, cardiac output; E_A, arterial elastance; EDP, end-diastolic pressure; EDPVR, end-diastolic pressure-volume relationship; EDV, end-diastolic volume; EF, ejection fraction; ESPVR, end-systolic pressure-volume relationship; HF, heart failure; IR, insulin resistance; IVS, interventricular septum; *Acadl*, long chain acyl CoA dehydrogenase; LV, left ventricle; MCT, monocrotaline; MHC, myosin-heavy chain; ND, normal diet; OGT, oral glucose tolerance; PH, pulmonary hypertension; RV, right ventricle; TC, total cholesterol; TL, tibial length; WD; Western-type diet.

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Materials and Methods

Animal model. Seven-week-old (180–200 g) male Wistar rats (Charles River; n = 192) randomly received either 60 mg·kg^{-1} s.c. MCT (Sigma Chemicals) (M) or an equal volume of vehicle (C). Both groups were randomly allocated 48 h later to consume ad libitum either a WD (F2685, BioServe Frenchtown; 5.4 kcal · g^{-1}, 35% animal fat, 35% simple carbohydrate, 20% protein, and 0.4% Na^+) or ND (A04, Scientific Animal Food & Engineering; 2.9 kcal · g^{-1}, 3% vegetable fat, 60% complex carbohydrate, 16% protein, and 0.25% Na^+). Nutrient sources were selected to reproduce a healthy diet and a WD (**Supplemental Table 1**). Rats were housed in groups of 5/cage in a controlled environment (12-h-light/-dark cycle, 22°C room temperature). BW, food ingestion, and mortality were recorded (n = 136 for survival analysis). IR and OGT were sequentially evaluated, with a 24-h interval, 20–23 d after injection. At d 24, rats underwent 24-h metabolic cage studies. Hemodynamic evaluation was carried out at d 28–32 after 24 h of ND. Investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the NIH (NIH Publication no. 85–23, revised 1996) and was approved by the ethics committee of the Faculty of Medicine of Porto.

Metabolic studies. IR and OGT (n = 7/group) were evaluated after 12-h feed deprivation, recording baseline, 15, 30, 45, 60, 90, and 120 min glycemia (Freestyle-Mini) after 0.5 U · kg^{-1} i.p. insulin and 1 g · kg^{-1} glucose gavage, respectively. BW gain, urine output, and energy intake were measured for 24 h (Techniplast, Buguggiate).

Hemodynamic evaluation. After sedation (100 μg · kg^{-1} and 5 mg · kg^{-1} i.p. fentanyl and midazolam, respectively), anesthesia (8 and 2.5–3% sevoflurane for induction and maintenance, respectively; Penlon Sigma Delta), endotracheal intubation, mechanical ventilation (model 683, Harvard Apparatus), 8 mL · kg^{-1} · h^{-1} i.v. warm Ringer's solution infusion (NE-1000, New Era Pump Systems), temperature maintenance at 38°C on a heating pad, left thoracotomy, LV and RV pressure-volume catheter insertion (SPR-838 and PVR-1045 Millar Instruments, respectively), and ascending aorta probe placement (200–367, Triton Technology) that allowed CO measurement (Active Redirection Transit Time Flowmeter, Triton Technology), signals were continuously acquired (MPVS 300, Millar Instruments), recorded at 1000 Hz (ML880 PowerLab 16/30, ADinstruments), and analyzed (PVAN 3.5, Millar Instruments). Recordings (n = 7/group) were obtained at suspended end-expiration. The LV catheter was advanced to record systemic BP. Parallel conductance was assessed with hypertonic saline. After killing (100 mg·kg^{-1} i.v. pentobarbital), blood was retrieved for storage (−80°C) and volume calibration (910–1048, Millar Instruments). Organs were weighed, RV and LV + IVS were weighed after dissection, and TL measured. The right upper lung lobe, mesenteric fat, and RV and LV free-walls were snap-frozen and stored (−80°C). Weights were normalized to TL, because BW fluctuations in MCT-induced PH make it unreliable (10).

Histology. Four-μm-thick, paraffin-embedded tissue sections (n = 7 additional rats/group) were evaluated for cardiomyocyte diameter, fibrosis, and medial hypertrophy of pulmonary arterioles (11).

DNA and protein content. After extraction (n = 7/group) from 10 mg of RV and LV (Cat. no. 80004, Qiagen), DNA, and protein concentrations were assayed by spectrophotometry at 260 nm (Eppendorf 6131000.012) and by bicinchoninic acid (Cat. no. 23250, Pierce), respectively (12).

mRNA. Two-step RT-PCR was performed in mesenteric fat, LV, and RV (n = 7/group) as reported (13), with specific primer pairs (**Supplemental Table 2**) for *Edn1*, a contributor to the progression of MCT-induced PH (13); *Bcl-2* and *Bax*, apoptosis regulators; the cytokines *Tnf* and *Il6*; the adipokines *Adipoq* and *Lep*, involved in CC pathophysiology (14); *Ppara* and the key enzymes it controls, namely *Acsl1*, *Acadm*, and, *Acadl*, involved in FFA oxidation; and *Pdk4*, involved in glucose oxidation (5). Results are presented as fold of C-ND. *Actb* and *Gapdh* were used as internal controls in the myocardium and adipose tissue, respectively, because groups did not differ.

MHC isoforms. In RV and LV samples (n = 6/group), 15 μg of protein underwent SDS-PAGE, as described (15). Staining was performed with Coomassie Brilliant Blue and scanning at 700 nm (Odyssey; LI-COR Biosciences).

Plasma TNFα, IL-6, adiponectin, and leptin. Enzyme immunoassays for TNFα (45-TNFRTU-E01, Alpco Diagnostics), IL-6 (DE 4845, Demeditec diagnostics GmbH), adiponectin (22-ADPRT-E01, Alpco Diagnostics), and leptin (27295, Immuno-Biological Laboratories) were performed according to manufacturer's instructions (n = 7/group).

Plasma TC and TG. Samples (n = 7/group) underwent TC (cholesterol CP, A11A01634, Horiba Medical) and TG quantification (TG CP, A11A01640, Horiba Medical) in a chemical analyzer (ABX Pentra 400, Horiba Medical).

Apoptosis. The extent of apoptosis was assessed in histological sections (n = 7/group) as percentage of terminal deoxynucleotidyl-transferase-mediated dUTP nick end-labeling-marked to total cardiomyocyte nuclei (15).

NF-κB and PPARα activity. Nuclear proteins (20 μg) extracted from RV and LV (No. 11906–100, Marligen Biosciences) were added to wells with specific dsDNA response elements for NF-κB and PPARα (nos. 1007889 and 10006915, Cayman Chemical). Binding was detected by an IRDye 800CW-conjugated secondary antibody (no. 926–32211, LI-COR biosciences) and reading at 800 nm (Odyssey; LI-COR Biosciences).

Statistical analysis. Data were analyzed by Kaplan-Meier survival analysis with the Gehan-Breslow statistic, 2-way repeated-measures ANOVA for BW and energy intake, and 2-way ANOVA elsewhere. Holm-Sidak's method was employed for post hoc comparisons between groups with adjusted P values. Unequal variances in 2-way ANOVA were assessed by Mauchly's test for assumption of sphericity and were dealt with by correcting d.f. to produce a valid F-ratio. Data are mean ± SEM. Differences were considered significant at $P < 0.05$.

Results

Survival, BW, energy intake, body composition, and metabolism. The mortality rate was greater for the M-WD group compared to the M-ND group (**Fig. 1***A*). Both M groups exhibited signs of HF, including labored breathing, lethargy, and pleural effusion, but M-WD rats were less lethargic.

Throughout the study, the M groups had lower BW gain (Fig. 1*B*) and energy intake than the C groups (Fig. 1*C*). The M-WD group, however, had significantly higher intake and BW than the M-ND group. Indeed, from the 4 groups, only M-ND did not gain BW and had lower urine output during metabolic cage studies compared with the C-ND group (**Table 1**). Furthermore, compared with the C-ND group, M-ND had lower gastrocnemius, liver, and perirenal and perigonadal fat pad weights, which were offset in the M-WD group, compared with M-ND (**Supplemental Table 3**). The C-WD group showed greater BW gain (Fig. 1*B*), energy intake (Fig. 1*C*), and adiposity than the C-ND group (Supplemental Table 3). Both WD groups, and also M-ND, showed increased basal glycemia and IR compared with their ND and C-ND counterparts (Table 1). Plasma TG concentrations were lower in the M-ND group than in the C-ND group and were restored in M-WD and TC was also lower in the M-ND group than in the C-ND group, but greater in both WD groups compared with their corresponding ND groups (Table 1).

Hemodynamics. The pulmonary artery E_A, RV systolic pressure, and ESPVR slopes were higher in the M-ND group than in the C-ND group, along with reduced EF, prolonged time constant of isovolumetric relaxation by logarithmic method,

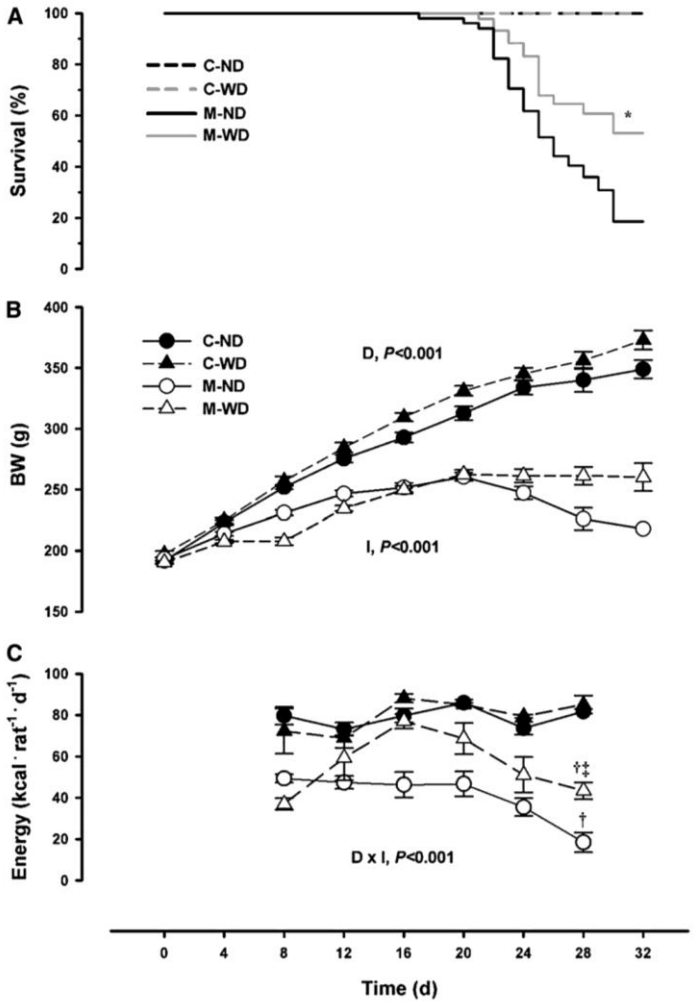

FIGURE 1 Survival (*A*), BW (*B*), and energy intake (*C*) in C- or M-injected rats fed a ND or WD. Data plotted as mean ± SEM, n = 17, 22, 53, and 44 rats in C-ND, C-WD, M-ND, and M-WD groups, respectively, for the purpose of survival analysis. BW and energy intake were recorded from rats still surviving at each time point. *P = 0.016 vs. M-ND, †P < 0.001 vs. corresponding C group, ‡P = 0.005 vs. M-ND. Significant P values are given for the main effects of D and I or for their interaction (D × I). BW, body weight; C, control; D, diet; I, injection; M, monocrotaline; ND, normal diet; WD, Western diet.

and increased RV EDV, EDP, and EDPVR slopes. All these changes were attenuated in the M-WD group that was either different from M-ND or did not differ from the C-WD group (**Table 2**). Heart rate, CO, and CI fell in the M-ND group compared with the C-ND group and were also preserved in the M-WD group compared with the M-ND group (Table 2).

TABLE 1 Metabolic studies, glucose metabolism, and plasma TC and TG in C- or M-injected rats fed a ND or WD diet for 5 wk[1]

	C-ND	C-WD	M-ND	M-WD	P value[2]
Final BW,[3] g	286 ± 4	300 ± 4†	263 ± 4*	268 ± 3*	I, D
BW gain, $\% \cdot d^{-1}$	2.48 ± 1.29	4.50 ± 1.18	−0.08 ± 1.36*	3.81 ± 0.73†	D x I
Energy intake, $kcal \cdot kg^{-1} \cdot d^{-1}$	0.246 ± 0.009	0.370 ± 0.031†	0.224 ± 0.024	0.326 ± 0.036†	D
Urine output, $mL \cdot kg^{-1} \cdot d^{-1}$	35.0 ± 3.4	33.1 ± 4.8	19.5 ± 2.8*	24.3 ± 4.5	I
Glycemia, $mmol \cdot L^{-1}$	5.9 ± 0.2	6.7 ± 0.3†	6.7 ± 0.3*	7.4 ± 0.2*†	I, D
OGT AUC, $mmol \cdot L^{-1} \cdot h$	19.4 ± 2.1	20.7 ± 1.9	19.5 ± 1.1	21.9 ± 0.9	NS
IR AUC, $mmol \cdot L^{-1} \cdot h$	9.0 ± 0.7	15.2 ± 0.5†	11.6 ± 1.1*	15.0 ± 1.0†	D x I
TC, $mmol \cdot L^{-1}$	0.90 ± 0.05	1.52 ± 0.09†	0.59 ± 0.08*	1.49 ± 0.12†	D x I
TG, $mmol \cdot L^{-1}$	14.6 ± 1.2	14.0 ± 2.6	4.6 ± 0.7*	14.0 ± 1.5†	D x I

[1] Metabolic cage studies were conducted at d 24 and plasma lipid measurements were performed at 5 wk. Values are mean ± SEM, n = 7. *Different from corresponding C group, $P < 0.05$; †different from the corresponding ND group, $P < 0.05$. BW, body weight; C, control; D, diet; I, injection; IR, insulin resistance test; M, monocrotaline; ND, normal diet; NS, not significant; OGT, oral glucose tolerance; TC, total cholesterol; WD, Western-type diet.
[2] Significant ($P < 0.05$) effects of D, I, and their interaction (D × I) are shown; NS, $P > 0.05$.

TABLE 2 RV and LV hemodynamics of C- or M-injected rats fed a ND or WD diet for 5 wk[1]

	C-ND	C-WD	M-ND	M-WD	*P* value[2]
Baseline			*fold of C-ND*		
HR,[3] min^{-1}	375 ± 22	397 ± 10	284 ± 23*	359 ± 15†	I,, D
CO, $mL \cdot min^{-1}$	57.8 ± 2.2	64.3 ± 7.2	28.3 ± 4.1*	43.6 ± 3.9*†	I, D
CI,[4] $mL \cdot min^{-} \cdot cm^{-2}$	0.127 ± 0.003	0.138 ± 0.014	0.079 ± 0.010*	0.116 ± 0.008†	I, D
Mean BP, *mm Hg*	116 ± 3	119 ± 4	84 ± 4*	97 ± 2*†	I, D
RV					
SP, *mm Hg*	38 ± 2	38 ± 4	74 ± 4*	59 ± 5*†	D x I
EDP, *mm Hg*	4 ± 0	4 ± 1	8 ± 3*	6 ± 1	I
EDV, μL	229 ± 16	241 ± 18	312 ± 36*	280 ± 41	I
EF, %	67 ± 2	66 ± 3	35 ± 5*	49 ± 5*†	D x I
τ_{log}, *ms*	11.7 ± 1.5	12.0 ± 0.6	15.8 ± 1.3*	11.1 ± 1.6†	D x I
E_A, $mm\ Hg \cdot \mu L^{-1}$	0.23 ± 0.01	0.25 ± 0.05	0.81 ± 0.14*	0.55 ± 0.06*†	D x I
IVC occlusion					
EDPVR, $mm\ Hg \cdot \mu L^{-1}$	0.014 ± 0.004	0.016 ± 0.002	0.024 ± 0.002*	0.022 ± 0.006	I
ESPVR, $mm\ Hg \cdot \mu L^{-1}$	0.20 ± 0.05	0.18 ± 0.07	0.73 ± 0.19*	0.56 ± 0.22	I
LV					
SP, *mm Hg*	129 ± 4	126 ± 5	95 ± 5*	111 ± 3*†	D x I
EDP, *mm Hg*	5 ± 1	4 ± 0	5 ± 0	6 ± 1	NS
EDV, μL	271 ± 19	258 ± 29	157 ± 27*	199 ± 20	I
EF, %	57 ± 5	62 ± 5	62 ± 3	58 ± 4	NS
Maximal rate of pressure rise, $mm\ Hg \cdot s^{-1}$	10200 ± 700	11200 ± 1200	5800 ± 600*	8100 ± 600*†	I, D
τ_{log}, *ms*	8.6 ± 0.8	7.6 ± 0.4	12.7 ± 1.1*	9.7 ± 0.6†	I, D
SW, $mm\ Hg \cdot \mu L$	14000 ± 1400	15900 ± 1800	6800 ± 800*	10800 ± 1300*†	I, D
E_A, $mm\ Hg \cdot \mu L^{-1}$	0.78 ± 0.05	0.76 ± 0.08	1.33 ± 0.27*	1.00 ± 0.11	I
IVC occlusion					
EDPVR, $mm\ Hg \cdot \mu L^{-1}$	0.038 ± 0.008	0.046 ± 0.008	0.099 ± 0.022*	0.066 ± 0.010	I
ESPVR, $mm\ Hg \cdot \mu L^{-1}$	0.75 ± 0.19	0.69 ± 0.28	2.86 ± 0.87*	3.16 ± 1.23*	I

[1] Values are mean ± SEM, $n = 7$. *Different from corresponding C group, $P < 0.05$; †different from corresponding ND group, $P < 0.05$. No differences were observed for the intercepts of indexes described by linear regression. These are not presented for the sake of simplicity. BP, blood pressure; C, control; CI, cardiac index; CO, cardiac output; E_A, arterial elastance; EDP, end-diastolic pressure; EDPVR, end-diastolic pressure-volume relationship slope; EDV, end-diastolic volume; EF, ejection fraction; ESPVR, end-systolic pressure-volume relationship slope; HR, heart rate; I, injection; IVC, inferior vena cava; LV, left ventricle; M, monocrotaline; ME, (time-varying) maximal elastance; ND, normal diet; NS, not significant; PH, pulmonary hypertensive; RV, right ventricle; SP, systolic or maximum pressure; SW, stroke work; τ_{log}, time-constant of isovolumic relaxation by logarithmic regression; WD, WD, Western-type diet.
[2] Significant ($P < 0.05$) effects of D, I, and their interaction (D × I) are shown; NS, $P > 0.05$.
[3] Body surface area was estimated as $9.1 \cdot (BW\ in\ g)^{2/3}$ for computation of CI.

Considering the LV, the M-ND group had lower LV systolic pressure, maximal rate of pressure rise, and stroke work and prolonged time constant of isovolumetric relaxation by logarithmic method compared with the C-ND group, and these were similarly attenuated in the M-WD group. The M-ND group, but not the M-WD group, also had lower LV EDV and elevated LV EDPVR slopes compared with their corresponding C groups (Table 2). Representative pressure-volume loops are given online (**Supplemental Fig. 1**).

Morphometry, histology, apoptosis, and MHC isoforms. The M-ND group (24.0 ± 0.6%) had hypertrophy ($P = 0.003$) of the media of lung arterioles (**Supplemental Fig. 2**) compared with the C-ND group (19.6 ± 1.4%) that was attenuated, compared with M-ND ($P = 0.002$), in M-WD (19.4 ± 0.4%). As for the myocardium, both M groups had significantly increased RV cardiomyocyte diameters compared with their respective C groups, whereas LV myocytes did not change (**Supplemental Table 4**). Nevertheless, whereas the RV weight:TL ratio was significantly greater in both M groups compared with their corresponding C groups, the TL-normalized weight of LV + IVS was lower in the M groups than in the C groups but significantly attenuated in M-WD compared with M-ND groups (Supplemental Table 4). Both the RV and LV of the M-ND group (3.5 ± 0.8 and 3.5 ± 0.7%, respectively) had higher ($P < 0.01$) fibrosis (**Supplemental Fig. 3**) than did the C-ND group (0.3 ± 0.1 and 0.5 ± 0.1%, respectively)m which was offset ($P = 0.02$), compared with the M-ND group, in the LV of the M-WD group (2.0 ± 0.2%). The extent of apoptosis was also higher ($P < 0.05$) in the RV and LV of the M-ND group (6.7 ± 1.6 and 4.8 ± 1.6%, respectively) compared with the C-ND group (0.7 ± 0.2 and 0.4 ± 0.0%, respectively), which was similarly attenuated ($P < 0.05$) in both ventricles of the M-WD group (3.2 ± 0.7 and 1.7 ± 0.2%, respectively) compared with the M-ND group, as documented by terminal deoxynucleotidyl-transferase-mediated dUTP nick end-labeling (**Supplemental Fig. 4**) and the *Bax:Bcl-2* expression ratio (**Table 3**). Analogously, the β-MHC isoform percentage (**Supplemental Fig. 5**) was higher ($P < 0.01$) in the RV and LV of the M-ND group (21 ± 4 and 19 ± 2%, respectively) than in the C-ND group (11 ± 1 and 7 ± 1%, respectively) and was also attenuated ($P < 0.01$) in the M-WD group (13 ± 1 and 11 ± 2%, respectively). Whereas there were no differences in the LV, both M groups had a higher RV protein content compared with their corresponding C groups, but the M-ND group had a lower protein:DNA ratio than the C-ND group due to a greater DNA content (Supplemental Table 4).

TABLE 3 RV, LV, and visceral adipose tissue gene expression in C- or M-injected rats fed a ND or WD diet for 5 wk[1]

	C-ND	C-WD	M-ND	M-WD	*P* value[2]
	fold of C-ND				
RV[3]					
Edn1	1.0 ± 0.3	1.2 ± 0.3	2.8 ± 0.1*	1.7 ± 0.4	I
Tnf	1.0 ± 0.3	0.8 ± 0.2	0.6 ± 0.3	1.2 ± 0.5	NS
Il6	1.0 ± 0.4	0.8 ± 0.2	0.6 ± 0.2	0.9 ± 0.3	NS
Bax:Bcl-2	1.0 ± 0.2	1.3 ± 0.4	2.1 ± 0.3*	1.5 ± 0.2	I
Ppara	1.0 ± 0.1	1.1 ± 0.1	0.6 ± 0.1*	0.7 ± 0.1*	I
Acsl1	1.0 ± 0.1	1.1 ± 0.1	0.4 ± 0.0*	0.5 ± 0.1*	I
Acadm	1.0 ± 0.1	0.8 ± 0.1	0.4 ± 0.1*	0.3 ± 0.1*	I
Acadl	1.0 ± 0.1	0.8 ± 0.1	0.5 ± 0.1*	0.5 ± 0.1*	I
Pdk4	1.0 ± 0.1	1.4 ± 0.2†	0.5 ± 0.1*	0.7 ± 0.2*	I, D
LV					
Edn1	1.0 ± 0.3	1.4 ± 0.3	10.7 ± 0.7*	4.8 ± 1.5*†	D x I
Tnf	1.0 ± 0.3	1.0 ± 0.5	3.2 ± 0.5*	0.6 ± 0.2†	D x I
Il6	1.0 ± 0.4	1.2 ± 0.3	11.8 ± 2.5*	3.3 ± 0.6†	D x I
Bax:Bcl-2	1.0 ± 0.3	1.2 ± 0.4	6.7 ± 1.5*	3.0 ± 0.3†	D x I
Ppara	1.0 ± 0.1	1.1 ± 0.1	0.6 ± 0.1*	0.8 ± 0.1	I
Acsl1	1.0 ± 0.0	1.5 ± 0.2†	1.0 ± 0.1	1.0 ± 0.1*	D x I
Acadm	1.0 ± 0.1	1.4 ± 0.3†	0.9 ± 0.1	1.1 ± 0.1	D
Acadl	1.0 ± 0.1	1.8 ± 0.2†	1.2 ± 0.2	1.3 ± 0.3	D
Pdk4	1.0 ± 0.0	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	NS
Visceral adipose tissue					
Tnf	1.0 ± 0.2	0.7 ± 0.1	6.1 ± 2.1*	3.8 ± 0.7	I
Il6	1.0 ± 0.3	0.4 ± 0.1	3.6 ± 1.5*	2.2 ± 1.0	I
Adipoq	1.0 ± 0.2	1.4 ± 0.3	6.5 ± 1.6*	3.1 ± 0.8*†	D x I

[1] Gene expression was normalized for *Actb* and *Gapdh* in the myocardium and adipose tissue, respectively. Values are mean ± SEM relative to C-ND (fold of C-ND), $n = 7$. *Different from corresponding C group, $P < 0.05$; †different from corresponding ND group, $P < 0.05$. No changes were observed for *lep* in visceral adipose tissue (data not shown). *Acadl*, long chain acyl CoA dehydrogenase; *Acadm*, medium-chain acyl CoA dehydrogenase; *Acsl1*, long-chain-fatty acid-CoA ligase 1; *Actb*, β-actin; *Adipoq*, adiponectin; *Bax*, Bcl-2–associated X protein; *Bcl-2*, B-cell lymphoma 2; D, diet; *Edn1*, endothelin-1; I, injection; *Lep*, leptin; LV, left ventricle; M, monocrotaline; NS, not significant; *Pdk4*, pyruvate-dehydrogenase kinase type 4; RV, right ventricle; WD, Western-type diet.

Significant ($P < 0.05$) effects of D, I, and their interaction (D × I) are shown; NS, $P > 0.05$.

Myocardium and visceral adipose tissue gene expression. *Edn1* was upregulated in both ventricles of the M-ND group compared with the group C-ND and was lower in the M-WD group compared with the M-ND group (Table 3). *Tnf* and *Il6*, however, were selectively upregulated in the LV compared with the C-ND group; these changes were also attenuated in the M-WD group compared with the M-ND group (Table 3). *Ppara* was downregulated in both ventricles of the M-ND group but only in the RV of the M-WD group compared with their corresponding C groups. Both M groups had lower expression of *Acsl1*, *Acadm*, *Acadl*, and *Pdk4* in the RV than the C groups, whereas *Acsl1*, *Acadm*, and *Acadl* LV expression was higher in the C-WD group than in the C-ND group, with no differences between M and C groups (Table 3). *Pdk4* mRNA did not vary in the LV (Table 3). In visceral adipose tissue, *Tnf* and *Il6* were also more expressed in the M-ND group than in the C-ND group and were alleviated in M-WD, that did not differ from its corresponding C group. *Adipoq* expression was higher in both M groups than in the C groups but increased less in the M-WD group than in the M-ND group (Table 3). *Lep* did not differ among the groups (data not shown).

Plasma mediators. TNFα and IL-6 concentrations were higher in the M-ND group than in the C-ND group, whereas the M-WD group had significantly lower concentrations than did the M-ND group (**Fig. 2***C*,*D*). Leptin plasma concentrations increased in the WD groups relative to their ND counterparts (Fig. 2*B*), whereas adiponectin was higher in both the C-WD and M-ND groups compared with C-ND but distinctly lower in the M-WD both than in the C-WD group (Fig. 2*A*).

Myocardial NF-κB and PPARα activities. The M-WD group had lower PPARα activity in the RV than both the C-WD and M-ND groups, whereas in the LV, the M-ND group had lower activity than in the C-ND group; this was reversed in the M-WD group (**Fig. 3***A*). As for NF-κB, the M-ND group had higher activity than the C-ND group in both ventricles, which was tapered in the RV and completely abrogated in the LV myocardium of the M-WD group compared with the M-ND group (Fig. 3*B*).

Discussion

The WD ameliorated survival, PH, inflammation, and CC in experimental PH. MCT-induced PH extensively activates neuroendocrine systems (13). Compensated RV hypertrophy is seen up to 3–4 wk after injection (15), but beyond this point, rats progress to HF, accompanied by CC (9). Our study was conducted at this later stage.

The M-ND group not only had PH but also increased RV EDP and reduced CO along with compromised LV diastolic function and lower BP, consistent with ventricular interdependence.

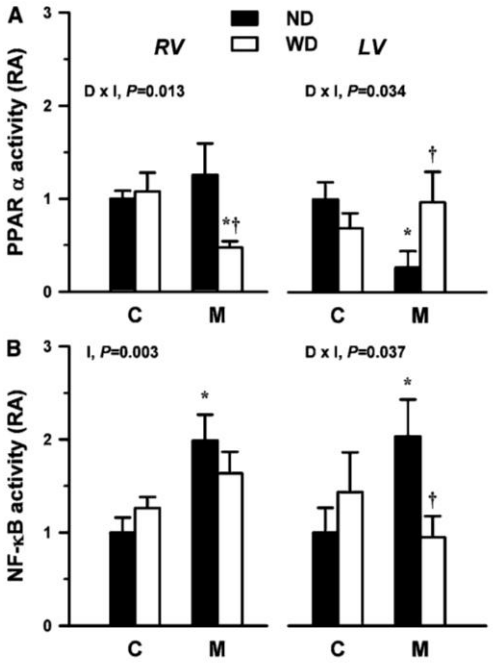

FIGURE 2 Plasma concentrations of adiponectin (*A*), leptin (*B*), TNFα (*C*), and IL-6 (*D*) in C- and M-injected rats injected rats fed a ND or WD for 5 wk. Bars represent mean ± SEM, $n = 7$. *Different from the corresponding C groups, $P < 0.05$; †different from corresponding ND groups, $P < 0.05$. Significant P values are given for the main effects of D and I or for their interaction (D × I). C, control; D, diet; I, injection; M, monocrotaline; ND, normal diet; WD, Western diet.

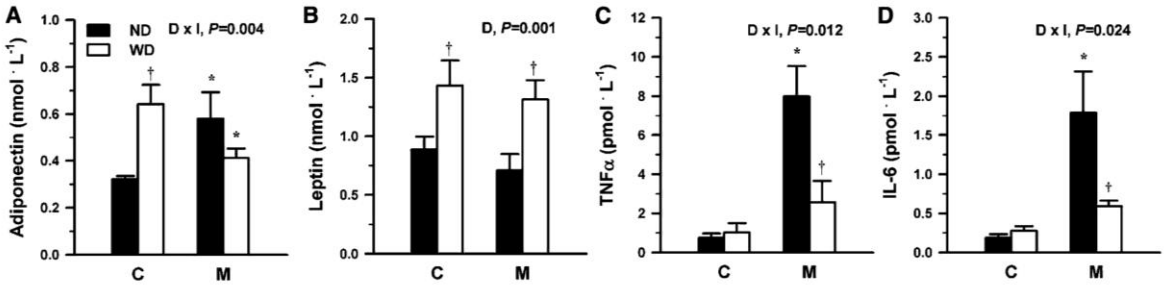

FIGURE 3 PPARα (*A*) and NF-κB transcription factor activities (*B*) in the RV and LV myocardium of C- and M-injected rats fed a ND or a WD for 5 wk. Data are mean ± SEM (fold of C-ND absorbance), $n = 7$. *Different from the corresponding C groups, $P < 0.05$; †different from corresponding ND groups, $P < 0.05$. Significant P values are given for the main effects of D and I or their interaction (D × I). C, control; D, diet; I, injection; LV, left ventricle; M, monocrotaline; ND, normal diet; RA, relative absorbance; RV, right ventricle; WD, Western diet.

Hemodynamic disturbances were accompanied by inflammatory activation. Antiinflammatory therapy has been successful in MCT-induced PH (16) and cachexia has been experimentally attenuated by long-term energy restriction (17). Surprisingly, the M-WD group had higher energy intake, preserved BW, and also attenuation of PH. The latter was nevertheless not accompanied by reduced RV hypertrophy or impaired systolic function, supporting improved ventriculovascular coupling. Moreover, the M-ND group had regression toward the β-MHC isoform and overexpression of ET-1, as reported (13,15), which were also attenuated. Analogously, increased apoptosis and a lower protein:DNA ratio were also blunted by the WD. We speculate the lower DNA content in the M-WD group might be due to prevention of apoptosis, as suggested for afterload-induced hypertrophy (18).

Regarding the LV, we found a lower LV+IVS mass with no change in cardiomyocyte diameters, consistent with previous reports (21). Indeed, only the LV cardiomyocyte length, but not width, is reduced after MCT in LV atrophic remodeling (10). The LV also had increased apoptosis and fibrosis along with surprisingly marked overexpression not only of *End1* but also of *Il6* and *Tnf*. We previously suggested that neuroendocrine mediators could trigger LV dysfunction and neuroendocrine activation in MCT-induced PH (13). Based on current results, we further propose that cytokine activation might also be explained by cachexia and unloading. Indeed, we have also described lower LV mass and *Tnf* overexpression in cachectic rats with nephrotic syndrome (12).

The M-WD group had improved survival and attenuated inflammatory activity with preservation of LV myocardial function, CO, and arterial BP. These remarkable findings can be supported by several lines of evidence. First, a WD is highly palatable and provides additional energy content, which is fundamental in critical illness (22). Although nutritional supplementation in HF is constrained by fluid and salt restriction and cannot circumvent anorexia, malabsorption, and catabolism, some small studies showed benefits, with a few cases of decompensation (23,24). Second, observational studies suggest risk factors such as obesity and hypercholesterolemia improve survival in HF (8). This "obesity paradox" has been attributed mostly to the lipoprotein-endotoxin hypothesis, which states lipoproteins neutralize LPS derived from intestinal bacterial translocation (3) attenuating inflammatory activation (25). Our data are consistent with this hypothesis, because TC concentrations were lower in cachectic M-ND rats and higher in the M-WD group, suggesting the higher lipoprotein content might have played an antiinflammatory role. Accordingly, NF-κB activity and cytokine activation were mitigated in the M-WD group.

NF-κB signaling has been linked to cytokine release, oxidative stress, and remodeling (26). LPS and cytokines activate translocation and binding of NF-κB to response elements in target genes (26). Some of these are cytokines that underlie many HF progression mechanisms (27) and also IR and cachexia due to their effects on metabolism (28).

Adiponectin, an adipokine with beneficial cardiovascular effects that paradoxically portends a bad prognosis in cachexia (29), was upregulated in the energy-depleted M-ND group, possibly through the fuel-sensing AMP-activated protein kinase (30), and was attenuated in the M-WD group.

Besides overload and neuroendocrine activity, mechanisms such as mitochondrial dysfunction, reduced energy production, and increased reactive oxygen species generation underlie RV compromise (19). Although it is acutely toxic to mitochondria, MCT has no longstanding actions after a single administration and mitochondrial disturbances only develop with PH progression (20).

Many metabolic adaptations accompany HF, namely impaired chemical energy conversion to mechanical work, decline in energy content, and loss of metabolic flexibility, with a switch of energy source to anaerobic glycolysis (31). Inactivation of PPAR plays a pivotal role in these modifications by transcriptional inactivation of FFA oxidation enzymes and *Pdk4* (32). PDK4 inhibits pyruvate dehydrogenase, an enzyme in control of glycolysis-derived pyruvate oxidation (5). Although enhanced glucose metabolism increases ATP production from O_2 (5), this is not entirely adaptive, because more ATP is produced per mole of FFA (32). Indeed, cardiac work is very dependent on FFA in HF (33). The combination of FFA and carbohydrates in the WD was able to restore plasma TG and might have contributed to the preservation of myocardial function. The M-ND group had decreased RV expression of *Ppara*, FFA oxidation enzymes, and *Pdk4*. LV PPARα expression and activity were also decreased in the M-ND group but not in the M-WD group, which could be partly explained by PPARα induction by TG (34). Although we did not find changes in LV FFA oxidation or PDK4 enzyme expression, expression does not translate enzymatic activity, and even though our methodology does not enable us to draw definitive conclusions (5), several findings support that the WD might have ameliorated substrate utilization through PPAR activation. Indeed, a high-fat diet prevented the decline in FFA oxidation and mitochondrial capacity in experimental LV overload (35) and medium-chain TG diet enrichment preserved

PPARα activity, FFA oxidation, and myocardial function in hypertensive rats (36).

We must point out that the preservation of liver and gastrocnemius mass suggests beneficial systemic effects that were not addressed but deserve future clarification. Additionally, similar favorable effects of hypercaloric diets have been recently reported in experimental kidney disease-associated cachexia (37).

To conclude, in a rapidly evolving model of PH and CC, a short-term WD regimen attenuated PH and improved survival, with concomitant attenuation of neuroendocrine activity, inflammation, and cachexia, possibly due to changes in metabolism and transcription factor activity. Nevertheless, longer diet courses might lead to cardiac TG accumulation and worsening function. Whether patients with CC and HF may benefit from WD regimens remains to be settled.

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Supplemental tables

Supplemental Table 1. Detailed normal (ND) and Western-type (WD) diet composition[1].

	ND	WD
Carbohydrates[2]		
Polysaccharides	91	59
Simple	3	42
Fibers	6	0
Aminoacids[3]		
Alanine	5.2	2.6
Arginine	6.2	3.7
Aspartic acid	8.3	6.3
Cystine	1.8	0.3
Glutamic acid	21.6	19.8
Glycine	5.6	2.4
Histidine	2.4	2.7
Isoleucine	3.8	5.4
Leucine	7.6	8.1
Lysine	5.0	7.2
Methionine	2.1	3.5
Phenylalanine	4.8	4.5
Proline	7.8	10.0
Serine	5.0	5.6
Threonine	3.8	4.4
Tryptophan	1.3	1.5
Tyrosine	3.1	5.6
Valine	4.7	6.4
FFA[4]		
Saturated		
16:0	19.3	24.8
18:0	2.1	12.3
Other	1.6	2.6
Monounsaturated		
16:1	0.7	3.1
18:1	18.5	45.1
20:1	1.2	1.3
Other	1	0.3
Polyunsaturated		
18:2 and 18:3	53.9	10
20:4	0.2	0.4
Other	1.6	0

[1]Values are given in percentage. Composition per kg of diet was similar for vitamins (6 mg calcium pantothenate, 1.3 g choline chloride, 0.75 mg folic acid, 0.63 mg menadione sodium bisulfate, 15 mg niacin, 40 μg vitamin B12, 5 mg pyridoxine, 2.25 mg riboflavin, 3 mg thiamin HCl, 0.9 mg vitamin A, 25 μg ergocalciferol, and 15 μg vitamin E) and minerals (5.53 g Ca, 0.86 g Cl, 5.57 mg Cu, 0.49 mg Cr, 0.3 mg I, 39.44 mg Fe, 0.49 g Mg, 52.24 mg Mn, 3.46 g P, 8.72 g K, 0.17 mg Se, 0.67 g S, and 12.51 mg Zn) except for added salt in the WD. [2]Derived from vegetable (wheat, barley, bran wheat and corn grain) in ND, and maltodextrin and corn starch with added sucrose (177.8 $g \cdot kg^{-1}$) in WD. [3]From vegetable and fish isolate origin in ND and from animal origin in WD. [4]Derived from vegetable origin and animal lard in ND and WD, respectively.

Supplemental Table 2. Gene specific real –time PCR primers.

Gene	Sequence
Bax	fw: 5' – TGG AGC TGC AGA GGA TGA TTG - 3'
	rev: 5' – ACG CGG CCC CAG TTG AAG T - 3'
Bcl-2	fw: 5' – GAG GGG CTA CGA GTG GGA TAC - 3'
	rev: 5' – GCG GGC GTT CGG TTG CTC TC - 3'
Edn1	fw: 5' – CGG GGC TCT GTA GTC AAT GTG - 3'
	rev: 5' – CCA TGC AGA AAG GCG TAA AAG - 3'
Tnf	fw: 5' – TGG GCT ACG GGC TTG TCA CTC - 3'
	rev: 5' – GGG GGC CAC CAC GCT CTT C - 3'
Il6	fw: 5' – GAA GTT GGG GTA GGA AGG AC - 3'
	rev: 5' – CCG TTT CTA CCT GGA GTT TG - 3'
Pdk4	fw: 5' – GAG GCC ACC GTC GTC TTG - 3'
	rev: 5' – ACA GGC GTT GGA GCA GTG G - 3'
Acsl1	fw: 5' – CTA CAG GCA ACC CCA AAG GAG - 3'
	rev: 5' – GGG CGA GAG GCA AGA AAG ATA - 3'
Acadm	fw: 5' – ACG ATA AAA GCG GGG AAT ACC - 3'
	rev: 5' – AGG CCA AGA CCA CCA CAA CTC - 3'
Acadl	fw: 5' – CAT TTT CCG GGA GAG TGT AA - 3'
	rev: 5' – CTT GCC AGC TTT TTC CCA GAG - 3'
Ppara	fw: 5' – TTC CAG CCC CTC CTC AGT CAG - 3'
	rev: 5' – AGC CCT TGC AGC CTT CAC AT - 3'
Adipoq	fw: 5' – GGG CTA CGG GCT GCT CTG A - 3'
	rev: 5' – TAT GGG GAA GGG GAC AAC AAT G - 3'
Lep	fw: 5' – CCG CCA GGC AGA GGG TCA C - 3'
	rev: 5' – TCT GCA GCA CGT TTT GGG AAG G - 3'
Actb	fw: 5' – ATC TGG GTC ATC TTT TCA CGG TTG G - 3'
	rev: 5' – GAT TTG GCA CCA CAC TTT CTA CA - 3'
Gapdh	fw: 5' – CCG CCT GCT TCA CCA CCT TCT - 3'
	rev: 5' – TGG CCT TCC GTG TTC CTA CCC - 3'

Acadm, medium chain acyl CoA dehydrogenase; *Acadl*, long chain acyl CoA dehydrogenase; *Acsl1*, Long-chain-fatty-acid-CoA ligase 1; *Actb,* β-actin; *Adipoq*, adiponectin; *Bax*, Bcl-2–associated X protein; *Bcl-2*, B-cell lymphoma 2; *Edn1*, endothelin-1; *Lep*, leptin; *Pdk4*, pyruvate-dehydrogenase kinase type 4.

Supplemental Table 3. Morphometry of control (C) or monocrotaline (M)-injected rats fed normal (ND) or a Western-type (WD) diet for 5 wk[1].

	C-ND	C-WD	M-ND	M-WD	*P*-Value[2]
BW[3], *g*	354 ± 6	361 ± 7	242 ± 7*	261 ± 7*†	I, *P*<0.001; D, *P*=0.043
TL, *mm*	40.0 ± 0.3	39.3 ± 0.4	38.5 ± 0.2*	38.6 ± 0.4	I, *P*=0.001
Lung : TL, *mg · mm*$^{-1}$	38 ± 1	42 ± 1	65 ± 3*	52 ± 3*†	D x I, *P*=0.005
Gastrocnemius : TL, *mg · mm*$^{-1}$	55 ± 1	51 ± 2	38 ± 2*	47 ± 1*†	D x I, *P*=0.002
Liver : TL, *mg · mm*$^{-1}$	325 ± 19	369 ± 33	214 ± 12*	284 ± 26*†	I, *P*<0.001; D, *P*=0.021
Perigonadal fat : TL, *mg · mm*$^{-1}$	80 ± 8	116 ± 7†	47 ± 3*	63 ± 4*†	I, *P*<0.001; D, *P*<0.001
Perirenal fat : TL, *mg · mm*$^{-1}$	80 ± 8	124 ± 16†	35 ± 4*	59 ± 4*†	I, *P*<0.001; D, *P*<0.001

[1]Values are mean ± SEM; *n*=7; *Different from corresponding C groups, *P*<0.05; †Different from corresponding ND group, *P*<0.05. [2]Main effects of diet (D) or injection (I), and interaction (D x I). [3]BW, body weight; TL, tibial length.

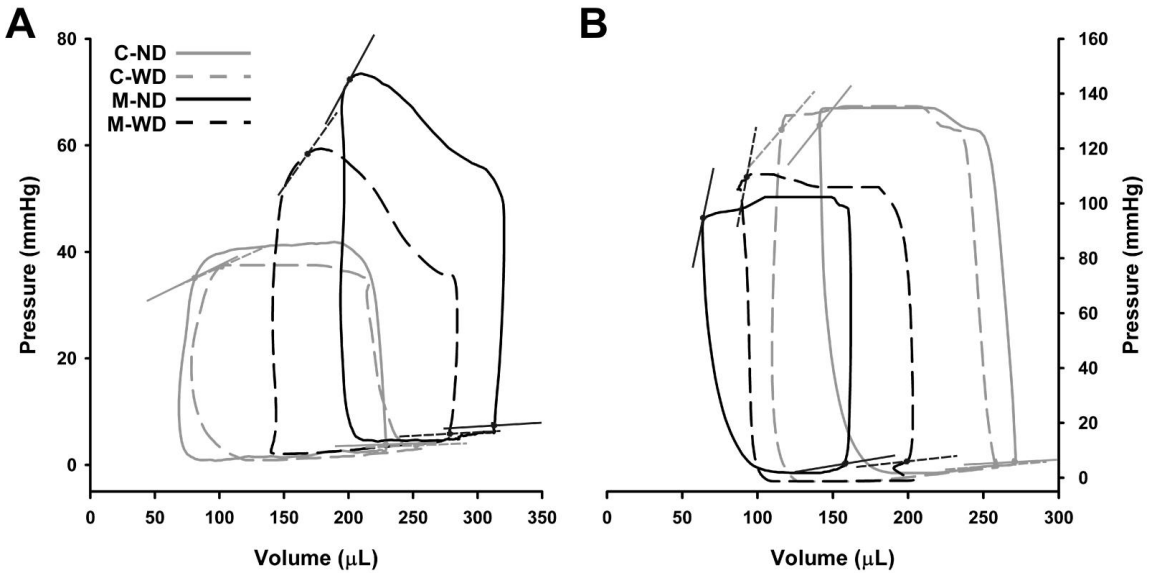
Supplemental Table 4. Myocardial morphometry, cardiomyocyte diameters and protein:DNA content in control (C) or monocrotaline (M)-injected rats fed normal (ND) or a Western-type (WD) diet for 5 wk[1].

	C-ND	C-WD	M-ND	M-WD	*P*-Values[2]
Morphometry					
RV[3]/TL, $mg \cdot mm^{-1}$	4.8 ± 0.2	5.1 ± 0.3	8.4 ± 0.2*	8.6 ± 0.5*	I, *P*<0.001
LV+IVS/TL, $mg \cdot mm^{-1}$	17.4 ± 0.8	17.7 ± 0.5	13.7 ± 0.6*	16.0 ± 0.4*†	D x I, *P*=0.048
Cardiomyocyte diameters					
RV, μm	15.6 ± 0.7	16.8 ± 0.3	18.5 ± 0.6*	18.7 ± 0.5*	I, *P*<0.001
LV, μm	16.9 ± 0.2	17.1 ± 0.8	17.0 ± 0.3	16.3 ± 0.5	NS
Protein and DNA[4]					
RV					
protein, $\mu g \cdot g^{-1}$	124 ± 5	119 ± 8	172 ± 14*	155 ± 15*	I, *P*=0.023
DNA, $ng \cdot g^{-1}$	296 ± 10	310 ± 11	565 ± 65*	342 ± 15†	D x I, *P*=0.030
protein/DNA, $\mu g \cdot ng^{-1}$	0.42 ± 0.00	0.38 ± 0.02	0.30 ± 0.01*	0.47 ± 0.07†	D x I, *P*=0.026
LV					
protein, $\mu g \cdot g^{-1}$	120 ± 12	120 ± 10	125 ± 5	130 ± 10	NS
DNA, $ng \cdot g^{-1}$	299 ± 15	274 ± 36	267 ± 26	261 ± 17	NS
protein/DNA, $\mu g \cdot ng^{-1}$	0.40 ± 0.05	0.46 ± 0.06	0.49 ± 0.06	0.51 ± 0.04	NS

[1]Values are mean ± SEM; *n*=7; *Different from correponding C group, *P*<0.05; †Different from corresponding ND group, *P*<0.05. [2]Main effects of diet (D) or injection (I), and interaction (D x I); NS, not-significant (*P*>0.050). [3]IVS, interventricular septum; LV, left ventricle; RV, right ventricle ; TL, tibial length. [4]DNA and protein to DNA concentration ratio were used as surrogates of cardiomyocyte number and size, respectively.

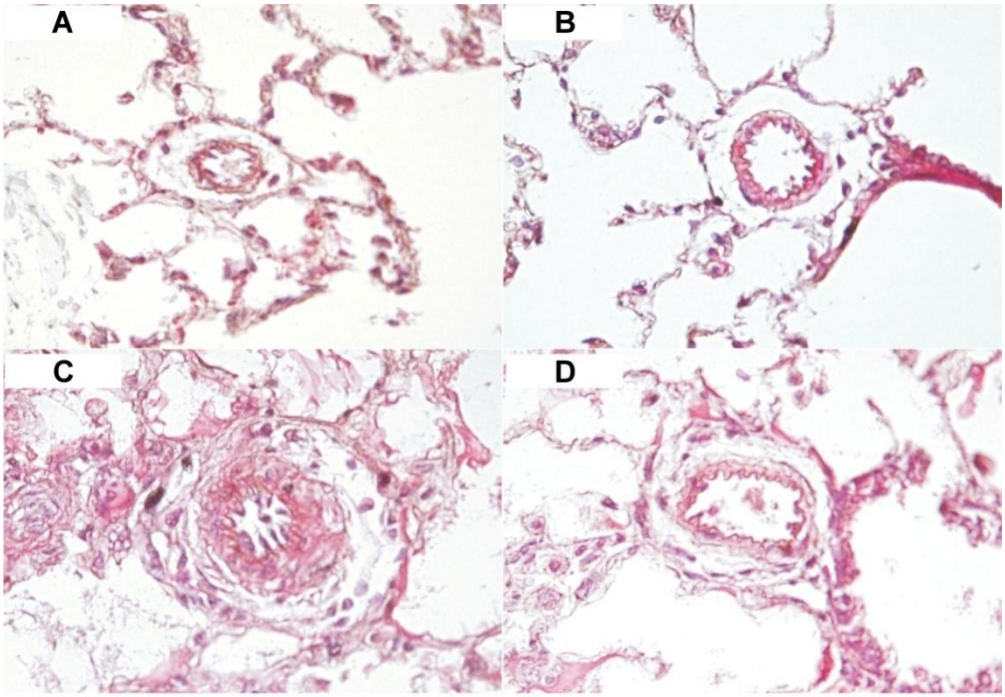
Supplemental figures

Supplemental Figure 1. ***Representative RV (A) and LV (B) PV loops with their respective ESPVR (upper-left corner of each loop) and EDPVR (lower-right corner) obtained from IVC occlusions.***

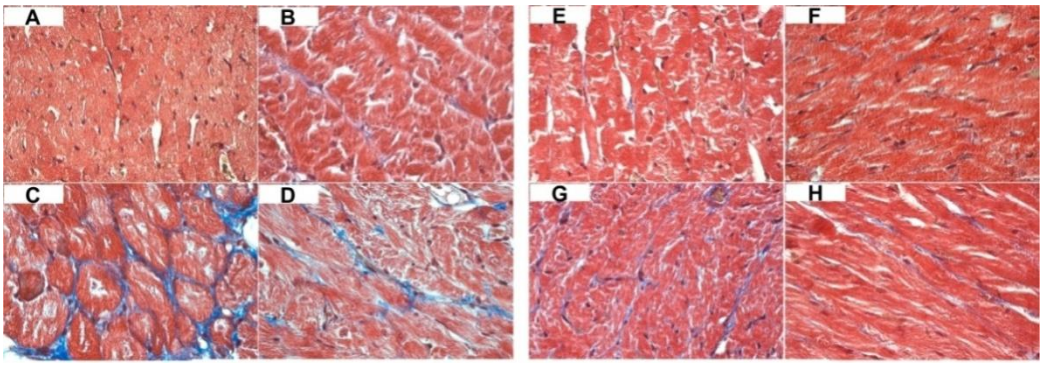


For significant differences please refer to table 2 of the manuscript. EDPVR, end-diastolic pressure-volume relationships; ESPVR, end-systolic pressure-volume relationships; IVC, inferior vena cava; LV, left ventricle; PV, pressure-volume; RV, right ventricle.

Supplemental Figure 2. ***Representative lung histological sections (Hematoxylin & Eosin) of pulmonary arterioles (200x) of control (C) and monocrotaline (M)-injected rats fed for 5 wk with either normal (ND), C-ND (A) and M-ND (panel C), respectively, or Western-type (WD) diet, C-WD (B) and M-WD (D), respectively.***

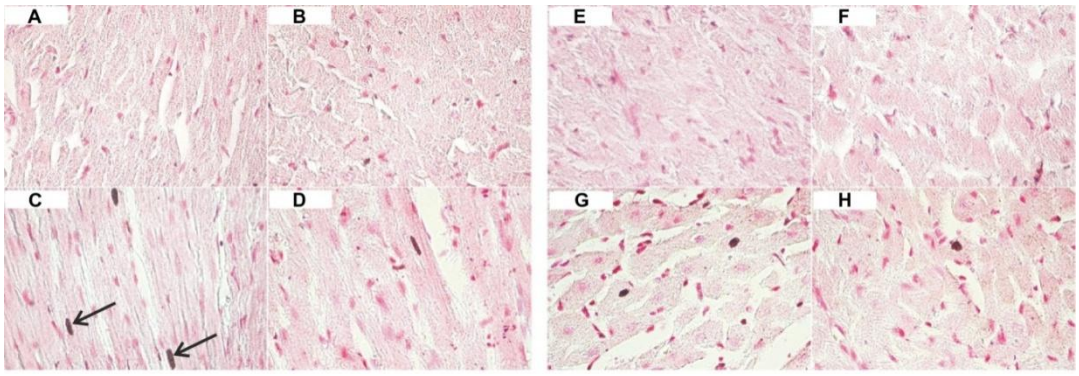


Supplemental Figure 3. ***Representative RV (A-D) and LV myocardial (E-H) histological sections of control (C) and monocrotaline (M)-injected rats fed for 5 wk with either normal (ND), C-ND (A and E) and M-ND (panel C and G), respectively, or Western-type (WD) diet, C-WD (B and F) and M-WD (D and H), respectively, stained with Masson's trichrome (400x).***



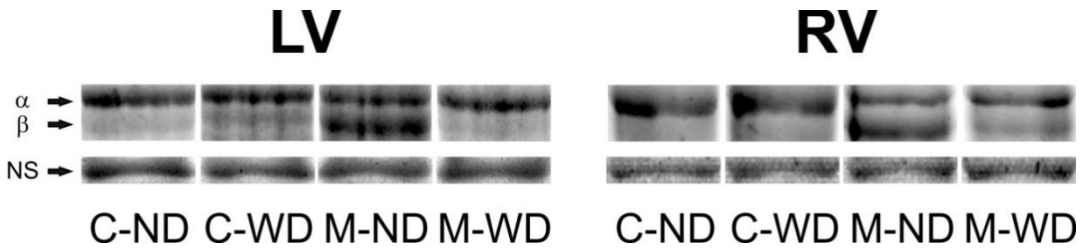
Fibrosis areas are stained blue. Notice also RV cardiomyocyte hypertrophy in M-ND (panel C). LV, left ventricle; RV, right ventricle.

Supplemental Figure 4. ***Representative RV (A-D) and LV myocardial (E-H) histological sections of control (C) and monocrotaline (M)-injected rats fed for 5 wk with either normal (ND), C-ND (A and E) and M-ND (panel C and G), respectively, or Western-type (WD) diet, C-WD (B and F) and M-WD (D and H), respectively, with TUNEL and hematoxylin counterstaining (400x).***



Dark staining nuclei of TUNEL-positive cardiomyocytes are signaled by arrows in panel C. LV, left ventricle; RV, right ventricle; TUNEL, terminal deoxynucleotidyl-transferase-mediated dUTP nick end-labeling.

Supplemental Figure 5. ***Representative blots of MHC isoforms in LV and RV myocardium of control (C) and monocrotaline (M)-injected rats fed either normal (ND) or a Western-type (WD) diet for 5 wk.***



Two distinctive protein bands (α MHC, and β MHC, isoforms) are indicated by arrows, along with a loading control (NS marking band). Percentage of β-isoform was assessed as a ratio to total absorbance. LV, left ventricle; MHC, myosin heavy-chain; NS; non-specific marking; RV, right ventricle.

Haemodynamic and neuroendocrine effects of tezosentan in chronic experimental pulmonary hypertension

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Abstract

Purpose. Chronic pulmonary hypertension (PH) therapy is poorly investigated in intensive care. Our aim was to evaluate haemodynamic and neuroendocrine effects of the dual endothelin-1 (ET-1) blocker tezosentan in monocrotaline (MCT)-induced PH.

Methods. Male Wistar rats (180-200g, n=194) randomly received 60mg.Kg^{-1} MCT or vehicle, subcutaneously, and 2 days later, a subgroup of MCT-injected rats was gavaged with 300mg.Kg^{-1}.d^{-1} bosentan (MCT BOS, n=46) while another (MCT, n=125) and control rats (Ctrl, n=23) received vehicle. At 25-30 days, 48h after interrupting bosentan, rats randomly underwent either a dose-response evaluation (0.5 to 20mg.Kg^{-1}, n=7 each group) or a 4h perfusion of tezosentan (20mg.Kg^{-1} in 10min + 10mg.g^{-1}.h^{-1}) or vehicle (n=8 per group, each). Haemodynamics were evaluated after thoracotomy under anaesthesia, as well as blood gas analysis. After plasma, right ventricle (RV) and lung collection, plasma ET-1, cytokines, nitrate and 6-keto-PGF1α, and lung and right ventricular gene expression and cyclooxygenase (COX) and nitric oxide synthase (NOS) activities were quantified.

Results. MCT resulted in PH, RV dilation and decreased cardiac output (CO) that were attenuated in MCT BOS. PH was attenuated by tezosentan without systemic hypotension. Tezosentan increased CO without changing ventilation-perfusion matching. Both bosentan and tezosentan reduced ET-1 and cytokine plasma levels and tissue expression, and inducible NOS and COX-2 RV activities. Bosentan increased nitrate plasma levels and non inducible NOS activities whereas tezosentan decreased circulating 6-keto-PGF1α but increased lung COX-1 activity.

Conclusions. Tezosentan may be useful for haemodynamic handling and bosentan replacement in critically ill PH exerting important beneficial neuroendocrine and anti-inflammatory actions.

Word count: 250

Introduction

Pulmonary hypertension (PH), the most serious chronic disease of the pulmonary circulation, defined by mean pulmonary artery pressure greater than 25mmHg, consists of an heterogeneous group of disorders characterized by vascular remodelling that leads to right ventricular (RV) failure. Currently, combined lung arteries vasodilators are the mainstay of treatment [1, 2]. Regrettably, acute therapy, which is of major importance to critical care practice, has been poorly investigated.[3] One of the most successful therapeutic approaches to PH, endothelin-1 (ET-1) blockade,[4] is mostly restricted to chronic oral administration and therefore of limited utility. Tezosentan, a dual ET-1 antagonist, however, was developed for intravenous use, and optimized to be rapidly-acting and short-lived, [5] and therefore suited for fine dose adjustment to a desired haemodynamic effect. Moreover, it has advantages over the cumbersome inhaled therapies. Nevertheless, most lung vessel vasodilators are negative inotropes, either by direct myocardial actions, systemic hypotension, decreased coronary perfusion [6] or simply by reducing afterload [7]. Additionally, disturbances in ventilation-perfusion (VQ) matching are also usual, and could lead to hypoxia [8]. ET-1 is a strong positive inotrope [9] but when chronically activated, as it happens in the failing heart, this supportive role is lost [10], suggesting ET-1 blockade could actually improve performance. As for VQ matching, acute ET-1 antagonism improved alveolar-arterial O_2 difference in lung injury [11] and did not disturb VQ matching in pulmonary thromboembolism [12]. Tezosentan has been found beneficial in acute experimental PH [13, 14] and also in an experimental model of left-right shunt in the newborn lamb [15], but a characterization of its pulmonary and myocardial effects in adult experimental chronic PH is lacking. Additionally, the myocardial and pulmonary vascular mechanisms involved in ET-1 blockade and whether tezosentan can be an effective replacement therapy for bosentan is also undefined. Our goal was to evaluate the haemodynamic, local myocardial and pulmonary and systemic neuroendocrine effects of acute ET-1 antagonism with tezosentan in chronic PH induced by monocrotaline (MCT), the most widely used adult chronic

PH experimental model, and to assess whether these were altered by previous chronic therapy with bosentan.

Methods

Male Wistar rats, 180-200g (Charles-River, Barcelona, Spain), randomly received 60mg.Kg^{-1} subcutaneous MCT (Sigma Chemical, St Louis, MO) or vehicle. Randomly, 48h later, some MCT-injected animals were gavaged 300mg.Kg^{-1}.d^{-1} bosentan (30mg.mL^{-1} in 5% gum Arabic; kindly provided by Actelion pharmaceuticals) (MCT BOS; n=46), while others (MCT; n=125) and controls (Ctrl; n=23) received vehicle. Rats were housed 5 per cage with controlled environment (22^{o}C, 12:12h light-dark). Experiments conformed to the Guide for Care and Use of Laboratory Animals (National Institutes of Health, Pub.No. 85-23, revised 1996). After bosentan interruption for 48h, at days 25-30, rats were anesthetized with 2.5-3% sevoflurane and 150μg.Kg^{-1} intraperitoneal fentanyl, mechanically ventilated (150.min^{-1}, 100% O_2, 14-16cmH_2O inspiratory pressure, with tidal volume adjusted to animal weight, and 4cmH_2O end-expiratory pressure; TOPO Small Animal Ventilator, Kent Scientific Inc.) and kept at 38^{o}C. Ringer's solution (30mL.Kg^{-1}.h^{-1}) was perfused through the femoral vein and arterial blood gas (ABG) samples collected (Stat Profile pHOx®, Nova Biomedical, Waltham, MA) from femoral artery. After thoracotomy, pressure-volume (P-V) catheters were implanted through the apex in the left ventricle (LV) and RV (SPR-838 and PVR-1045, Millar Instruments, Houston, TX, respectively) and a transit-time flow-probe in the ascending aorta (200-367, Triton Technology, San Diego, CA). Data were continuously acquired (MPVS 300, Millar Instruments), digitally recorded at 1000Hz (ML880 PowerLab 16/30, Millar Instruments, Houston, TX), and analyzed (PVAN 3.5™, Millar Instruments). After 15min stabilization, recordings were done at sustained end-expiration. Parallel conductance and field inhomogeneity were estimated from 50μL 10% saline injections and cardiac output (CO) measurement (Active Redirection Transit Time Flowmeter, System 6, Triton Technology), respectively. First, 0.5, 1, 5, 10, and 20 mg.Kg^{-1} intravenous tezosentan doses (in saline; kindly

provided by Actelion pharmaceuticals) were assessed (n=7 each group), then rats randomly received either an intravenous 20mg.Kg^{-1} dose followed by 10mg.Kg^{-1}.h^{-1} tezosentan perfusion (TEZO; n=8 per group), or saline (Vehicle; n=8 per group), and recordings and ABG collection were repeated 1h after stable effect. A 2mL venous sample was withdrawn 4h after. Animals were euthanized by exsanguination, the heart, RV and LV and interventricular septum (IVS) and the lungs were weighed and stored (-80^{o}C). A flowchart with experimental methods can be found in Supplemental Fig. 5. Lung and RV free-wall mRNA underwent two-step real-time reverse transcription-polymerase chain reaction as described [16]. Results, normalized for β-actin, are presented relative to Ctrl Vehicle. Primers are given in Supplemental Table 1. Cyclooxygenase (COX) and nitric oxide synthase (NOS) activities were quantified in homogenates (760151 and 760871, Cayman Chemical Company, respectively) with or without specific COX-2, DuP-697 (70645, Cayman Chemical Company), and inducible NOS (iNOS), aminoguanidine (396494, Sigma-Aldrich), inhibitors. Circulating ET-1 (S-1171, Peninsula laboratories, San Carlos, CA), interleukin-6 (IL-6; DE4845, Demeditec diagnostics GmbH, Germany) and tumor necrosis factor-α (TNF-α; 45-TNFRTU-E01, Alpco Diagnostics™, Salem, NH) were quantified by enzyme immunoassay. Nitrates and 6-keto-$PGF_{1\alpha}$, stable nitric oxide (NO) and prostacyclin (PGI_2) metabolites, were also quantified (Cat. No. 760871 and Cat. No. 515211, Cayman Chemical Company, Ann Arbor, MI, respectively) after ultrafiltration (Amicon® Ultra 30K, Millipore, Ireland).

Statistical analysis

Analysis by two-way repeated-measures ANOVA for dose-response, two-way ANOVA for haemodynamics, ABG, gene expression, neuroendocrine and enzyme activities, and paired *t*-test for perfusion, with Holm-Sidak's method for *post hoc* comparisons. ANOVA on ranks was used

for non-normally distributed data. Chi-square was used to compare mortality. Two-tailed $P<0.05$. Variables: mean±SEM.

Results

Animal model

None of Ctrl animals died during follow-up or haemodynamic evaluation while mortality rates were 77% and 43% ($P<0.001$) and another 6 and 3 rats were lost during haemodynamic evaluation in MCT and MCT BOS, respectively. MCT and MCT BOS presented lower body weights. MCT showed increased RV weight and RV to LV+IVS weight ratio which was attenuated in MCT BOS. No differences were observed between TEZO and Vehicle (Supplemental Table 2).

Dose-response evaluation

MCT and MCT BOS showed increased RV maximal pressures that were dose-dependently reduced by TEZO, while no effect was observed in Ctrl. Distinctly, TEZO only reduced LV pressure in Ctrl, not in MCT or MCT BOS. MCT however had lower LV maximal pressures compared with both Ctrl and MCT BOS (Supplemental Fig. 6). Higher doses elicited no additional effects (not shown).

Haemodynamic effects of tezosentan

Maximal RV pressure and arterial elastance (E_A), surrogates of PH and RV afterload, respectively, were increased in MCT, which was accompanied by prolonged time constant of relaxation τ, increased end-diastolic volume and pressure, reduced ejection fraction (EF) (Table 1), and lower heart rate (HR) and CO (Fig. 1a) compared with Ctrl, while MCT BOS showed an overall improvement. Though no changes were observed with vehicle, TEZO attenuated maximal and end-diastolic RV pressure and E_a rise both in MCT and MCT BOS and also τ, end-diastolic volume,

EF and CO in MCT without changing HR (Fig. 1a). Load-independent indexes obtained from inferior vena cava occlusions are given in Table 2 and representative RV PV loops in Supplemental Fig. 7. End-diastolic PV relationships were upward-shifted in MCT, not in MCT BOS, and were also normalized by TEZO, whereas end-systolic PV relationships and other load-independent contractility indexes were similarly increased in MCT and MCT BOS, with no changes after TEZO. Since end-systolic PV relationship slope was preserved and E_A was decreased, ventriculo-vascular coupling was improved both in MCT BOS and MCT TEZO compared with MCT (Fig. 1b). As for the LV, MCT showed reduced maximal pressures, reduced end-diastolic volume and EF along with prolonged τ (Table 1), while MCT BOS did not. TEZO decreased maximal pressure and maximum rate of LV pressure rise only in Ctrl, while it increased LV end-diastolic volumes and EF in MCT. LV preload recruitable stroke work was unchanged in MCT or after chronic and acute ET-1 blockade. MCT showed upward-shifted LV end-diastolic PV relationships that were restored to normal both by chronic BOS therapy in MCT BOS and by acute TEZO therapy in MCT TEZO (Table 2).

Respiratory effects of tezosentan

Animals were normoventilated and maintained acid-base balance throughout perfusion. Arterial O_2 pressure under stable ventilation was used as surrogate of oxygenation. Both MCT and MCT BOS showed decreased arterial O_2 pressures that were not altered by TEZO (Supplemental Table 3).

Effects of tezosentan on endothelin-1 and cytokine production

MCT showed increased plasma levels of ET-1, IL-6 and TNF-α (Fig. 2c), that were accompanied by increased local expression of TNF-α and ET-1 in the RV (Fig. 2b) and TNF-α and IL-6 in the lung (Fig. 2a). These were partly prevented by chronic ET-1 antagonism and markedly attenuated with acute antagonism in MCT BOS and MCT TEZO, respectively. TEZO also reduced plasma

levels of TNF-α and IL-6 in MCT BOS, whereas it raised ET-1 RV expression and plasma levels in Ctrl compared with Vehicle.

Effects of tezosentan on prostaglandin and NO production

PGI_2 metabolite 6-keto-$PGF_{1\alpha}$ was increased both in MCT and MCT BOS compared with Ctrl, which was attenuated after TEZO perfusion, compared with Vehicle (Fig. 3c). Contrastingly, TEZO increased 6-keto-$PGF_{1\alpha}$ in Ctrl. These plasma changes were accompanied by increased RV activities of both COX-1 and -2 and also by increased gene expression of COX-1 in MCT, that were attenuated by chronic and acute ET-1 antagonism in MCT BOS and MCT TEZO, respectively. While gene expression of COX-2, contrarily, was decreased in MCT with no changes after either BOS or TEZO (Fig. 3b). As for the lung, no changes were observed in either the expression or enzymatic activity of COX-2, whereas COX-1 showed increased gene expression in MCT, that was attenuated by either chronic or acute ET-1 antagonism, and markedly decreased activity both in MCT and MCT BOS, which was attenuated only by acute ET-1 antagonism in MCT TEZO (Fig. 3a). Plasma levels of nitrates were lower in MCT and restored to Ctrl values or higher in MCT BOS. TEZO had no effect compared with Vehicle (Fig. 4c). Plasma changes were paralleled by lower non-inducible NOS activity in the lung (Fig. 4a) and RV (Fig. 4b) of MCT that was attenuated by chronic ET-1 antagonism in MCT BOS but only abrogated in the RV, not in the lung, by TEZO, whilst iNOS was overactive both in the lungs and RV of MCT. TEZO markedly attenuated both lung and RV activities whereas BOS only attenuated RV activity. As for gene expression, while in the RV no changes were observed in either eNOS or iNOS, in the lung eNOS was upregulated in MCT and MCT BOS, with no change after TEZO, and iNOS was downregulated in MCT, which was attenuated both by short-term and chronic ET-1 antagonism.

Discussion

We demonstrate that an acute intravenous infusion of the short acting dual ET-1 antagonist tezosentan attenuates PH, without compromising VQ matching or systemic pressure and even

improving CO and ventriculo-vascular coupling, while concomitantly blunting inflammatory and vasoconstrictor mediator production in chronic experimental PH induced by MCT. These effects were also observed after previous therapy with bosentan.

MCT-induced PH is a well-established model with extensive neuroendocrine and inflammatory activation that rapidly progresses to RV failure [16]. In MCT we observed PH and increased afterload that were accompanied not only by RV hypertrophy but also by disturbed ventriculo-vascular coupling, RV dilation, decreased EF and CO output, and compromised diastolic function. As for the LV, MCT showed decreased end-diastolic volumes, compromised diastolic function and lower LV maximal pressures, as expected by ventricular interaction [17] and possibly also due to intrinsic myocardial dysfunction [16]. Regarding gas exchange, under normoventilation MCT showed lower arterial O_2 pressures as expected [18]. Also as described, circulating levels of ET-1 and RV gene expression were increased [16], which was accompanied by marked inflammatory activation, as assessed by TNF-α and IL-6 gene expression and plasma concentrations [19]. PH is an inflammatory condition with endothelial dysfunction and loss of vascular control mechanisms. The imbalance between vasodilators and vasoconstrictors, namely an altered local ratio of thromboxane A_2 (TxA_2) to PGI_2, decreased expression of eNOS, and increased ET-1 plays an important part in its pathophysiology [20]. Inflammatory activation also compromises myocardial function. As previously reported, plasma NO levels and lung activity of eNOS were reduced in MCT-induced PH [21]. Contrastingly, PGI_2 production was increased in MCT-induced PH [22], which could be due to concomitant heart failure, with platelet activation and systemic production [23], since it is normally decreased in human PH [24]. Nevertheless, prostanoid lung synthesis was compromised, since COX-1 activity was markedly reduced, as previously reported in chronically hypoxic pigs that showed a markedly decreased PGI_2 to TxA_2 ratio [25]. On the other hand, in the RV of MCT, we observed high iNOS and inducible COX-2 activation. iNOS is known to mediate the negative inotropic effects of cytokines, generating high quantities of NO that profoundly depress myocardial function [26]. COX-2, also activated by

cytokines, produces high levels of prostanoids , mediates TNF-α responsiveness through TxA_2 production [27], and was shown to be upregulated in the failing heart [28]. Additionally, in the failing RV of MCT we also observed decreased eNOS activity and increased COX-1 activity. eNOS, the major NO source in the normal heart, is typically reduced in heart failure and restoration of its activity exerts protective actions [29], whereas COX-1 is typically increased by oxidative stress and its antagonism exerts beneficial effects in heart failure [30].

RV haemodynamic disturbances were attenuated in MCT BOS and ventriculo-vascular coupling was improved, as previously reported [31]. Systemic hypotension, as assessed by LV maximal pressures, was not observed which could be attributed to preserved CO due to restored LV preload and reduced ventricular interaction, but also to improved myocardial function [16]. Chronic ET-1 antagonism did not alter overall VQ matching. Curiously, and contrarily to what has been reported for earlier stages of disease in MCT-induced PH [32], chronic ET-1 antagonism reduced plasma concentration and RV expression of ET-1, probably due to attenuation of PH and disease progression [16, 31] and not to ET-1 antagonism itself, since plasma ET-1 levels were increased in Ctrl by acute ET-1 antagonism with tezosentan, as described [5]. Alongside haemodynamic benefits and reduced ET-1 activity, MCT BOS also showed increased NO plasma levels and increased eNOS lung activity, as previously reported for bosentan-treated PH patients [33], which could partly account for the attenuation in PH.

Nevertheless, our main goal was to evaluate the effects of the short-acting intravenous dual ET-1 antagonist tezosentan in chronic PH. Since perfusion doses were not previously established for this model we carried out a dose-response evaluation. Tezosentan dose-dependently reduced RV maximal pressures up to $20mg.Kg^{-1}$, doses higher than previously described [34], without significant reduction of LV pressure, not only in MCT but also in MCT BOS, suggesting an additional benefit of acute ET-1 blockade even after chronic bosentan therapy. Based on previous works that have shown molecular and neuroendocrine changes as soon as 4h after drug perfusion [35], we then evaluated haemodynamic, respiratory and neuroendocrine

responses to tezosentan perfusion. While vehicle had no effects, tezosentan attenuated PH and RV afterload, both in MCT and MCT BOS. Ventriculo-vascular coupling, EF and CO however were only improved in MCT. Similar to chronic ET-1 antagonism, tezosentan perfusion did not disturb VQ matching. Moreover, tezosentan perfusion was able to acutely reduce circulating levels and RV expression of ET-1 in MCT, as observed after chronic bosentan therapy in MCT BOS. Both chronic and acute dual ET-1 antagonism with bosentan and tezosentan, respectively, attenuated inflammatory activation in MCT, namely TNF-α and IL-6 circulating concentrations and tissue expression, which could be due not only to direct ET-1 antagonism, since ET-1 increases the production of inflammatory mediators [36], but also to the beneficial haemodynamic effects and improved CO and thus better tissue perfusion. Additionally, it should be mentioned that the anti-inflammatory effects of ET-1 antagonism might have been partly responsible for improved haemodynamics and lower ET-1 activity in MCT after acute and chronic ET-1 antagonism, as cytokines also induce ET-1 production [37]. Indeed, we also observed a marked attenuation of inflammation induced iNOS and COX-2 activation in the RV of MCT after either an acute tezosentan perfusion or chronic bosentan therapy. The fast haemodynamic, neuroendocrine and anti-inflammatory effects observed with tezosentan perfusion are not surprising, since it has repeatedly been tested with success in experimental models of septic shock and acute lung injury [11]. As additional effect, tezosentan perfusion increased COX-1 activity in the lungs of MCT compared with Vehicle. Although surprising, since ET-1 induces the expression of COX-1 [38], we interpret this upregulation of COX-1 activity after ET-1 blockade as a consequence of decreased pulmonary vascular load and increased flow, as observed in cell culture experiments [39].

Concerning the effects of acute ET-1 antagonism in PH animals that had previously undergone chronic ET-1 blockade. We must stress that even though bosentan was withdrawn for 48h its molecular effects surely will last considerably longer and therefore tezosentan was not expected to exert major actions. Still, although we found no amelioration in CO, we did observe reduced

PH, improved ventriculo-vascular coupling and further anti-inflammatory effects, which supports its use as a replacement drug.

To conclude, we have demonstrated in chronic experimental MCT-induced PH, that acute ET-1 antagonism with tezosentan attenuates PH and improves CO with concomitant reduction of ET-1 and inflammatory cytokine levels, and increased vasodilator production. Part of these beneficial effects were also observed after previous chronic ET-1 antagonism with bosentan, suggesting it may be a good replacement drug when the enteric route of administration is not tolerated or a precise real-time control of haemodynamics is required.

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Figure Legends

Fig. 1 Cardiac output (**a**) and right ventricular (RV) ventriculo-vascular coupling (**b**) before and after perfusion of saline (Vehicle) or tezosentan (TEZO) in vehicle-injected (Ctrl), monocrotaline-injected (MCT) and monocrotaline-injected bosentan-treated rats (MCT BOS); Ctrl (circular symbols), MCT (triangular symbols) and MCT BOS (quadrangular symbols) were given either a 20mg.Kg^{-1} TEZO intravenous loading dose during 10min, followed by a perfusion of 10mg.Kg^{-1}.h^{-1} (white symbols) or the corresponding volume of Vehicle (black symbols). E_{max}, maximal elastance; E_A, arterial elastance. $^{*}P$<0.01 *vs* Ctrl, $^{\dagger}P$<0.05 *vs* MCT and $^{\ddagger}P$=0.028 *vs* Vehicle on two-way ANOVA; $^{\S}P$<0.001 *vs* before on paired *t*-test; n=8 per group

Fig. 2 Lung (**a**) and right ventricular myocardium gene expression (**b**) of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and endothelin-1 (ET-1) and corresponding plasma levels (**c**) in vehicle-injected (Ctrl), monocrotaline-injected (MCT) and monocrotaline-injected bosentan-treated rats (MCT BOS) undergoing either perfusion of vehicle (black bars) or tezosentan (TEZO; white bars); gene expression was normalized for β-actin and is presented in an arbitrary unit (AU), set as the average of Ctrl Vehicle. $^{*}P$<0.05 *vs* Ctrl, $^{\dagger}P$<0.05 *vs* MCT, and $^{\ddagger}P$<0.05 *vs* vehicle; n=7 per group

Fig. 3 Lung (**a**) and right ventricular myocardium (**b**) cyclooxygenase gene expression and enzymatic activity and 6-keto-$PGF_{1\alpha}$ plasma concentrations (**c**); gene expression and enzymatic activities of cyclooxygenase-1 (COX 1) and -2 (COX 2) and plasma concentrations of 6-keto-$PGF_{1\alpha}$ in vehicle-injected (Ctrl), monocrotaline-injected (MCT) and monocrotaline-injected bosentan-treated rats (MCT BOS) given vehicle (black bars) or tezosentan (TEZO, white bars). Gene expression was normalized for β-actin and is presented in an arbitrary unit (AU), set as the average of Ctrl Vehicle. $^{*}P$<0.05 *vs* Ctrl, $^{\dagger}P$<0.05 *vs* MCT, and $^{\ddagger}P$<0.05 *vs* vehicle; n=7 per group

Fig. 4 Lung (**a**) and right ventricular myocardium (**b**) nitric oxide synthase gene expression and enzymatic activity and plasma concentrations of nitrates (**c**); gene expression of inducible (iNOS) and endothelial nitric oxide synthases (eNOS) and enzymatic activities of iNOS and non inducible NOS in vehicle-injected (Ctrl), monocrotaline-injected (MCT) and monocrotaline-injected bosentan-treated rats (MCT BOS) given vehicle (black bars) or tezosentan (TEZO, white bars). Gene expression was normalized for β-actin and is presented in an arbitrary unit (AU), set as the average of Ctrl Vehicle. $^{*}P$<0.05 *vs* Ctrl,$^{\dagger}P$<0.05 *vs* MCT, and $^{\ddagger}P$<0.05 *vs* vehicle; n=7 per group

Tables

Table 1. Haemodynamics before and after vehicle and tezosentan perfusion.

	Before			After		
	Ctrl	**MCT**	**MCT BOS**	**Ctrl**	**MCT**	**MCT BOS**
			Vehicle			
RV						
P_{max}, mmHg	34.6 ± 1.5	56.8 ± 3.9*	43.5 ± 3.8*†	30.8 ± 1.6	55.8 ± 2.5*	43.7 ± 4.3*†
EDP, mmHg	2.6 ± 0.6	4.4 ± 0.4*	3.4 ± 1.0	3.1 ± 1.2	3.8 ± 0.4	3.2 ± 0.8
EDV, µL	229 ± 22	279 ± 32*	239 ± 39	226 ± 23	281 ± 29	259 ± 43
EF, %	68 ± 5	31 ± 4*	43 ± 4*†	66 ± 5	30 ± 4*	45 ± 4*†
τ, ms	8.88 ± 0.58	10.03 ± 0.77*	9.85 ± 0.79	9.76 ± 0.43	10.57 ± 0.60	9.65 ± 1.17
E_A, mmHg.µL^{-1}	0.24 ± 0.03	0.82 ± 0.17*	0.54 ± 0.09*†	0.23 ± 0.03	1.02 ± 0.05*	0.44 ± 0.07*†
LV						
P_{max}, mmHg	128.0 ± 2.4	98.0 ± 7.7*	122.3 ± 9.4†	122.9 ± 2.3*	98.5 ± 7.4*	121.4 ± 7.6†
EDP, mmHg	4.5 ± 0.8	5.4 ± 0.9	4.2 ± 0.6	3.7 ± 0.6	5.7 ± 1.3	6.4 ± 0.9
EDV, µL	244 ± 28	194 ± 31*	180 ± 19	232 ± 22	191 ± 24	209 ± 32
EF, %	62 ± 3	51 ± 11*	53 ± 5	64 ± 5	47 ± 4*	46 ± 3*
dP/dt_{max}, mmHg.s^{-1}	9831 ± 606	8042 ± 812	9524 ± 1191	8778 ± 1102	7716 ± 1121	9534 ± 1484
τ, ms	6.99 ± 0.22	8.90 ± 0.29*	7.86 ± 0.56†	7.44 ± 0.44	8.70 ± 0.61*	8.80 ± 0.84
E_A, mmHg.µL^{-1}	0.93 ± 0.09	1.37 ± 0.36*	1.28 ± 0.17*	0.89 ± 0.09	1.28 ± 0.22*	1.35 ± 0.20*
HR, min^{-1}	429 ± 15	384 ± 23*	419 ± 11	419 ± 12	372 ± 16*	399 ± 17
			Tezosentan			
RV						
P_{max}, mmHg	33.4 ± 0.9	60.3 ± 4.2*	52.1 ± 5.4*†	28.6 ± 1.1‡	49.7 ± 2.5*‡	42.3 ± 2.6*‡
EDP, mmHg	2.4 ± 0.4	4.2 ± 0.3*	3.9 ± 0.8	1.7 ± 0.4	3.1 ± 0.4‡	2.6 ± 0.7‡
EDV, µL	239 ± 14	289 ± 11*	231 ± 30	248 ± 20	274 ± 40	256 ± 32
EF, %	68 ± 3	32 ± 3*	48 ± 3*†	63 ± 4	48 ± 4*‡	45 ± 3*
τ, ms	8.85 ± 0.47	11.32 ± 0.44*	9.78 ± 0.99	8.57 ±0.50	9.76 ± 0.44‡	9.67 ± 1.17
E_A, mmHg.µL^{-1}	0.21 ± 0.01	0.77 ± 0.10*	0.60 ± 0.10*†	0.20 ± 0.01	0.45 ± 0.05‡	0.40 ± 0.06‡
LV						
P_{max}, mmHg	124.8 ± 2.6	98.7 ± 3.2*	125.7 ± 8.5†	110.9 ± 6.6‡	94.7 ± 3.3*	116.9 ± 7.5†
EDP, mmHg	4.0 ± 0.7	5.1 ± 0.7	5.3 ± 0.8	4.6 ± 0.5	4.2 ± 0.5	4.4 ± 0.7
EDV, µL	247 ± 17	189 ± 9*	213 ± 22	234 ± 24	202 ± 20	230 ± 30
EF, %	64 ± 3	49 ± 5*	52 ± 6	66 ± 4	58 ± 3‡	52 ± 3
dP/dt_{max}, mmHg.s^{-1}	8220 ± 713	6765 ± 616	8750 ± 849	6777 ± 629‡	6396 ± 674	8601 ± 1326
τ, ms	7.38 ± 0.17	9.96 ± 0.78*	8.33 ± 0.53†	7.55 ± 0.39	9.35 ± 0.56*	8.59 ± 0.59
E_A, mmHg.µL^{-1}	0.83 ± 0.04	1.16 ± 0.15*	1.25 ± 0.10*	0.69 ± 0.06‡	0.83 ± 0.10‡	1.01 ± 0.11‡
HR, min^{-1}	409 ± 13	354 ± 13*	399 ± 23	404 ± 16	338 ± 17*	383 ± 26

Right (RV) and left ventricular (LV) haemodynamic parameters in vehicle-injected (Ctrl), monocrotaline-injected (MCT) and monocrotaline-injected bosentan-treated rats (MCT BOS). P_{max}, maximal or systolic pressure; EDP, end-diastolic pressure; EDV, end-diastolic volume; EF, ejection fraction; dP/dt_{max}, maximum rate of pressure rise; τ, time constant of isovolumetric relaxation; E_A, arterial elastance; HR, heart rate. $^{*}P<0.01$ *vs* Ctrl and $^{\dagger}P<0.05$ *vs* MCT on two-way ANOVA; $^{\ddagger}P<0.01$ *vs* before on paired t-test; n=8 per group.

Table 2. Inferior vena cava occlusions before and after vehicle and tezosentan perfusion.

	Before			After		
	Ctrl	**MCT**	**MCT BOS**	**Ctrl**	**MCT**	**MCT BOS**
			Saline			
RV						
EDPVR (exponential)						
k_1	0.006 ± 0.001	0.015 ± 0.003*	0.011 ± 0.003	0.007 ± 0.001	0.016 ± 0.004*	0.010 ± 0.003
k_2	1.24 ± 0.29	0.25 ± 0.10	0.68 ± 0.21	0.92 ± 0.22	0.33 ± 0.20	0.90 ± 0.46
ESPVR (linear)						
Slope (E_{max}), mmHg.µL^{-1}	0.22 ± 0.04	0.50 ± 0.09*	0.50 ± 0.07*	0.24 ± 0.03	0.56 ± 0.12*	0.45 ± 0.09*
Intercept, µL	-74.5 ± 34.8	71.5 ± 42.1*	33.2 ± 26.1*	7.0 ± 15.5	87.0 ± 37.9*	43.3 ± 11.47*
dP/dt_{max}-EDV, mmHg.s^{-1}.µL^{-1}	7.0 ± 0.9	19.2 ± 5.6*	19.4 ± 3.3*	7.2 ± 1.5	23.8 ± 3.2*	18.3 ± 3.0*
PRSW, mmHg	20.8 ± 2.9	27.6 ± 3.3*	29.4 ± 3.4*	17.0 ± 2.4	28.6 ± 5.0*	29.4 ± 2.7*
Time var E_{max}, mmHg.µL^{-1}	0.66 ± 0.06	0.92 ± 0.13*	0.99 ± 0.15*	0.62 ± 0.07	1.08 ± 0.18*	0.96 ± 0.21*
LV						
EDPVR (exponential)						
k_1	0.015 ± 0.004	0.025 ± 0.005*	0.014 ± 0.003†	0.013 ± 0.003	0.036 ± 0.003*	0.011 ± 0.002†
k_2	0.92 ± 0.47	2.70 ± 1.02	1.07 ± 0.53	2.69 ± 1.31	1.72 ± 0.73	1.09 ± 0.48
ESPVR (linear)						
Slope, mmHg.µL^{-1}	0.97 ± 0.23	2.42 ± 0.55*	1.89 ± 0.15	1.18 ± 0.24	2.52 ± 0.62*	1.76 ± 0.10
Intercept, µL	-28.4 ± 52.2	41.4 ± 17.8	39.9 ± 4.8	-35.0 ± 21.4	14.7 ± 12.0	23.5 ± 4.5
dP/dt_{max}-EDV, mmHg.s^{-1}.µL^{-1}	56.6 ± 8.0	111.4 ± 17.1*	100.6 ± 15.9*	60.1 ± 10.2	134.5 ± 17.2*	121.4 ± 18.5*
PRSW, mmHg	105.6 ± 22.2	126.5 ± 27.1	124.9 ± 11.7	106.8 ± 16.0	106.2 ± 7.6	144.4 ± 8.6
Time var E_{max}, mmHg.µL^{-1}	3.76 ± 0.52	4.74 ± 1.04	5.12 ± 0.96	3.26 ± 0.64	5.83 ± 1.05	4.77 ± 1.47
			Tezosentan			
RV						
EDPVR (exponential)						
k_1	0.007 ± 0.002	0.015 ± 0.002*	0.013 ± 0.002	0.007 ± 0.001	0.012 ± 0.002‡	0.013 ± 0.002
k_2	0.84 ± 0.17	0.25 ± 0.06	0.90 ± 0.54	0.81 ± 0.20	0.60 ± 0.11‡	0.40 ± 0.22
ESPVR (linear)						
Slope(E_{max}), mmHg.µL^{-1}	0.21 ± 0.04	0.50 ± 0.06*	0.60 ± 0.03*	0.22 ± 0.04	0.52 ± 0.06*	0.47 ± 0.07*
Intercept, µL	-58.2 ± 51.1	45.9 ± 20.2*	83.3 ± 17.1*	-13.2 ± 20.4	24.1 ± 9.6*	76.3 ± 35.7*
dP/dt_{max}-EDV, mmHg.s^{-1}.µL^{-1}	5.4 ± 0.7	19.9 ± 4.2*	19.4 ± 3.6*	6.4 ± 0.9	16.0 ± 3.3*	20.6 ± 6.0*
PRSW, mmHg	17.6 ± 1.8	29.5 ± 2.6*	34.4 ± 1.4*	15.0 ± 2.0	33.6 ± 3.6*	34.5 ± 3.9*
Time var E_{max}, mmHg.µL^{-1}	0.63 ± 0.08	0.94 ± 0.14*	1.02 ± 0.07*	0.46 ± 0.07	0.81 ± 0.09*	0.87 ± 0.08*
LV						
EDPVR (exponential)						
k_1	0.012 ± 0.002	0.023 ± 0.002*	0.013 ± 0.004†	0.013 ± 0.002	0.012 ± 0.001‡	0.009 ± 0.003‡
k_2	0.85 ± 0.24	0.38 ± 0.32	0.88 ± 0.37	0.67 ± 0.28	1.15 ± 0.18‡	1.04 ± 0.37‡
ESPVR (linear)						
Slope, mmHg.µL^{-1}	1.04 ± 0.14	2.11 ± 0.50*	1.56 ± 0.28	0.94 ± 0.12	2.25 ± 0.74*	1.70 ± 0.16
Intercept, µL	-21.2 ± 33.8	3.9 ± 24.8	44.4 ± 39.2	-25.5 ± 25.3	9.6 ± 22.6	38.5 ± 12.7
dP/dt_{max}-EDV, mmHg.s^{-1}.µL^{-1}	56.1 ± 12.3	108.2 ± 17.8*	96.2 ± 19.5*	45.9 ± 8.4	96.3 ± 13.1*	98.3 ± 23.0*
PRSW, mmHg	104.7 ± 17.8	121.3 ± 8.8	110.8 ± 17.7	130.9 ± 25.8	136.4 ± 17.5	121.3 ± 19.6
Time var E_{max}, mmHg.µL^{-1}	3.25 ± 0.36	4.29 ± 0.73*	3.92 ± 0.74	3.18 ± 0.60	3.77 ± 0.83	3.48 ± 0.77

Right (RV) and left ventricular (LV) load-independent indexes derived from inferior vena cava occlusions in vehicle-injected (Ctrl), monocrotaline-injected (MCT) and monocrotaline-injected bosentan-treated rats (MCT BOS). EDPVR, end-diastolic pressure-volume relationship; k_1 and k_2, indexes of exponential function; ESPVR, end-systolic pressure-volume relationship; E_{max}, maximal elastance; dP/dt_{max}-EDV, slope of preload (EDV, end diastolic volume)-induced increase in maximum rate of pressure rise (dP/dt_{max}); PRSW, slope of preload recruitable stroke work; Time var E_{max}, time varying maximal elastance. *P<0.01 *vs* Ctrl and †P<0.05 *vs* MCT on two-way ANOVA; ‡P<0.01 *vs* before on paired t-test; n=8 per group.

Figures

Fig. 1

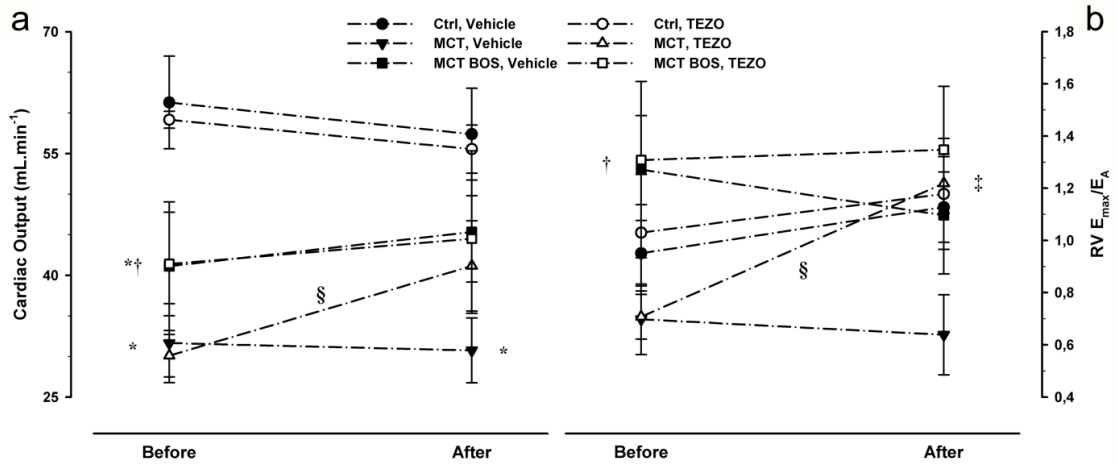


Fig. 2

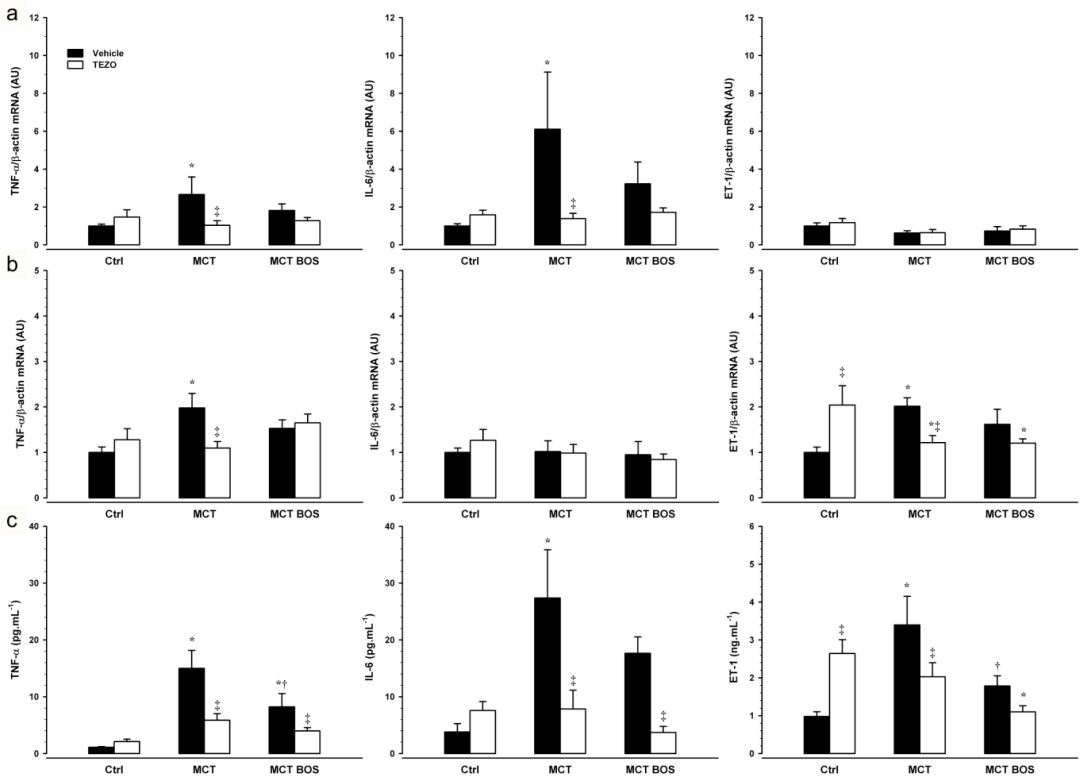

a
b
c
Vehicle
TEZO
TNF-α/β-actin mRNA (AU)
IL-6/β-actin mRNA (AU)
ET-1/β-actin mRNA (AU)
TNF-α (pg.mL-1)
IL-6 (pg.mL-1)
ET-1 (ng.mL-1)
Ctrl
MCT
MCT BOS

Fig. 3

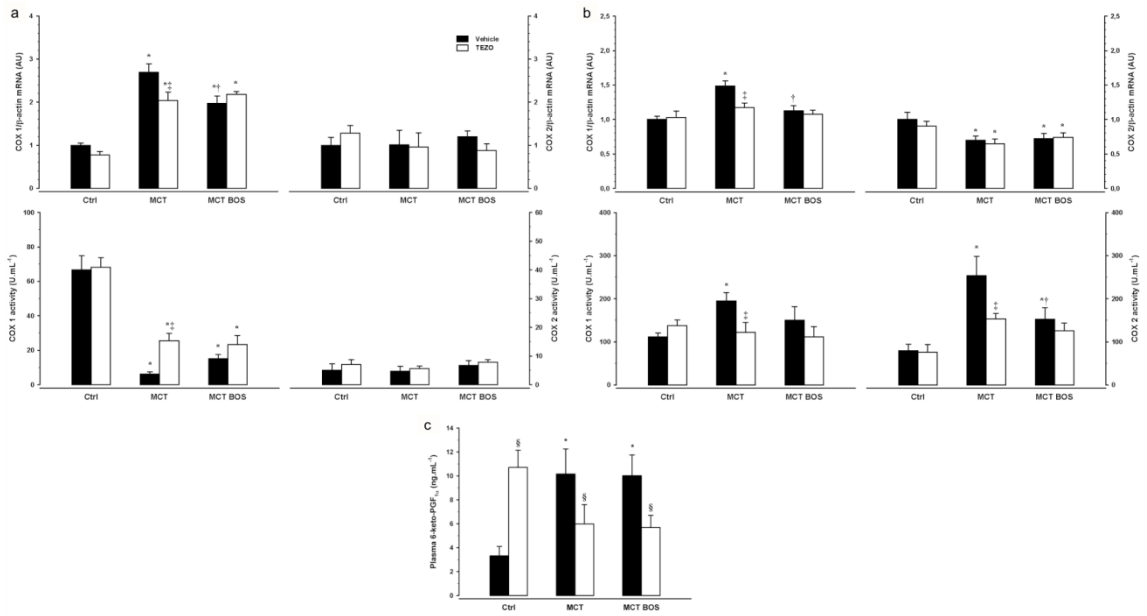
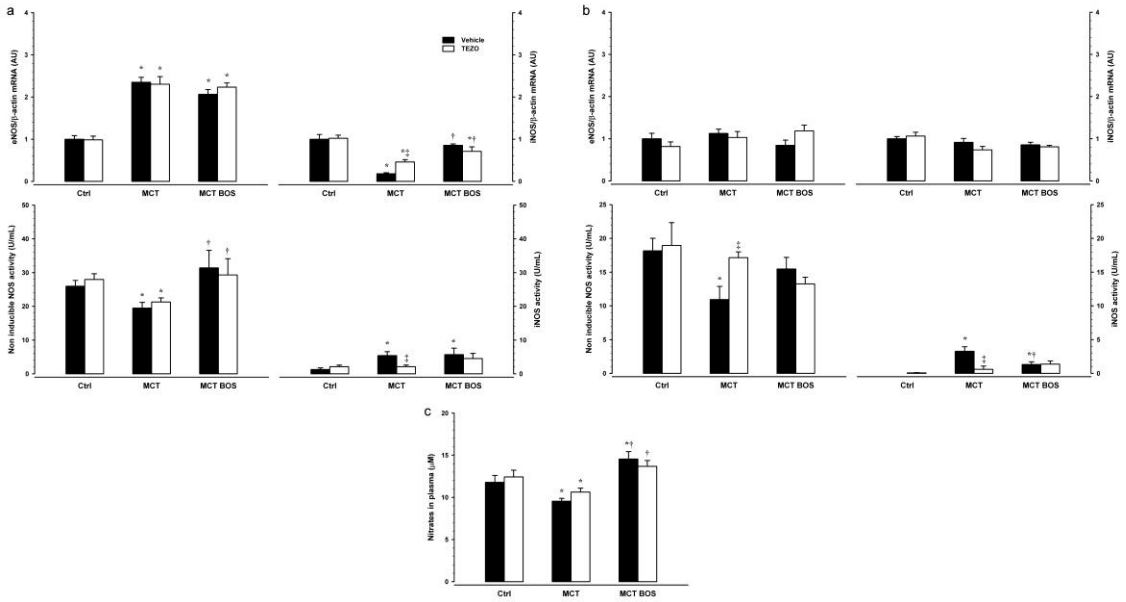

a
b
c
Vehicle
TEZO
COX 1/β-actin mRNA (AU)
COX 2/β-actin mRNA (AU)
COX 1 activity (U.mL⁻¹)
COX 2 activity (U.mL⁻¹)
Plasma 6-keto-PGF1α (ng.mL⁻¹)
Ctrl
MCT
MCT BOS

Fig. 4


a
b
c
Vehicle
TEZO
eNOS/β-actin mRNA (AU)
iNOS/β-actin mRNA (AU)
Non inducible NOS activity (U/mL)
iNOS activity (U/mL)
Nitrates in plasma (μM)
Ctrl
MCT
MCT BOS

Supplemental Figure Legends

Supplemental Fig. 5. Flowchart of experimental procedures; for details on experimental protocols please refer to the manuscript. ABG, arterial blood gas analysis

Supplemental Fig. 6 Acute dose-response haemodynamic effects of tezosentan (TEZO) in right (RV) and left ventricular (LV) maximal pressures of vehicle-injected (Ctrl), monocrotaline-injected (MCT) and monocrotaline-injected bosentan-treated rats (MCT BOS); recordings of RV (black symbols) and LV maximal pressures (white symbols) obtained in Ctrl (circular symbols, solid lines), MCT (triangular symbols, long dash) and MCT BOS (quadrangular symbols, short dash) are shown at baseline and after stable effects of 0.5, 1, 2, 5, 10 and 20mg.Kg^{-1} cumulative intravenous TEZO bolus administrations. $^{*}P<0.05$ *vs* baseline, $^{\dagger}P<0.001$ *vs* Ctrl, $^{\ddagger}P<0.01$ *vs* Ctrl and MCT BOS on two-way repeated-measures ANOVA; n=7 per group

Supplemental Fig. 7 Representative right ventricular pressure-volume (PV) loops before and after perfusion of vehicle (**a** and **b**, respectively) and tezosentan (**c** and **d**, respectively) in vehicle-injected (Ctrl), monocrotaline-injected (MCT) and monocrotaline-injected bosentan-treated rats (MCT BOS); PV loops and linear, end-systolic, and exponential, end-diastolic, PV relationships are presented as solid lines for Ctrl (dark gray), MCT (black) and MCT BOS (light gray). Dashed lines represent inferior vena cava occlusions. For differences between groups please refer to tables 1 and 2

Supplemental Tables

Supplemental Table 1. Gene specific real -time PCR primers.

Gene	Sequence
ET-1	fw: 5' – CGG GGC TCT GTA GTC AAT GTG - 3' rev: 5' – CCA TGC AGA AAG GCG TAA AAG - 3'
TNF-α	fw: 5' – TGG GCT ACG GGC TTG TCA CTC - 3' rev: 5' – GGG GGC CAC CAC GCT CTT C - 3'
IL-6	fw: 5' – GAA GTT GGG GTA GGA AGG AC - 3' rev: 5' – CCG TTT CTA CCT GGA GTT TG - 3'
COX-1	fw: 5' – AGT ACC ACC TGC GGC TCT TCA - 3' rev: 5' – CTT TTC GGG CGG GAC ACC T - 3'
COX-2	fw: 5' – GCT GCT GCC GGA CAC CTT - 3' rev: 5' – CAA CCC GGC CAG CAA TCT - 3'
eNOS	fw: 5' – GTC GGG CCC CTA CAA CAG C - 3' rev: 5' – TGC GCC GCC AAG AGG ATA - 3'
iNOS	fw: 5' –CCC AGC CCA ACA ACA CAG GA- 3' rev: 5' –GGC GGG TCG ATG GAG TCA- 3'
β-actin	fw: 5' – ATC TGG GTC ATC TTT TCA CGG TTG G - 3' rev: 5' – GAT TTG GCA CCA CAC TTT CTA CA - 3'

ET-1, endothelin-1; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; COX-1, cyclooxygenase type 1; COX-2, cyclooxygenase type 2; eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase.

Supplemental Table 2. Morphometry.

		BW, g	RVW/BW, $mg.g^{-1}$	RVW/(LVW+IVSW), %
Ctrl	**Vehicle**	328 ± 13	0.56 ± 0.03	28 ± 1
	TEZO	317 ± 16	0.62 ± 0.03	31 ± 2
MCT	**Vehicle**	236 ± 9*	1.46 ± 0.10*	60 ± 4*
	TEZO	247 ± 9*	1.33 ± 0.09*	62 ± 4*
MCT BOS	**Vehicle**	257 ± 9*	1.19 ± 0.11*†	53 ± 4*†
	TEZO	252 ± 10*	1.16 ± 0.08*†	52 ± 4*†

Morphometric evaluation of vehicle-injected (Ctrl), monocrotaline-injected (MCT) and monocrotaline-injected rats chronically treated with Bosentan (MCT BOS) that later underwent either vehicle or tezosentan perfusion (TEZO). BW, body weight; RVW, right ventricular weight; LVW, left ventricular weight; IVSW, interventricular septum weight.

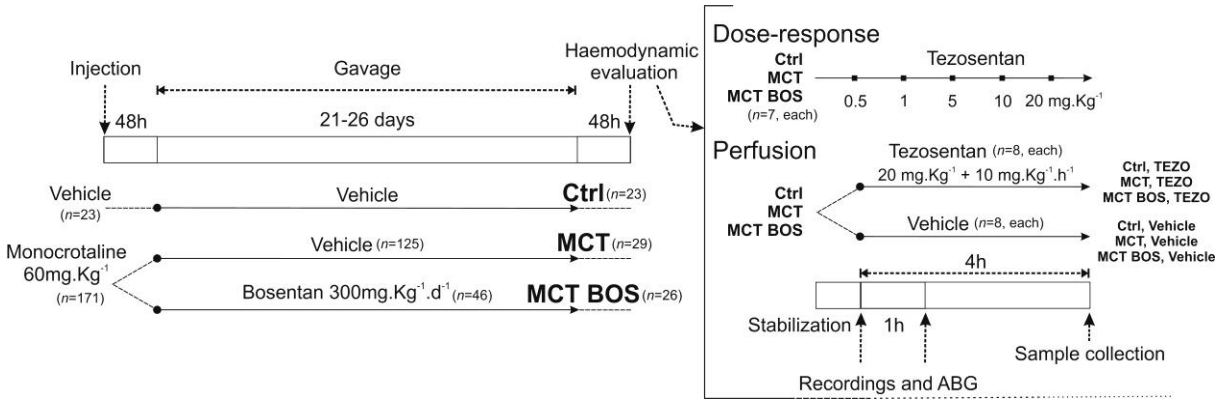
Supplemental Table 3. Blood gas values before and after vehicle and tezosentan perfusion.

	Before			After		
	Ctrl	**MCT**	**MCT BOS**	**Ctrl**	**MCT**	**MCT BOS**
			Vehicle			
*p*H	7.46 ± 0.03	7.50 ± 0.05	7.44 ± 0.01	7.48 ± 0.02	7.50 ± 0.05	7.41 ± 0.02
P_aO_2, mmHg	324 ± 36	178 ± 36*	164 ± 17*	343 ± 28	223 ± 38*	251 ± 48*
P_aCO_2, mmHg	32.8 ± 3.1	28.0 ± 2.9	28.3 ± 2.7	30.8 ± 2.3	28.0 ± 2.4	28.0 ± 2.5
			Tezosentan			
*p*H	7.44 ± 0.05	7.43 ± 0.04	7.44 ± 0.01	7.42 ± 0.06	7.39 ± 0.03	7.41 ± 0.02
P_aO_2, mmHg	320 ± 29	225 ± 23*	183 ± 38*	279 ± 41	230 ± 27*	174 ± 24*
P_aCO_2, mmHg	31.7 ± 2.9	29.2 ± 3.0	32.1 ± 2.6	28.0 ± 4.2	28.2 ± 1.6	28.7 ± 3.7

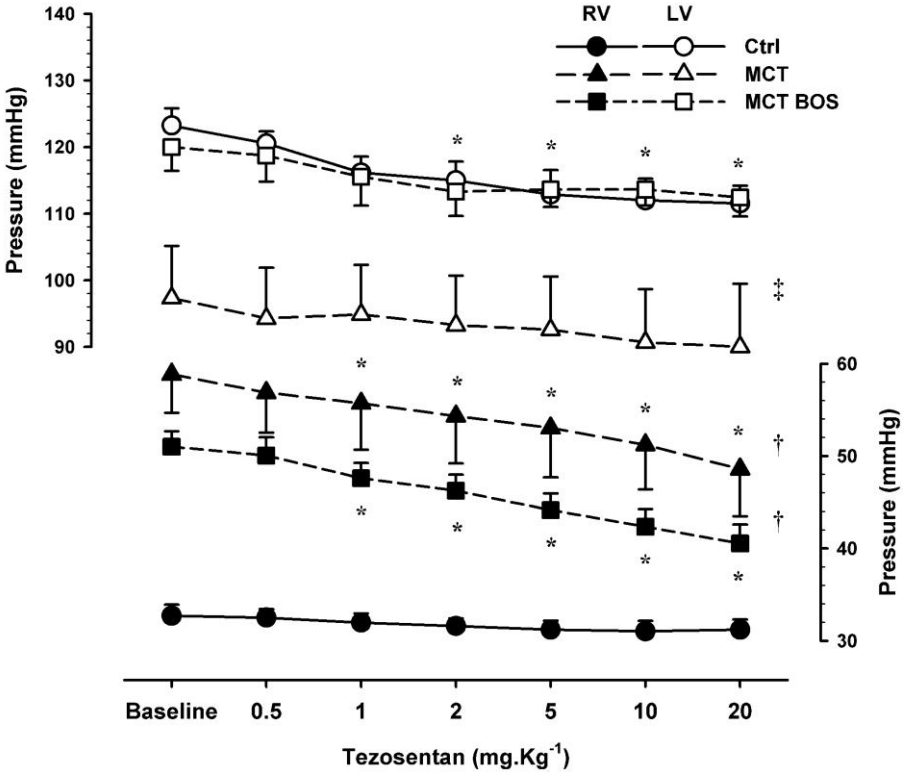
Arterial blood gas values before and after saline (Vehicle) or tezosentan perfusion in vehicle-injected (Ctrl), monocrotaline-injected (MCT) and monocrotaline-injected bosentan-treated rats (MCT BOS). P_aO_2, arterial O_2 pressure; P_aCO_2, arterial CO_2 pressure. *P<0.01 *vs* Ctrl on two-way ANOVA; n=8 per group.

Supplemental Figures

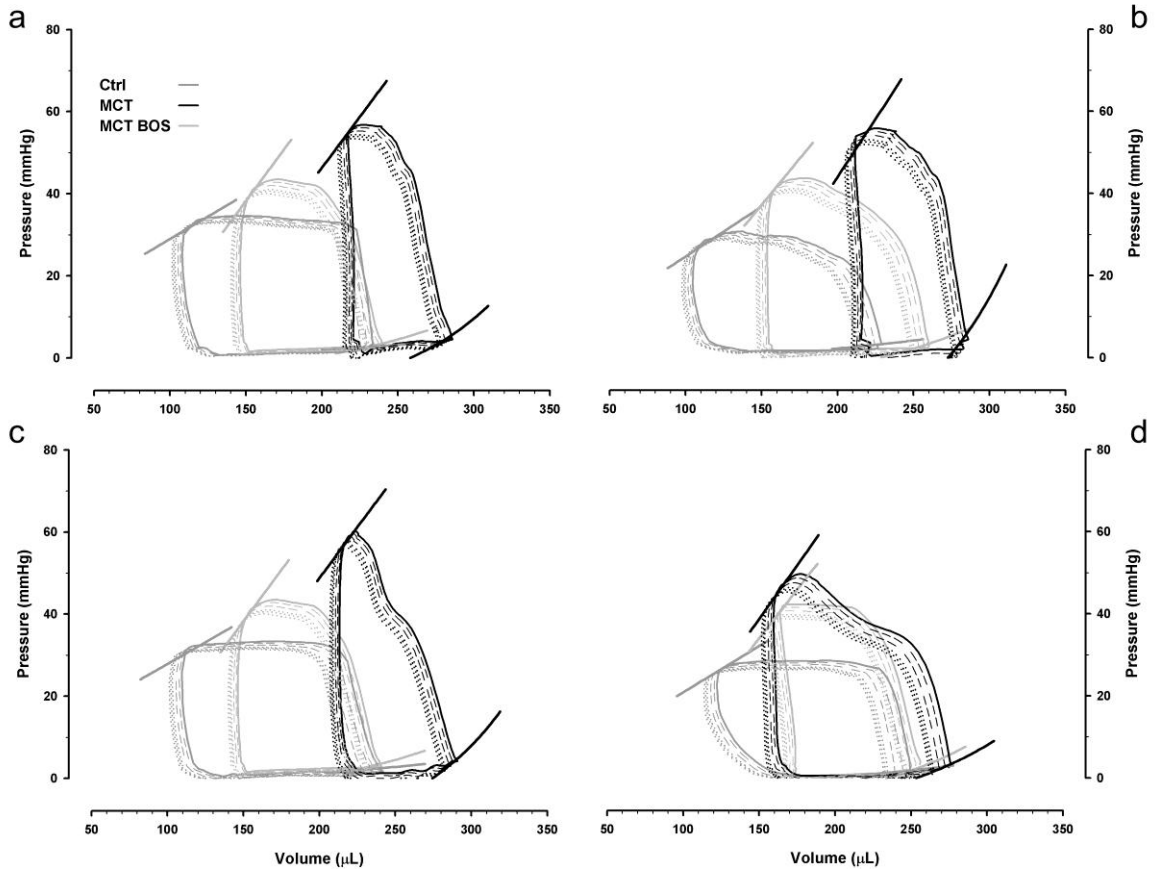
Supplemental Fig. 5



Supplemental Fig. 6



Supplemental Fig. 7



Diastolic tolerance to systolic pressures closely reflects systolic performance in patients with coronary heart disease

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Leite-Moreira: Diastolic function and systolic performance

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ABSTRACT

Background. In animal experiments, elevating systolic pressures induces diastolic dysfunction and may contribute to congestion, a finding not yet translated to humans.

Methods and Results. Coronary surgery patients (63±8yrs) were studied with left ventricular (LV) pressure (n=17) or pressure-volume (n=3) catheters, immediately before cardiopulmonary bypass. Single-beat graded pressure elevations were induced by clamping the ascending aorta. Protocol was repeated after volume loading (n=7). Consecutive patients with a wide range of systolic function were included. Peak isovolumetric LV pressure (LVP_{iso}) ranged from 113-261 mmHg. With preserved systolic function, LVP elevations neither delayed relaxation nor increased filling pressures. With decreasing systolic function, diastolic tolerance to afterload progressively disappeared: relaxation slowed and filling pressures increased (diastolic dysfunction). In severely depressed systolic function, filling pressures increased even with minor LVP elevations, suggesting baseline load dependent elevation of diastolic pressures. The magnitude of filling pressure elevation induced in isovolumetric heartbeats was closely and inversely related to systolic performance, evaluated by LVP_{iso} (r=-0.96), and directly related to changes in the time constant of relaxation τ (r=0.95). The maximum tolerated systolic LVP (without diastolic dysfunction) was similarly correlated with LVP_{iso} (r=0.99). Volume loading itself accelerated relaxation, but augmented afterload-induced upward shift of filling pressures (7.9±3.7 mmHg vs. 3.0±1.5; p<0.01).

Conclusion. The normal human response to even markedly increased systolic pressures is no slowing of relaxation and preservation of normal filling pressures. When cardiac function deteriorates, the LV becomes less tolerant, responding with slowed relaxation and increased filling pressures. This increase is exacerbated by volume loading.

Keywords: diastole, diastolic dysfunction, afterload, systolic function

INTRODUCTION

Ventricular remodeling in coronary heart disease includes myocyte loss, changes in myocyte biology and extracellular matrix, and alterations in chamber geometry. These aspects contribute to diastolic dysfunction[1, 2],which is characterised by impaired ventricular filling and an upward shift of the diastolic pressure-volume relation [3, 4]. The main cause of diastolic dysfunction is increased late-diastolic stiffness [5-7]. Accumulating evidence shows that, in addition to long-term structural changes that underlie myocardial stiffness, there might also be short-term functional determinants such as ischemia[8], titin phosphorylation status [9] and neuroendocrine mediation[10].

Impaired myocardial relaxation may result in sustained pressure at end-diastole and may thus contribute to increased left ventricular (LV) stiffness, mainly in failing hearts[7]. One of the possible causes for impaired myocardial relaxation and diastolic dysfunction in animal models is excessive afterload[11]. This load-dependence might be relevant as well for the interpretation of diastolic dysfunction resulting from arterial hypertension, from increased arterial stiffening and from early wave reflection[12]. In order to clarify the relevance of these concepts to human disease, the present study analysed the effects on diastolic filling pressures of graded elevations of systolic LV pressures (LVP) induced by aortic clamping. The study was performed during coronary artery bypass grafting (CABG).

MATERIALS AND METHODS

Study population

Twenty consecutive adult patients with 3-vessel disease undergoing elective on-pump CABG were enrolled. Exclusion criteria included: unstable angina, pericardial disease, LV hypertrophy defined as mean wall thickness > 1.1 cm, evidence of calcified ascending aorta in preoperative exams or in intra-operative assessment both by palpation and epivascular

ultrasonography, previous stroke or transient ischemic event, as assessed by clinical interview or preoperative exams, significant carotid artery disease based on pre-operative evaluation, and previous cardiac surgery. All patients underwent routine preoperative evaluation including coronary angiogram and echocardiographic evaluation. Left ventricular ejection fraction (EF) was calculated by 2D-echcardiography using the Simpson's rule. The study was approved by the ethics committee of the University Hospital São João in Porto and conforms with the principles of the Declaration of Helsinki. All patients gave their written informed consent.

Procedure

Preoperative, anaesthetic and surgical procedures were standard. Briefly, regular medication was continued until the morning of surgery, 0.1mg/Kg oral diazepam was used as an anxiolytic in the morning and on the night before the intervention. On arrival to the operation room, the EKG and pulse oximetry were monitored. Patients were then premedicated with 0.1 mg/Kg intravenous diazepam, and invasive blood pressures were monitored after radial artery catheterization under local anaesthesia. General anaesthesia was induced with 10-25μg/Kg fentanyl, 0.1mg/Kg etomidate and 0.1mg/Kg vecuronium, and maintained with 0.2-0.4% isoflurane on a 50% O_2:N_2O gas mixture, and additional fentanyl and vecuronium boluses. A central venous line was then placed and central venous pressure was continuously monitored, as well as capnography, central and peripheral temperature. An additional bolus of 20-25μg/Kg fentanyl was given before sternotomy. Before anaesthesia mean blood pressure was 86.4 $\pm$ 12.0 mmHg and decreased to 77.7 $\pm$ 13.2 mmHg at the start of the measurements. During surgical preparation for cardiopulmonary bypass, just before the insertion of the venous cannula, a 3F catheter with a high-fidelity transducer (SPC-330A, Millar Instruments, Houston, Texas) was inserted through a needle puncture in the LV apical dimple and held in place by a teflon felt pledgeted purse-string suture with 4/0 polypropilene in order to record LV pressures. Before

insertion, the catheter was calibrated and stabilized for 30 minutes in 37°C saline. The catheter was connected to a pressure amplifier and a differentiator to evaluate the first derivative of the pressure recording. To strengthen our findings, in three patients we simultaneously recorded LV pressure and volume using an equipment transiently available at our institution. For this purpose a 5-Fr combined pressure-volume catheter with 1-cm inter-electrode spacing (SPC-551, Millar Instruments, Houston, Texas) was inserted instead.

Data acquisition and analysis

EKG (DII), LV pressure and its first derivative were digitized with a sample rate of 500Hz. The mean heart rate (HR) value was 72±5 bpm and remained stable. Haemodynamic recordings were done with ventilation suspended at end-expiration. The R wave of the EKG tracing was used to define end-diastole. The following parameters were obtained: LV end-diastolic pressure (LVEDP), peak systolic LVP, maximum velocity of LV pressure rise (dP/dt_{max}) and fall (dP/dt_{min}), and the logistic time constant of isovolumetric relaxation τ[13, 14].

Beat-to-beat systolic LVP elevations were induced by constricting the ascending aorta with an aortic clamp, above the sinotubular junction. Variable degrees of constriction were performed and isovolumetric heartbeats were obtained with complete aortic occlusions. Constrictions were started during diastole and sustained for 2 to 5 cycles. An interval of 2 to 3 minutes of rest and stabilization was observed between maneuvers. No electrocardiographic signs of ischemia or haemodynamic instability were observed before, during or after the interventions. The first heartbeat after the clamp was analysed. From physiology and previous experiments in animal models, we know that aortic clamps increase systolic pressure and systolic volumewhile decreasing systolic wall thickness. The three parameters of Laplace's law concur to increase systolic wall stress and hence afterload. In the given experimental conditions, systolic pressure can therefore be considered a surrogate of afterload. The clamp technique

selectively increases afterload with no preload changes, no changes in long term load history, and no neurohumoral adaptations[15].

Effects of systolic LVP elevations on filling pressures were assessed by subtracting LVEDP at the end of the test beat from LVEDP of the previous control beat (diastolic dysfunction). We previously showed in animal experiments that single beat afterload elevations do not alter LV end-diastolic volume and therefore increases in LVEDP denote a true upward shift of the end-diastolic pressure-volume relation [11]. This was confirmed in the present study with pressure-volume measurements.

In each patient the maximum systolic LVP, which did not slow relaxation and did not increase subsequent filling pressures was determined, referred to as maximum tolerated pressure. In 3 patients, in whom no additional systolic LVP could be developed without slowing of relaxation, systolic pressure was carefully decreased by transient caval occlusion. As LVP progressively decreased, the time constant τ initially decreased, then increased. This was monitored on-line on a dP/dt versus LVP phase-plane plot, as previously described [15],and confirmed by off-line analysis. The level of systolic LVP at which the time constant τ was minimal in these patients was deemed to be the maximum tolerated systolic LVP in this subgroup.

In 7 patients with EF≥0.30, 500 mL of extracorporeal circulation priming solution were administered. Recordings of baseline and isovolumetric heartbeats were repeated after stabilization.

Statistical analysis

Quantitative results are presented as mean±SD. Systolic LVP is given in absolute values (mmHg) or as a percentage of the isovolumetric pressure of each patient. Effects of LVP elevations were analysed with repeated measurements one-way ANOVA and Holm-Sidak *post hoc* test. Volume loading was compared to baseline with paired *t*-test. Linear regression was

performed on normally distributed data by least squares regression and the Pearson correlation coefficient was obtained. Statistical significance was set at $P<0.05$.

RESULTS

The mean patient age was 63±8 years, 19 were men. Other preoperative data including drug therapy and comorbidities are summarized in table 1. These consecutive patients constitute a representative sample of CABG patients at our institution.

Individual haemodynamic data are presented in supplemental table 1. Patients were pooled in three groups according to their preoperative EF as assessed by 2D-echocardiography (normal ≥0.50; moderately decreased 0.30-0.49; severely decreased<30). Twelve patients had a normal and eight patients a decreased EF.

All patients underwent graded aortic constrictions resulting in beat-to-beat elevations of systolic LVP, ranging from a small elevation of a few mmHg to full isovolumetric beats. A representative LVP tracing illustrating the increase in LVEDP after complete aortic occlusion is presented in Figure1. Corresponding tracings from other patients are presented in supplemental figure 1. Tracings, representative of each of the three EF patient categories, are presented in Figure 2. In each panel, three pressure-time curves are superimposed: a control, an isovolumetric beat and one intermediate LVP elevation. Peak isovolumetric LVP was higher in the left panel (normal EF) and lower in the right panel (EF<0.30). In the left panel, LVP elevations did not affect subsequent filling pressures, while the right panel, illustrates a patient with poor EF, who already presented elevated filling pressure at rest and showed further increases with both the intermediate and isovolumetric LVP elevations. As to the middle panel (EF 0.30-0.49), filling pressures were increased in the isovolumetric beat but not in the intermediate-afterloaded heartbeat. When we consider the patients with normal EF (n=12), they operate at baseline (under anaesthesia) at a systolic pressure corresponding to 47.8±5.0% of peak isovolumetric pressure (Supplemental table 2).

Increasing filling pressures, induced by beat-to-beat interventions, occured without concomitant increases of diastolic volume in the recordings obtained with pressure-volume catheters, representing a true upward shift of the diastolic pressure-volume relation, hence afterload dependent diastolic dysfunction. Representative pressure-volume tracings are presented for a patient with an EF of 0.30-0.49 in supplemental figure 2.

The shift in LVEDP induced by the first isovolumetric heartbeat after complete aortic occlusion was then correlated with haemodynamics and with LV function. Significant negative correlations were found with systolic function evaluated by dP/dt_{max} (r=-0.68) or peak isovolumetric LVP (r=-0.96; Figure 3, left panel). There was no correlation with the baseline value of the time constant τ or baseline LVEDP, but a close inverse correlation with the % change in τ induced by isovolumetric heartbeats (r=0.95; Figure 3, right panel) was observed. Accordingly, the changes were more prominent in reduced EF categories.

For assessing the maximum systolic LVP that the heart could tolerate without slowing of relaxation and elevation of filling pressures, we analyzed multiple graded systolic pressures in each patient. Tolerated systolic LVP ranged from 60 to 100% of peak isovolumetric LVP (Supplement table 3). In 3 patients with normal EF, afterload values of more than 230 mmHg were tolerated. In 3 other patients with EF<0.30, no pressure elevation was tolerated, even when baseline systolic LVP was lower than 90 mmHg. In these patients, the maximum tolerated systolic LVP was derived by caval occlusion (see methods). The remaining 14 patients tolerated intermediate levels of systolic LVP. Similarly to the magnitude of shift in diastolic pressure volume relation induced by isovolumetric heartbeats, tolerated systolic LVP was also strongly correlated with peak isovolumetric LVP (r=0.99, *P*<0.001; Figure 4) and dP/dt_{max} (R=0.71, P<0.001, not shown). Of note, the data of the 3 patients, in whom the maximum tolerated pressure was obtained with caval occlusion are well aligned with the other data.

In 7 patients the experimental protocol was repeated after volume loading (Table1). Volume loading increased diastolic and systolic LVP and accelerated myocardial relaxation (shortened time constant τ). Peak isovolumetric LVP was higher after volume loading (Frank-Starling). Afterload-induced prolongation of the time constant τ was not exacerbated by volume loading, but the shift in LVEDP more than doubled. This is illustrated in Figure 5, which shows in addition that, after volume loading, the onset of pressure fall was delayed (time to dP/dt_{min} increased, Table 1) and that, at matched HR, the duration of diastole decreased.

None of the patients died or suffered from neurological complications in the perioperative or early postoperative period (30 days). One patient was reinterventioned due to immediate post-operative bleeding, one patient needed continuous renal replacement therapy and two patients needed an intra-aortic balloon pump and prolonged cardiovascular support in the intensive care unit (ICU). The lengths of stay were 1.6 ± 1.3 and 8.0 ± 3.9 days in the ICU and in the hospital, respectively.

DISCUSSION

The present investigation describes how human LV diastole responds to systolic pressure elevation, and defines for the first time how much systolic pressure is tolerated without diastolic dysfunction. The study illustrates a close coupling between the diastolic response and systolic performance, evaluated by peak isovolumetric pressure.

In healthy animals, the physiological response of the myocardium to a moderate increase in afterload is a slight acceleration of relaxation[15] and no elevation of filling pressures[11]. This is valid up to a load level that corresponds to a high percentage of peak isovolumetric load[15, 16].

The analysis of isovolumetric heartbeats allows to refine earlier physiological observations and to apply them to patients with coronary heart disease. There is no precedent of a similar study conducted in humans, except maybe for the analysis of left ventricular systolic

stiffness in 7 patients by Ritter et al.[17] From an ethical point of view it is important to note that we didn't observe complications, carefully selected patients and performed intra-operative epivascular ultrasonography for excluding atherosclerotic aortic plaques. Given the increasing evidence of cerebrovascular complications with manipulations of the aorta, we however decided not to expand this series of patients. This is a "proof of concept", translational study, which allows us to draw some important conclusions even with 20 observations. This study describes how the better functioning human LV of CABG patients is able to develop a peak systolic LVP of more than 230 mmHg, with no slowing of relaxation and no increase in LVEDP, hence no diastolic dysfunction. Such a ventricle operates at rest at a systolic LVP that is 40-50% of peak isovolumetric LVP. This baseline load level corresponds to an optimal ventriculo-arterial hydraulic and energetic matching [18, 19]. It confers a surprisingly high afterload reserve to the healthy human LV, allowing it to face stress and exercise without compromising filling. This is the case even in the presence of severe coronary heart disease, during anaesthesia and surgery. The remaining patients responded to afterload with variable degrees of slowing of myocardial relaxation and diastolic dysfunction, manifest as an upward shift of the end-diastolic pressure-volume relation. This shift closely and inversely correlated with dP/dt_{max} and peak isovolumetric LVP: the better the systolic performance, the more limited the shift. In addition, this shift closely correlated with the changes in time constant τ, suggesting that slowing of relaxation was responsible for the observed shift of the end-diastolic pressure-volume relation. This confirms in cardiac patients a close relation between systolic and diastolic function, previously described in various animal studies[11, 16, 20]. A limitation of the present study is that we performed volume measurements in only 3 patients. However, the effects of aortic clamping and the relation between delayed relaxation and increased filling pressures were also previously demonstrated in those animal models[11, 16, 21].

By performing multiple graded aortic constrictions, we determined the maximum level of systolic LVP that the heart could tolerate without elevating its filling pressures and slowing

relaxation. The maximum tolerated LVP is highly predictive of the peak isovolumetric LVP and ranges from 60 to 100% of the isovolumetric LVP. The tolerated systolic LVP ranged from isovolumetric in some patients to baseline and even less in others. The observations on the maximum tolerated systolic pressures expand the knowledge on the effects of unphysiological isovolumetric pressures and provide a clinically useful translation of the concepts related to load dependence of diastolic function. The findings are consonant with previous studies documenting in failing hearts the reversal of diastolic dysfunction in response to decreasing systolic pressures[22, 23]. This was observed in heart failure patients with severely depressed systolic function (low EF's)[22], and in dogs with pacing induced cardiomyopathy[23]**.** These findings imply that elevated filling pressures in patients with advanced HF with reduced EF include a load dependent and potentially reversible component. A patient with a severely depressed EF and lower blood pressures works at a high percentage of isovolumetric load and mandatorily has load dependent diastolic dysfunction, which can be limited with even small decreases of systolic pressure. This adds an additional pathophysiological mechanism for the beneficial effects of vasodilators and diuretics in this frequently occurring clinical condition.

The effects of increased load on diastolic function were previously acknowledged by other groups. Vatner's group analyzed the effects of increased systolic pressure and showed that in developing hypertensive heart disease in dogs, diastolic dysfunction was present. This dysfunction was not attributable to structural changes and could be mimicked in healthy animals by acutely increasing preload and afterload [24]. Similarly, aged dogs with renal hypertension and increased LV systolic and arterial stiffness presented impaired LV relaxation, but no increase in the coefficient of LV diastolic stiffness. Nevertheless, filling pressures increased with hypertensive episodes due to load-dependent impairment of relaxation[25].

Even if one should be aware of shifts in the end-diastolic pressure-volume relation, related to pericardial constraint and ventricular interdependence[26], these mechanisms were most likely not quantitatively important in the present open chest and open pericardium study

conditions. Although these conditions might be well suited for the study of the pathophysiological effects of acute overload in the myocardium, they preclude extrapolation to long term pressure elevations and to a clinical setting without anaesthesia and surgery.

By studying the effects of selective beat-to-beat afterload elevations on both relaxation rate and diastolic function we were able to better control for confounding alterations of global haemodynamics due to decreased stroke volume, acute backward failure, increased LV filling pressures, neurohumoral responses and possibly afterload-induced myocardial ischemia [11]. Furthermore, as myocardial hypertrophy reduces tolerance to ischemia and coronary vasodilator reserve leading to diastolic dysfunction [27], we selected patients with a similar degree of stable 3-vessel coronary artery disease and excluded those with other than mild LV hypertrophy. Extent of myocardial ischemia and hypertrophy are therefore unlikely to have contributed significantly to diastolic dysfunction during transient aortic clamps with analysis of the first clamped heartbeat.

In 7 patients with a normal or moderately depressed EF, afterload elevations were repeated after volume loading. Volume loading itself accelerated myocardial relaxation, which challenges earlier reports of slowed myocardial relaxation in volume loaded anaesthetized open-chest dogs[28]. This observation is nevertheless consonant with what was shown in the better responding subgroup of coronary patients after leg elevation[29]. These apparently contradictory results might relate to anaesthesia and operative conditions of those earlier canine observations.

After volume loading, the upward shift of the end-diastolic pressure-volume relation was aggravated and this was not attributable to slower myocardial relaxation. This corroborates the recent demonstration in healthy dogs that changes in peak early-diastolic mitral annulus velocity (e') are not dependent on myocardial relaxation, as assessed by time constant τ, after acute preload manipulations[30]. Therefore it must be due (at least in part) to prolongation of systole

and abbreviation (at comparable HR) of the duration of diastole, as previously demonstrated in animal experiments[21]. Other potential mechanisms still are largely speculative and require further investigation.

The negative inotropic effects of anaesthetics may have influenced the magnitude of effects, thus our results cannot be readily extended to unanaesthetized patients. The present study is applicable to patients with coronary heart disease with various EF's but not necessarily to other patients groups. The data however could be relevant for patients with HF with preserved EF (HFpEF) in whom increased vascular load and ventricular systolic stiffness were shown to enhance the sensitivity of systolic pressures to volume changes[31]. Many patients with HFpEF have concomitant systolic dysfunction abnormalities, particularly in the long axis[32, 33], which may limit their tolerated systolic pressures. Beyond the profound structural changes underlying diastolic dysfunction and volume loading, limited tolerance to systolic pressures may provide additional mechanistic information on hypertensive pulmonary edema [5, 34].

In summary, the present manuscript extends and refines previous experimental work on load and diastolic function. The physiological response of the human heart to increased systolic pressures (and to volume loading) is the preservation of relaxation velocity and normal filling pressures. When cardiac function deteriorates, however, the LV becomes less tolerant to increased systolic pressures and reacts with slowed relaxation and increased filling pressures even at lower pressure levels. Volume loading further exacerbates such intolerance.

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DISCLOSURES

None.

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FIGURE LEGENDS

Figure 1. A representative left ventricular pressure (LVP) tracing of patient 1 illustrating the increase in left ventricular end-diastolic pressure (LV EDP) after complete aortic occlusion.

LVP tracing of a control and isovolumetric cycle obtained in patient 1 after complete aortic occlusion during diastole. The upward shift in LV EDP from control to isovolumetric heartbeat, is represented.

Figure 2. The effects of left ventricular pressure (LVP) elevation on diastolic pressures depend on systolic function.

LVP tracings in baseline conditions (solid line), during a moderate LVP elevation (dotted line) and during an isovolumetric heartbeat (dashed line). Recordings of three representative patients are displayed: normal systolic function and normal ejection fraction (EF) on the left; moderately depressed systolic function and EF 30-49% in the middle; severely depressed systolic function and EF<30% on the right. See text for details.

Figure 3. The magnitude of the elevation of diastolic pressures closely correlates with systolic performance and with slowing of relaxation.

The afterload induced elevation of diastolic pressures, expressed as the upward shift of the left ventricular end-diastolic pressure (LVEDP) is plotted as a function of the peak systolic pressure of an isovolumetric heartbeat (LVP_{ISO}) (left panel) and as the percentage change of the logistic time constant τ, induced by this isovolumetric beat (right panel). Symbols in gray scale correspond to different categories of ejection fraction (EF), as indicated.

Figure 4. Tolerated systolic left ventricular pressures (LVP) strongly correlate with peak isovolumetric LVP.

Tolerated systolic LVP derived from the analysis of multiple graded afterloaded heartbeats are plotted as a function of the peak systolic LVP of the isovolumetric beat (LVP_{ISO}). Symbols in gray scale correspond to categories of ejection fraction (EF), as indicated.

Figure 5. Volume loading exacerbates afterload induced diastolic dysfunction.

Left ventricular pressure (LVP) tracings of baseline (black lines) and isovolumetric heartbeats (grey lines).Data before (solid lines) and after (dashed lines) volume loading. After volume loading, pressure fall is delayed, but the rate of pressure fall is similar. Filling pressures rise much more in response to the isovolumetric condition after volume loading.

TABLES

TABLE 1. Sample characteristics.

Patient characteristics	20
Age	63.2±8.0
Female gender	1 (5%)
BMI (Kg/m^2)	27.0±2.6
Previous MI	8 (40%)
Diabetes mellitus	2 (10%)
Arterial hypertension	12 (60%)
COPD	1 (5%)
Hemoglobin (g/dL)	13.9±1.58
Creatinine (mg/dL)	1.00±0.29
Chronic medication	
β-blockers	15 (75%)
ACEi/ARB	10 (50%)
Diuretics	7 (35%)
Nitrates	16 (84%)
CCB	10 (20%)

BMI, body mass index; COPD, chronic obstructive pulmonary disease; ACEi, angiotensin converting enzyme inhibitors; ARB, angiotensin receptor blockers; CCB, calcium channel blockers.

TABLE 2. Effects of volume loading in baseline and isovolumetric beats.

	Normal Filling	Volume loading
Baseline heartbeat		
LVEDP (mmHg)	11.4±1.1	17.0±3.7*
dP/dt_{max}(mmHg/s)	1363±241	1453±384
LVP_{max}(mmHg)	100±8	115±26*
τ (ms)	35.1±6.1	28.0±9.3*
Isovolumetric heartbeat		
LVP_{ISO}(mmHg)	207±77	220±53*
τ_{ISO} (ms)	46.7±6.1	40.1±11.1*
Time to dP/dt_{min}(ms)	405±66	461±108*
Upward shift in LVEDP (mmHg)	3.0±4.0	7.9±9.8*

LVEDP, left ventricular end-diastolic pressure; dP/dt_{max}, peak rate of left ventricular pressure rise; LVP_{max}, maximum developed left ventricular pressure; τ, time constant of isovolumetric relaxation; LVP_{ISO}, peak isovolumetric left ventricular pressure; τ_{ISO}, τ in the isovolumetric beat; dP/dt_{min}, peak rate of left ventricular pressure fall. n=7.

FIGURES

FIGURE 1

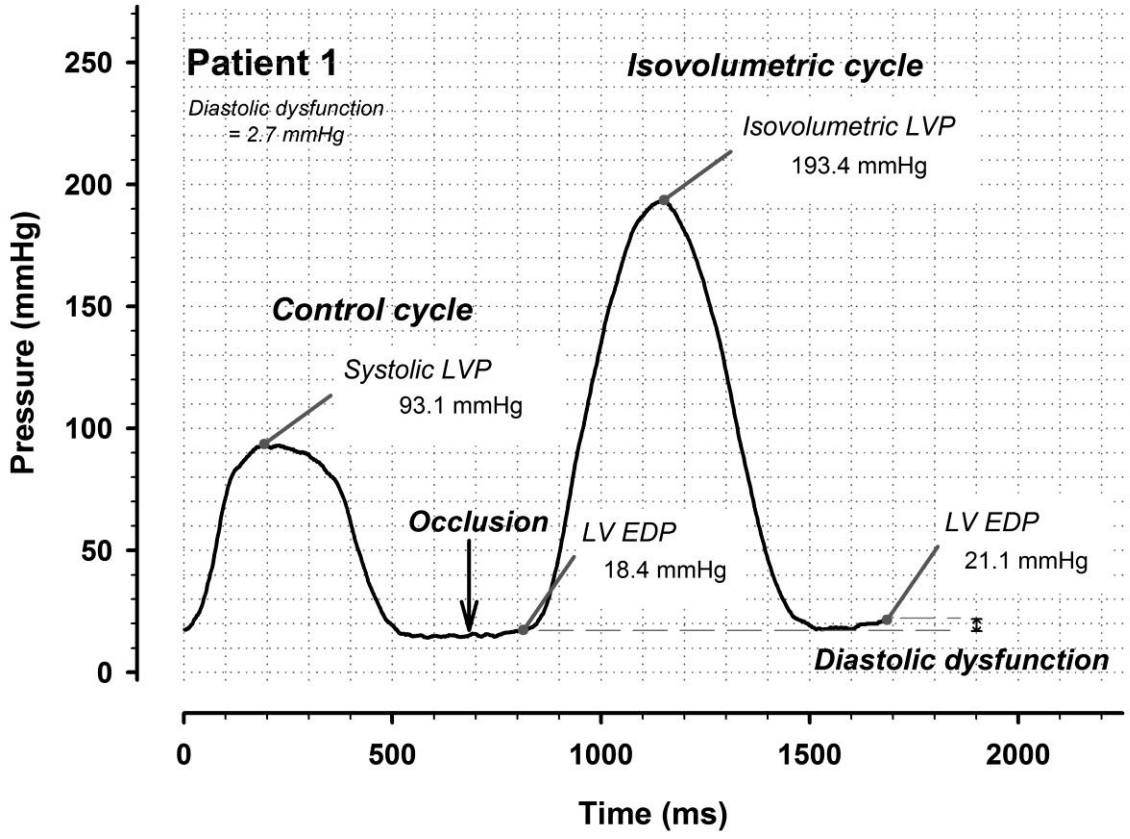


FIGURE 2

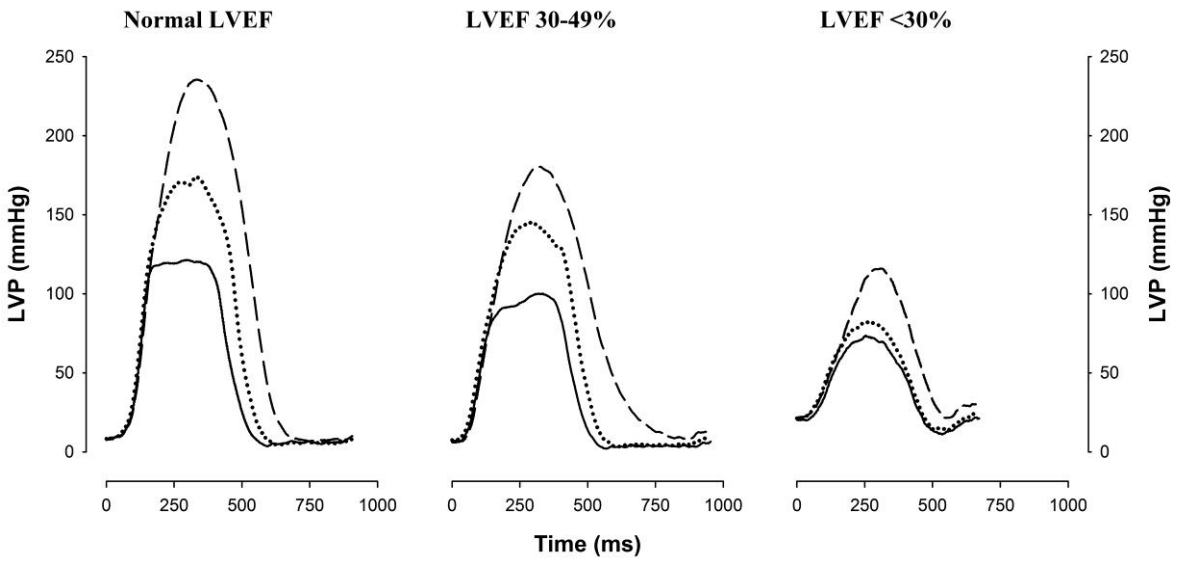

Normal LVEF
LVEF 30-49%
LVEF <30%
LVP (mmHg)
LVP (mmHg)
250
200
150
100
50
0
0
250
500
750
1000
Time (ms)

FIGURE 3

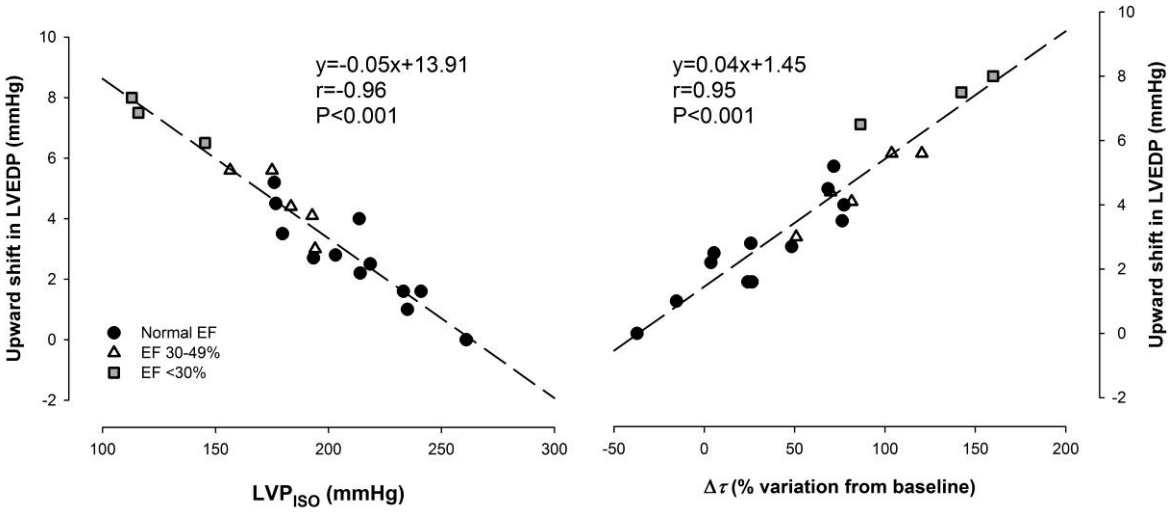

y=-0.05x+13.91
r=-0.96
P<0.001
Normal EF
EF 30-49%
EF <30%
Upward shift in LVEDP (mmHg)
LVP$_{ISO}$ (mmHg)
y=0.04x+1.45
r=0.95
P<0.001
Upward shift in LVEDP (mmHg)
$\Delta\tau$ (% variation from baseline)

FIGURE 4

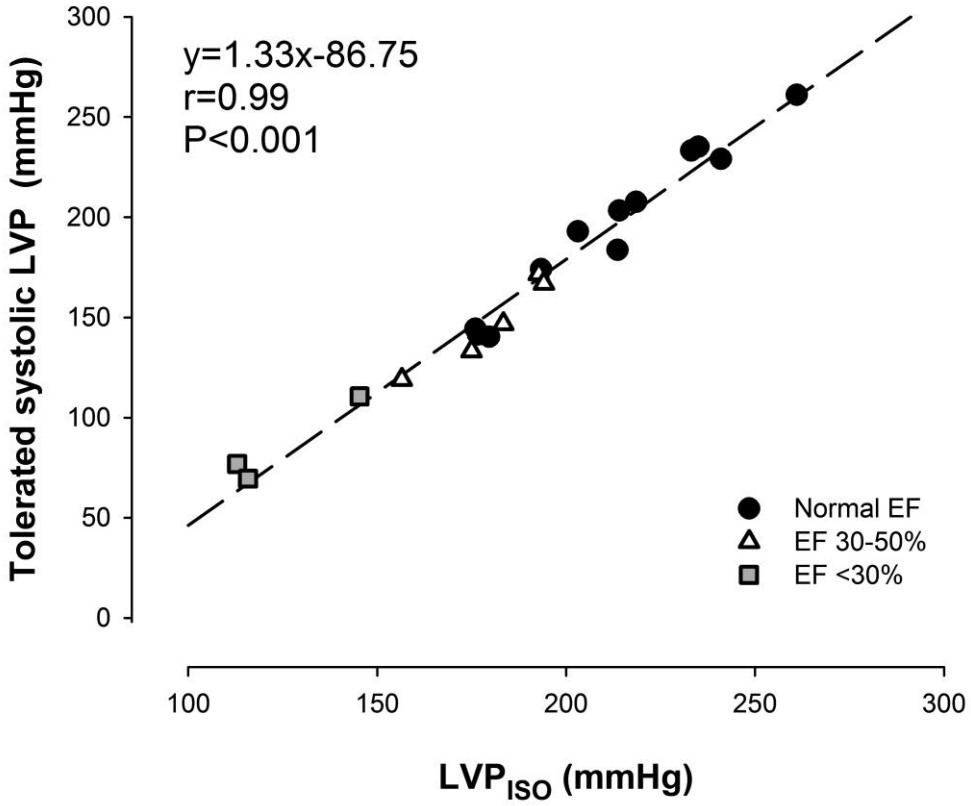
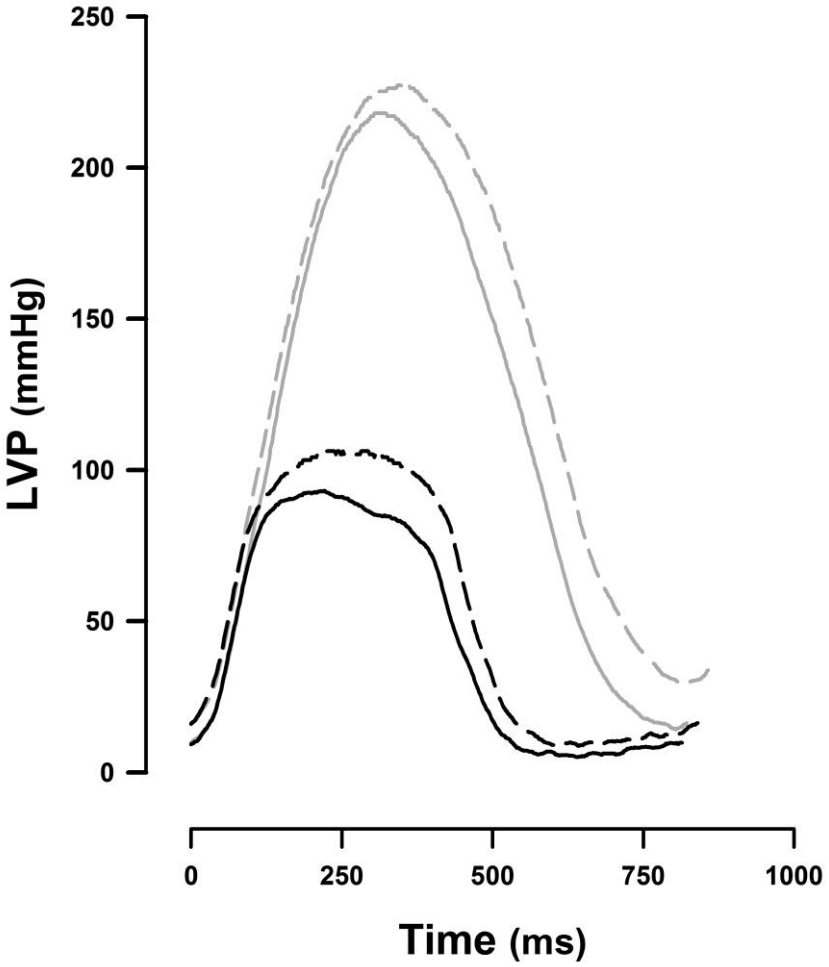

y=1.33x-86.75
r=0.99
P<0.001
Tolerated systolic LVP (mmHg)
LVP$_{ISO}$ (mmHg)
Normal EF
EF 30-50%
EF <30%
0
50
100
150
200
250
300
100
150
200
250
300

FIGURE 5



SUPPLEMENTAL MATERIAL

TABLES

SUPPLEMENTAL TABLE 1. Summary of baseline cardiovascular function parameters.

Patient	Ejection Fraction	dP/dt_{max} (mmHg/s)	mean BP (mmHg)	τ (ms)	LV EDP (mmHg)
1	Normal	987	100.0	40.1	18.4
2	Normal	1654	91.7	41.7	9.8
3	Normal	1002	86.7	38.5	6.5
4	Normal	948	68.3	29.2	13.2
5	Normal	922	93.2	36.8	7.9
6	Normal	1653	66.6	57.1	9.8
7	Normal	2399	78.4	28.6	10.6
8	Normal	1111	91.5	49.8	10.8
9	Normal	1245	71.8	44.6	11.1
10	Normal	1312	66.7	32.1	10.4
11	Normal	1552	80.1	39.4	12.8
12	Normal	1139	83.3	37.9	6.0
13	30-50%	1037	103.2	40.2	13.8
14	30-50%	1970	83.4	25.6	9.9
15	30-50%	1265	93.1	25.5	14.3
16	30-50%	1062	78.5	34.0	14.1
17	30-50%	972	76.7	34.8	5.3
18	<30%	645	61.8	23.4	19.4
19	<30%	522	96.5	26.9	20.7
20	<30%	764	98.4	11.0	14.5

dP/dt_{max}, peak rate of left ventricular pressure rise ; BP, blood pressure; τ, time constant of isovolumetric relaxation; LV EDP, left ventricular end-diastolic pressure.

SUPPLEMENTAL TABLE 2. Systolic pressures at baseline and after graded aortic clamping.

Patient	Baseline Systolic LVP mmHg	Tolerated Systolic LVP mmHg	Isovolumetric LVP mmHg
1	93.1 (48%)	174.1 (90%)	193.4
2	122.7 (52%)	235.0 (100%)	235.0
3	100.1 (57%)	144.3 (82%)	176.0
4	94.0 (53%)	141.4 (80%)	176.7
5	77.1 (43%)	140.2 (78%)	179.7
6	103.2 (40%)	261.0 (100%)	261.0
7	121.0 (50%)	229.0 (95%)	241.0
8	93.1 (43%)	207.6 (95%)	218.5
9	99.2 (49%)	192.9 (95%)	203.1
10	104.3 (49%)	203.3 (95%)	214.0
11	99.9 (43%)	233.1 (100%)	233.1
12	98.6 (46%)	183.7 (86%)	213.6
13	102.1 (53%)	171.6 (89%)	192.8
14	91.5 (50%)	146.7 (80%)	183.4
15	90.4 (58%)	118.9 (76%)	156.5
16	86.7 (45%)	166.9 (86%)	194.1
17	117.6 (67%)	133.0 (76%)	175.0
18	82.3 (73%)	76.8 (68%)	113.0
19	73.6 (64%)	69.5 (60%)	115.9
20	116.9 (80%)	110.5 (76%)	145.4

LVP, left ventricular pressure. Baseline and tolerated systolic LVP are presented both in absolute values and as a percentage of the peak isovolumetric LVP.

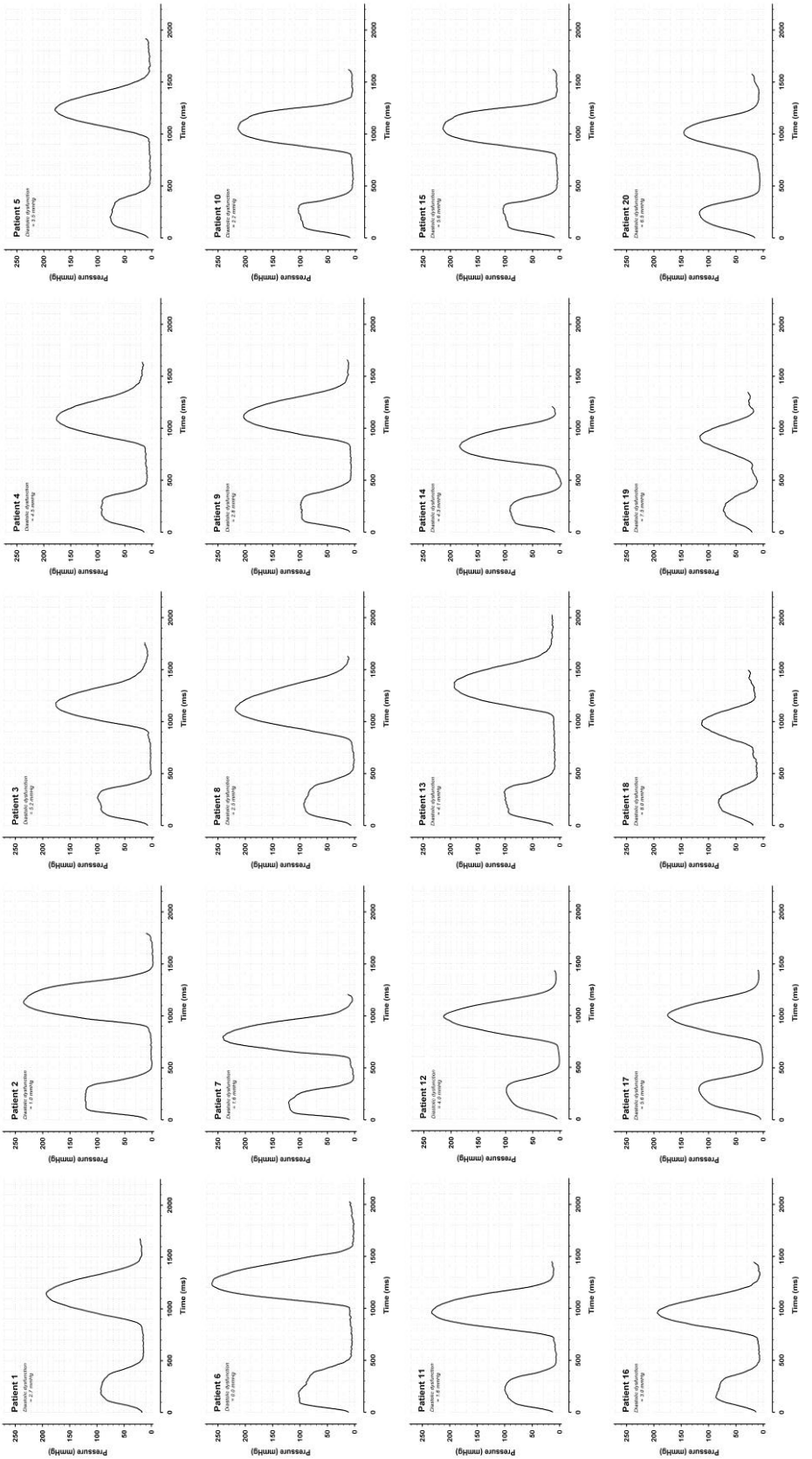
SUPPLEMENTAL FIGURE LEGENDS

Supplemental figure 1. Left ventricular pressure (LVP) tracings illustrating the increase in left ventricular end-diastolic pressure (LV EDP) after complete aortic occlusion.

Control and isovolumetric cycles obtained after complete aortic occlusion are presented. Diastolic dysfunction, the shift in LV EDP from control to isovolumetric cycle, is indicated for every patient.

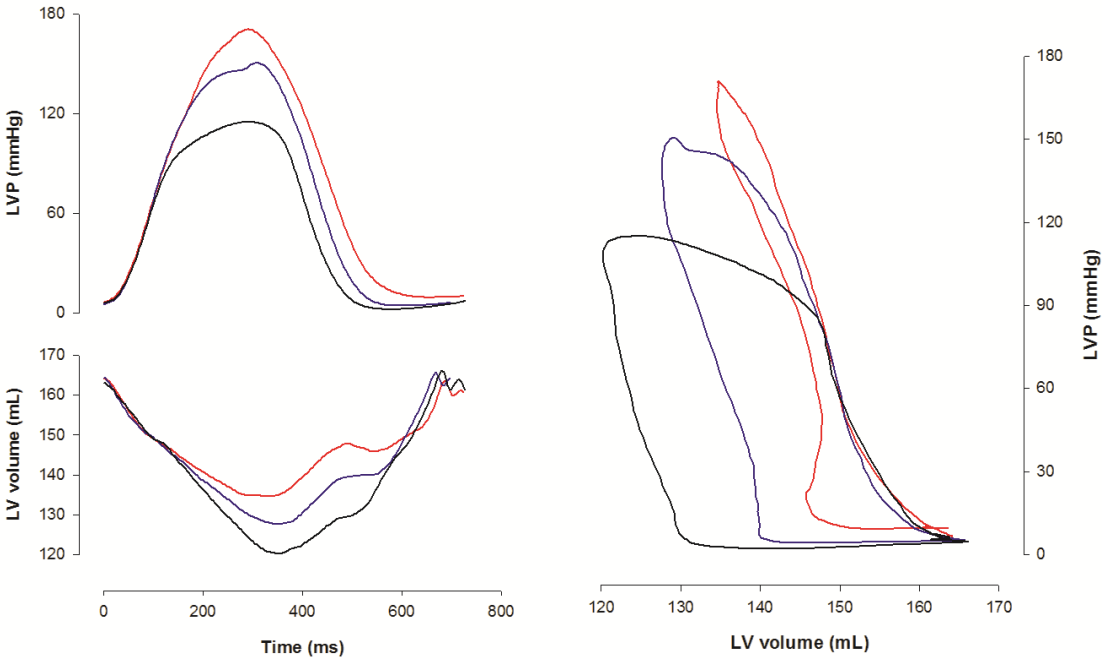
Supplemental figure 2. The elevation of diastolic pressures induced by systolic left ventricular pressure (LVP) elevations represents true diastolic dysfunction.

LVP (upper left), LV volume (lower left) and LV pressure-volume loops (right) in a representative patient with moderate LV systolic dysfunction. Tracings in baseline conditions (black line), during a moderate LVP elevation (blue line) and during an isovolumetric heartbeat (red line) are displayed. The isovolumetric beat elevates filling pressures (upper left) without increasing diastolic volume (lower left). Accordingly, the diastolic segment of the pressure-volume loop is shifted upwards. This corresponds to afterload induced diastolic dysfunction.



SUPPLEMENTAL FIGURE 1. Representative LVP tracing of aortic occlusions in every patient.

SUPPLEMENTAL FIGURE 2. The elevation of diastolic pressures induced by systolic left ventricular pressure (LVP) elevations represents true diastolic dysfunction.



Discussão

Miocárdio ventricular esquerdo na hipertensão pulmonar

Com os trabalhos incluídos nesta dissertação demonstrámos que, num modelo experimental de hipertensão pulmonar severa e insuficiência cardíaca, o miocárdio ventricular esquerdo desenvolve disfunção intrínseca.

Apesar dos índices hemodinâmicos independentes da carga obtidos com cateteres de pressão-volume sugerirem isto mesmo, tanto mais que as intervenções foram conduzidas em animais com tórax aberto e pericardiectomizados, por forma a minimizar os fenómenos de interacção ventricular (Slinker e Glantz 1986), a observação experimental que nos permite retirar conclusões sólidas advém das relações força-frequência negativas, próprias do miocárdio insuficiente, verificadas nos feixes musculares ventriculares esquerdos. Este achado original contraria experiências anteriores levadas a cabo, no mesmo modelo, numa fase de hipertrofia ventricular direita compensatória (Kogler et al. 2003). Kögler *et al*. avaliaram a relação força-frequência no miocárdio ventricular esquerdo e direito do modelo de hipertensão pulmonar induzida pela monocrotalina, observando relações negativas apenas no ventrículo direito concluíram que a sobrecarga de pressão seria o principal determinante de disfunção ventricular, uma vez que ambos os ventrículos se encontrariam sob uma influência neuroendócrina comum (Kogler et al. 2003). Esta conclusão contraria, no entanto, um paradigma clínico. Foi o antagonismo de sistemas neuroendócrinos que se revelou mais eficaz no tratamento da falência miocárdica (Remme 1994). De facto, na terapêutica crónica com o antagonista não selectivo da endotelina-1, *Bosentan*, observámos uma reversão da disfunção miocárdica esquerda. Curiosamente, um relato do caso clínico de um paciente com hipertensão pulmonar severa e disfunção ventricular esquerda sugere que o *Bosentan* possa de facto ter benefícios clínicos, uma vez que após terapêutica prolongada com *Bosentan* este paciente apresentou melhoria funcional a nível miocárdico e tolerou transplante pulmonar sem complicações cardíacas subsequentes (Brauchlin et al. 2005). No miocárdio ventricular esquerdo documentámos, igualmente, aumento da apoptose cardiomiocitária, comutação para as isoformas-β das cadeias pesadas de miosina, algum grau de fibrose e activação neuroendócrina e inflamatória local. Todos os referidos são marcos de remodelagem e regressão para o fenótipo fetal que constituem marcadores de, e contribuem para a, disfunção miocárdica (Bernardo et al. 2010; Borbely et al. 2009; Dhalla et al. 2009; Diwan e Dorn 2007). Adicionalmente, a inclusão de dois pontos temporais de avaliação permitiu-nos esclarecer que algumas destas alterações são mais precoces, acompanhando mesmo estádios de hipertrofia compensatória, enquanto outras,

designadamente a sobre-expressão e activação local da endotelina-1 nos cardiomiócitos, surgem tardiamente.

Com base nestes resultados, propomos que, para além da interacção ventricular, também alterações intrínsecas do fenótipo contráctil e funcional dos cardiomiócitos sejam um mecanismo fisiopatológico de disfunção ventricular esquerda na hipertensão pulmonar severa.

Mecanismos fisiopatológicos de disfunção ventricular esquerda intrínseca

Vários mecanismos podem condicionar um ventrículo não sujeito a sobrecarga de pressão, como o ventrículo esquerdo na hipertensão pulmonar, a seguir um trajecto de disfunção contráctil e remodelagem.

O primeiro que considerámos foi, desde logo, o da mediação neuroendócrina. Baseados na hipótese de Gomez *et al*. de que um mediador produzido pelo ventrículo direito, sobrecarregado pudesse actuar, por via endócrina ou parácrina, no ventrículo esquerdo (Gomez et al. 1993) e admitindo que neste modelo experimental, e na hipertensão pulmonar clínica, ocorre um processo de activação neuroendócrina sistémica (Brunner 1999; Leineweber et al. 2002; Nootens et al. 1995), levantámos a hipótese de que a mediação endócrina pudesse estar envolvida nas transformações desenroladas no miocárdio ventricular esquerdo. Para este efeito estudámos os níveis plasmáticos e a produção local da endotelina-1 e das citocinas inflamatórias factor de necrose tumoral-α e interleucina-6, verificámos, como seria expectável, uma elevação no plasma (Miyauchi et al. 1993; Steffen et al. 2008), mas verificámos também uma elevação da produção local no miocárdio ventricular esquerdo. Esta activação neuroendócrina e inflamatória local fez-se acompanhar de modificações de factores de transcrição importantes na regulação da função miocárdica, como o factor de transcrição nuclear-κB (Hall et al. 2006), e do metabolismo, caso do receptor dos peroxissomas activado pelos estímulos proliferativos (Berger e Moller 2002).

A activação neuroendócrina e inflamatória local no ventrículo esquerdo é surpreendente, visto que este ventrículo não se encontra sobrecarregado, sendo a sobrecarga tida como um estímulo preponderante na activação neuroendócrina e inflamatória (Opie 2002). É ainda mais surpreendente porque, no caso das citocinas, esta não se verificou no ventrículo contralateral e, no caso da endotelina-1, esta foi mais pronunciada no ventrículo esquerdo que no direito. Nos trabalhos desenvolvidos procurámos esclarecer quais os mecanismos que presidem a esta

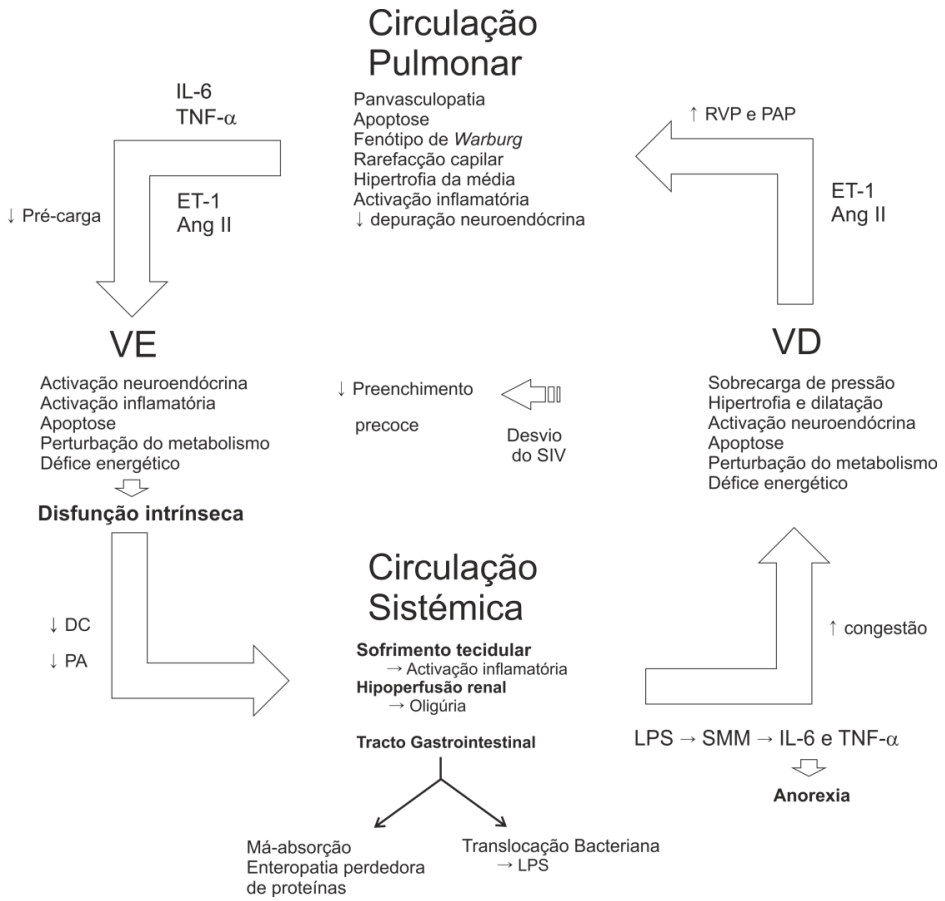
activação. Levantamos várias hipóteses, a seguir enunciadas, e, posteriormente, resumidas na Figura 2:

i. que mediadores produzidos pelo ventrículo direito, sobrecarregado, como a endotelina-1 e a angiotensina II (a enzima de conversão da angiotensina apresenta expressão aumentada no ventrículo direito), pelo próprio pulmão e por outros órgãos, como o tecido adiposo, no caso das citocinas, possam actuar em elevada concentração no ventrículo direito após passagem através da circulação pulmonar. Esta hipótese é muito plausível uma vez que os mecanismos de depuração da circulação pulmonar estão usualmente deprimidos na hipertensão pulmonar, particularmente no caso da endotelina-1 (Brunner 1999), mas sobretudo porque estes mediadores induzem activação neuroendócrina e inflamatória no miocárdio. A angiotensina II, por exemplo, induz a produção de endotelina-1 (Ito et al. 1993). Do mesmo modo, já havíamos observado indução da transcrição do factor de necrose tumoral-α no ventrículo esquerdo, após constrição aguda da artéria pulmonar (Roncon-Albuquerque et al. 2006), e num modelo experimental de transplantação cardíaca heterotópica de corações previamente enfartados também se observou indução endócrina do mesmo factor no coração, previamente saudável, do receptor (Nakamura et al. 2003).

ii. que a redução da pré-carga ventricular esquerda possa ter desencadeado activação neuroendócrina e remodelagem, como se verifica na constrição crónica da veia cava inferior (Lisy et al. 2000). No entanto, a redução de pré-carga descrita no modelo de hipertensão pulmonar induzida pela monocrotalina descrita por outros autores (Hardziyenka et al. 2011) e por nós é de magnitude substancialmente inferior, o que nos leva a crer que este mecanismo não seja fundamental.

iii. que a hipoxemia pudesse induzir a activação neuroendócrina. Efectivamente, um grau ligeiro de hipoxemia já foi descrito neste modelo experimental (Yuyama et al. 2004) e a hipóxia é um estímulo que reconhecidamente se pode associar a maior expressão génica de mediadores neuroendócrinos, nomeadamente da endotelina-1, por intermédio de vias intracelulares que envolvem o factor de transcrição indutível pela hipóxia-1α (Kakinuma et al. 2001). No entanto, a hipoxemia ligeira, que também pudemos observar, e a ausência de alterações na expressão do factor de transcrição indutível pela hipóxia-1α levam-nos a concluir que este mecanismo não será fisiologicamente relevante.

Figura 1. Mecanismos conducentes à disfunção ventricular esquerda na hipertensão pulmonar.



Ang II, angiotensina II; DC, débito cardíaco; ET-1, endotelina-1; IL-6, interleucina-6; LPS, lipopolissacarídeo; PA, pressão arterial; PAP, pressão arterial pulmonar; RVP, resistência vascular pulmonar; SIV, septo interventricular; SMM, sistema monocítico-macrofágico; TNF-α, factor de necrose tumoral- α; VD, ventrículo direito; VE, ventrículo esquerdo.

Modulação nutricional da hipertensão pulmonar, disfunção miocárdica e caquexia cardíaca

Demonstrámos que um regime alimentar hipercalórico, rico em lípidos saturados, glícidos simples e elevado teor de sal, ou seja, uma dieta Ocidental típica, reverteu parcialmente a hipertensão pulmonar, insuficiência cardíaca e caquexia cardíaca num modelo animal. Estes resultados são particularmente controversos, atendendo a que já havíamos usado o mesmo tipo de regime alimentar para induzir experimentalmente síndrome metabólico (Roncon-Albuquerque et al. 2008). De facto, as dietas do tipo Ocidental são factores de risco cardiovascular bem estabelecidos (Hu e Willett 2002). No entanto, as nossas observações podem ser facilmente interpretados à luz do "paradoxo da obesidade" e conferem-lhe um suporte

experimental e fisiopatológico. Vários mecanismos são sugeridos, que passaremos a abordar em seguida.

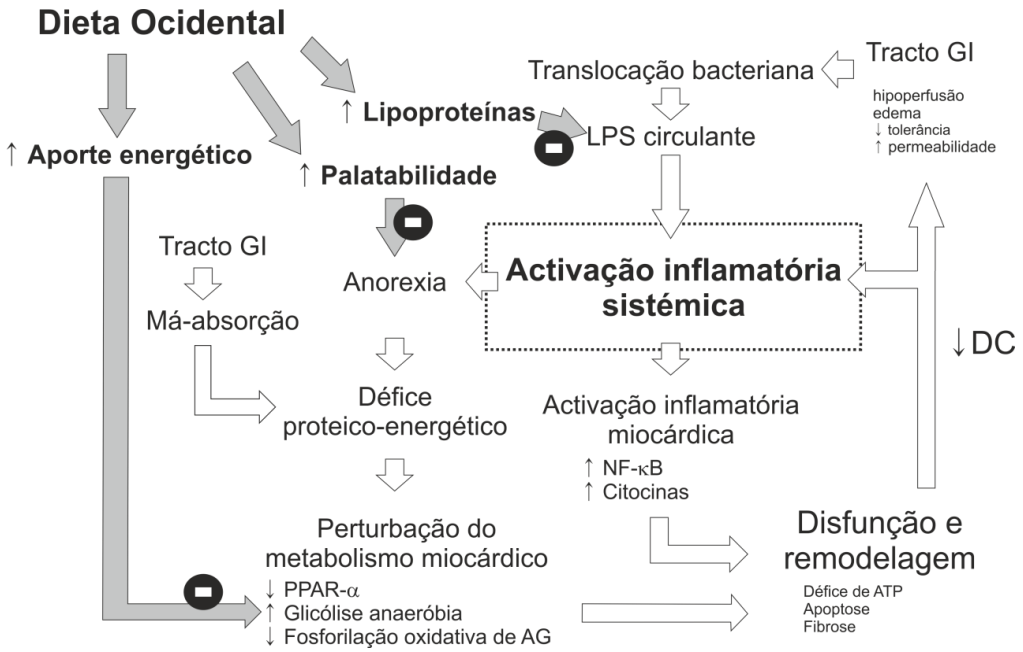
As dietas do tipo ocidental têm elevada palatabilidade e estimulam o apetite (Erlanson-Albertsson 2010). Na caquexia cardíaca experimental esta dieta aumentou a ingestão calórica, ou seja, atenuou a anorexia. O aporte proteico-energético suplementar seguramente protegeu os animais da perda ponderal e contribui para a manutenção da massa magra e gorda, que também foram observadas.

No entanto, os benefícios observados foram bem mais alargados do que uma mera alteração da composição corporal. Surpreendentemente, apesar da dieta do tipo Ocidental induzir disfunção endotelial e stress oxidativo em animais previamente saudáveis (Ketonen et al. 2010), na hipertensão pulmonar severa com caquexia cardíaca, pelo contrário, verificámos uma redução das resistências vasculares pulmonares e uma atenuação marcada da actividade inflamatória sistémica e local. Este achado apesar de paradoxal é, contudo, face aos resultados observados, extremamente coerente com a hipótese lipoproteína-endotoxina que tem vindo a ser sustentada para justificar a melhoria de sobrevida dos doentes insuficientes cardíacos com índices de massa corporal mais elevados e níveis de colesterol mais altos e que já exploramos na introdução desta dissertação. De acordo com esta hipótese, a elevação da colesterolemia nos animais com hipertensão pulmonar, que ingeriram dieta do tipo Ocidental, poderá ter contribuído para a inactivação dos lipopolissacarídeos (Rauchhaus et al. 2000a). Estes produtos bacterianos, que entram em circulação por translocação no tracto gastrointestinal, têm sido apontados como o principal motor para a activação inflamatória na insuficiência cardíaca (Rauchhaus et al. 2000a). Procurando fazer uma translação destes resultados para a clínica, muitos dos doentes com insuficiência cardíaca, que têm índices de massa corporal e valores de colesterolemia mais elevados, porventura terão um regime alimentar mais próximo da típica dieta Ocidental. Muitos autores, aliás, advogam que as estatinas, atendendo ao seu efeito redutor da colesterolemia, podem ter efeitos deletérios na insuficiência cardíaca e, particularmente, na caquexia cardíaca (Leite-Moreira e Castro-Chaves 2006; von Haehling 2009). Assim, as estatinas não melhoraram a sobrevida na insuficiência cardíaca (Kjekshus et al. 2007), mas uma sub-análise dos doentes com proteína C reactiva elevada, um marcador inflamatório, revelou melhoria na maior parte dos pontos avaliados, incluindo a sobrevida (McMurray et al. 2009), insinuando que os seus efeitos anti-inflamatórios pleiotrópicos possam ser benéficos e compensar eventuais efeitos deletérios, como a redução da colesterolemia (von Haehling e Anker 2005).

A atenuação da actividade inflamatória pela dieta do tipo Ocidental parece ter desempenhado um papel fundamental na melhoria da caquexia. A activação miocárdica local do factor de necrose tumoral-α e da interleucina-6 foi substancialmente atenuada. O factor de necrose tumoral-α é provavelmente a citocina mais importante na progressão da caquexia, activando a degradação proteassómica, favorecendo o catabolismo, a remodelagem e a disfunção cardiomiocitária (von Haehling et al. 2004). Curiosamente, num modelo experimental de síndrome nefrótico com caquexia já havíamos demonstrado que a activação miocárdica local deste factor se associava a disfunção (Moreira-Rodrigues et al. 2007). Não podemos deixar de mencionar, igualmente, que um regime alimentar hipercalórico e hiperlipídico com restrição moderada de proteínas preveniu as vias de progressão da lesão renal, atenuou a inflamação e melhorou a caquexia num modelo experimental de nefrectomia (Kim et al. 2010). A abordagem anti-inflamatória na modulação da gravidade da hipertensão pulmonar (Henriques-Coelho et al. 2008), bem como a terapêutica com hormonas orexigénicas e promotoras do eixo da hormona de crescimento (Henriques-Coelho et al. 2004), já havia, por nós, sido testada com sucesso.

Um mecanismo adicional que permite interpretar a melhoria de função miocárdica é a maior disponibilidade de substratos energéticos e a modulação de vias intracelulares relacionadas com o metabolismo. A actividade do receptor dos peroxissomas activado pelos estímulos proliferativos é inibida pelo factor de necrose tumoral-α, tanto na patogenia da insulino-resistência e da aterosclerose como na insuficiência cardíaca, resposta inflamatória e caquexia (Ye 2008). Pelo contrário, é estimulada pelos regimes alimentares hiperlipídicos (Sharma et al. 2007). No grupo de animais com hipertensão pulmonar que foram alimentados com dieta ocidental observámos aumento dos triglicerídeos plasmáticos, atenuação da activação inflamatória, nomeadamente do factor de necrose tumoral-α e modificação da expressão e actividade do receptor dos peroxissomas activado pelos estímulos proliferativos, apontando para um resgate das alterações metabólicas que acompanham a insuficiência cardíaca e caquexia. Efectivamente, a activação deste factor de transcrição normaliza o metabolismo miocárdico ventricular direito na progressão para a insuficiência cardíaca (Jucker et al. 2007). Mais recentemente, surgem também evidências de que a activação do mesmo factor pode inibir vias inflamatórias no miocárdio, como a via do factor de transcrição nuclear κB (Alvarez-Guardia et al. 2011; Rodriguez-Calvo et al. 2008). Uma hipótese integradora justificativa da melhoria da caquexia e função miocárdica pelo regime nutricional Ocidental é apresentada na Figura 3.

Figura 2. Mecanismos de atenuação da caquexia e disfunção miocárdica pela dieta Ocidental.



AG, ácidos gordos; ATP, trifosfato de adenosina; DC, débito cardíaco; GI, gastrointestinal; LPS, lipopolissacarídeo; NF-κB, factor nuclear κ de células B activadas; PPAR-α, receptor dos peroxissomas activado por estímulos proliferativos-α.

Para além da recuperação da função miocárdica, a atenuação da resposta inflamatória pode ter contribuído para uma melhoria da função vascular (Brasier 2010) e, deste modo, para mitigar a hipertensão pulmonar. Adicionalmente, foi também recentemente demonstrado que agonistas do receptor dos peroxissomas activado pelos estímulos proliferativos podem atenuar a hipertensão pulmonar (Hansmann et al. 2007), sendo este um mecanismo alternativo pelo qual a dieta Ocidental, rica em lípidos, pode ter melhorado as resistências vasculares pulmonares.

Efeitos e mecanismos de acção dos antagonistas da endotelina-1 na hipertensão pulmonar e insuficiência cardíaca direita

Demonstrámos que mesmo o antagonismo agudo da endotelina-1, e não só o antagonismo crónico, tem acções hemodinâmicas e moleculares relevantes e benéficas num modelo experimental de hipertensão pulmonar crónica. Caracterizámos alguns dos mecanismos moleculares subjacentes pela avaliação da expressão génica, níveis plasmáticos de mediadores e actividades enzimáticas, após apenas 4 horas de perfusão. Esta abordagem metodológica é invulgar. Usualmente não se analisam alterações moleculares após tão curto período de acção farmacológica. Todavia, assumimos o risco de proceder deste modo atendendo à experiência

prévia com protocolos experimentais agudos (Guerra et al. 2006; Roncon-Albuquerque et al. 2006) e à modulação da expressão génica descrita após 4 horas de perfusão de *Fenoldopam* (Aravindan et al. 2006). Tendo observado melhoria hemodinâmica e modificação significativa, quer da expressão génica quer da actividade enzimática e níveis plasmáticos de mediadores, podemos inferir que a activação da endotelina-1 na hipertensão pulmonar crónica tem um efeito agudo importante na modulação das resistências vasculares pulmonares e na perturbação da função miocárdica por intermédio de mecanismos moleculares que também esclarecemos parcialmente.

Como achados mais importantes, com potencial translação para a prática clínica, destacamos que o antagonismo agudo da endotelina-1, para além da redução das resistências vasculares pulmonares, aumentou o débito cardíaco e melhorou o acoplamento ventrículo-vascular do ventrículo direito, o que é compatível com manutenção ou melhoria da contractilidade. De facto, embora fisiologicamente a endotelina-1 exerça um efeito inotrópico positivo (Brunner et al. 2006) na activação crónica do sistema, como sucede na hipertensão pulmonar, esta passa a exercer efeitos inotrópicos negativos (Zolk et al. 2004), contribuindo para a remodelagem e disfunção (Drimal et al. 2003; Iwanaga et al. 1998; Rothermund et al. 2000). Parte destes efeitos havia já sido descrito num modelo experimental de hipertensão pulmonar por hiperfluxo (Rondelet et al. 2003). Acresce que ao contrário do habitualmente descrito (McMurray et al. 2007; O'Connor e Colaboradores 2003), e verificado no grupo controlo, constituído por animais saudáveis, o antagonismo da endotelina-1 não reduziu as pressões arteriais sistémicas na hipertensão pulmonar crónica. Esta observação é particularmente relevante porque a hipotensão arterial pode associar-se quer a insuficiência renal quer a isquemia miocárdica. Verificámos que o antagonismo agudo da endotelina-1 com *Tezosentan* manteve efeitos mesmo após terapêutica crónica prévia com *Bosentan*, sugerindo que o primeiro fármaco pode ser utilizado na prática clínica sempre que o segundo tem que ser descontinuado, por exemplo, quando a via oral deixa de estar disponível, no período perioperatório ou durante o internamento em unidades de cuidados intensivos. Nestes contextos, constatámos, finalmente, que o *Tezosentan* pode ser titulado em perfusão para atingir os objectivos hemodinâmicos desejados, dada a acção dependente da dose registada no protocolo de avaliação dose-resposta. Por último, nem o antagonismo crónico nem o antagonismo agudo da endotelina-1 melhoraram ou comprometeram o acoplamento ventilação-perfusão, o que contraria parcialmente os relatos de melhor oxigenação na lesão pulmonar aguda da endotoxemia (Geiger 2008; Rossi 2004), embora esta diferença possa dever-se ao tipo de modelo experimental.

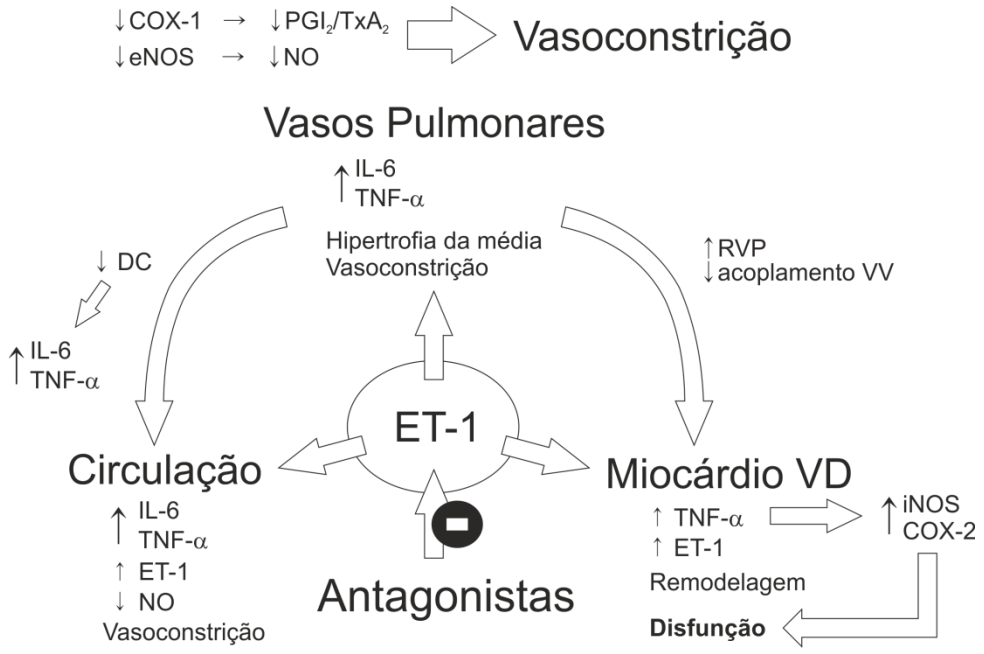
Quanto aos mecanismos celulares e moleculares associados à melhoria hemodinâmica, testemunhámos potentes acções anti-inflamatórias. A activação inflamatória sistémica de citocinas (avaliámos o factor de necrose tumoral-α e a interleucina-6) na hipertensão pulmonar experimental induzida pela monocrotalina, foi atenuada quer pelo antagonismo crónico quer pelo antagonismo agudo da endotelina-1, com *Bosentan* e *Tezosentan*, respectivamente, e com a associação dos dois observámos uma redução ainda mais acentuada. Atribuímos este efeito não só à melhoria hemodinâmica global e melhor perfusão tecidular, mas também à acção pró-inflamatória da endotelina-1 (Gamze et al. 2007; Juergens et al. 2008). De facto, a terapêutica crónica com *Bosentan* atenua a actividade inflamatória na hipertensão pulmonar clínica (Karavolias et al. 2010). As citocinas referidas modulam a reactividade vascular (Brasier 2010; Steiner et al. 2009) e a função miocárdica (Hedayat et al. 2010; Wu et al. 2011). Parte das acções agudas na regulação da função vascular e miocárdica poder-se-ão dever à modulação de enzimas que sintetizam quantidades elevadas de óxido nítrico e prostaglandinas quando activadas por estímulos inflamatórios, concretamente a síntase indutível do óxido nítrico (Alexander 1998; Funakoshi et al. 2002) e a ciclooxigenase do tipo 2 (Grandel et al. 2000; Vane et al. 1998), respectivamente. De facto, a activação destas a nível miocárdico acompanha e contribui para a progressão da insuficiência cardíaca (Drexler et al. 1998; Hare e Colucci 1995; Wong et al. 1998), resultando em efeitos inotrópicos negativos (Elahi et al. 2007; Grandel et al. 2000).

Também a síntese de óxido nítrico e prostaglandinas pelas enzimas constitutivas, ou seja, não induzidas pela inflamação, foi modulada pelo antagonismo da endotelina-1. Tanto a síntase do óxido nítrico endotelial como a ciclooxigenase 1, ambas constitutivas, produzem vasodilatadores pulmonares, óxido nítrico e prostaciclina, respectivamente, importantes no tono basal e vasoreactividade do leito vascular pulmonar. A síntese pulmonar de prostaciclina depende crucialmente da ciclooxigenase 1 tanto no desenvolvimento fetal e embrionário (Brannon et al. 1994) como na resposta à hipóxia (North et al. 1994), enquanto que a síntese de óxido nítrico, o principal determinante do tono vasodilatador pulmonar (Fagan et al. 1999; Hagan e Pepke-Zaba 2011), depende da síntase endotelial (Fiscus 1988). Na hipertensão pulmonar, a desregulação dos mecanismos de controlo do tono vascular, com alteração do equilíbrio entre o tromboxano A_2 e a prostaciclina, favorecendo o primeiro (Christman et al. 1992), e a menor síntese de óxido nítrico por redução da actividade da síntase endotelial estão bem estabelecidas (Crosswhite e Sun 2010; Giaid e Saleh 1995). Estas alterações foram também observadas no pulmão dos

animais com hipertensão pulmonar induzida pela monocrotalina, embora os níveis sistémicos de metabolitos estáveis da prostaciclina aumentassem, o que poder-se-á dever ao desenvolvimento de insuficiência cardíaca (Santilli et al. 2010). No antagonismo da endotelina-1 observámos elevação dos níveis plasmáticos de óxido nítrico e maior actividade pulmonar de síntase endotelial, o que já havia sido descrito em pacientes com hipertensão pulmonar tratados com *Bosentan* (Girgis et al. 2005), assim como recuperação da actividade da ciclooxigenase 1, constitutiva.

Na figura 3 apresentamos um resumo dos mecanismos de acção miocárdicos e vasculares pulmonares dos antagonistas da endotelina-1.

Figura 3. Papel fisiopatológico da endotelina-1 na hipertensão pulmonar e mecanismos farmacológicos dos seus antagonistas.



COX-1 e -2, ciclooxigénases tipo 1 e 2; DC, débito cardíaco; eNOS, síntase constitutiva, ou endotelial, do óxido nítrico; ET-1, endotelina-1; IL-6, interleucina-6; iNOS, síntase indutível do óxido nítrico; NO, óxido nítrico; PGI_2, prostaciclina; RVP, resistências vasculares pulmonares; TNF-α, factor de necrose tumoral-α; TxA_2, tromboxano A_2; VD, ventrículo direito; VV, ventriculo-vascular.

Tolerância diastólica à pós-carga como teste funcional da função miocárdica

Esta metodologia experimental consiste na elevação selectiva da pós-carga, sem alteração da pré-carga, sem interferir com as condições de carga ao longo da intervenção, sem induzir isquemia subendocárdica e sem suscitar adaptações neuroendócrinas (Leite-Moreira e Gillebert 1994). A obtenção de registos por elevações gradativas da pós-carga ou de ciclos isovolumétricos tornam esta manipulação independente das condições prévias de pós-carga. Deste modo, estão reunidas algumas das características de um bom índice funcional. Recorrendo a esta metodologia invasiva de avaliação funcional, demonstrámos pela primeira vez que enquanto o miocárdio normofuncionante de animais controlo respondeu à elevação abrupta de pós-carga com aceleração do relaxamento e pressões tele-diastólicas inalteradas, o miocárdio disfuncionante (ventrículo esquerdo na hipertensão pulmonar) responde com lentificação do relaxamento e elevação das pressões tele-diastólicas, ou seja, disfunção diastólica. A avaliação da elevação das pressões tele-diastólicas após uma oclusão súbita, num ciclo único, da aorta, com imposição de pós-carga elevada, pode ser utilizada, assim, como teste da função miocárdica. Para além do referido, utilizando elevações gradativas da pós-carga, também em ciclos isolados, pudemos verificar que a transição para a lentificação do relaxamento ocorreu a cargas relativas inferiores, ou seja para elevações de pós-carga relativas à elevação máxima (do ciclo isovolumétrico) inferiores, denotando menor reserva de pós-carga (Gillebert et al. 1997), no miocárdio insuficiente. Curiosamente, na avaliação de dois grupos de animais com doença progressiva, constatámos que este teste discriminou a diferença de gravidade e identificou doença incipiente, comparativamente com outros índices hemodinâmicos.

Embora a natureza invasiva exclua a aplicação como teste funcional na prática clínica, o contexto particular da cirurgia cardíaca com clampagem da aorta durante a circulação extracorporal permitiu-nos validar as observações animais no ser humano. Demonstrámos pela primeira vez que o miocárdio humano saudável tolera a imposição de cargas isovolumétricas sem desenvolver disfunção diastólica, denotando uma grande reserva funcional, e provámos também que no caso do miocárdio insuficiente, em que o mesmo não se verificou, o grau de disfunção diastólica se correlaciona estreitamente com a função sistólica. Esta última constatação, que valida este teste como indicador funcional, corrobora a estreita relação entre os fenómenos sistólicos e diastólicos que já havíamos proposto e demonstrado no animal (Gillebert et al. 1997; Leite-Moreira et al. 1999) e que também foi sugerida por De Hert *et al*., igualmente em doentes submetidos a cirurgia coronária, recorrendo a metodologias alternativas de avaliação (De Hert et al. 1999), e por Eichorn *et al*. (Eichhorn et al. 1992). O inotropismo e a lusitropia estariam de tal

modo relacionados que as perturbações de um se associariam previsivelmente e reprodutivelmente a alterações do outro (Eichhorn et al. 1992), pela dependência de ambas dos mesmos mecanismos reguladores, nomeadamente a cinética do cálcio intracelular e a interacção entre cálcio e pontes cruzadas.

Um resultado importante, e igualmente com potencial relevância clínica, é a constatação que os doentes com disfunção sistólica grave, apesar de apresentarem níveis baixos de pressão arterial sistémica, não toleraram qualquer elevação de pós-carga sem que o relaxamento se prolongasse. De facto, nestes doentes foi necessário reduzir a pré-carga, por oclusão da veia cava inferior, para registar o valor de carga relativa abaixo do qual o relaxamento acelerava, indiciando que, mesmo no nível de pós-carga e trabalho usual, parte da lentificação do relaxamento se deve à pós-carga excessiva, nestes corações com insuficiência cardíaca severa e sem qualquer reserva de pós-carga. Este resultado suporta experimentalmente a observação clínica de melhoria da função diastólica após redução da pré-carga em doentes com função sistólica severamente comprometida (Eichhorn et al. 1992).

No subgrupo de doentes em que se procedeu a elevação da volemia e, portanto, da pré-carga, observamos uma acentuação da disfunção diastólica induzida pela pós-carga, de acordo também com o previamente demonstrado no animal (Leite-Moreira e Correia-Pinto 2001).

Conclusões gerais

Na hipertensão pulmonar experimental severa o miocárdio ventricular esquerdo apresenta disfunção intrínseca, remodelagem (fibrose, apoptose, comutação das cadeias pesadas de miosina e modificações da matriz extracelular) e activação neuroendócrina e inflamatória locais. Este poderá ser um mecanismo fisiopatológico complementar à interacção ventricular na génese da disfunção ventricular esquerda que acompanha a hipertensão pulmonar clínica.

A activação neuroendócrina e inflamatória locais no miocárdio ventricular esquerdo, não sobrecarregado, poder-se-ão dever à acção de mediadores circulantes e à redução dos mecanismos de depuração pulmonares. Os mediadores produzidos pelo ventrículo direito sobrecarregado e pelos vasos pulmonares remodelados poderão exercer efeitos selectivamente no miocárdio ventricular esquerdo em elevada concentração.

Na hipertensão pulmonar com insuficiência cardíaca e caquexia cardíaca experimental a ingestão de um regime alimentar hipercalórico, rico em lípidos saturados e glícidos simples (dieta Ocidental), paradoxalmente, atenuou a hipertensão pulmonar, melhorou a hemodinâmica e a sobrevida, contribuiu para a manutenção do peso corporal, da massa magra e adiposa, reduziu a activação inflamatória e neuroendócrina e alterou a actividade de factores de transcrição ligados quer ao metabolismo quer à activação inflamatória.

A elevação da colesterolemia pode justificar a menor activação inflamatória segundo a hipótese lipoproteína-endotoxina. De igual modo, a elevação da trigliceridemia pode explicar a indução do receptor dos peroxissomas activado por estímulos proliferativos e, desta forma, a modulação do metabolismo. O maior aporte calórico e palatabilidade da dieta compensaram parcialmente a anorexia e défice proteico-energético.

O antagonismo agudo da endotelina-1 na hipertensão pulmonar crónica experimental exerce efeitos hemodinâmicos relevantes, atenuando a hipertensão pulmonar, melhorando o débito cardíaco e preservando o acoplamento ventrículo-vascular direito, assim como uma marcada acção anti-inflamatória, que modula quer a função miocárdica quer a reactividade vascular pulmonar. Estes efeitos verificam-se tanto no antagonismo crónico com *Bosentan*, como na acção aguda do *Tezosentan*. Adicionalmente, o *Tezosentan* tem efeitos benéficos mesmo após antagonismo crónico. Constatámos ainda um efeito aditivo dos dois fármaco em vários destes mecanismos de acção farmacológicos.

Demonstrámos, pela primeira vez, que a intolerância diastólica à pós-carga discrimina o miocárdio insuficiente do miocárdio normofuncionante, num modelo animal e também em

doentes submetidos a cirurgia cardíaca. A lentificação do relaxamento e a elevação das pressões tele-diastólicas correlacionam-se com a gravidade da disfunção sistólica do miocárdio.

Trabalhos em curso e perspectivas futuras

Vários aspectos da fisiopatologia da hipertensão pulmonar e hipertrofia e posterior falência ventricular direita encontram-se ainda por esclarecer. Vários trabalhos em curso procuram elucidá-los.

Hipertensão pulmonar secundária a doença respiratória crónica

A doença pulmonar obstrutiva crónica, definida como um estado mórbido caracterizado por limitação do fluxo das vias aéreas com irreversibilidade, normalmente progressivo e associado a uma resposta inflamatória pulmonar (Gold 2009), tem uma prevalência a nível Europeu de 6,2% (Boutin-Forzano et al. 2007), mas muito provavelmente esta é uma subestimativa, uma vez que o diagnóstico é usualmente feito em fases avançadas (Pauwels e Rabe 2004). É, presentemente, a 5ª causa de morte (Pauwels e Rabe 2004) mas a maior parte das estimativas aponta para que venha a ser a 3ª em 2020 (Murray e Lopez 1997).

A hipertensão pulmonar é um dos factores independentes de prognóstico de maior importância, mas a caracterização da sua epidemiologia e a compreensão dos seus mecanismos fisiopatológicos têm ainda grandes lacunas. O diagnóstico é difícil, requerendo meios invasivos e a prevalência oscilará entre 90% e 35% dependendo do estádio da doença (Barbera e Blanco 2009; Weitzenblum et al. 1981). Na maior parte dos doentes, os valores de pressão média na artéria pulmonar são relativamente baixos, mas, nos casos de doença mais grave podem ultrapassar os 40mmHg (Scharf et al. 2002), para além do mais, reconhece-se a existência de subgrupos de doentes com hipertensão pulmonar desproporcionadamente grave relativamente à doença pulmonar subjacente (Chaouat et al. 2005). De qualquer modo, a presença de hipertensão pulmonar, ainda que de grau ligeiro, tem impacto na sobrevida (Weitzenblum et al. 2009). Vários mecanismos participarão na patogenia da hipertensão pulmonar na doença pulmonar obstrutiva crónica, entre os quais vasoconstrição hipóxica e a muscularização da arteríolas, mas destaca-se essencialmente a activação inflamatória e neuroendócrina (Wright et al. 2005). De facto, os níveis plasmáticos de interleucina-6 correlacionam-se com o grau de hipertensão pulmonar e diferenças nos genótipos predispõem ao desenvolvimento de hipertensão pulmonar (Chaouat et al. 2009). Acompanhando a activação inflamatória, cerca de 25% dos doentes, e até 50% nos casos mais graves, desenvolve caquexia (Wagner 2008). Como consequência da sobrecarga crónica de pressão o ventrículo direito acaba por sofrer remodelagem e, posteriormente, disfunção, que recebem usualmente a designação de *cor pulmonale*. Esta é a complicação mais grave da doença pulmonar obstrutiva crónica (Szilasi et al.

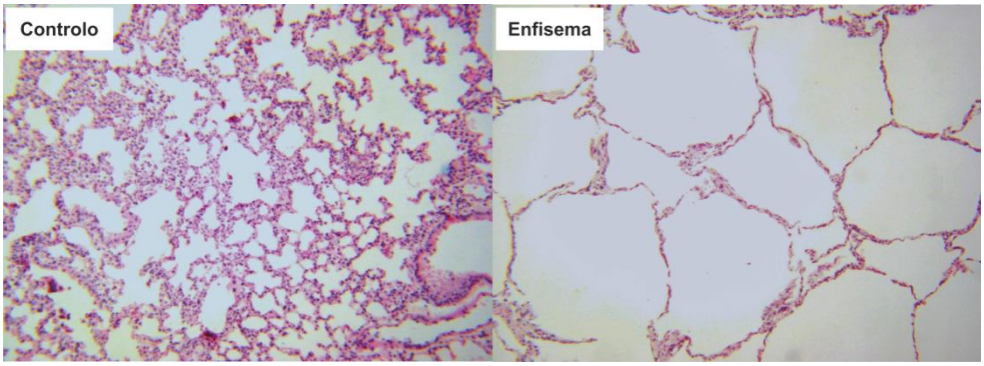
2006) e é responsável por até 30% das admissões por descompensação de insuficiência cardíaca (MacNee 1994). Tal como descrito para outras causas de hipertensão pulmonar, também ocorre disfunção ventricular esquerda na doença pulmonar obstrutiva crónica. Para além da doença coronária e hipertensão arterial, frequentemente coexistentes, a própria interacção ventricular contribui como mecanismo fisiopatológico para a disfunção ventricular esquerda (Jorgensen et al. 2007a; Schena et al. 1996). A insuflação hiperdinâmica do pulmão também pode contribuir para a redução de pré-carga ventricular esquerda (Barr et al. 2010; Jorgensen et al. 2007b).

Atendendo aos trabalhos e conclusões desta dissertação, e em linha com a sugestão de Gomez et al. que levantou a hipótese de que a função ventricular esquerda pudesse estar intrinsecamente comprometida em cães enfisematosos por acção neuroendócrina (Gomez et al. 1993).

Propusemo-nos investigar o desenvolvimento de hipertensão pulmonar e disfunção ventricular esquerda num modelo experimental de doença pulmonar obstrutiva crónica. Os modelos de exposição crónica ao fumo de tabaco reproduzem melhor a etiologia da doença, no entanto, a maior variabilidade no desenvolvimento de hipertensão pulmonar e a necessidade de longos cursos de exposição tornam-nos pouco práticos (Wright et al. 2008). Modelos mais práticos, reprodutíveis e de curso mais rápido são os modelos de enfisema induzidos por instilação intra-traqueal de elastase, embora não reproduzam por completo o espectro das alterações da doença pulmonar obstrutiva crónica humana. Na maior parte dos trabalhos anteriores estes animais não desenvolveram hipertensão pulmonar significativa, contudo, recentemente, Luhtje *et al*. descreveram um modelo no murganho que, por via de instilações reiteradas, progride para hipertensão pulmonar e caquexia (Luthje et al. 2009). Estes autores, todavia, não se debruçaram sobre a função miocárdica, quer ventricular direita quer esquerda. Aplicando as metodologias de avaliação descritas nesta dissertação procuraremos avaliar se ocorre disfunção miocárdica intrínseca ventricular esquerda e qual o papel da mediação neuroendócrina e inflamatória. Adicionalmente, como este é ainda um modelo de caquexia, estudaremos, num passo subsequente, o papel da modulação nutricional. Deste modo, tentaremos generalizar as observações correntes nesta dissertação a patologias mais prevalentes, a hipertensão pulmonar e caquexia secundárias à doença respiratória crónica.

Neste momento completámos um estudo piloto em que comprovámos o desenvolvimento de hipertensão pulmonar e caquexia, estes resultados preliminares, sumariados nas tabelas e figura seguintes, foram comunicados no Congresso Português de Cardiologia.

Figura 4. Secções histológicas pulmonares representativas de murganhos com enfisema e respectivos controlos, após coloração com hematoxilina e eosina (x400).



Destaca-se o alargamento marcado dos espaços alveolares e a rarefacção vascular nas secções de murganhos enfisematosos.

Tabela 1. Morfometria de murganhos com enfisema e respectivos controlos.

	Controlo	**Enfisema**
Peso corporal (g)	33,6 ± 1,2	27,2 ± 0,8*
Ventrículo direito (mg)	34,0 ± 2,1	37,2 ± 1,6*
Gastronémio (mg)	184 ± 4	152 ± 9*

Dados apresentados como média ± erro padrão da média. *P<0,05 *vs* Controlo no teste *t* de *Student*; n=7 em cada grupo.

Tabela 2. Hemodinâmica de murganhos com enfisema e respectivos controlos.

	Controlo	**Enfisema**
Frequência cardíaca (min^{-1})	470 ± 35	406 ± 39*
Débito cardíaco ($mL.min^{-1}$)	6,9 ± 0,9	6,6 ± 1,1
P_{max} VD (mmHg)	23 ± 1	31 ± 3*
P_{max} VE (mmHg)	78 ± 4	61 ± 6*

Dados apresentados como média ± erro padrão da média. P_{max} VD e VE, pressões máximas desenvolvidas pelos ventrículos direito e esquerdo. *P<0,05 *vs* Controlo no teste *t* de *Student*; n=7 em cada grupo.

Terapêutica crónica com levosimendan na hipertensão pulmonar

O *Levosimendan*, um sensibilizador para o cálcio da classe III, que actua por estabilização da troponina C, tem efeitos inotrópicos positivos sem perturbar o relaxamento ou induzir sobrecarga citoplasmática de cálcio (Parissis et al. 2009), uma vez que a interacção com a troponina é dependente do cálcio e, portanto, mais fraca durante a diástole (Jorgensen et al. 2008). Comparativamente com outros agentes inotrópicos, não aumenta o consumo de oxigénio do miocárdio nem aumenta a incidência de arritmias (Kivikko e Lehtonen 2005), em boa parte porque activa canais de potássio sensíveis ao trifosfato de adenosina na membrana mitocondrial dos cardiomiócitos, protegendo-os da apoptose e desequilíbrios energéticos e oxidativos

(Parissis et al. 2009). Estes canais são activados igualmente no músculo liso vascular, contribuindo para vasodilatação sistémica, pulmonar e coronária (Parissis et al. 2009). Atendendo aos seus efeitos farmacológicos, o *Levosimendan* teve grande sucesso na insuficiência cardíaca aguda, comparativamente com outros inotrópicos (Adamopoulos et al. 2006), e as orientações mais recentes da Sociedade Europeia de Cardiologia recomendam a sua utilização em pacientes insuficientes cardíacos sintomáticos com fracção de ejecção comprometida e baixo débito mas sem hipotensão grave (Parissis et al. 2009). Quanto à farmacocinética, embora tenha uma semi-vida de apenas de 1 hora, os seus metabolitos activos têm semi-vidas de cerca de 80 horas e são responsáveis por um efeito sustentado (Louhelainen et al. 2010). Apesar de ser mais frequentemente administrado por via oral, o *Levosimendan* tem boa disponibilidade oral e pode ser usado em terapêutica crónica. Na terapêutica crónica da insuficiência cardíaca, por via oral, obtiveram-se bons resultados quer em modelos animais (Louhelainen et al. 2009; Louhelainen et al. 2007) quer em doentes com insuficiência cardíaca grave (Nieminen et al. 2008). No entanto, os efeitos a longo prazo na remodelagem e mortalidade cardiovascular ainda não estão documentados (Louhelainen et al. 2009). No caso de doentes com compromisso de função ventricular direita no contexto de insuficiência cardíaca também foram observadas melhorias, o que não surpreende, face às suas acções farmacológicas vasodilatadoras pulmonares e inotrópicas positivas (Yilmaz et al. 2009). Ainda no que diz respeito à função ventricular direita, nos contextos experimentais de disfunção aguda por enfarte do miocárdio (Missant et al. 2007) e por tromboembolismo pulmonar (Kerbaul et al. 2007) o *Levosimendan* melhorou a hemodinâmica e o acoplamento ventrículo-vascular. Como contrapartida clínica, doentes com hipertensão pulmonar severa submetidos a cirurgia mitral que receberam *Levosimendan* apresentaram redução dos níveis de pressão da artéria pulmonar e protecção da função ventricular direita (Cicekcioglu et al. 2008). Mais recentemente, observou-se também uma melhoria da remodelagem vascular pulmonar após terapêutica crónica com *Levosimendan* no modelo experimental de hipertensão pulmonar induzida pela monocrotalina (Revermann et al. 2011). No entanto, encontram-se por avaliar os efeitos na sobrevida e hemodinâmica, com particular foco na função miocárdica, bem como os mecanismos moleculares envolvidos numa potencial melhoria. Atendendo aos efeitos farmacológicos que não se limitam à vasodilatação pulmonar mas também abarcam a função miocárdica, à biodisponibilidade oral e aos resultados promissores, o *Levosimendan* poderá ser uma alternativa terapêutica muito útil na hipertensão pulmonar crónica humana.

Após submissão de projecto e obtenção gratuita do fármaco junto da companhia farmacêutica produtora, temos em curso um projecto ambicioso que visa caracterizar os efeitos funcionais e

moleculares da terapêutica crónica e aguda com *Levosimendan* nos modelos experimentais de hipertensão pulmonar crónica induzida pela monocrotalina e hipertensão pulmonar aguda induzida por tromboembolismo pulmonar. Neste projecto recorreremos à avaliação hemodinâmica *in vivo* e à avaliação funcional *in vitro* de feixes musculares miocárdicos e anéis arteriais pulmonares. Por forma a melhor caracterizar os efeitos miocárdicos implementámos já um modelo de constrição da artéria pulmonar, que reproduz sobrecarga de pressão ventricular direita, sem hipertensão pulmonar, (Tarnavski et al. 2004).

Num estudo piloto constatámos não só uma atenuação da hipertensão pulmonar e melhoria de débito cardíaco e função ventricular direita, mas uma tendência considerável para melhoria de sobrevida no modelo experimental de hipertensão pulmonar induzida pela monocrotalina. No entanto, estes resultados terão que ser confirmados pela avaliação de animais adicionais.

Modelo experimental de sobrecarga de pressão por constrição da aorta ascendente

Este modelo experimental é classicamente empregue quer para avaliar a resposta hipertrófica do miocárdio quer para estudar a transição de hipertrofia compensatória para insuficiência cardíaca, mimetizando razoavelmente a estenose aórtica humana (Tarnavski et al. 2004). Recentemente, foi também demonstrado o desenvolvimento de caquexia cardíaca (Helies-Toussaint et al. 2005). Adicionalmente, durante a fase de hipertrofia compensatória estes animais apresentam disfunção diastólica e, durante o curso da doença, também apresentam disfunção endotelial pulmonar e elevação das resistências vasculares pulmonares (Yin et al. 2011). A caracterização da transição da disfunção diastólica para a sistólica em termos moleculares e a correlação funcional destas alterações moleculares é ainda uma incógnita. Nos últimos anos, várias abordagens moleculares permitiram estudar alterações de expressão génica e composição proteica de inúmeros reguladores biológicos. Estas têm sido aplicadas para discriminar, por exemplo, vias moleculares envolvidas na transição entre hipertrofia compensatória e disfunção (Buermans et al. 2005) ou entre mediação neuroendócrina e sobrecarga (Schott et al. 2005). Até ao momento, estas metodologias não foram ainda aplicadas ao estudo da transição entre disfunção diastólica e sistólica. Propomo-nos levar a cabo esta avaliação, conjuntamente com uma caracterização funcional detalhada. Para este efeito implementámos um modelo de sobrecarga crónica de pressão no murganho por constrição da aorta ascendente. Os animais serão acompanhados por ecocardiografia transtorácica seriada por forma a definir dois pontos temporais de avaliação, um numa fase de hipertrofia concêntrica

com fracção de ejecção preservada e outro subsequente à dilatação e comprometimento da fracção de ejecção.

Como neste modelo experimental está também descrita evolução para caquexia cardíaca, procuraremos reproduzir os efeitos de regimes alimentares do tipo Ocidental na insuficiência cardíaca esquerda. Recorrendo à metodologia molecular já mencionada procuraremos também caracterizar as transformações moleculares que acompanham o desenvolvimento da caquexia, validando e complementando algumas das alterações já descritas num modelo transgénico (Wellner et al. 2005).

Presentemente, encetámos já um estudo piloto em que acompanhámos os murganhos ecocardiograficamente e delimitamos temporalmente um período de hipertrofia miocárdica com preservação de débito e da fracção de ejecção, mas alterações importantes em parâmetros ecocardiográficos que avaliam a função diastólica, e um período subsequente em que a fracção de ejecção está já comprometida.

Avaliação da tolerância diastólica à pós-carga ventricular direita na patologia humana

A avaliação hemodinâmica do ventrículo direito esteve sempre um passo atrás da do ventrículo esquerdo. Muitos dos conceitos e conhecimentos obtidos da análise de ansas pressão-volume ventriculares esquerdas não foram ainda validados para o ventrículo direito (Burkhoff et al. 2005; Sunagawa 2010). De facto, apenas nos últimos anos esta metodologia tem vindo a ser validada em modelos animais (Yerebakan et al. 2010; Yerebakan et al. 2009) e na prática clínica (La Vecchia et al. 2006; Uebing et al. 2011). Propomo-nos avaliar ansas pressão-volume ventriculares direitas em pacientes submetidos a cirurgia cardíaca com e sem hipertensão pulmonar prévia, adquirindo índices independentes da carga por oclusão parcial e transitória da veia cava inferior e por oclusões gradativas até ao ciclo isovolumétrico da artéria pulmonar, em batimentos isolados. Obteremos simultaneamente índices ecocardiográficos de função ventricular direita. Correlacionaremos os índices funcionais obtidos invasivamente, quer com parâmetros ecocardiográficos, por forma a validar estes últimos para avaliação não-invasiva, quer com características clínicas e evolução pós-operatória destes pacientes.

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