Additional lack of iNOS attenuates diastolic dysfunction in aged ET-1 transgenic mice[1]

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Abstract: Endothelin-1 (ET-1) exhibits potent proinflammatory and profibrotic properties. Moreover, inflammation is a potent stimulus for inducible NO synthase (iNOS), which has been shown to contribute to cardiac injury. We thus hypothesized that ET-1-induced cardiac injury is attenuated by concomitant lack of iNOS. We established crossbred animals of ET-1 transgenic mice ($ET^{+/+}$) and iNOS knockout mice ($iNOS^{-/-}$). At 13 months of age, mice were allocated according to their genotype to one of 4 study groups: wild type (WT) controls (n = 8); ET-1 transgenic ($ET^{+/+}$) mice (n = 10); iNOS knockout ($iNOS^{-/-}$) mice (n = 7); and crossbred ($ET^{+/+}$ $iNOS^{-/-}$) mice (n = 15). Left ventricular function was determined in vivo by using a tip catheter. Animals were subsequently euthanized and hearts were harvested for weight assessment and histologic evaluation. No cardiac hypertrophy was present, as evidenced by similar mean cardiac weight and myocyte diameter in all groups. Cardiac perivascular fibrosis was significantly increased in $ET^{+/+}$ and $iNOS^{-/-}$ groups versus WT, whereas $ET^{+/+}$ $iNOS^{-/-}$ mice did not differ from WT. Regarding left ventricular function, plasma B-type natriuretic peptide was elevated in $ET^{+/+}$ and $iNOS^{-/-}$ mice, but again in crossbred animals this effect was blunted. Heart catheterization revealed a significantly increased stiffness constant in both ET-overexpressing groups versus WT, but this increase was significantly attenuated in the $ET^{+/+}iNOS^{-/-}$ group versus the $ET^{+/+}$ group. Parameters indicating systolic heart failure (EF, cardiac output), however, were not different between all study groups. Our study demonstrates that ET transgenic mice develop left ventricular stiffening with subsequent diastolic dysfunction in a slow, age-dependent manner. Additional knock out of iNOS significantly attenuates cardiac injury. We thus conclude that ET-1-induced cardiac injury is at least partially mediated by iNOS.

Key words: NO, endothelin, cardiac injury.

Résumé : L'endothéline-1 (ET-1) présente de puissantes propriétés pro-inflammatoires et pro-fibrotiques. Toutefois, des travaux ont montré que l'inflammation est aussi un puissant stimulus de l'expression de la NO synthase inductible (iNOS) et que celle-ci contribue à la lésion cardiaque. Nous avons donc émis l'hypothèse que la lésion cardiaque induite par l'ET-1 pourrait être atténuée par l'absence de iNOS. Nous avons produit des animaux croisés à partir de souris transgéniques ET-1 ($ET^{+/+}$) et de souris knock-out iNOS ($iNOS^{-/-}$). À l'âge de 13 mois, les souris ont été réparties dans 4 groupes en fonction de leur génotype : type sauvage (TS; n = 8), transgénique ET-1 ($ET^{+/+}$; n = 10), knock-out iNOS (iNOS; n = 7) et animaux croisés ($ET^{+/+}$ $iNOS^{-/-}$; n = 15). La fonction ventriculaire gauche a été déterminée in vivo à l'aide d'un cathéter. Les animaux ont ensuite été sacrifiés et leur cœur mis en culture pour une évaluation pondérale et histologique. Aucune hypertrophie cardiaque n'a été notée, et aucune différence n'a été observée entre le poids cardiaque et le diamètre des myocytes des divers groupes. La fibrose périvasculaire cardiaque a augmenté significativement chez les groupes $ET^{+/+}$ et $iNOS^{-/-}$ vs le groupe TS, mais elle n'a pas différé entre les souris $ET^{+/+}$ $iNOS^{-/-}$ et les souris de type sauvage témoins. Concernant la fonction ventriculaire gauche, le BNP plasmatique a augmenté chez les souris $ET^{+/+}$ et $iNOS^{-/-}$, mais a diminué chez les animaux croisés. Le cathétérisme cardiaque a révélé une augmentation significative de la constante de rigidité chez les deux groupes surexprimant l'ET versus le groupe TS, mais cette augmentation a été significative-

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ment atténuée chez le groupe $ET^{+/+}$ $iNOS^{-/-}$ versus le groupe $ET^{+/+}$. Toutefois, les paramètres indiquant une insuffisance cardiaque systolique (FÉ, débit cardiaque) n'ont pas différé entre les groupes. Notre étude démontre que les souris transgéniques ET développent une rigidité ventriculaire gauche et une dysfonction diastolique subséquente lentement avec l'âge. L'inactivation de iNOS atténue significativement la lésion cardiaque. Ainsi nous concluons que la lésion cardiaque induite par l'ET-1 est au moins en partie véhiculée par iNOS.

Mots-clés : NO, endothéline, lésion cardiaque.

[Traduit par la Rédaction]

Introduction

Endothelin-1 (ET-1) exhibits potent proinflammatory and profibrotic properties in lung and kidney (Hocher et al. 1997, 2000*b*). Regarding the cardiac phenotype, it has been shown (Yang et al. 2004) that ET-1 overexpression increases cardiac fibrosis and inflammation. Moreover, it is known that ET-1 upregulates inducible NO synthase (iNOS) in cardiomyocytes (Shimojo et al. 2006). Data indicate a detrimental effect of iNOS in cardiac disease via a markedly enhanced NO production, with subsequent nitrative stress, toxic peroxynitrite generation, and enhanced apoptosis (Li et al. 2006). We thus hypothesized that ET-1-induced cardiac injury is mediated by iNOS.

To test this hypothesis we crossbred ET-1-overexpressing mice and iNOS knockout mice and established a novel mouse model exhibiting both features. As the detrimental effects of both ET-1 overexpression and iNOS on cardiac phenotype increase with age (Yang et al. 2004; Li et al. 2006), we used aged crossbred animals and their respective single-transgenic and wild-type counterparts for extensive histologic and functional cardiac assessment.

Materials and methods

Study design

Animal studies were carried out in accordance with German law governing the use and care of laboratory animals. We established crossbred animals of ET-1 transgenic mice ($ET^{+/+}$) and iNOS knockout mice ($iNOS^{-/-}$). At 13 months of age, the animals were allocated according to their genotype to one of 4 study groups: WT, wild-type controls (n = 8); ET-1 transgenic $ET^{+/+}$ mice (n = 10); iNOS knockout $iNOS^{-/-}$ mice (n = 7); or crossbred $ET^{+/+}$ $iNOS^{-/-}$ mice carrying both ET-1 overexpression and iNOS deficiency (n = 15).

Animals were housed under standardized conditions and with water and food ad libitum. Blood pressure was characterized by using the tail-cuff method as recently described (Quaschning et al. 2007).

Surgical procedures and LV hemodynamic measurements

The animals were anesthetized (thiopental 125 μg/g i.p.), intubated, and artificially ventilated. Left ventricular (LV) assessment was performed as recently described (Westermann et al. 2006).

A 1.4 F microconductance pressure catheter (ARIA SPR-719; Millar-Instruments, Inc., Tex.) was positioned in the LV for continuous registration of LV pressure–volume (PV) loops in a closed-chest model. Calibration of the volume signal was obtained by a hypertonic saline (10%, 5 μL i.v.) wash-in technique. Indices of cardiac function were derived from PV data obtained both at steady state and during transient preload reduction by occlusion of the abdominal vena cava. Systolic function was quantified by ejection fraction (EF), stroke volume (SV), cardiac output (CO), and heart rate (HR).

Diastolic function was measured by LV end-diastolic pressure (LVEDP) and the stiffness constant β, an indicator for LV stiffness, determined from an exponential fit to the end-diastolic pressure–volume points.

Histological studies

After invasive heart function assessment, animals were euthanized and hearts were harvested for further study. Cardiac tissue samples were all embedded in paraffin, cut into 3- and 1-micrometre sections, and subjected to hematoxylin–eosin, Sirius red, and elastica van Gieson staining. Quantitative stereology (that is, media/lumen ratio and lumen area of cardiac arteries) was analyzed by using a computer-aided image analysis system as previously described (Hocher et al. 1999). The heart morphology (interstitial fibrosis, perivascular fibrosis, and myocyte diameter) was measured as recently described (Hocher et al. 2000*a*; Haffner et al. 2005). In brief, interstitial fibrosis was evaluated after Sirius red staining by using computer-aided histomorphometry devices. We measured the relation of Sirius red-stained area (connective tissue) to total area of the whole section. The data thus obtained were analyzed with the Image program version 1.61 (shareware of the NIH).

Perivascular fibrosis was graded in Sirius red staining via a scoring system by two independent investigators who were blinded to the study groups of the animals.

For calculation of the media/lumen ratio of cardiac arteries, we measured the area contents of the media and the lumen after elastica – van Gieson staining using the NIH Image 1.61 program.

Assessment of myocyte diameter was performed by means of 1-micrometre cardiac tissue sections in hematoxylin–eosin staining. Myocyte diameter was quantified with the NIH Image software.

Plasma parameters and B-type natriuretic peptide

Blood samples were obtained after invasive cardiac assessment, and routine parameters like creatine kinase (CK), potassium, alanine transaminase (ALT), and aspartate transaminase (AST) were measured by using a standard autoanalyzer. Sodium and chloride were not measured because the values may have been inaccurate due to previous heart catheterisation, which requires administration of a small amount of hypertonic saline (see below). Plasma concentra-

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Table 1. Synopsis of body weight and plasma parameters in 4 genotypes of mice.

Parameter	WT	$ET^{+/+}$	$iNOS^{-/-}$	$ET^{+/+}$ $iNOS^{-/-}$
Body weight, g	38.9±7.7	35.1±3.3	38.9±6.2	35.9±3.9
K, mmol/L	4.4±1	4.4±1	4.2±1	5.2±2
CK, U/L	1213±691	932±734	977±323	1639±1339
ALT, U/L	36±17	42±35	39±16	36±11
AST, U/L	80±52	57±20	69±33	68±32
BNP, pg/mL	46±4	66±6*,†	69±6*,†	43±2

Note: WT, wild type (n = 8); $ET^{+/+}$, ET-1 transgenic (n = 10); $iNOS^{-/-}$, iNOS knockout (n = 7); $ET^{+/+}$ $iNOS^{-/-}$, crossbred; K, potassium; CK, creatine kinase; ALT, alanine transaminase; AST, aspartate transaminase ; BNP, B-type natriuretic peptide. Values are means ± SE. Significant at *, $p < 0.05$ vs. WT controls. †, $p < 0.05$ vs. $ET^{+/+}$ $iNOS^{-/-}$.

Table 2. Synopsis of blood pressure, cardiac histology, and left ventricular function in 4 genotypes of mice.

Parameter	WT	$ET^{+/+}$	$iNOS^{-/-}$	$ET^{+/+}$ $iNOS^{-/-}$
Systolic blood pressure, mm Hg	118±8	109±5†	112±4†	119±7
Heart weight, % BW	0.49±0.08	0.56±0.17	0.52±0.09	0.52±0.06
Myocyte diameter, μm	9.6±0.7	9.6±0.9	10.1±0.9	10.5±1.2
Perivascular fibrosis score	1.36±0.26	1.67±0.39*	1.63±0.29*	1.50±0.34
Interstitial fibrosis, %	1.00±0.16	1.51±0.32*	1.81±0.86*	1.44±0.51*
Media/lumen ratio	1.27±0.32	1.14±0.36	1.22±0.29	1.32±0.41
Heart rate, bpm	426±13	432±15	402±16	426±15
Ejection fraction, %	40±4	42±3	45±4	42±2
Stroke volume, mm Hg/s	27±2	28±4	31±2	28±2
Cardiac output, mL/min	11.3±1.3	12.3±1.1	12.3±0.8	12.5±1.1
LVEDP, mm Hg	5.7±0.7	7.2±0.9†	8.1±0.8*,†	4.9±0.4

Note: WT, wild type (n = 8); $ET^{+/+}$, ET-1 transgenic (n = 10); $iNOS^{-/-}$, iNOS knockout (n = 7); $ET^{+/+}$ $iNOS^{-/-}$, crossbred; BW, body weight; LVEDP, left ventricular end-diastolic pressure. Values are means ± SE. Significant at *, $p < 0.05$ vs. WT controls. †, $p < 0.05$ vs. $ET^{+/+}$ $iNOS^{-/-}$.

tions of B-type natriuretic peptide (BNP) were measured by using a commercially available radioimmunoassay (Biotrend, Cologne, Germany).

Statistical analysis

The data were expressed as means ± SE. To screen for differences between the study groups, ANOVA was used; the unpaired Student's t test was used to compare two groups of interest. Statistical significance was assumed with a value of $p < 0.05$.

Results

Screening of common plasma parameters like potassium, transaminases, and CK did not reveal any significant differences between wild-type controls and our new double-transgenic $ET^{+/+}$ $iNOS^{-/-}$ model or the single-transgenic groups ($ET^{+/+}$ and $iNOS^{-/-}$) (Table 1). Neither were there any differences between the study groups regarding body weight. Tail-cuff measurement revealed that $ET^{+/+}$ $iNOS^{-/-}$ mice exhibited a slightly (about 10 mm Hg), but significantly, higher systolic blood pressure than either of the single-transgenic groups, but no significant difference was observed versus wild-type controls.

With respect to plasma BNP levels, however, we observed a significant elevation in $ET^{+/+}$ and $iNOS^{-/-}$ mice versus wild-type controls, whereas in $ET^{+/+}$ $iNOS^{-/-}$ mice this effect was absent.

Further investigation of the cardiac phenotype revealed no differences in cardiac weight between the groups, neither were there any differences regarding histologic parameters like myocyte diameter and media/lumen ratio of cardiac arteries (Table 2). Cardiac interstitial fibrosis, however, was significantly greater in all transgenic groups than in wild-type controls (Fig. 1 and Table 2). Perivascular fibrosis was likewise enhanced in $ET^{+/+}$ and $iNOS^{-/-}$ mice versus wild-type controls, whereas this effect was absent in $ET^{+/+}$ $iNOS^{-/-}$ mice.

Invasive left ventricular function assessment using a conductance catheter showed no difference between the study groups with respect to systolic function parameters, like ejection fraction, cardiac output, and stroke volume, or to heart rate (Table 2). On the other hand, parameters indicative of diastolic function were different. The left ventricular end-diastolic pressure (LVEDP) was significantly lower in $ET^{+/+}$ $iNOS^{-/-}$ mice than in either single-transgenic group ($ET^{+/+}$ or $iNOS^{-/-}$).

Assessment of the stiffness constant of the left ventricle revealed a significant increase in all transgenic groups versus wild-type controls, but in $ET^{+/+}$ $iNOS^{-/-}$ mice this increase was significantly attenuated compared with that of $ET^{+/+}$ mice (Fig. 2).

Discussion

Our study demonstrated that aged mice overexpressing

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Fig. 1. Representative sections of cardiac tissue in 4 genotypes of mice: WT, wild type; $ET^{+/+}$, ET-1 overexpression; $iNOS^{-/-}$, iNOS knockout; *and* $ET^{+/+}$ $iNOS^{-/-}$, crossbred. Sirius red staining indicates fibrotic tissue. Magnification × 200.

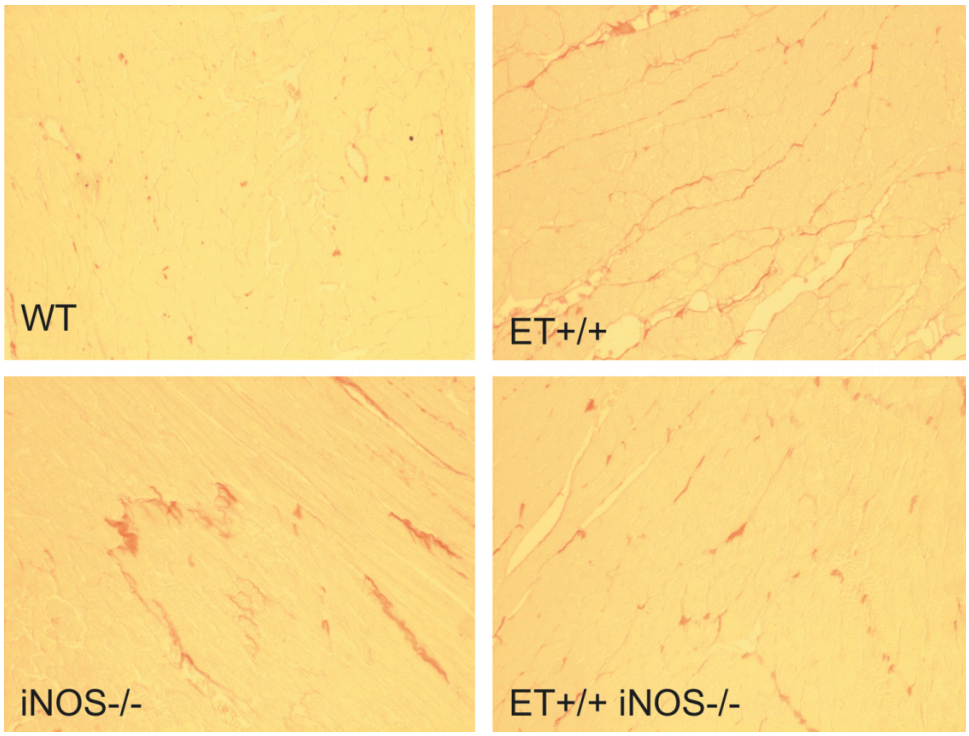
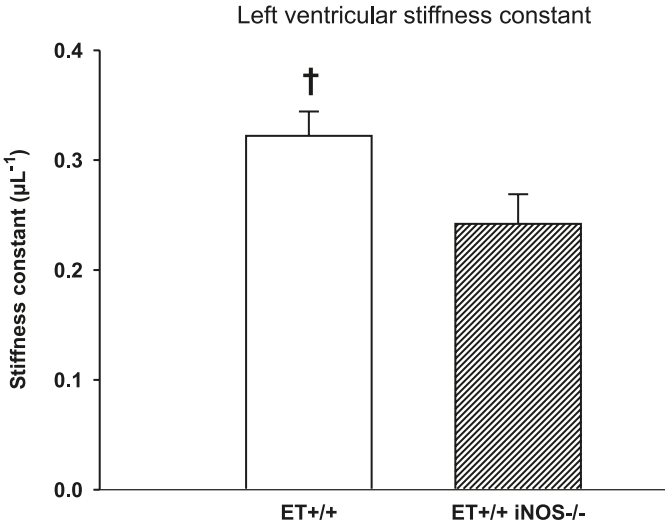


Fig. 2. Left ventricular stiffness constant in $ET^{+/+}$ and $ET^{+/+}$ $iNOS^{-/-}$ mice. Values are means ± SE. Dagger (†) indicates significant difference ($p < 0.05$) from $ET^{+/+}$ $iNOS^{-/-}$.



ET-1 develop cardiac injury, as indicated by higher BNP, increased cardiac fibrosis, and subsequent diastolic dysfunction. We showed that additional lack of iNOS in $ET^{+/+}$ mice significantly attenuates cardiac injury, as indicated by absence of perivascular fibrosis and subsequent reduction in ventricular stiffness and lowering of BNP.

As we know that cardiac fibrosis is not present at the age of 9 months in ET-1-overexpressing mice (unpublished observations), the presence of cardiac fibrosis in our 13-month-old ET-1 transgenic mice indicates that cardiac overexpression of ET-1 leads to an age-dependent development of cardiac fibrosis. This is consistent with data from a mouse model with conditional, heart-specific ET-1 overexpression, which exhibits cardiac fibrosis only when the transgenic overexpression is activated (Yang et al. 2004).

The role of iNOS in heart disease is controversial: on one hand, it is ardently portrayed as a cytoprotective agent in the heart—for example under conditions of ischaemia–reperfusion injury (Jones and Bolli 2006). On the other hand, transgenic mice overexpressing iNOS in the heart develop cardiac inflammation and fibrosis (Mungrue et al. 2002). This ambivalent role of iNOS in the heart is reflected in the present study: iNOS deficiency alone led to deleterious cardiac effects (that is, BNP elevation, enhanced LVEDP, and left ventricular stiffness constant), whereas in ET-1-overexpressing mice, concomitant lack of iNOS attenuated cardiac injury. The reason for the ambivalence of iNOS is the delicate balance between physiological, protective NO production and excess NO formation that leads to cytotoxic products such as peroxynitrite (Stoclet et al. 1999). Whether the former or the latter situation occurs is therefore highly dependent on such circumstances as redox state, substrate supply, age (Li et al. 2006), or ET-1, all of which have an important impact on iNOS expression and NO processing. This explains why in our study the single lack of physiological iNOS lead to cardiac injury, whereas in ET-1 transgenic mice—representing a constantly upregulated iNOS setting (Hocher et al. 2004)—additional lack of iNOS decreased cardiac injury.

Interestingly, in our study perivascular fibrosis appeared to correlate more closely with the results on diastolic dysfunction (LVEDP, stiffness constant) than with interstitial fibrosis. Considering that perivascular fibrosis is closely related to inflammation, while interstitial fibrosis is regarded

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as a reparative mechanism (Guerin et al. 2004), this is suggestive of a vital role for inflammation in our model. This hypothesis of ET-1 overexpression causing cardiac inflammation with subsequent cardiac fibrosis is also supported by findings in the above-mentioned mouse model with conditional, heart-specific ET-1 overexpression (Yang et al. 2004).

In conclusion, our study demonstrates that ET transgenic mice develop left ventricular stiffening with subsequent diastolic dysfunction in a slow, age-dependent manner. Additional knock out of iNOS significantly attenuates cardiac injury. We thus conclude that ET-1-induced cardiac injury is at least partially mediated by iNOS.

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References

Guerin, A.P., Adda, H., London, G.M., and Marchais, S.J. 2004. Cardiovascular disease in renal failure. Minerva Urol. Nefrol. **56**: 279–288. PMID:15467506.

Haffner, D., Hocher, B., Muller, D., Simon, K., Konig, K., Richter, C.M., et al. 2005. Systemic cardiovascular disease in uremic rats induced by 1,25(OH)2D3. J. Hypertens. **23**: 1067–1075. doi:10.1097/01.hjh.0000166849.72721.1c. PMID:15834294.

Hocher, B., Thone-Reineke, C., Rohmeiss, P., Schmager, F., Slowinski, T., Burst, V., et al. 1997. Endothelin-1 transgenic mice develop glomerulosclerosis, interstitial fibrosis, and renal cysts but not hypertension. J. Clin. Invest. **99**: 1380–1389. doi:10.1172/JCI119297. PMID:9077548.

Hocher, B., George, I., Rebstock, J., Bauch, A., Schwarz, A., Neumayer, H.H., and Bauer, C. 1999. Endothelin system-dependent cardiac remodeling in renovascular hypertension. Hypertension, **33**: 816–822. PMID:10082493.

Hocher, B., George, I., Diekmann, F., Zart, R., Rebstock, J., Schwarz, A., et al. 2000*a*. ETA receptor blockade induces fibrosis of the clipped kidney in two-kidney-one-clip renovascular hypertensive rats. J. Hypertens. **18**: 1807–1814. doi:10.1097/00004872-200018120-00015. PMID:11132605.

Hocher, B., Schwarz, A., Fagan, K.A., Thone-Reineke, C., El Hag, K., Kusserow, H., et al. 2000*b*. Pulmonary fibrosis and chronic lung inflammation in ET-1 transgenic mice. Am. J. Respir. Cell Mol. Biol. **23**: 19–26. PMID:10873149.

Hocher, B., Schwarz, A., Slowinski, T., Bachmann, S., Pfeilschifter, J., Neumayer, H.H., and Bauer, C. 2004. In-vivo interaction of nitric oxide and endothelin. J. Hypertens. **22**: 111–119. doi:10.1097/00004872-200401000-00020. PMID:15106802.

Jones, S.P., and Bolli, R. 2006. The ubiquitous role of nitric oxide in cardioprotection. J. Mol. Cell. Cardiol. **40**: 16–23. doi:10.1016/j.yjmcc.2005.09.011. PMID:16288777.

Li, D., Qu, Y., Tao, L., Liu, H., Hu, A., Gao, F., et al. 2006. Inhibition of iNOS protects the aging heart against beta-adrenergic receptor stimulation-induced cardiac dysfunction and myocardial ischemic injury. J. Surg. Res. **131**: 64–72. doi:10.1016/j.jss.2005.06.038. PMID:16154595.

Mungrue, I.N., Gros, R., You, X., Pirani, A., Azad, A., Csont, T., et al. 2002. Cardiomyocyte overexpression of iNOS in mice results in peroxynitrite generation, heart block, and sudden death. J. Clin. Invest. **109**: 735–743. PMID:11901182.

Quaschning, T., Voss, F., Relle, K., Kalk, P., Vignon-Zellweger, N., Pfab, T., et al. 2007. Lack of endothelial nitric oxide synthase promotes endothelin-induced hypertension: lessons from endothelin-1 transgenic/endothelial nitric oxide synthase knockout mice. J. Am. Soc. Nephrol. **18**: 730–740. doi:10.1681/ASN.2006050541. PMID:17287431.

Shimojo, N., Jesmin, S., Zaedi, S., Soma, M., Kobayashi, T., Maeda, S., et al. 2006. EPA effect on NOS gene expression and on NO level in endothelin-1-induced hypertrophied cardiomyocytes. Exp. Biol. Med. (Maywood), **231**: 913–918. PMID:16741023.

Stoclet, J.C., Muller, B., Gyorgy, K., Andriantsiothaina, R., and Kleschyov, A.L. 1999. The inducible nitric oxide synthase in vascular and cardiac tissue. Eur. J. Pharmacol. **375**: 139–155. doi:10.1016/S0014-2999(99)00221-6. PMID:10443572.

Westermann, D., Rutschow, S., Van Linthout, S., Linderer, A., Bucker-Gartner, C., Sobirey, M., et al. 2006. Inhibition of p38 mitogen-activated protein kinase attenuates left ventricular dysfunction by mediating pro-inflammatory cardiac cytokine levels in a mouse model of diabetes mellitus. Diabetologia, **49**: 2507–2513. doi:10.1007/s00125-006-0385-2. PMID:16937126.

Yang, L.L., Gros, R., Kabir, M.G., Sadi, A., Gotlieb, A.I., Husain, M., and Stewart, D.J. 2004. Conditional cardiac overexpression of endothelin-1 induces inflammation and dilated cardiomyopathy in mice. Circulation, **109**: 255–261. doi:10.1161/01.CIR.0000105701.98663.D4. PMID:14718401.

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