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Regional heterogeneity of myocardial norepinephrine and lipid peroxidation levels in patients with end-stage heart failure

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Abstract

Objectives **-** Regional alterations in norepinephrine (NE) and lipid peroxidation in the myocardium of patients with heart failure is not well known; this study was designed to investigate regional myocardial NE levels and the lipid peroxidation index and their relationship with the functional parameters in two pathologies (dilated and ischemic cardiomyopathies: DCM and ICM).

Methods and Results – Biopsied heart samples were obtained from 13 DCM and 10 ICM patients (orthotopic cardiac transplantation). Non-failing hearts (n=4) were included in this study (controls: C). Left ventricular dysfunction was present at rest with a mean of LVEF of 19.1 ± 2.6 % (DCM) and 17.4 ± 2.0 % (ICM). The amounts of NE in the control hearts (4 patients) were significantly lower ($p<0.05$) than those in patients with DCM or ICM. In all patients, there were several differences in the distribution of NE among the subdivisions of the atria and ventricles studied. The NE content was significantly higher in the right atria than in the left atria or in ventricles. A significant correlation between LVEF and NE concentrations was observed in the left septum of ICM and DCM patients and in the left ventricles of the ICM group. In DCM and ICM patients, some parts of the left ventricle showed high levels of lipid peroxides.

Conclusions **-** It is the first demonstration of a correlation between values of the preoperative LVEF and cardiac NE concentrations in some specific parts of the myocardium; this effect is not generalized to the whole heart.

Key Words: autonomic nervous system, heart failure; neurotransmitters ; oxygen radicals, transplantation

Introduction

The final stages of human heart failure due to cardiomyopathy or other heart diseases are accompanied by a great number of metabolic defects. Previous studies indicate that heart failure in its terminal stage is characterized by extensive myocardial damage with altered structure of the myocardium [1]. Myocardial fibrosis appears to be a major component of some heart diseases, such as dilated cardiomyopathy, and may contribute to the development of cardiac dysfunction. One important compensatory mechanism for maintaining adequate tissue perfusion during functional impairment of the heart is the augmentation of the failing cardiac output by a compensatory increase in the activity of the sympathetic nervous system. Inability to respond to β-adrenergic stimulation is a hallmark of the failing human heart. Many years ago, it was shown that the myocardial levels of norepinephrine (NE) were depleted in patients with congestive heart failure, even though norepinephrine turnover was increased [2]. Using C^{11}-hydroxyepinephrine position emission tomography (HED-PET), Ungerer et al [3] investigated the regional tissue content of NE in cardiomyopathic human heart tissue. There were marked regional variations in the quantity of NE and its uptake by sympathetic neurons in cardiomyopathic left ventricles. Several experimental studies have shown that catecholamines induced oxidative stress (OS) in the heart. It has been reported that isoproterenol increased lipid peroxidation levels and that NE generated hydroxyl free radicals in animal hearts [4]. Recently, several investigators have suggested that OS may be involved in the development of heart failure [5,6]

Thus it is of interest to examine whether products of free radical reactions, i.e., lipid peroxides, are elevated throughout the myocardium or only in some specific areas. End-stage heart failure may be the final outcome of different cardiomyopathies, particularly dilated cardiomyopathy and ischemic pathologies. Previous studies have presented evidence that

these two clinical situations are characterized by a great number of metabolic and histological defects that occur during different stages of the disease are not chronologically the same [7,8,9].

As little is known about regional alterations in norepinephrine and lipid peroxidation in the myocardium of patients with different stages of heart failure, the purpose of this study was to investigate in two pathologies (dilated and ischemic cardiomyopathies: DCM and ICM) regional myocardial NE and lipid peroxidation levels and their relationships with functional parameters.

Methods

Myocardial tissues

The study conformed to the Declaration of Helsinki and institutional ethical regulations. Informed consent for the use of human tissue for research was obtained in all cases. Explanted hearts were obtained from patients undergoing cardiac transplantation for end-stage coronary heart failure. These patients had New York Heart Association class IV CHF. The study design was approved by the Ethics Committee for Organ Transplant before the study began.

Myocardial and vascular tissues from the heart were obtained from 13 consenting patients with DCM, and from10 patients with ICM who underwent orthotopic cardiac transplantation. Non-failing hearts (n=4) that were rejected for transplantation for clinical reasons were also included in this study (controls). In ischemic hearts, samples of the left ventricle were obtained from parts outside of the infarcted region. The hearts were arrested in situ with ice-cold St Thomas' Hospital cardioplegic solution. The explanted heart was placed on ice immediately after removal from the body and transported to the laboratory within 20 min in

ice-cold cardioplegic solution. Selected clinical features of the transplant recipients are shown in Table 1.

The hearts were sectioned for analysis. In each one, the right ventricle (basal and apical), left atrium and right atrium were removed, and the septum was excised (interventricular septum). The left ventricle was divided into four parts. Transmural tissue samples (epicardium and endocardium) derived from the left ventricular free wall (apex and base) were collected. Portions of the left coronary artery and aorta were taken.

Frozen specimens were crushed under liquid nitrogen and a resultant powder was obtained. In order to reduce the effect of variations between specimens the contents of NE and of lipid peroxidation, levels were expressed as nM/g protein. Protein levels were measured according to the Lowry method.

Measurement of norepinephrine

Tissue NE was assayed by high-pressure liquid chromatography with electrochemical detection [10]. Each fragment was homogenized on ice for 1 minute in a solution containing 0.2 M perchloric acid, 2.7 mM ethylene diamine tetracetate (EDTA), 5.26 mM sodium metabisulfite, and 100 µl of an internal standard, 3,4-dihydroxybenzylamine hydrobromide. The samples were centrifuged at 13,000g for 6 minutes at 4°C. The supernatants were analyzed by high-performance liquid chromatography (HPLC) with a reverse phase column (60 RP Select B, 250 x 4 mm, 5 µm), with electrochemical detection. The potential of the electrochemical detector was set at +0.85V. The mobile phase consisted of 0.08 M sodium phosphate, 0.27 mM EDTA, 3.7 mM octan-1-sulfonic acid at pH 3.5.

Measurement of lipid peroxidation

The hearts were homogenized in 5 vol of 50 mM phosphate buffer at pH 7.4 containing 0.1 mM EDTA or in 5 vol of a 0.25 M sucrose solution. Tissue lipid peroxidation (malondialdehyde: MDA) was evaluated by the thiobarbituric acid (TBA) reaction; using spectrofluorimetry [11]. Cardiac homogenates were combined with the trichloracetic acid (TCA)-TBA-hydrochloric acid (13.5% w/v, 0.33% w/v, 0.85 *N* respectively) reagent and thoroughly mixed. The tubes were boiled for 15 min and cooled in a bucket of ice. TCA (70%) was then added and after 20 min of incubation, the flocculent precipitate was removed by centrifugation. The fluorescence was measured at 515 nm excitation and 553 nm emission. Thiobarbituric acid reactive substances (TBARS) were expressed as nmol of MDA per gram of proteins.

Statistical analysis

Comparisons between control tissue and tissue from DCM and ICM hearts were made using a t-test for two means. A probability of ≤ 0.05 was considered significant. A one-way analysis of variance was used to determine whether there were differences between control and DCM for NE or lipid peroxide levels, and between control and ICM for the various myocardial and vascular samples. If significance was attained, paired comparisons between control and DCM or ICM were performed using significance set at $P \leq 0.05$. All data are reported as the mean ± S.E.M.

The correlations between the values of each group were determined using Pearson correlation coefficients. A p value of <0.05 was considered to be significant.

Results

The hemodynamic parameters in each group are given in Table 1. There were no differences between the patients with DCM and those with ICM regarding the left ventricular ejection fraction (LVEF), cardiac index (CI) and pulmonary capillary wedge pressure (PCWP). At cardiac catheterization, left ventricular dysfunction was present at rest with a mean PCWP of 26.2 ± 2.9 mmHg in the DCM group and 24.5 ± 1.8 mmHg in the ICM group and a mean LVEF of 19.1 ± 2.6 % and 17.4 ± 2.0 %. The CI in the patients with DCM or ICM was lower than those conventionally used to estimate myocardial function (50-60%), but there was no difference between the two groups of patients.

The data for NE content in the different parts of the hearts and vessels are summarized in Table 2 and Figure 1.

Our data provide a direct comparison between DCM and ischemic ICM. There were some significant differences in the concentration of NE in the parts of myocardium in both types of heart. NE levels tended to be lower in LV regions in the ICM group in comparison with the values obtained in the DCM group. There were major differences between the amounts of NE in the control hearts (4 patients) and those in patients with DCM or ICM. The hearts of these two groups demonstrated a decrease in comparison with those of patients without cardiomyopathy.

There were several differences in the distribution of NE among the various subdivisions of the atria and ventricles studied. The NE content was consistently and significantly higher in the right atria that in the left atria (Fig. 1) or in the ventricles (right atrium 6.91 ± 2.36 ng/mg prot in ICM group and 7.09 ± 1.27 ng/mg prot in DCM group). A gradient in the concentration of NE from the endocardium to the epicardium is evident (DCM group: LV basal epi: 4.15 ± 0.68 ng/mg, LV basal endo: 2.30 ± 0.31 ng/mg $p \leq 0.05$; LV apical epi: 3.31 ± 0.62 ng/mg, LV apical endo 2.05 ± 0.37 ng/mg). The gradient from base to apex was not significant. The

NE content in the right ventricle was higher than that of the corresponding segments in the left ventricle ($p < 0.05$ in the DCM group). The NE content of the coronary artery and aorta was very low.

Tissue levels of MDA for the regions of the heart and vascular samples are given in Table 2. , MDA levels in patients with DCM varied little from one region to another, whereas in ICM patients there was considerable variation. The concentrations of lipid peroxidation compounds ranged from 62 ± 13 nM/g (aortic samples) to 176 ± 45 nM/g (septum parts) in DCM and from 49 ± 8 nM/g (aortic samples) to 329 ± 174 nM/g (LVA epi). There was considerable variation in MDA concentrations between the hearts of the patients with DCM and those with ICM. The pattern of myocardial MDA distribution did not follow that of NE distribution. MDA values in the left or right atria and vessels (coronary and aortic arteries) were similar in the three groups of patients.

The preoperative LVEF were reduced in all patients and correlated with the concentration of norepinephrine in some areas of the myocardium. A significant correlation between LVEF and NE concentrations in the left septum was observed in the two groups of patients (ICM and DCM) (Fig 2). A significant correlation ($p< 0.05$) was also noted in the samples of LV (endo and epi) of ICM patients (Fig 3). However, compared to control, there was no significant difference in the LV of DCM patients or in the RV of ICM and DCM patients, and we observed no correlation between the MDA levels in the different parts of the myocardium and functional parameters from the same patient.

Discussion

To our knowledge, this is the first demonstration of a correlation between the values of preoperative LVEF with cardiac NE concentrations in specific parts of the myocardium in ICM and DCM. These results reinforce the concept that chronic heart failure is characterized by excess adrenergic activity and accompanied by a decrease in tissue levels of NE in specific areas.

We observed a basal-apical gradient of NE concentrations in all human hearts .Tissue NE content showed a regional variation that was apparent in all hearts (controls or DCM and ICM groups). The distribution of NE within the left ventricle showed higher concentrations at the base decreasing toward the apex. We also observed a higher norepinephrine concentration in the right than in the left ventricle. NE levels in the arteries (coronary and aorta) were low in comparison with those found in the ventricle or atrial samples. Our study is in accordance with previous results showing that NE concentrations in the heart may vary significantly, not only between right and left ventricles, but also within the left ventricle itself [12-16].

The heterogeneity of regional function has been described in patients with hypertrophic cardiomyopathy using magnetic resonance imaging [17]. This heterogeneity may reflect the regional variation in myocardial dysfunction and fibrosis that is characteristic of this pathology. A potential mechanism implicated in this regional variation is the heterogeneous nature of regional wall stress in these hearts. Heng et al. [18] suggested the importance of the geometric determinant of altered intramyocardial strains in some area of the myocardium. It has been reported that hypertrophic cardiomyopathy involves sympathetic dysinnervation [19] and that dilated cardiomyopathy is frequently associated with regional neuronal dysfunction[20].

Heterogeneous regional myocardial uptake of exogenous glucose has been reported in patients with cardiomyopathy. Perrone-Filardi et al [21] reported that regional

^{18}fluorodeoxyglucose (FDG) activity differed between the septum and lateral wall, even though there were no changes in myocardial blood flow. The data suggested that this difference was related, at least in part, to differences in regional left ventricular systolic function. With the advent of magnetic resonance tissue tagging with spatial modulation of magnetization, regional heterogeneity of ventricular function has been found in patients with cardiomyopathy [14]. This may reflect variations in wall stress, but also the regional and heterogeneous loss of neuronal tissue and an increase in tissue peroxidation.

Little is known about regional alterations in lipid peroxidation in the myocardium of patients with different stages of heart failure. The present study demonstrates that in failing human myocardium, some parts of LV myocardium (basal and apical epicardium) showed higher levels of lipid peroxides compared with controls ($p < 0.05$).). In our study, the myocardial MDA contents were very heterogeneous, and interpretation is complicated by the possibility that the disease process associated with heart failure might not affect all areas of the myocardium equally. On the other hand, the variations might be related to a loss of myocardium associated with an increase in new tissue with increased lipid content. Our results are in agreement with some others that showed an increase in myocardial MDA content in the failing human myocardium [22]. In the study reported by Maack et al, [22] lipid peroxidation was measured in only one part of the ventricular myocardium. In our group of patients with ICM, we observed large variability in the amounts of peroxide. These data indicate that the samples were not homogeneous.

In our study, the right ventricles of cardiomyopathy patients appeared to have depleted levels of NE. Previous results demonstrated a similar phenomenon in both ventricles for a variety of biochemical changes in heart failure. There is increasing evidence of the role played by

oxidative stress, mediated by the generation of oxygen free radicals, in the physiopathology of heart failure and cardiomyopathy.[5,6]. Several experimental studies have shown that catecholamines induced OS in the heart. It has been reported that isoproterenol increased lipid peroxidation levels and that NE generated oxygen free radicals in animal hearts [4].

Gene expression of antioxidants in the human heart with end-stage failure has been investigated [6].The results of these studies clearly demonstrate that no differences in gene expression of MnSOD, Cu2n, SOD and GXP exist between failing and non failing hearts, whereas the expression and activities of the catalase gene in the failing heart were twice that of the non-failing heart. It appears that the only effort made by the heart to protect itself from oxidative stress is to increase endogenous catalase. Finally, endogenous protection against oxidative stress is important in the heart, as myocardial antioxidant reserves are not significantly diminished during end-stage heart failure [11,23]. On the other hand, one of the profound mechanisms influencing myocardial function in heart failure is the structural adaptation of the small vessels. Cardiac failure of the hypertrophied heart may be attributed, in part, to disorders in microcirculation [24]. The varying functional adaptation of each part of the myocardium may reflect not only the localized changes in wall stress in these hearts, but also a regional modification of metabolic changes associated with impaired local coronary flow and myocardial remodelling [25,26].

This vicious circle may result in myocardial functional and structural damage in heart failure. One of the inevitable limitations of this study using human samples is the fact that patients receive treatment with adrenergic modulators and other medications which might affect metabolic and functional parameters.

In conclusion, in end-stage heart failure, a significant loss of myocardial NE is found in ICM and DCM pathologies. A significant correlation between LVEF decrease and loss of NE

levels was observed, not in all parts of the myocardium, but only in some areas. Data in this current investigation demonstrated the uneven depletion of NE during heart failure. Results concerning lipid peroxidation showed that there were considerable variations in MDA concentrations depending on whether the patients had DCM or ICM. The pattern of myocardial MDA distribution did not follow that of NE distribution.

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Figure Legends

Figure 1: Tissue levels of norepinephrine (NE) in the left and right atrium of patients: controls (C) dilated cardiomyopathy (DCM), ischemic cardiomyopathy (ICM).

Norepinephrine (NE) levels: ng/mg protein

Means ± SEM

[a] $p < 0.05$: C vs DCM or ICM; [b] $p < 0.05$: C vs ICM; [c] $p < 0.05$: C: right atrium vs left atrium

[d] $p < 0.05$: ICM vs DCM; [e] $p < 0.05$: C vs DCM; * $p < 0.05$: left atrium vs right atrium

Figure 2: Correlation between NE levels and LVEF values in the interventricular septum (left: LS, right: RS) of the heart (Ischemic cardiomyopathy: ICM and dilated cardiomyopathy: DCM patients) NE: norepinephrine ng/mg protein, LVEF: Left Ventricular Ejection Fraction (%)

Figure 3: Correlation between NE levels and the values of LVEF in the left and right ventricles. (LV: endocardium: ENDO; epicardium: EPI, RV) of the heart (ICM and DCM patients); NE: norepinephrine ng/mg protein, LVEF: Left Ventricular Ejection Fraction (%)

Table 1: Clinical characteristics of heart transplants recipients

	Dilated cardiomyopathy DCM	**Ischemic cardiomyopathy ICM**	**Controls**
Sex M/F	12/1	9/1	4/0
Age (yr)	53 ± 2	51 ± 2	18 -25- 27- 37
Left ventricular ejection fraction (%)	19.1 ± 2.6	17.4 ± 2.0	50, 50,55,60
Cardiac Index (liters/min per m^2)	1.9 ± 0.1	1.9 ± 0.1	ND
Pulmonary capillary wedge pressure (mmHg)	26.2 ± 2.9	24.5 ± 1.8	ND

Means ± SEM

ND: not determined

Table 2: Tissue levels of norepinephrine (NE) in different regions of the heart

	Dilated cardiomyopathy DCM		**Ischemic cardiomyopathy ICM**		**Controls C**	
Left ventricular regions		n		n		n
Basal endocardium	2.30 ± 0.31	13	3.49 ± 0.42[c]	10	7.22 ± 0.26[a]	4
Basal epicardium	4.15 ± 0.68	13	5.19 ± 1.01	10	9.32 ± 0.27[d]	4
Apical endocardium	2.05 ± 0.37	12	1.81 ± 0.60	7	4.27 ± 0.15[a]	4
Apical epicardium	3.31 ± 0.62	12	1.33 ± 0.44[c]	6	6.12 ± 0.09[a]	4
Interventricular septum	5.12 ± 1.40	12	2.59 ± 0.42	9	7.37 ± 0.22[b]	4
Basal	4.38 ± 0.56[e]	12	3.77 ± 0.96	8	7.97 ± 0.24[d]	4
Apical	4.83 ± 0.69[e]	11	5.53 ± 1.51	9	8.17 ± 0.09[d]	4
Left coronary artery	1.66 ± 0.33	10	1.03 ± 0.26	7	2.32 ± 0.24	4
Aortic artery	1.34 ± 0.16	8	0.75 ± 0.17[c]	7	1.95 ± 0.05[b]	4

Norepinephrine (NE) levels: ng/mg protein

Means ± SEM

[a] $p < 0.05$ C vs DCM or ICM

[b] $p < 0.05$ C vs ICM

[c] $p < 0.05$ ICM vs DCM

[d] $p < 0.05$ C vs DCM

[e] $p < 0.05$ RV basal vs LV basal endo

RV apical vs LV apical endo

Table 3: Tissue levels of malondialdehyde (MDA) in different regions of the heart

	Dilated cardiomyopathy DCM		Ischemic cardiomyopathy ICM		Controls C	
Basal endocardium	157 ± 46	10	290 ± 84	7	74 ± 8	4
Basal epicardium	128 ± 23	10	275 ± 175	7	65 ± 6[a]	4
Apical endocardium	156 ± 34	9	275 ± 175	4	75 ± 11	4
Apical epicardium	130 ± 31	9	329 ± 174	4	74 ± 15[a]	4
Interventricular septum	176 ± 45	10	297 ± 113	7	108 ± 10	4
Basal	160 ± 35	10	241 ± 70	7	123 ± 9	4
Apical	130 ± 31	10	244 ± 78	7	166 ± 18	4
Left atrium	158 ± 55	9	195 ± 93	7	116 ± 8	4
Right atrium	130 ± 39	10	124 ± 32	7	187 ± 4	4
Left coronary artery	77 ± 15	8	72 ± 34	5	60 ± 2	4
Aortic artery	62 ± 13	7	49 ± 8	4	61 ± 2	4

Malondialdehyde levels: nM/g protein

Means ± SEM

[a] $p < 0.05$ Controls vs DCM

Figure 1

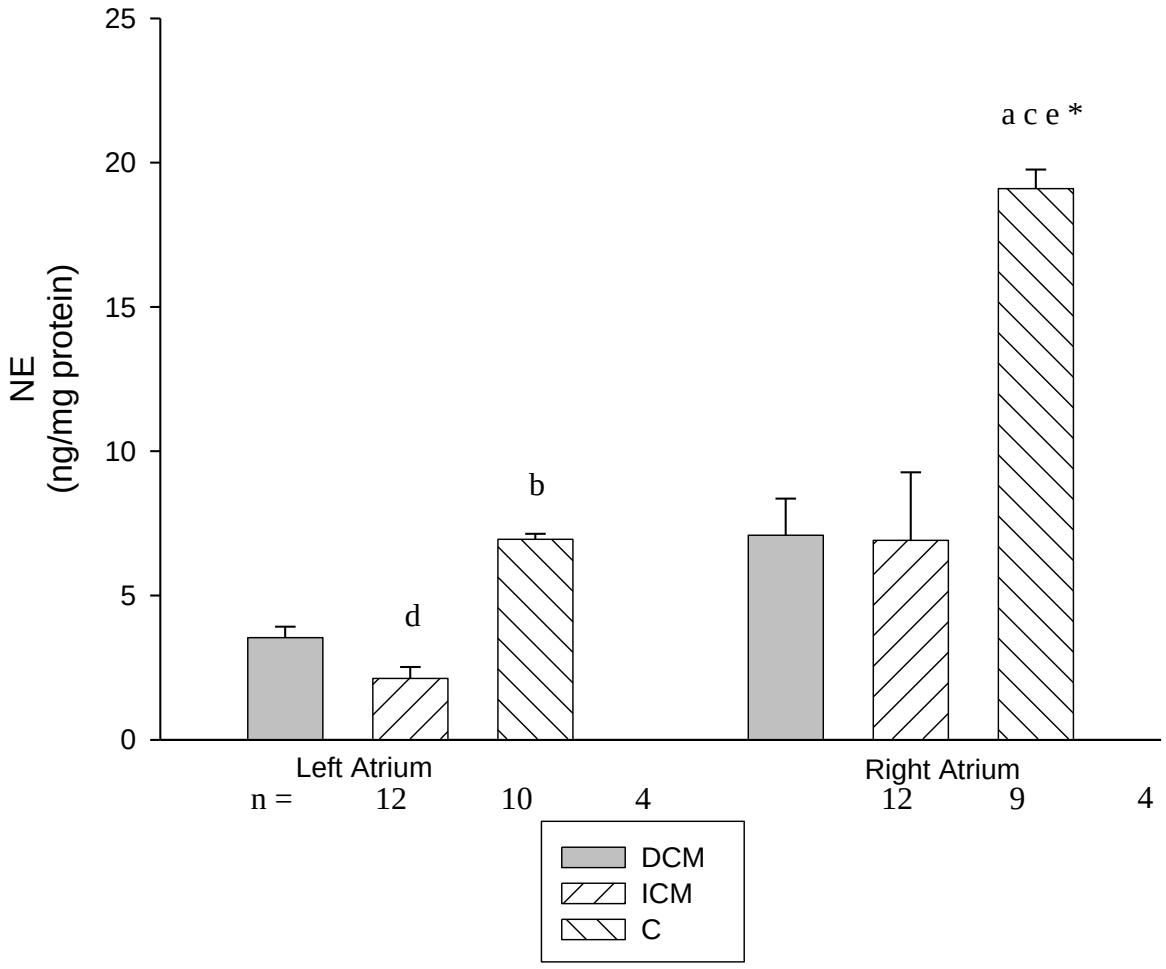

25
20
15
10
5
0
NE
(ng/mg protein)
a c e *
b
d
Left Atrium
Right Atrium
n =
12
10
4
12
9
4
DCM
ICM
C

Figure 2

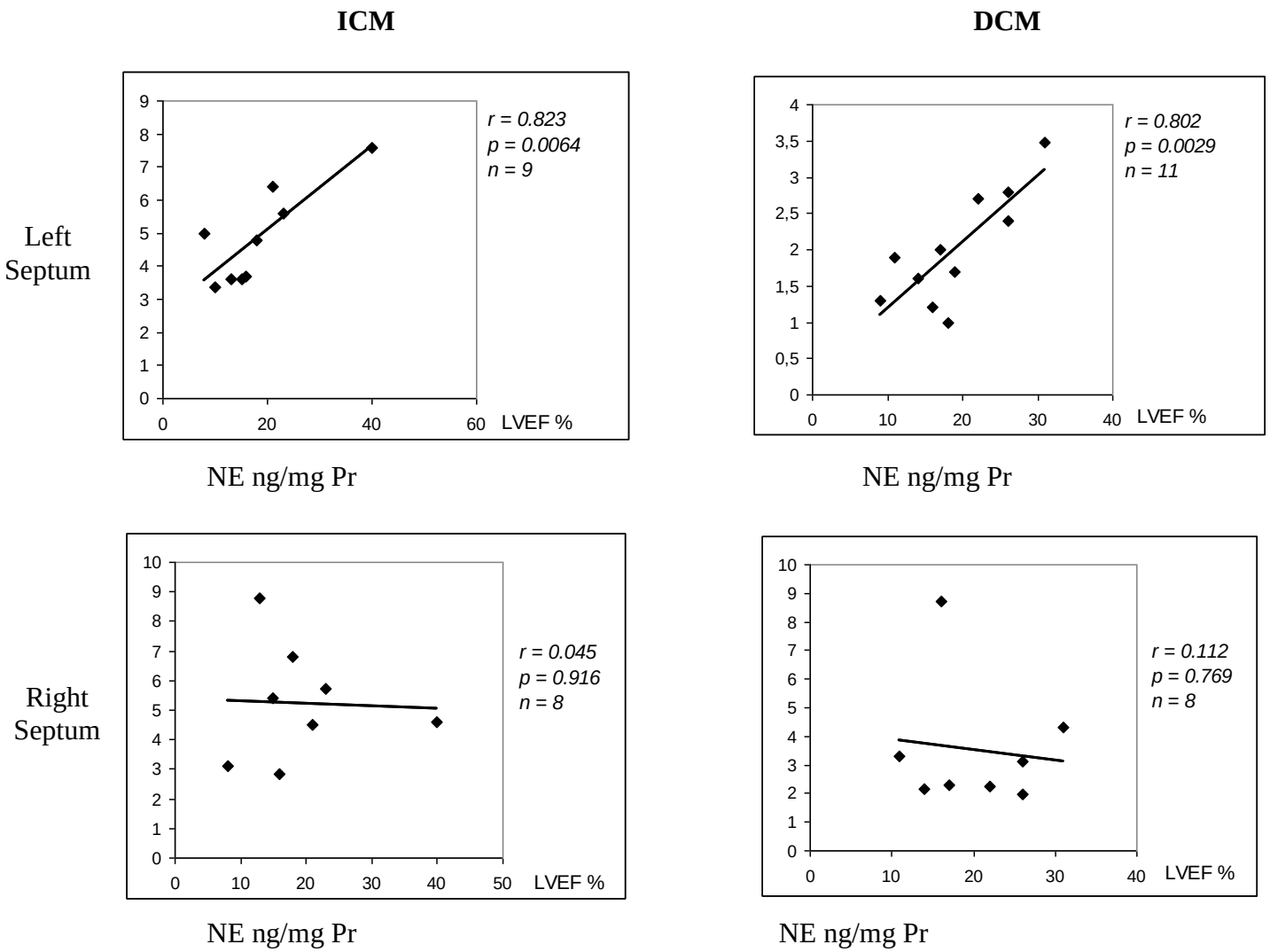
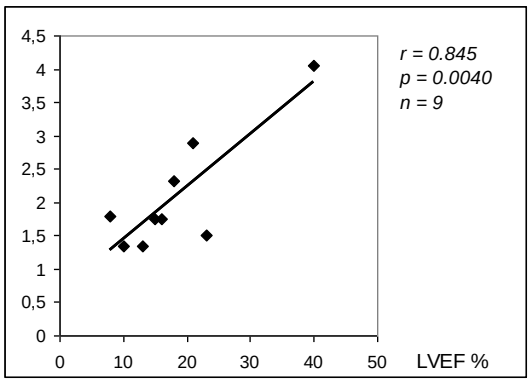
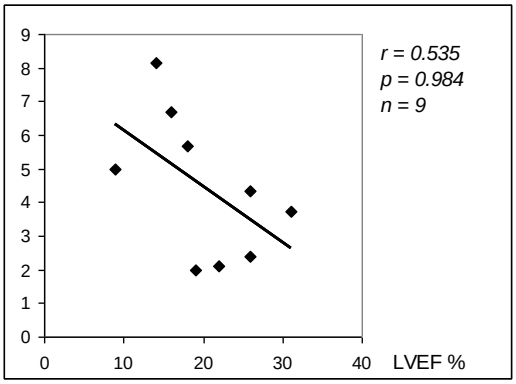

ICM
DCM
Left Septum
r = 0.823
p = 0.0064
n = 9
LVEF %
NE ng/mg Pr
r = 0.802
p = 0.0029
n = 11
LVEF %
NE ng/mg Pr
Right Septum
r = 0.045
p = 0.916
n = 8
LVEF %
NE ng/mg Pr
r = 0.112
p = 0.769
n = 8
LVEF %
NE ng/mg Pr

Figure 3

ICM **DCM**

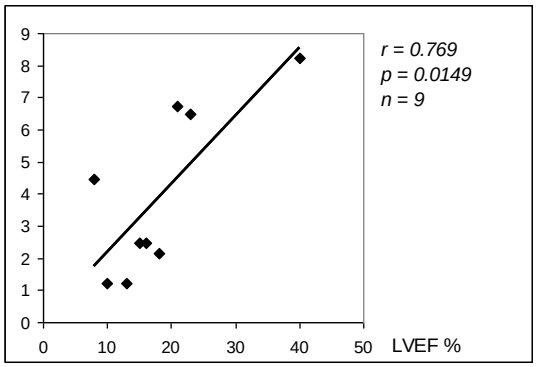
LV ENDO

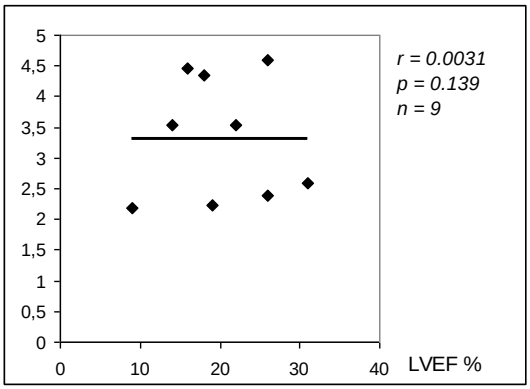




NE ng/mg Pr NE ng/mg Pr

LV EPI





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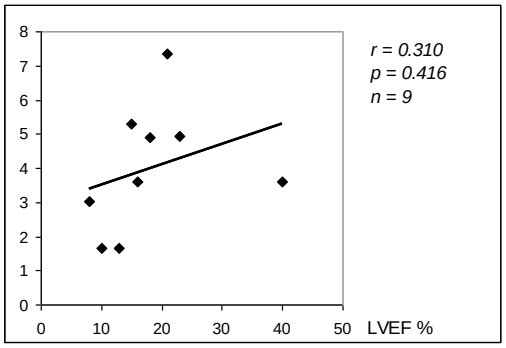

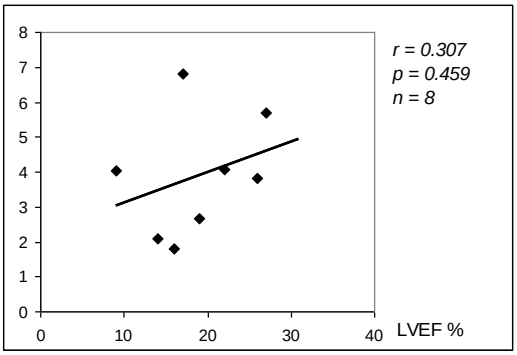

NE ng/mg Pr NE ng/mg Pr