Intraventricular placement of a spring expander does not attenuate cardiac atrophy of the healthy heart induced by unloading via heterotopic heart transplantation

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Running head: isovolumic loading and cardiac atrophy

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Summary

An important complication of the prolonged left ventricle assist device support in patients with heart failure is unloading-induced cardiac atrophy which proved resistant to various treatments. Heterotopic heart transplantation (HT_x) is the usual experimental model to study this process. We showed previously that implantation of the newly designed intraventricular spring expander can attenuate the atrophy when examined after HT_x in the failing heart (derived from animals with established heart failure). The present study aimed to examine if enhanced isovolumic loading achieved by implantation of the expander would attenuate cardiac post-HT_x atrophy also in the healthy heart. Cardiac atrophy was assessed as the ratio of the transplanted-to-native heart weight (HW) and its degree was determined on days 7, 14, 21 and 28 after HT_x. The transplantation resulted in 32±3, 46±2, 48±3 and 46±3% HW loss when measured at the four time points; implantation of the expander had no significant effect on these decreases. We conclude that enhanced isovolumic loading achieved by intraventricular implantation of the expander does not attenuate the development of cardiac atrophy after HT_x in the healthy heart. This indicates that such an approach does not represent a useful therapeutic measure to attenuate the development of unloading-induced cardiac atrophy.

Key Words: cardiac atrophy, heterotopic heart transplantation, mechanical heart unloading, spring expander.

Introduction

Heart transplantation (HT_x) is in practice the only treatment approach that substantially improves survival rate in patients with end-stage heart failure (HF) (Braunwald 2015, Kassi *et al.* 2018, Moayedi and Ross 2017). However, application of HT_x in this patient group is limited due to the scarcity of donor supply. Therefore implantation of the left ventricle assist device (LVAD) has emerged as an alternative treatment approach and is increasingly used (Drakos and Mehra 2016, Kassi *et al.* 2018). Application of LVAD results in unloading of the left ventricle (LV) and was reported to reverse the pathological cellular, structural and functional changes in the myocardium of patients with HF ("remodeling process") and therefore described as "reverse remodeling" (Birks 2013, Chaggar *et al.* 2016, Ibrahim *et al.* 2015). It has been proposed that the improvement of cardiac function after LVAD-induced mechanical unloading could eventually lead to a successful weaning from LVAD support (Birks 2013, Chaggar *et al.* 2016, Ibrahim *et al.* 2015). Unfortunately, even though the biological signs of reverse remodeling are present in the majority of patients after LVAD implantation, a clinical recovery of myocardial function that would allow termination of LVAD support was observed in 4 – 8% only (Drakos and Mehra 2016, Chaggar *et al.* 2016, Kassi *et al.* 2018). The reason(s) for the discrepancy between biological and functional outcome of LVAD treatment have not been elucidated. It is notable that prolonged use of LVAD has also some detrimental effects: the unloading results in significant cardiac atrophy which is now considered the main counterindication to the successful weaning from LVAD support (Benke *et al.* 2017, Birks 2013, Fu *et al.* 2016, Heckle *et al.* 2016, Pokorný *et al.* 2014).

Many attempts have been made to minimize the said detrimental effect of long-term LVAD use, however, the therapeutic effects were not satisfactory, which underscores the need for further search for new treatment approaches (Benke *et al.* 2017, Fu *et al.* 2016, Geen *et al.* 1994, Heckle *et al.* 2016, Navarathnarajah *et al.* 2014, Pokorný *et al.* 2014, Pokorný *et al.* 2018a). We reported recently that implantation of the stainless steel spring expander into the LV (it provides sufficient isovolumic loading without impairing LV ejection function) attenuated the process of unloading-induced cardiac atrophy after heterotopic HT_x onto the abdominal aorta of an isogenic rat recipient (a recognized model to study effects of mechanical heart unloading). This improvement was observed in the failing heart i.e. the one derived from animals with established advanced HF (Pokorný *et al.* 2018b). Moreover, we

have also found that the natural course of unloading-induced cardiac atrophy is different in the healthy and failing hearts (Pokorný *et al.* 2018a). On the whole, the basic question is whether an enhancement of isovolumic loading can be used as a general therapeutic approach to prevent or at least attenuate the development of unloading-induced cardiac atrophy. All the aspects considered, we decided to examine if also in the healthy heart, isovolumic loading achieved by implantation of the spring expander into LV would attenuate the process of unloading-induced cardiac atrophy after heterotopic HT_x.

Our previous study employing implantation of the spring expander into the LV had a major limitation because it was not assessed if the long-term implantation of the expander does not impair cardiac function and structure of the LV in the heart after heterotopic HT_x (Pokorný *et al.* 2018b). Therefore, we performed an additional comprehensive series of experiments in animals after heterotopic HT_x with and without implantation of the spring expander into the LV, to evaluate the cardiac structure and function by echocardiography.

METHODS

Ethical approval, animals, heart transplantation (HT_x).

The studies were performed in accordance with the guidelines and practices established by the *Animal Care and Use Committee of the Institute for Clinical and Experimental Medicine*, Prague. The present study used adult male Lewis rats (an inbred strain in which no need for post-transplantation immunosuppression is required) at the initial age of 10 – 11 weeks and body weight 290–320 g. The rats were purchased from Charles River Laboratories (Velaz, Prague, Czech Republic). The heterotopic HT_x, originally described by Ono and Lindsey (Ono and Lindsey 1969) and employed by many groups including ours, was used as the model to mimic the consequences of mechanical heart unloading (Benke *et al.* 2017, Brinks *et al.* 2014, Fu *et al.* 2016, Geen *et al.* 1994, Klein *et al.* 1991, Kolář *et al.* 1996, Navarathnarajah *et al.* 2014, Pokorný *et al.* 2014, Pokorný *et al.* 2018a, Pokorný *et al.* 2018b).

Experimental design

Series 1: Effects of enhanced isovolumic loading induced by implantation of the spring expander into the LV on the cardiac atrophy after heterotopic HT_x in healthy hearts.

HT_x of healthy heart was performed and, in appropriate groups, implantation into the left ventricle of the stainless steel three-branch expander (briefly: “expander”) was performed through LV apex incision. For healthy hearts the expanders with branch length of 6 mm were used (Figure 1). The same types of expanders were used in our recent study with failing hearts, except that the branch was now shortened from 9 to 6 mm, based on the assessment of the LV dimensions in healthy animals. The characteristics of the stainless wire were identical as employed in our previous study: 0.17 mm in diameter, 316 LVM, Fort Wayne Metal was used. Its chemical composition was as follows (%): carbon 0.023, manganese 1.84, silicon 0.37, phosphorus 0.014, sulphur 0.001, chromium 17.57, nickel 14.68, molybdenum 2.79 , copper 0.03 , nitrogen 0.03 and iron to balance of 100%. Elastic and mechanical properties of spring expanders were measured on the miniaturized compression device and the stress-strain relationship was analyzed as described by Lossef *et al.* (Lossef *et al.* 1994).

The degree of cardiac atrophy was evaluated as the weight of total heart and, separately, of its individual structural components [i.e. LV + septum and right ventricle (RV)]. The degree of cardiac atrophy was expressed as percent decreases in the whole heart weight, and LV and

RV weights of the hearts after the HT_x. This approach was chosen in order to maintain the same mode of presentation as in our recent study which explored the effects of implantation of the expander on the course of unloading-induced cardiac atrophy in failing hearts (Pokorný *et al.* 2018b). Unfortunately, for evaluation of the degree of cardiac atrophy we could not use HW of the donor´s heart before and after HT_x. This was so because with the classical heterotopic HT_x the donor's heart is immediately placed in cold cardioplegia solution, which precludes precise determination of heart weight. The following experimental groups were examined (n = 11 in each group):

1. Lewis rats (recipient) + HT_x of healthy donor's heart (7 days),
2. Recipient + HT_x of healthy heart (14 days),
3. Recipient + HT_x of healthy heart (21 days),
4. Recipient + HT_x of healthy heart (28 days),
5. Recipient + HT_x of healthy heart + implantation of expander (7 days),
6. Recipient + HT_x of healthy heart + implantation of expander (14 days),
7. Recipient + HT_x of healthy heart + implantation of expander (21 days),
8. Recipient + HT_x of healthy heart + implantation of expander (28 days).

The experimental design used in this series is outlined in Figure 2. At the end of experiments, the hearts were excised, blood was removed from the chambers by gentle compression, and the hearts´ wet weight was determined. In separate appropriately matched experimental groups (n = 9 in each) the hearts were harvested for histological examination of the myocardium as described previously (Kolář *et al.* 1996, Pokorný *et al.* 2018a). Analysis of LV and RV fibrosis was performed in sections stained with Picrosirius red (Direct Red 80, Sigma Aldrich, MO, USA) as described in detail previously (Hampl *et al.* 2015, Kolář *et al.* 1996, Pokorný *et al.* 2018a). Briefly, the interstitial collagen was analyzed in polarized light using 10 images of the LV and 5 images of a RV scanned from a midmyocardium, without perivascular areas (magnification 200x, microscope Nikon eclipse Ni-E, camera Nikon DS-L3, Tokyo, Japan). The per cent area of myocardial fibrosis was calculated semiquantitatively, using the imaging software NIS-Elements Ar (LIM, Prague, Czech Republic).

Series 2: Echocardiographic assessment of the effects of enhanced isovolumic loading induced by implantation of the spring expander into the LV on the basal cardiac function parameters after heterotopic HT_x (native and transplanted hearts)

Animals were prepared as described in series 1 and the primary aim of this series was to evaluate the cardiac function and structure of the native and transplanted hearts and effects of expander implantation on these cardiac parameters. Echocardiographic examination was performed 7 and 14 days after HT_x, by methods described previously and also employed by our group (xxx). Briefly, the animals were anaesthetized with 4% isofluran combined with 3L/min oxygen; the ventral thorax and abdomen area was shaved. During the image acquisition, the rats were maintained under isoflurane anesthesia (2 – 2.3 %, at oxygen flow of 1 L/min; if necessary, the dosage was slightly altered, depending on the animal's weight, its reaction and breathing). and fixed in the supine position. Before acquisition of standardized B-Mode and M-Mode images, the implanted hearts were screened for the presence of thrombi (defined as hyperechogenic structures inside heart cavities), for aortic insufficiency (defined as any regurgitation detected below aortic wave by color Doppler), and for any other major abnormalities. For standard measurements of cardiac parameters, B-Mode and M-Mode images were recorded in parasternal long axis view (PSLAX) and parasternal short axis view (PSAX) at the papillary muscle level. Morphological parameters of the LV, including dimensions of LV inner diameter; anterior and posterior walls at systole and diastole were measured in M-mode from long and short axis sections as previously described (Hanton *et al.* 2008) and employed in our laboratory (Beneš *et al.* 2011, Červenka *et al.* 2015a, Červenka *et al.* 2015b) The function of the LV was assessed in the native and transplanted hearts. For each parameter the mean of 3 optimally obtained measurements was used. All ultrasound studies were done by Vevo® 2100 Imaging System with the MS250S transducer (13 - 24 MHz), (FUJIFILM VisualSonics, Inc., Toronto, Ontario, Canada). Immediately after echocardiography the animals were sacrificed by intraperitoneal injection of thiopenthal in the lethal dose, and LV weights (LV + septum weights) were assessed in the native and transplanted hearts. The following experimental groups were examined (n = 7 in each group):

1. Recipient + HT_x of healthy heart (7 days),
2. Recipient + HT_x of healthy heart (14 days),

3. Recipient + HT_x of healthy heart + implantation of expander (7 days),

4. Recipient + HT_x of healthy heart + implantation of expander (14 days),

The experimental design used in this series is outlined in Figure 3.

Statistical Analyses

All values are expressed as mean ± SEM. Using the Graph-Pad Prism software (Graph Pad Software, San Diego, CA, USA), statistical analysis was done by Student´s t-test, Wilcoxon´s signed-rank test for unpaired data, or one-way analysis of variance (ANOVA) when appropriate. Values exceeding 95% probability limits ($p<0.05$) were considered statistically significant.

Results

Series 1: Effects of enhanced isovolumic loading induced by implantation of the spring expander into the LV on the cardiac atrophy after heterotopic HT_x in healthy hearts

Table 1 summarizes HW, LV weight (LVW) and RV weight (RVW) of native (used as 100% controls) and transplanted hearts, in absolute values, measured 7, 14, 21 and 28 days after HT_x.

As shown in Figure 4A, 7 days´ unloading obtained by HT_x in healthy hearts caused a significant decrease in whole HW (-32 ± 3%), which became more pronounced by day 14 (-46 ± 2%); thereafter no further progress was seen on days 21 and 28 after HT_x.

As shown in Figure 4B, the dynamics of LV atrophy displayed an almost identical pattern as that of the whole heart.

As shown in Figure 4C, RV atrophy after HT_x exhibited a pattern similar as observed in the whole heart; surprisingly, the change was more pronounced already on day 7 after HT_x.

The data of Figure 4 shows that in healthy animals implantation of the expander did not have any significant effect (at all-time points) on the decreases in whole HW, LVW and RVW .

Figure 5 summarizes the data on the index of myocardial fibrosis (%) in the LV (Figure 5A) and RV (Figure 5B). It is seen that the degree of fibrosis was significantly lower in the LV as compared with the RV. The degree of myocardial fibrosis in the LV of the healthy native hearts (i.e. the native hearts of the recipient) was significantly lower than in the RV throughout the 28-day observation period and was not altered after HT_x, nor did implantation of the expander after HT_x alter it.

Figure 6 shows representative images of myocardial fibrosis in the LV and the RV of the healthy native heart.

Series 2: Echocardiographic assessment of the effects of enhanced isovolumic loading induced by implantation of the spring expander into the LV on the basal cardiac function parameters after heterotopic HT_x.

Table 2 summarizes the evaluation of cardiac function by echocardiography. These data show that throughout the experimental period the native hearts exhibited normal parameters characteristic for healthy rats, as repeatedly shown in our previous studies (Beneš *et al.* 2011, Červenka *et al.* 2015a, Červenka *et al.* 2015b). Neither HT_x, nor HT_x with implantation of the expander changed the cardiac function parameters of the native hearts. The function of the post-HT_x heart exhibited all the characteristics that were already reported for the volume-unloaded heterotopic heart transplants (Didié *et al.* 2013). Specifically, there was a decrease in LV diameter in diastole, in the stroke volume, cardiac output, LV anterior and LV posterior wall thickness in systole, LV fractional shortening and LV ejection fraction. In addition, implantation of the expander resulted in a significant increase in LV diameter in systole as compared with values obtained in heterotopic heart transplants without the expander. In addition, in each of the experimental group, there was one animal which showed moderate (hemodynamically unimportant) aortal insufficiency in the heterotopic heart transplant. Moreover, in each experimental group, two animals showed thrombi either in the LV or RV (in one case in both) in heterotopic heart transplant, irrespective of the expander implantation.

As shown in Figure 7A, assessment of the LV weight by echocardiography revealed excellent correlation with values obtained by direct weighing of the native hearts. In the heterotopic heart transplant the correlation between values obtained by direct weighing and by echocardiography was good, however, the scatter of values obtained by echocardiography

was more pronounced; nevertheless, the values were not altered by implantation of the expander.

As shown in Figure 7B, when estimated on day 7, the extent of LV atrophy was overestimated by echocardiography as compared with direct weighing, however, on day 14 almost identical respective values were obtained.

Figures 8 and 9 show representative images of echocardiographic assessment of the hearts after HT_x, including those in some pathological conditions, e.g. aortic regurgitation and the thrombi in the LV or RV of the transplanted hearts.

Discussion

The first critically important finding of the present study is that enhancement of isovolumic loading induced by implantation of the spring expander into the LV of the healthy heart did not attenuate the development post- HT_x cardiac atrophy. This was documented by determination of decreases in whole HW, LV and RV weights of the transplanted heart when compared with weights of the native heart. This finding is surprising and in striking contrast to our recent discovery that application of the expander markedly attenuated the development of post-HT_x cardiac atrophy in the failing heart (Pokorný *et al.* 2018b). We cannot provide any clear explanation for such discrepant effects of the implantation on the course of unloading-induced cardiac atrophy in healthy and failing hearts.

In this context, it is important to acknowledge that the idea to increase isovolumic loading by implantation of the expander into the LV, as employed in our present and previous study (Pokorný *et al.* 2018b), was inspired by a pioneering study by Klein and co-workers (Klein *et al.* 1991) who found that inflation of a latex balloon in the LV provided an isovolumic load which prevented the development of cardiac atrophy after HT_x in healthy heart. Unfortunately this approach is not applicable in the clinic: in patients with LVAD this procedure would cause obstruction of the LV and failure of LVAD function.

It is admitted that the lack of "anti-atrophic" actions of the expander as observed here is extremely surprising, considering the sound knowledge that the cardiac work is the major determinant of the heart mass (Korecký and Masika 1991, Lee *et al.* 2016). It would be expected that enhancing this work by isovolumic loading (induced by implantation of the expander, a procedure that does not impair LV function) should attenuate the development of unloading-induced cardiac atrophy in the healthy just as it did in the failing heart (Pokorný *et al.* 2018b). Regardless of the exact explanation, the present findings indicate that evaluation of the effectiveness of pharmacological and/or non-pharmacological measures aimed at attenuation of unloading-induced atrophy should be based on studies of the healthy as well as failing hearts. Evidently, our present and previous results show that cardiac atrophy after HT_x is more prominent in the failing heart (Pokorný *et al.* 2018a, Pokorný *et al.* 2018b). Similarly, the responses to attempts to prevent or attenuate this process might differ. This view has now found growing recognition among investigators interested in various aspects of

the process of unloading-induced myocardial remodeling after HT_x (Benke *et al.* 2017, Brinks *et al.* 2014, Fu *et al.* 2016).

Of special interest is our observation that the decreases in RV weights of the transplanted heart reached the maximum on day 7 after HT_x whereas those of the LV were delayed and did not reach the maximum value until the day 14, We cannot provide any satisfactory explanation for this difference. In our recent study, a similar one but performed with the failing hearts, the course of unloading-induced cardiac atrophy was identical in the RV and LV (Pokorný *et al.* 2018a, Pokorný *et al.* 2018b). Evidently, the course of unloading-induced cardiac atrophy might significantly differ, depending on the condition of the heart (healthy or failing), and may also show differences between individual heart structural compartments; such possible differentiation should be considered in designing future studies.

The second important set of findings relates to results of studies evaluating the effect of long-term implantation of the expander on the cardiac function and structure of the native and, in particular, transplanted hearts. Notably, earlier studies revealed that permanent placement of an inflated balloon in the LV not only caused obstruction of the LV, but also elicited major structural changes of the transplanted heart (Galiňanes *et al.* 1991, Klein *et al.* 1991). Therefore, our present findings are critically important for several reasons.

First, we found that the function of the native hearts of the recipient was within physiological range for healthy animals (Beneš *et al.* 2011, Červenka *et al.* 2015a, Červenka *et al.* 2015b). Thence forward, the HT_x with implantation of the expander did not alter cardiac function of the orthotopic native hearts as compared with animals that underwent only HT_x; this excludes any possible indirect effects (e.g. due to secondary changes in hormonal environment) on the course of cardiac atrophy after HT_x.

Second, the transplanted hearts studied did not reveal any important mechanical damage of the aortic valve and there were no signs of serious congestion of the LV , similarly in HT_x without or with expander implantation. The relatively high occurrence of thrombi, both in the LV or RV of the transplanted heart, was the only complication which was unrelated to expander implantation and obviously due to the absence of anticoagulation therapy in this experimental setting. In any case, the presence of thrombi did not apparently have any important influence on the course of cardiac atrophy after HT_x.

Third, our results show a good correlation between the assessment of the LV weight by echocardiography and by direct weighing of the transplanted heart. Admittedly, the variability of the values obtained by echocardiography was somewhat greater (irrespective of expander implantation), which further strengthens our opinion that for precise evaluation of the unloading-induced cardiac atrophy after HT_x, direct weighing of the heart and its individual compartments should be standard procedure.

Fourth, our echocardiographic data are typical for the volume-unloaded heart after HT_x, with markedly decreased stroke volume and cardiac output. (Didié *et al.* 2013). The slightly higher LV diameter in systole in the transplanted heart with the expander suggests that its implantation increased cardiac work in this experimental group. In addition, our data show that the LV fractional shortening and the LV anterior and posterior wall thickness in systole, the parameters of global and regional cardiac function, respectively, were reduced in the transplanted as compared with native hearts. These findings are important in that they suggest that in our experimental setting the contractile function of the LV of the chronically unloaded heart after HT_x is impaired. It will be noticed that the results of earlier studies evaluating the contractile function of the heart after HT_x were not consistent: some studies reported a decrease (Galiňanes *et al.* 1991, Galiňanes *et al.* 1995, Ito *et al.* 2003, Soppa *et al.* 2008) but the majority (those employing an array of imaging and functional assessment techniques) showed that despite considerable atrophy, the intrinsic contractile properties of the transplanted heart remained normal (Brinks *et al.* 2009, Didié *et al.* 2013, Geenen *et al.* 1994, Liu *et al.* 2015, Kolář *et al.* 1993, Kolář *et al.* 1995, Korecký and Rakušan 1983, Takaseya *et al.* 2004, Welsch *et al.* 2001) or even were improved as compared with the native control hearts (Ritter *et al.* 2000). However, it is now acknowledged that the measures of cardiac parameters have to be normalized for morphological parameters. If not, decreased contractile performance in the atrophied hearts is shown, whereas normalized parameters (in vitro evaluation of mechanical performance of the papillary muscles or in vivo pressure-volume analysis of LV function) suggest intact or even enhanced cardiac contractile function (Benke *et al.* 2017, Fu *et al.* 2016).

In conclusion, even with echocardiographic evaluation of the cardiac function, a limitation of our present study was that we did not perform a rigorous hemodynamic evaluation of the cardiac function by employing analyses of the LV pressure-volume

relationship of the transplanted heart (Pacher *et al.* 2008). Only such assessment could definitely characterise the effects of expander implantation on the cardiac work and cardiac contractility of the transplanted heart. However, such evaluation would require insertion of a conductance catheter into the LV to obtain pressure-volume relationship data. While such approach is standardly employed in our laboratory in healthy rats as well as in rats with HF (Červenka *et al.* 2015a, Červenka *et al.* 2015b), we have not yet succeeded to apply it in the heart after HT_x (the impediment here is the state of volume-unloading).

Another limitation of our present study is related to the model of HT_x. It is important to recognize that normally the transplanted heart is exposed to the same hormonal environment as the control (i.e. ortotopic native) heart: the transplanted organ is supplied by the recipient. This means that the direct or indirect effects of metabolic and hormonal factors on the course of unloading-induced cardiac atrophy after HT_x can be disregarded. This is regarded a major advantage of the heterotopic HT_x model (Benke *et al.* 2017, Fu *et al.* 2016, Ono and Lindsey 1969). However, in our latest study which showed that implantation of the expander attenuated cardiac atrophy in the failing heart, this heart was transplanted to a healthy recipient (Pokorný *et al.* 2018b). The reason was that the HF model employed was one of volume overload induced by aorto-caval fistula (ACF); the model is well-characterized and standardly employed in our laboratory (Červenka *et al.* 2015a, Červenka *et al.* 2015b). Animals 10 weeks after ACF creation were used as heart donors, because at that time ACF animals are in the stage of advanced but still compensated HF, then they soon progress toward decompensation (Červenka *et al.* 2015a, Červenka *et al.* 2015b) and at this stage would not survive the surgical procedure of HT_x. Moreover, transplantation would have to be done to the site where ACF is already located. Thus, in that study the heart from the animal with advanced HF and marked activation of the circulatory hormonal systems was suddenly placed in the normal hormonal environment of a healthy animal. This situation could have some modulatory action on the course of unloading-induced cardiac atrophy and particularly on the effect of expander implantation on this process. This line of reasoning is supported by the clinical evidence that the "reverse remodeling" and functional recovery of the LV function in patients after implantation of LVAD was significantly enhanced when intensive adjunctive pharmacological therapies aimed at normalization of the neurohormonal environment were employed (Birks *et al.* 2011, Catino *et al.* 2018). All these aspects considered, it is apparent

that more studies are needed to address the above limitations and our current study provides a necessary basis for such investigations.

In conclusion, the results of the present study show that implantation of the spring expander into the LV does not attenuate the development of cardiac atrophy after HT_x in the healthy heart. Considering the reservations discussed above we believe that increasing isovolumic loading using the expander may not represent a generally useful therapeutic measure to attenuate the development of unloading-induced cardiac atrophy, however, it might be of value in selected clinical conditions.

Conflict of Interest.

Authors declare no conflict of interest.

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Figure Legends

Figure 1. Diagramatic presentation of the spring expander with branch lengths of 6 mm (A) and the general view of the device (B).

Figure 2. An outline of the set of experimental groups for series 1: healthy animals after heterotopic heart transplantation (HT_x) either without (A) or with (B) implantation of the expander.

Figure 3. An outline of the set of experimental groups for series 2: healthy animals after heterotopic heart transplantation (HT_x) either without (A) or with (B) implantation of the expander.

Figure 4. Effect implantation of the spring expander on the course of cardiac atrophy in response to mechanical heart unloading induced by heterotopic heart transplantation (HT_x) in healthy animals. Data are expressed as percent decreases from the values for the native healthy heart (100%): (A) changes in whole heart weight, (B) changes in left ventricle weight, (C) changes in right ventricle weight. **#** $P<0.05$ versus the values for the animals studied 7 days after HT_x.

Figure 5. The index of myocardial fibrosis for the left ventricle (LV) (A) and the right ventricle (RV) (B) in the native hearts, and effects of heterotopic heart transplantation (HT_x) without and with spring expander implantation.

Figure 6. Representative images of the left (A) and right (B) ventricle of the native heart of the healthy animals. Sections are stained with Picrosirius red (magnification 200x); in these bright-field microscopy images the collagen is red against a pale yellow background. The fibrosis in the left ventricle of this particular healthy animal was estimated at 1.93%. The fibrosis in the

right ventricle of this particular healthy animal was estimated at 3.74%. Scale bar in the figure is 100 µm.

Figure 7. (A) The left ventricle weight evaluated by direct weighing and indirectly by echocardiography: effect implantation of the spring expander on the course of weight change in native hearts and in unloaded hearts after heterotopic heart transplantation (HT_x) in healthy animals. (B) Effect implantation of the spring expander on the course of left ventricle atrophy in response to mechanical unloading (after HT_x) in healthy animals: data are expressed as percent decreases from the values for the native left ventricle weight (100%).* $P<0.05$ versus native hearts at the same time point. # $P<0.05$ versus values obtained by direct weighing at the same time point.

Figure 8. Representative echocardiographic images of the heterotopic heart (A) recorded 7 days after heterotopic heart transplantation without spring expander implantation and heterotopic heart (B) recorded 7 days after heterotopic heart transplantation with spring expander implantation. Red arrows show the position of the spring expander branches in the heterotopic heart.

Figure 9. Representative echocardiographic images of the heterotopic heart (A) recorded 7 days after heterotopic heart transplantation with spring expander implantation (red arrow), showing slight aortic regurgitation (yellow asterisks) and (B) the heterotopic heart recorded 7 days after heterotopic heart transplantation with spring expander implantation, showing a thrombus inside the left ventricle inner branches of the spring expander (yellow arrow)

Table 1. The weights of the native heart (i.e. recipient heart) and the transplanted heart (i.e. donor´s heart) and of the individual structural components after heterotopic heart transplantation (HT_X). Native heart values served as basal values (100 %) for evaluation of the process of cardiac atrophy in animals after HT_X.

	Parameter					
	HW (mg) (native)	**HW (mg) (HT_X)**	**LVW (mg) (native)**	**LVW (mg) (HT_X)**	**RVW (mg) (native)**	**RVW (mg) (HT_X)**
Group						
Recipient + HT_X of healthy donor´s heart without expander (7 days after HT_X)	1055 ± 37	717 ± 21*	673 ± 21	478 ± 17*	219 ± 9	114 ± 6*
Recipient + HT_X of healthy donor´s heart without expander (14 days after HT_X)	1014 ± 31	548 ± 19*#	677 ± 19	359 ± 17*#	204 ± 11	88 ± 7*#
Recipient + HT_X of healthy donor´s heart without expander (21 days after HT_X)	1126 ± 34	586 ± 22*#	695 ± 22	375 ± 18*#	214 ± 9	96 ± 8*#
Recipient + HT_X of healthy donor´s heart without expander (28 days after HT_X)	1090 ± 29	589 ± 21*#	701 ± 22	323 ± 24*#	223 ± 11	100 ± 11*#
Recipient + HT_X of healthy donor´s heart + implantation of expander (7 days after HT_X)	1056 ± 39	771 ± 29*	674 ± 26	498 ± 28*	212 ± 15	115 ± 9*
Recipient + HT_X of healthy donor´s heart + implantation of expander (14 days after HT_X)	1045 ± 43	606 ± 19*#	667 ± 25	367 ± 18*#	209 ± 16	100 ± 7*#
Recipient + HT_X of healthy donor´s heart + implantation of expander (21 days after HT_X)	1161 ± 42	662 ± 29*#	719 ± 33	395 ± 21*#	218 ± 18	109 ± 11*#
Recipient + HT_X of healthy donor´s heart + implantation of expander (28 days after HT_X)	1117 ± 41	670 ± 34*#	724 ± 35	369 ± 27*#	218 ± 20	111 ± 12*#

Values are means ± SEM. HT_x, heterotopic heart transplantation; HW, heart weight; LVW, left ventricle weight; RVW, right ventricle weight; * $P<0.05$ vs. values from native heart at the same time point. # $P<0.05$ vs. values observed 7 days after HT_x.

Table 2. Echocardiography analyses of left ventricle function and morphology in native heart (i.e. recipient heart) and transplanted heart (i.e. donor´s heart) after heterotopic heart transplantation (HT_x).

	Group 1		**Group 2**		**Group 3**		**Group 4**	
Parameter	**native**	**HT_x**	**native**	**HT_x**	**native**	**HT_x**	**native**	**HT_x**
LVDD (mm)	6.49±0.14	5.09±0.14*	6.61±0.11	5.14±0.09*	6.72±0.09	5.11±0.11*	6.71±0.14	5.15±0.10*
LVSD (mm)	3.49±0.12	4.19±0.11	3.52±0.12	4.46±0.08*	3.57±0.18	4.19±0.12	3.49±0.15	4.51±0.09*
LVFS (%)	45.1±1.9	16.8±2.3*	47.9±2.1	13.1±2.1*	48.1±2.5	17.1±2.8*	48.8±2.2	14.1±1.9*
LVEF (%)	75.5±1.8	32.4±3.1*	77.5±1.4	25.5±3.7*	75.7±2.4	31.8±3.6*	78.5±1.6	24.1±2.1*
SV (µl)	160±9.2	41±6.8*	180±6.4	31±5.1*	182±5.4	44±8.9*	186±9.2	35±3.1*
CO (ml/min)	64.9±3.4	13.1±1.9*	67.7±2.6	9.6±1.1*	70.6±1.6	15.1±3.1*	71.5±3.2	8.8±0.9*
LWAWTd (mm)	1.68±0.06	1.54±0.11	1.79±0.04	1.74±0.11	1.66±0.05	1.81±0.16	1.70±0.05	1.56±0.1
LWPWTd (mm)	1.76±0.10	1.41±0.11	1.76±0.08	1.51±0.12	1.81±0.06	1.57±0.10	1.79±0.06	1.46±0.08
LWAWTs/LWAWTd	1.51±0.06	1.10±0.06*	1.60±0.07	1.04±0.02*	1.68±0.06	1.11±0.03*	1.72±0.05	1.09±0.02*
LWPWTs/LWPWTd	1.59±0.08	1.07±0.04*	1.65±0.5	1.07±0.03*	1.62±0.06	1.10±0.03*	1.70±0.04	1.05±0.02*

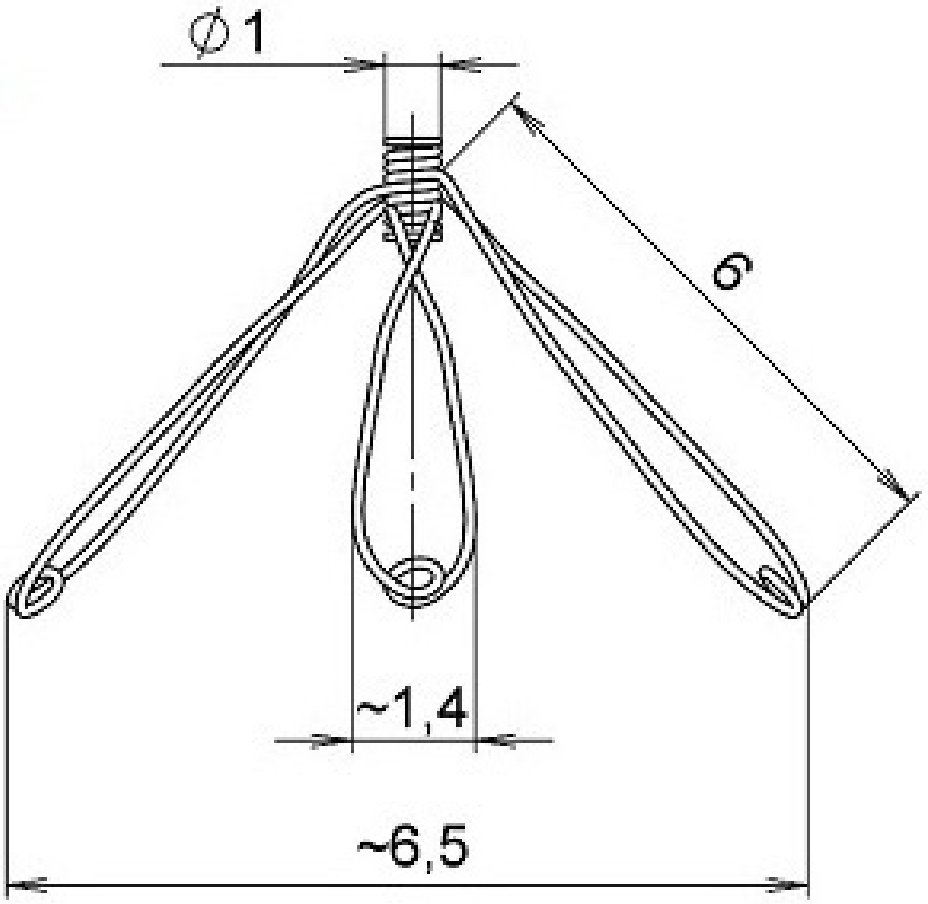
Values are means ± SEM. HT_x, heterotopic heart transplantation.

Group 1, Recipient + HT_x of healthy donor´s heart without expander (7 days after HT_x); Group 2, Recipient + HT_x of healthy donor´s heart without expander (14 days after HT_x); Group 3, Recipient + HT_x of healthy donor´s heart + implantation of expander (7 days after HT_x); Group 4, Recipient + HT_x of healthy donor´s heart + implantation of expander (14 days after HT_x).

LVDD, left ventricular diastolic diameter; LVSD, left ventricular systolic diameter; LVFS, left ventricular fractional shortening; LVEF, left ventricular ejection fraction; SV, stroke volume; CO, cardiac output; LVAWTd, left ventricular anterior wall thickness in diastole; LVPWTd, left ventricular posterior wall thickness in diastole; LVAWTs, left ventricular anterior wall thickness in systole; LVPWTs, left ventricular posterior wall thickness in systole. * $P<0.05$ vs. values from the native heart at the same time point.

Figure 1

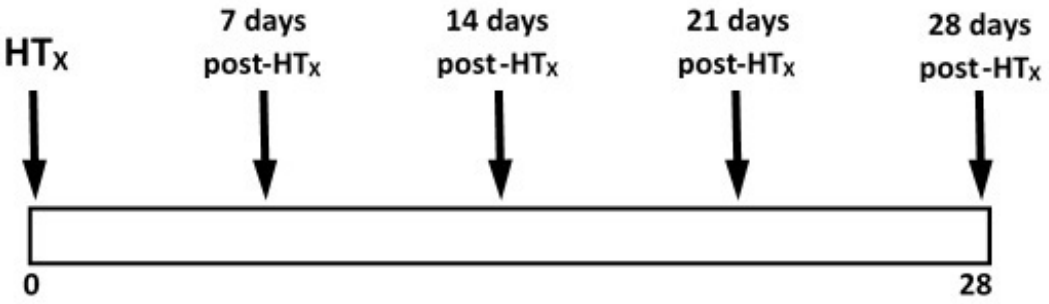
A



B

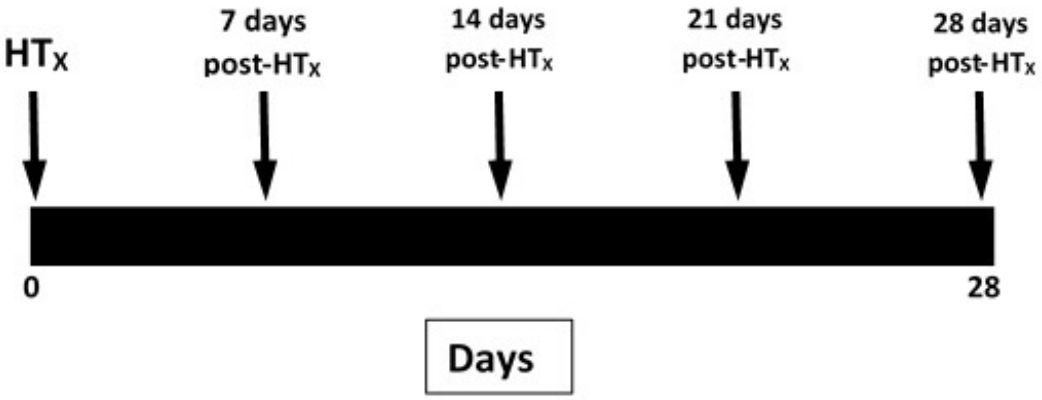
Figure 2

A



Recipient + **HTX of healthy heart**

B



Recipient + **HTX of healthy heart + expander**

Figure 3

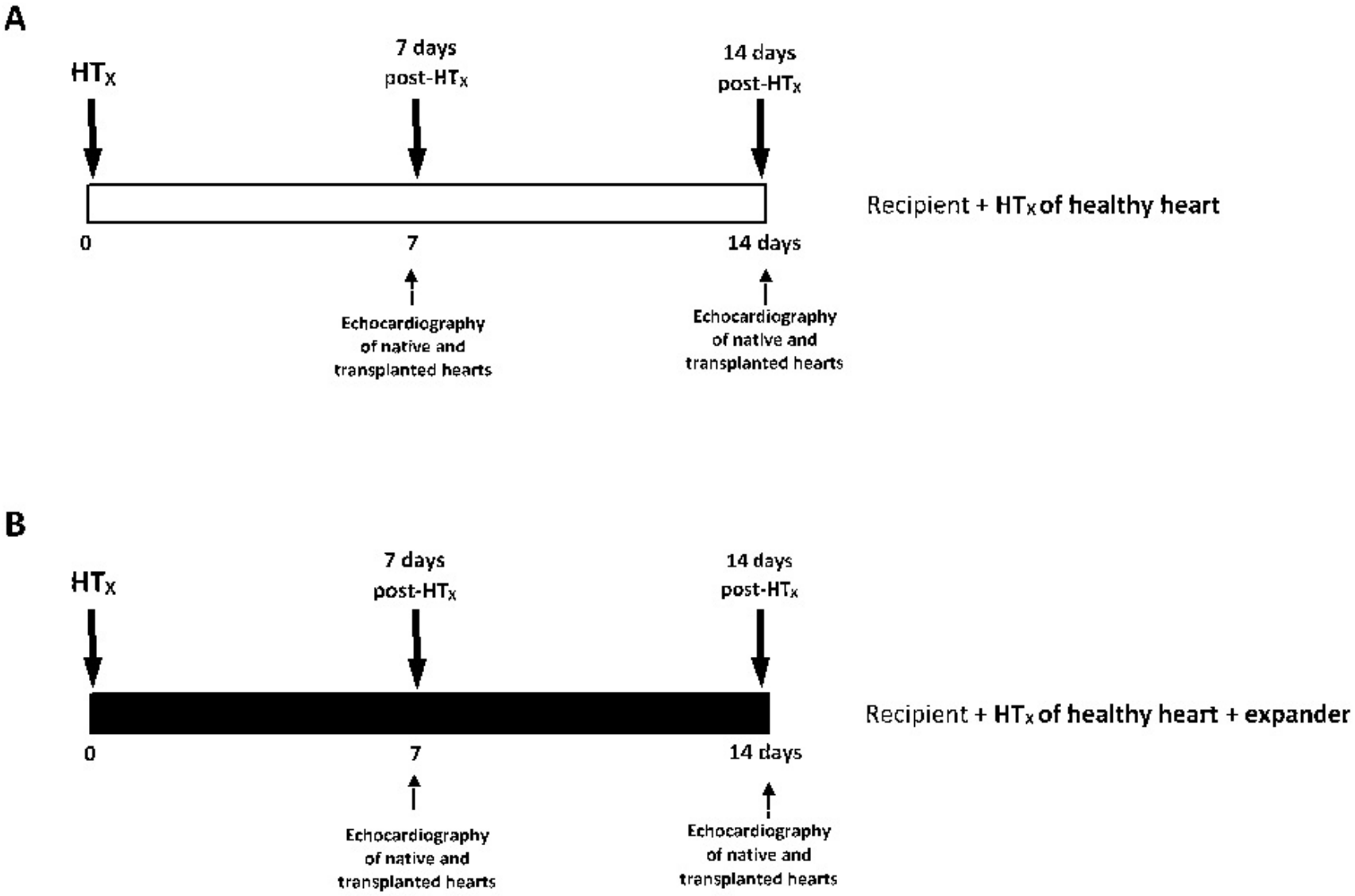

A
HTX
7 days post-HTX
14 days post-HTX
0
7
14 days
Echocardiography of native and transplanted hearts
Echocardiography of native and transplanted hearts
Recipient + HTX of healthy heart
B
HTX
7 days post-HTX
14 days post-HTX
0
7
14 days
Echocardiography of native and transplanted hearts
Echocardiography of native and transplanted hearts
Recipient + HTX of healthy heart + expander

Figure 4

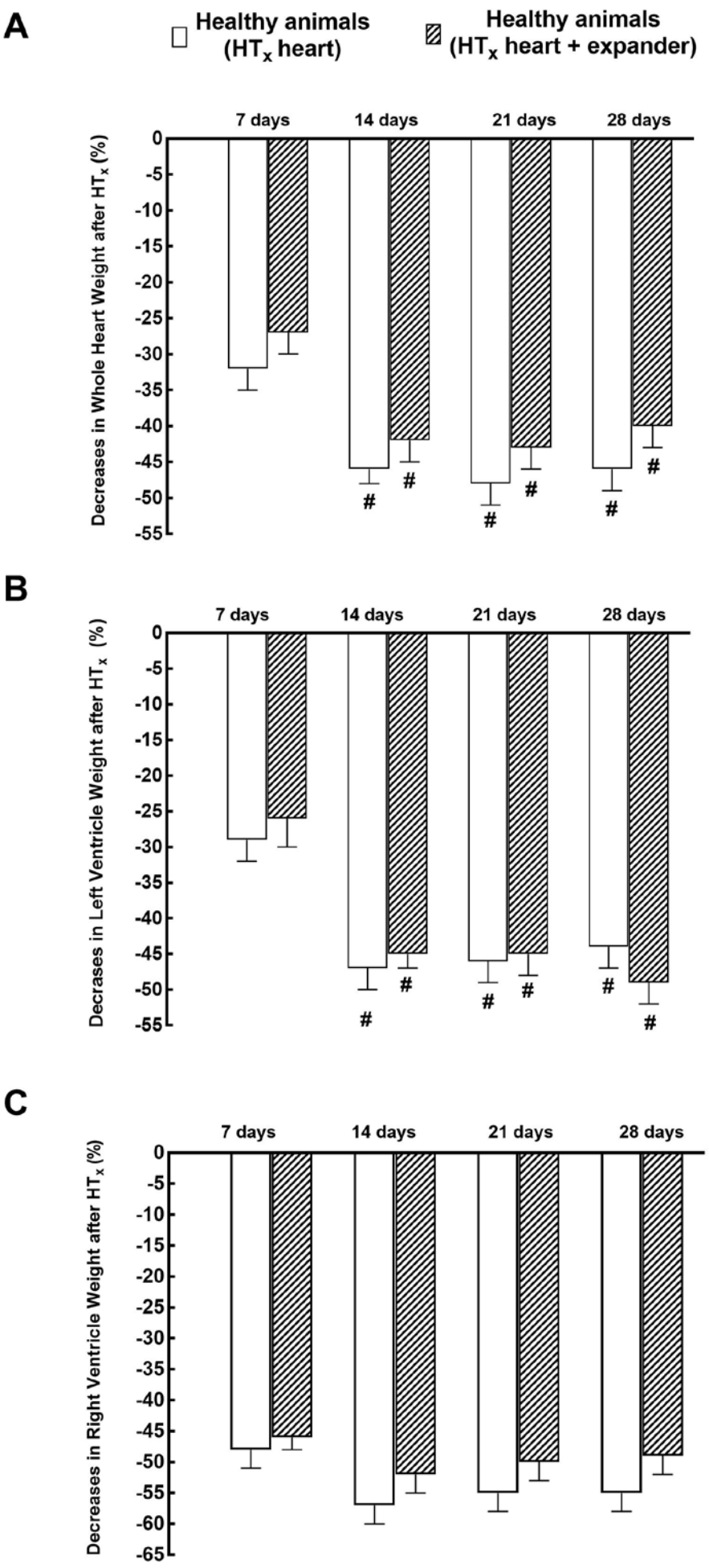

A
Healthy animals (HTx heart)
Healthy animals (HTx heart + expander)
7 days
14 days
21 days
28 days
Decreases in Whole Heart Weight after HTx (%)
0
-5
-10
-15
-20
-25
-30
-35
-40
-45
-50
-55
#
B
Decrases in Left Ventricle Weight after HTx (%)
C
Decreases in Right Ventricle Weight after HTx (%)
-60
-65

Figure 5

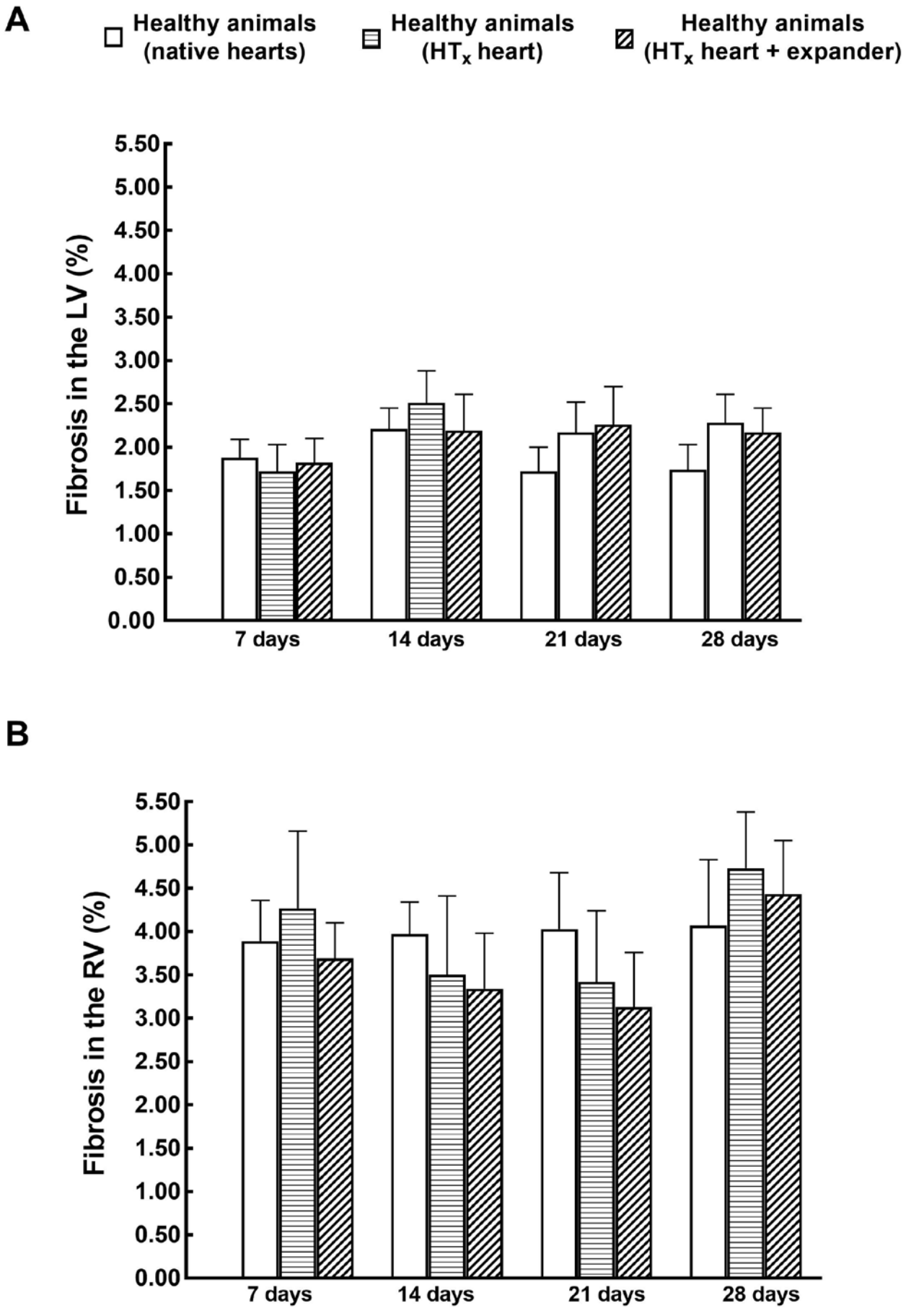
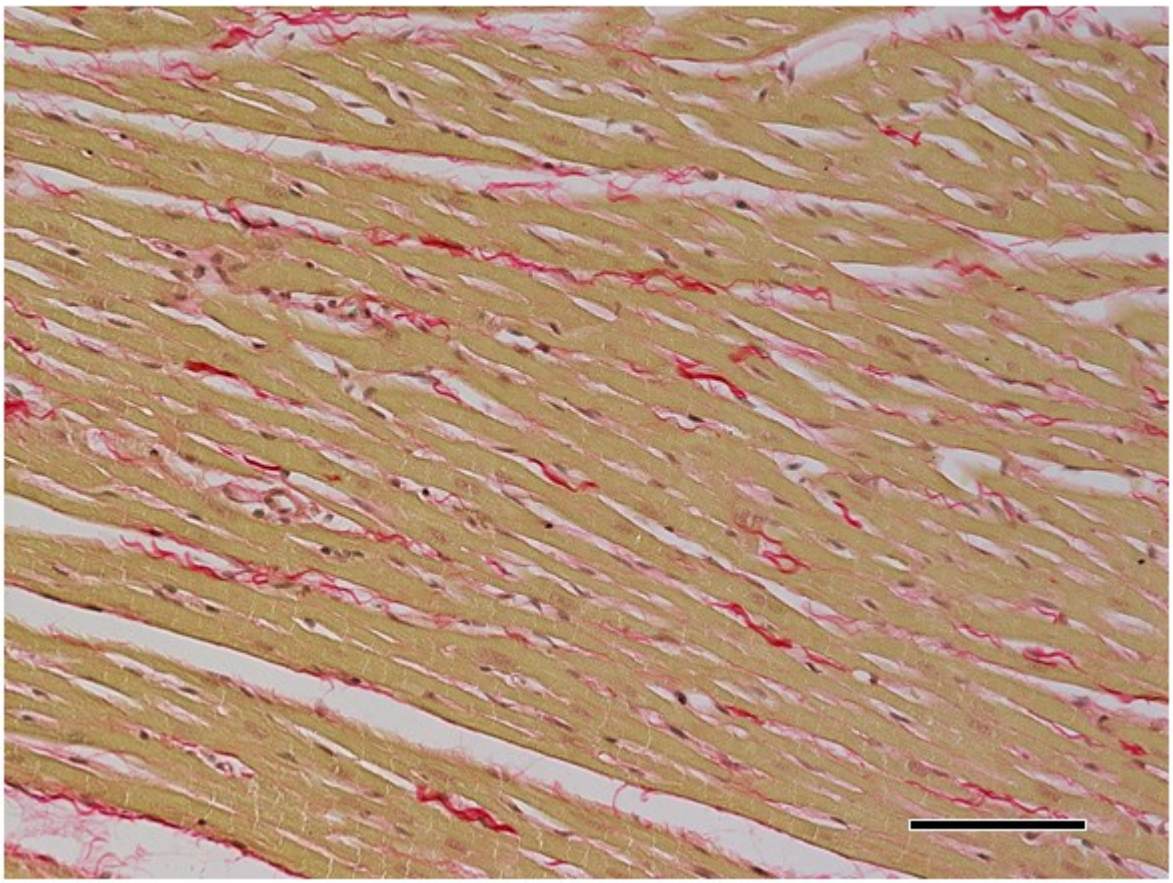

A
Healthy animals (native hearts)
Healthy animals (HT_x heart)
Healthy animals (HT_x heart + expander)
Fibrosis in the LV (%)
5.50
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
7 days
14 days
21 days
28 days
B
Fibrosis in the RV (%)
5.50
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
7 days
14 days
21 days
28 days

Figure 6

A



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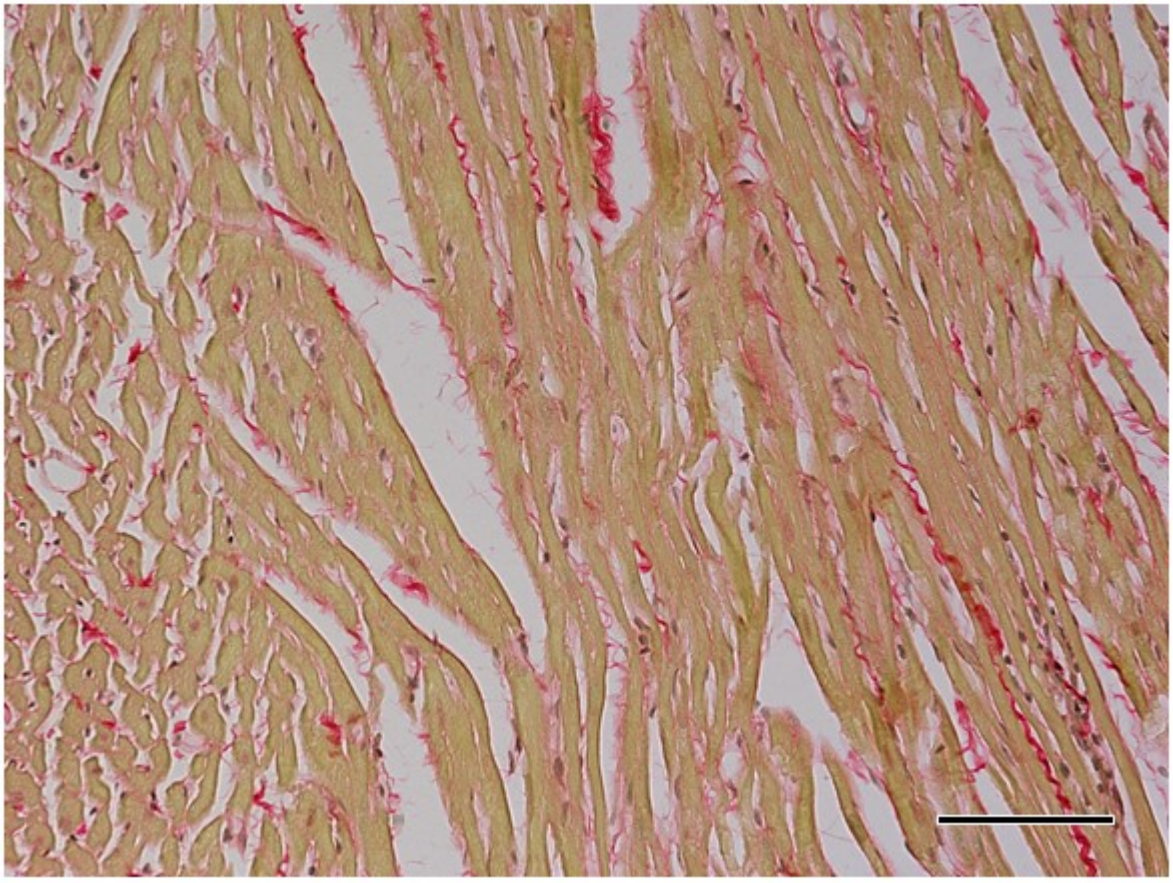


Figure 7

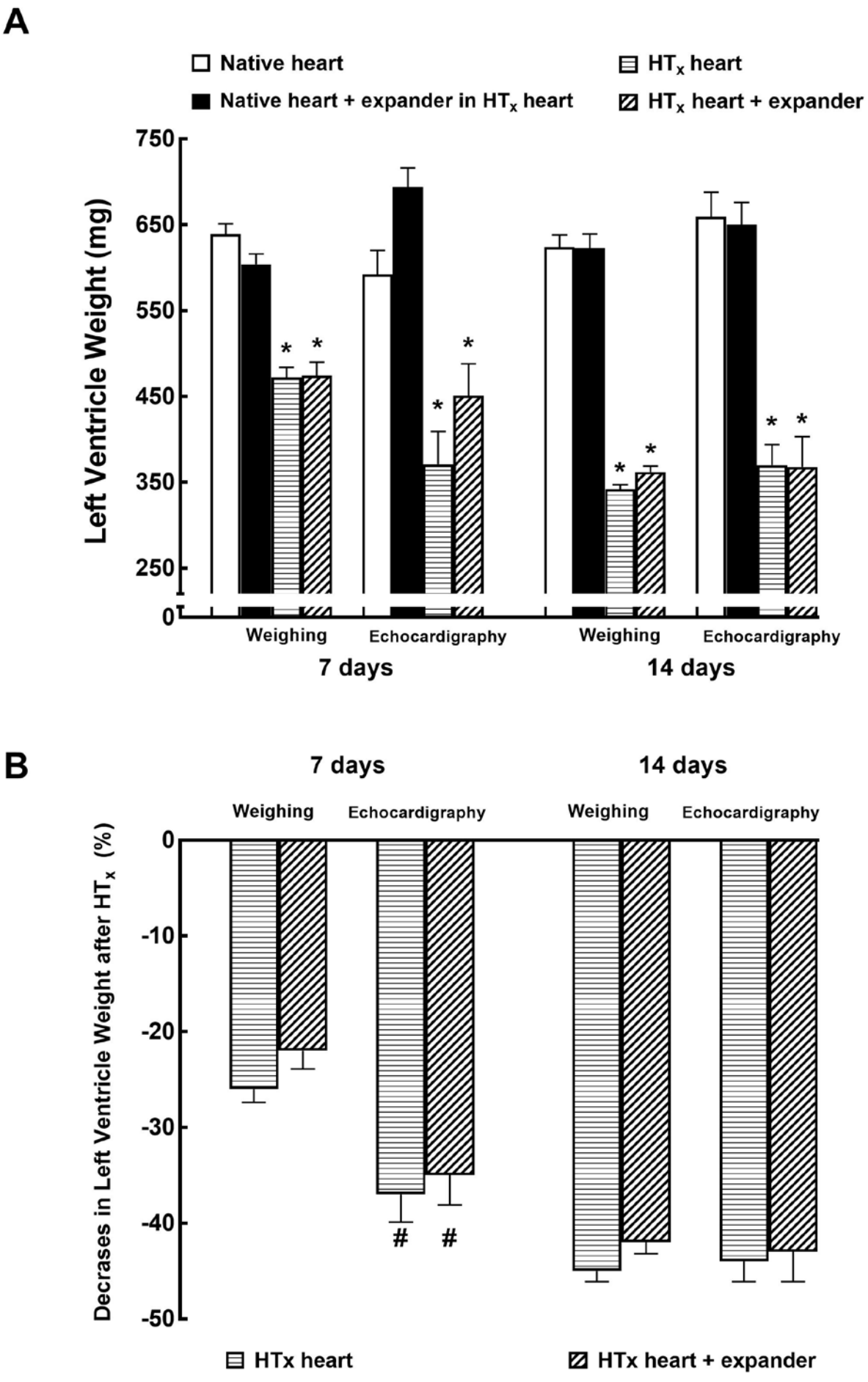
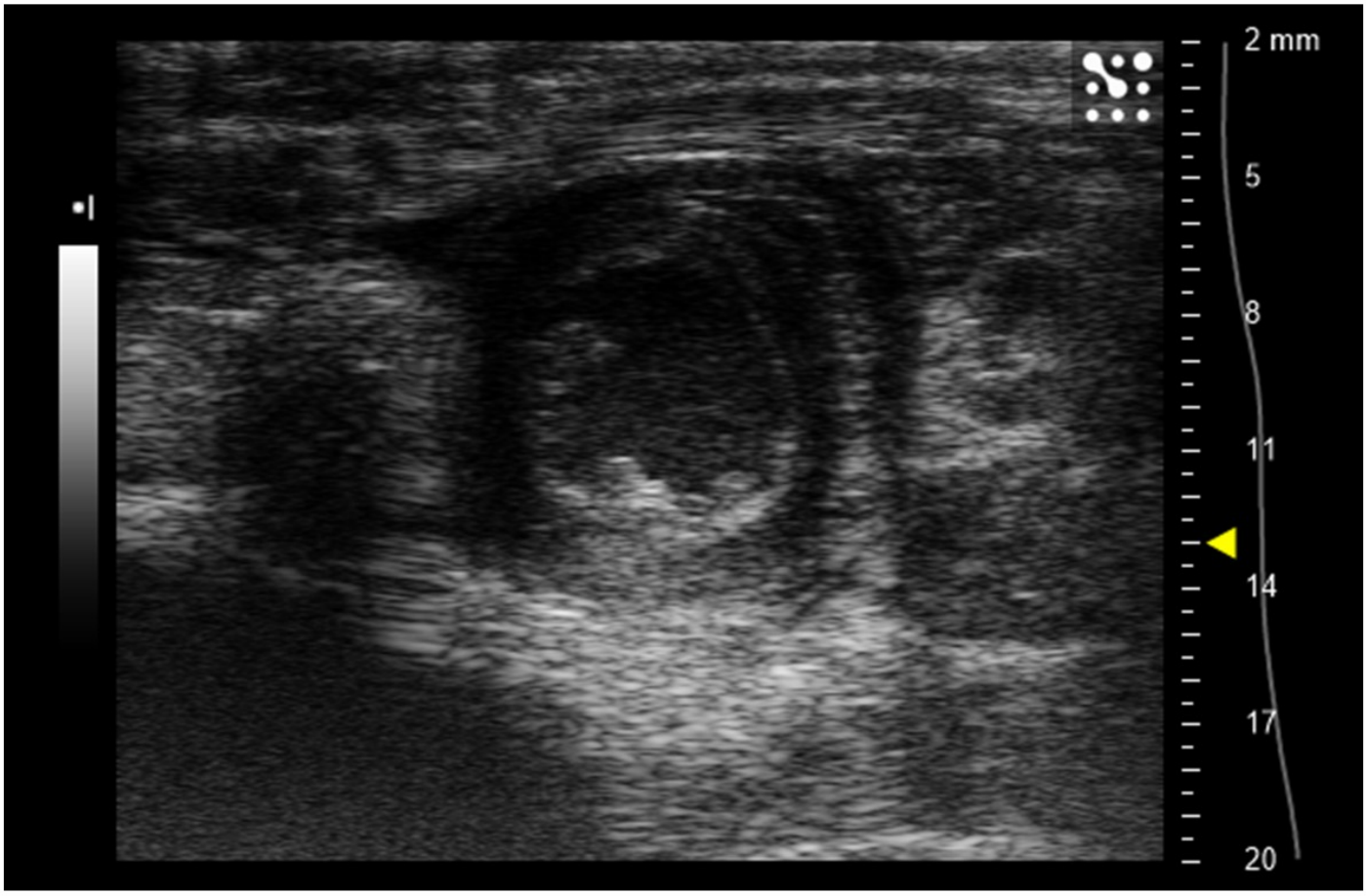

A
Native heart
Native heart + expander in HTx heart
HTx heart
HTx heart + expander
Left Ventricle Weight (mg)
750
650
550
450
350
250
0
Weighing
Echocardigraphy
Weighing
Echocardigraphy
7 days
14 days
B
7 days
14 days
Weighing
Echocardigraphy
Weighing
Echocardigraphy
Decrases in Left Ventricle Weight after HTx (%)
0
-10
-20
-30
-40
-50
#
#
HTx heart
HTx heart + expander

Figure 8

A



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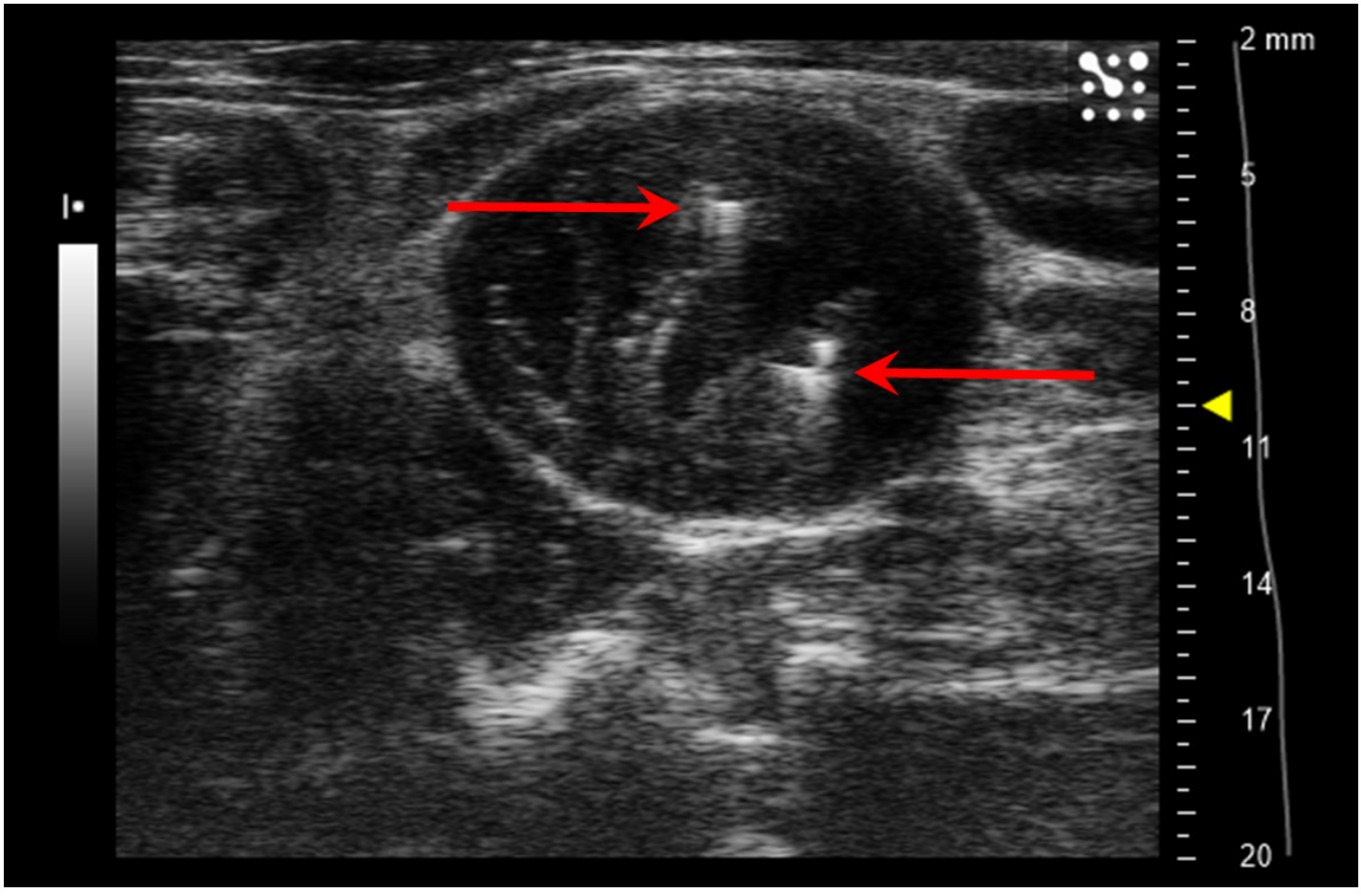
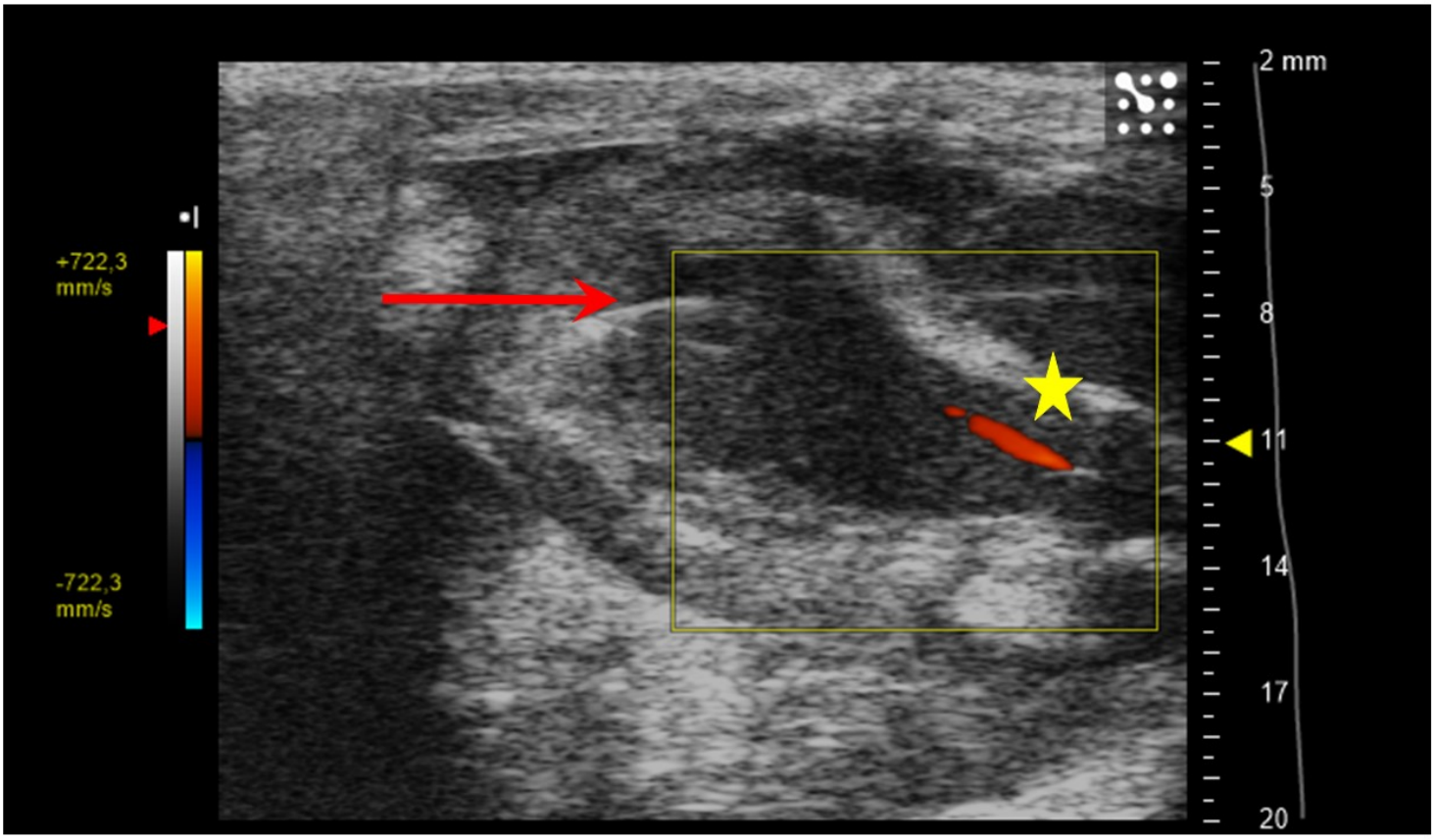


Figure 9

A



B