Effects of high-altitude exercise training on contractile function of rat skinned cardiomyocyte

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EFFECTS OF HIGH-ALTITUDE EXERCISE TRAINING ON CONTRACTILE FUNCTION OF RAT SKINNED CARDIOMYOCYTE.

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1. Introduction

Aerobic exercise induces cardiac morphological remodelling (increase in myocardial mass and ventricular chamber dimensions) and functional adaptations (for review see {Moore, 1995 #43}) leading to **increased** maximal stroke volume and subsequently cardiac output. Part of the training effect on the cardiac performances is due to **increased** myocyte contractile function possibly through changes in their intrinsic active properties {Moore, 1995 #43}. Various cellular parameters have been investigated to account for the beneficial effect of exercise on contraction. Training does not appear to modify intracellular Ca^{2+} transient {Natali, 2001 #2; Palmer, 1998 #11; Laughlin, 1992 #29}. However, **it increased** Ca^{2+} sensitivity of the myofilaments in intact {Wisloff, 2001 #4} and skinned cardiomyocytes {Diffee, 2001 #25}. **The aforementioned experiments used** various training and/or experimental conditions that could lead to some heterogeneity in the results. Cardiomyocyte properties are not uniform within the myocardium. In the rat heart, there are regional differences in electrical {Aimond, 1999 #45} as well as in mechanical properties. We have described **previously** that intact {Cazorla, 2000 #33}, and skinned {Cazorla, 2005 #1} myocytes isolated from the sub-endocardium (Endo) are more responsive to stretch than those from the sub-epicardium (Epi), with higher passive and active tensions developed in Endo at the same sarcomere length (SL). Natali et al. {Natali, 2002 #12} showed that voluntary exercise increases the steepness of the active tension–SL relationship of attached intact cardiomyocytes exclusively in Endo but not in Epi. However, Diffee et al. reported **that training increases** Ca^{2+} sensitivity in both Endo and Epi skinned myocytes {Diffee, 2003 #5}. The latter experiments were performed at 2.35 µm SL, while the intact cells in the former work could be stretched experimentally to 2.05 µm SL at the best.

In their constant search to improve performance, many athletes train at high altitude because hypoxia exposure enhances erythropoiesis and subsequently oxygen carrying capacity. However, there is now clear evidence in both animal models and human that training at high altitude does not provide advantages over training at sea level as regards maximal oxygen uptake and aerobic performance {Henderson, 2001 #30; Reboul, 2005 #7}. This altitude-training paradox is in part due to changes in cardiac morphological and functional parameters determining stroke volume {Reboul, 2005 #7}. We showed previously that rats trained under hypobaric hypoxic conditions presented a specific cardiac remodelling characterised by **increased** left ventricular wall thickness without chamber

enlargement. Moreover, contrary to sea-level training, cardiac function **was not improved** when training was conducted under these experimental conditions. **In particular resting and** maximal stroke volumes **were not improved,** leading to the assumption that high-altitude training depressed heart and especially myocyte contractile properties.

This study was specifically designed to test the hypothesis that high-altitude training limits the beneficial effects classically observed after training at sea level. Considering that cardiomyocyte properties, including the response to exercise training, are not uniform within the myocardium, we investigated the effects of training under normoxic and hypobaric hypoxic conditions on myofilament Ca^{2+} sensitivity and its stretch-dependency in single skinned cardiomyocytes, taking into account their localization in the sub-endocardium and sub-epicardium.

2. Methods

2.1. Training procedure

Male Wistar rats (300-350 g) were randomly assigned to one of the four groups (7 animals per group): rats living continuously either in normoxic conditions without or with aerobic training sessions (N and NT, respectively) and rats living continuously in hypoxic conditions without or with aerobic training sessions (H and HT, respectively). High altitude was simulated by a hypobaric hypoxia environment obtained with a vacuum pump as described previously {Reboul, 2005 #7}. Rats were maintained for 5 weeks at a barometric pressure of 760 mmHg ($PIO_2 \approx 159$ mmHg, altitude ≈ 80 m) for N and NT, or at a pressure of 475 mmHg ($PIO_2 \approx 90$ mmHg, altitude ≈ 4000 m) for H and HT. Some were trained under sea-level (NT) or high-altitude (HT) conditions for 5 weeks in a driven wheel as described previously {Reboul, 2005 #7}. Briefly, the training program consisted of 5 sessions (45-min) per week at 80% of the maximal aerobic velocity (MAV) determined in each environmental condition. MAV was evaluated using a driven wheel during a maximal exercise test {Gonzalez, 1993 #22} in each environment and for each rat before the study, during the 3rd week, and at the end of the protocol. All procedures were performed in agreement with the *Guide for the Care and Use of Laboratory Animals*

published by the US National Institutes of Health (NIH Publications No. 85-23, revised 1996) and with the approval of the French Ministry of Agriculture.

2.2. Echocardiography and heart weighing.

Rats were anesthetized with intraperitoneal ketamine HC1 (75 mg/kg) and xylazine (10 mg/kg). A two-dimensional short-axis view of the left ventricle was obtained at the level of the papillary muscles using an echocardiograph (5-8 MHz micro-convex transducer HDI 3000, ATL, Phillips Company, Bothell, USA) as reported previously {Reboul, 2005 #7}. End-diastolic anterior and posterior wall thicknesses (AWT and PWT, respectively), and left ventricular end-diastolic (LVEDd) and end-systolic (LVESd) diameters were measured. The diastolic relative wall thickness (RWT= AWT+PWT/LVEDd) was calculated as an index of cardiac remodelling. Left ventricular stroke volume (LVSV) and heart mass were evaluated as described previously {Reboul, 2005 #7}. Measurements from at least three consecutive cardiac cycles were averaged.

2.3. Single cardiomyocyte sarcomere length and force measurements

The Ca^{2+}-activated force of single skinned myocyte was measured as described previously {Cazorla, 2005 #1; Cazorla, 2006 #56}. Myocytes were isolated by mechanical dissociation from pre-skinned left ventricular strips. Some cells were kept on ice and used within a day for mechanical experiments; the others were solubilized for gel electrophoresis.

Skinned myocytes were attached to a piezoresistive strain gauge (AE801 sensor, Memscap, Crolle, France) and to a stepper motor driven micromanipulator with thin needles and UV-polymerized optical glue (NOA 63, Norland products Inc, North Brunswick, NJ). Sarcomere length (SL) was determined online throughout the experiment (Ionoptix Boston, USA). Force was normalized to cross-sectional area measured from the imaged cross-section as described previously {Cazorla, 2000 #9}. Slack SL was measured before attachment to serve as zero reference and did not differ significantly between myocytes from normoxic (1.87±0.01 µm SL, n=45) and hypoxic (1.86±0.01 µm SL, n=43) rats. The pCa (=-log[Ca^{2+}]i)-force relationships were established at two SL: 1.9 then 2.3 µm, at 22°C. Cells that did not maintain 80% of the first maximal tension or a visible striation pattern were discarded. We continued with cells that were well attached with minimal SL changes (< 0.1 µm) during activation.

When required, cell length was varied during contraction in order to keep SL constant. Active tensions at submaximal activations were normalized to maximal isometric tension (classically obtained at pCa 5) at the same SL. The relation between force and pCa was fitted to the following equation:

$$force = \frac{\left[Ca^{2+}\right]^{n_H}}{\left(K + \left[Ca^{2+}\right]^{n_H}\right)}$$

where n_H is the Hill coefficient and pCa_{50}, pCa for half-maximal activation equals –(log K)/n_H.

2.4. Solutions

Solutions were similar to those used previously {Cazorla, 2005 #1}. For cell dissociation, the Ca^{2+}-free Hanks-HEPES buffered solution contained (in mM): NaCl 117, KCl 5.7, $NaHCO_3$ 4.4, KH_2PO_4 1.5, $MgCl_2$ 1.7, HEPES 21, glucose 11.7, taurine 20, 2,3-butanedione monoxime 15, pH 7.15 adjusted with NaOH, and was bubbled with 100 % O_2.

For myocyte force measurements, activating solutions were prepared daily by mixing relaxing and maximal activating solutions containing (in mM): Na-acetate 10, phosphocreatine 12, imidazole 30, free Mg^{2+} 1, EGTA 10, Na_2ATP 3.3 and dithiothreitol 0.3 with either pCa 9.0 (relaxing solution) or pCa 4.5 (maximal activating solution), protease inhibitors (PMSF 0.5; leupeptin 0.04 and E64 [trans-epoxysuccinyl-1-leucine-guanidobutylamide] 0.01), pH 7.1 adjusted with KOH. Ionic strength was adjusted to 180 mM with K-acetate.

2.5. Biochemistry

Myosin heavy chain (MHC) isoforms were separated following a procedure adapted from Warren et al. {Warren, 2003 #41}. The myocytes were dissolved in a SDS lysis buffer (TRIS-HCl 50 mM, 2% (w/v) SDS, urea 8 M, EGTA 1 mM, EDTA1 mM, DTT 80 mM, 10% (v/v) glycerol, pH 6.8, protease inhibitors) and heated at 60°C for 5 min. Proteins (≈0.1 μg) were separated on a 6% SDS-PAGE at 16 mA constant current for 20 h at 4°C (Shelton Scientific gel system). The gels were silver stained and analysed with an imaging system (Kodak Image Station 2000R). β-MHC content was expressed relative to the total amount of the MHC protein.

Citrate synthase (CS) activity was assayed by measuring absorbance over 3 min at 412 nm at 25°C in soleus muscle homogenate. Muscle was first homogenized in buffer (in mM: 210 sucrose, 2

EGTA, 40 NaCl, 30 HEPES, 5 EDTA, and 2 PMSF, pH 7.4) and then mixed with 15 µM acetylCoA, 0.5 mM oxaloacetate, 0.1 mM DNTB {Srere, 1969 #48}.

2.7. Statistics

Data are presented as mean ± SEM. The effects of hypoxia and training were examined by using a 2-way analysis of variance (factor 1 : hypoxia vs normoxia, factor 2 : sedentary vs trained) with or without repeated measures (i.e. time or sarcomere length) depending on variables. The effect of the cellular localization on the stretch-dependent Ca^{2+} sensitization in the different conditions was tested by a 3-way ANOVA (Statview, Abacus concepts, Inc., Berkeley, CA). Post-hoc tests of Fisher's Protected Least Significant Difference were used when appropriate. Linear regression analyses performed on raw data examined relationships between ΔpCa_{50} and β-MHC content or passive tension. Comparison of linear regressions (Y intercepts and slopes) was examined using Statgraphics Centurion XV. Statistical significance was defined as $P \leq 0.05$.

3. Results

3.1 Training features and cardiac morphological parameters.

Efficiency of the training program in NT and HT rats was evaluated from maximal aerobic velocity (MAV) and skeletal muscle citrate synthase activity. Both increased significantly after training, irrespective of environmental conditions (Fig. 1A). LVSV increased significantly in NT but was unchanged in HT (Table 1). No changes were reported for N and H rats for all parameters.

Right ventricular mass increased significantly with hypoxic exposure. Left ventricular mass did not differ between the two sedentary groups, but it increased significantly to the same extent in the two groups after exercise training performed at sea level or high altitude (Table 1).

Echocardiographic morphological parameters were **affected differently** by training depending on environmental conditions (Fig. 1B). Namely, LVEDd increased in NT but decreased in HT rats. AWT and PWT **increased significantly** with training conducted under normoxic as well as hypoxic conditions. The cardiac remodelling was harmonious in NT rats (unchanged RWT values) but

disharmonious (marked increase in RWT highlighting a concentric LV hypertrophy) in HT rats. LV dimensions were not affected throughout the study period in N and H rats.

3.2. Stretch-dependent contractile properties

The Ca^{2+} sensitivity of myofilament activation (pCa_{50}) at 1.9 µm SL was similar across the ventricular wall in N, H and NT rats. However **Ca^{2+} sensitivity decreased significantly** at this SL in HT rats for both Epi and Endo cells (Fig. 2). Stretching cells to 2.3 µm SL induced a leftward shift of the tension-pCa curve in all groups, indicative of **increased** myofilament sensitivity to Ca^{2+}. For both Epi and Endo cells, Ca^{2+} sensitivity at 2.3 µm SL increased with training in normoxic conditions but decreased when training was performed in hypoxic conditions (Fig. 2C).

The stretch-dependent modulation of activation or Ca^{2+} sensitization (ΔpCa_{50}, difference in pCa_{50} obtained at 2.3 and 1.9 µm SL) is an important factor accounting for the Frank-Starling mechanism of the heart. It increased significantly with chronic exercise at sea level in Endo and in Epi cells (Fig. 3). Chronic hypoxia exposure did not **modify significantly** ΔpCa_{50} although a slight decrease was observed in Endo H cells compared to Endo N cells (p=0.09). High-altitude endurance training had no beneficial effect on stretch-dependent Ca^{2+} sensitization. Rather it even decreased in Endo cells from HT when compared to N and NT (Fig. 3 and Table 2). Maximal active tension did not differ between the various groups and myocardium localization (Table 2). The Hill coefficient increased significantly only in Epi from the HT group at both SL, but this effect was not investigated further.

3.3. Involvement of MHC isoform

The heart co-expresses two isoforms of MHC (α- or β-MHC). The change in α/β-MHC ratio influences cardiac function and occurs under various stresses including exercise (see for review {Schiaffino, 1996 #47}). As shown previously rat ventricles used in our study contained mostly α-MHC (Fig. 4A). After training in normoxic conditions, β-MHC expression was **reduced significantly** in both Epi and Endo cells. Chronic hypoxia exposure increased β-MHC expression particularly in Endo cells. β-MHC expression was similar in N and HT rats in both Endo and Epi cells. Thus, chronic exercise affected β-MHC expression in both myocardial layers and by chronic hypoxia mostly in Endo cells. Accordingly,

we investigated the relationship between the stretch-dependent Ca^{2+} sensitization and β-MHC expression level. A significant negative correlation was obtained in Endo and Epi when data from the 4 groups were pooled (Fig. 4B). When groups were analysed separately, the correlation between ΔpCa_{50} and β-MHC expression levels was still evident in N and NT rats (Fig. 4C). After normoxic training, ΔpCa_{50} was higher and associated with lower β-MHC expression in both myocardial layers**.** However under hypoxic conditions, the correlation was no longer obtained in Endo cells. **Values in Epi** cells from sedentary and trained rats were mixed and spread similarly from 20 to 40 % of β-MHC, resulting in similar average values.

3.4. Passive tension-dependent contractile properties.

In the relaxing solution Endo cells developed significantly higher passive tension than Epi cells after a stretch from slack length up to 2.3 µm SL (Table 2). **Chronic hypoxic exposure or exercise training did not affect the transmural gradient of passive tension**.

ΔpCa_{50} is closely related to passive tension rather than to sarcomere length {Cazorla, 2001 #13; Cazorla, 1999 #57} and there is a transmural gradient of stretch-sensitization {Cazorla, 2005 #1}. We thus established the relationships between ΔpCa_{50} and the passive tension developed at 2.3 µm SL by individual cells (Fig. 5). A positive correlation was found across the free wall in N and NT rats. Higher passive tensions and ΔpCa_{50} were obtained in NT when compared to N rat cells but individual values from the 2 groups were distributed along the same regression lines (Fig. 5A). A positive correlation between passive tension and ΔpCa_{50} was also observed for H rat cells. The regression line (slope and intercept) of H rats was not significantly different to the ones observed in N and NT rats although its slope was slightly lower (Fig. 5B). When rats were trained under a hypoxic environment, the relationship between passive tension and ΔpCa_{50} disappeared, mostly due to a diminished ΔpCa_{50} of Endo cells. When the stretch-dependence of Ca^{2+} sensitivity was indexed by $\Delta[Ca^{2+}]_{50}$ (*i.e.* difference in $[Ca^{2+}]_i$ required for half-maximal activation at 1.9 and 2.3 µm SL, Table 2) similar correlations were obtained between the groups (data not shown).

4. Discussion

To gain insight into the effects of exercise training at high altitude on cardiac function, we subjected rats to chronic hypobaric hypoxia and endurance training. Hypoxic stress had a moderate negative impact on the contractile properties of the myofilaments, affecting preferentially cardiomyocytes from the endocardium. The transmural gradient of cardiomyocyte contractile properties, classically observed within the normal ventricular wall, was improved by sea-level training but disappeared after high-altitude training. This depressed cardiac myofilament calcium responsiveness may contribute to the lack of improvement in ventricular function observed after high-altitude training.

4.1. Effect of training at sea level

Sea-level training improved the contractile properties of the myofilaments in both Endo and Epi layers. This is in accordance with previous studies reporting an increase in contraction and Ca^{2+} sensitivity in cardiomyocytes isolated from endurance-trained rats {Wisloff, 2001 #4; Diffee, 2001 #25}. Diffee & Nagle {Diffee, 2003 #6} reported an increase of the length-dependence of maximal and sub-maximal tension in LV cardiomyocytes, mostly due to increased Ca^{2+} sensitivity at long sarcomere length. Furthermore, we **reported recently** a gradient of contractile properties across the LV wall. After stretch, Endo cells developed more passive tension than Epi cells and the stretch-dependent Ca^{2+} sensitization of the myofilaments (ΔpCa_{50}) was higher {Cazorla, 2005 #1}. Sea-level training shifted the stretch-induced Ca^{2+} sensitization–passive tension relationships of Epi and Endo cells to higher values. Cells from NT rats were more responsive to stretch than those from N rats but the reasons for this improvement are still unclear (see below). These results may however account for the steeper slope of the active tension-SL relationship observed by Natali et al. {Natali, 2002 #12} in attached intact cardiac myocytes from trained rats .

The changes in cellular properties observed in the present study might contribute to the enhanced ventricular function (i.e. higher LV stroke volume) reported in NT rats. This is in line with

Reboul et al {Reboul, 2005 #7} who observed a greater increase in LVSV following volume overloading in endurance-trained rats compared to sedentary ones, indicative of an exercise-induced improvement of the Frank-Starling mechanism of the whole heart. In accordance with previous results from our group {Reboul, 2005 #7} and others {Wisloff, 2001 #4}, sea-level training induced LV hypertrophy, accounted for increased wall thickness and internal dimensions, with also important significance for cardiac performance. Thus, the enhanced LVSV in NT rats was likely due to greater ventricular filling associated with improved contractile properties of the myofibrils across the ventricular wall.

4.2. Effect of training at altitude

High-altitude training did not improve LVSV (Table 1). From the present results, we suggest that this is partly due to specific cardiac remodelling (reduced LVEDd) and deteriorations of the myofilament contractile properties. The marked wall thickening may be a compensatory mechanism for the depressed contractile function, allowing maintenance of the cardiac output and a response to the exercise-induced increase in energetic demand.

Chronic hypoxia exposure alone did not alter significantly Ca^{2+} sensitivity of the myofilaments. However high-altitude exercise training reduced the Ca^{2+} sensitivity of the myofilaments in both Epi and Endo cells whatever the sarcomere length. Moreover, the transmural gradient of stretch-induced Ca^{2+} sensitization of the myofilaments was **abolished completely** in HT rats. Whether haemodynamic factors and/or hypoxia per se are responsible for this effect cannot be ascertained. On one hand, assuming similar LV end-diastolic pressure in NT and HT rats and considering the cardiac remodelling in these 2 groups, lower end-diastolic wall stress is to be expected in HT when compared to NT rats. On the other hand, we could postulate that episodes of severe myocardium hypoxemia occurred as a result of exercise at high-altitude thus affecting the contractile machinery. In this context, it is interesting to note that similar alterations in cardiomyocyte contractile properties were obtained previously by our group in a rat model of chronic myocardial ischemia {Cazorla, 2005 #1}**.** Long-term exposure to high-altitude hypoxemia of foetal sheep has also been reported to decrease contraction of intact papillary muscle {Browne, 1997 #19}. Finally, 4 weeks of chronic hypoxia has been shown to **reduce Ca^{2+} transient** in association with impaired Ca^{2+} release and uptake in rat cardiomyocytes {Pei,

2003 #16}. The loss of the contractile transmural gradient in HT rats should contribute to the lack of improvement in LVSV after training at simulated high altitude. Similarly the present cellular data can account for the lack of improvement after high-altitude training in the Frank-Starling mechanism investigated with a volume overloading protocol {Reboul, 2005 #7}.

4.3. Molecular basis of the stretch-dependent Ca^{2+} sensitivity

The stretch-induced Ca^{2+} sensitization of the myofilaments relates to passive tension rather than to SL {Cazorla, 2001 #13; Cazorla, 1999 #57; Cazorla, 2005 #1}. This relationship across the wall was enhanced in NT rats but no longer existed in HT rats. Over the physiological range of SL, titin is the major component of the cellular passive properties {Cazorla, 2001 #13}. No change in titin content or isoform expression was observed in the present study (data not shown). Although not significant, the opposite passive tension changes observed in NT and HT rats could be due to other mechanisms that may have participated in the fine tuning of passive tension-dependent modulation of activation across the wall such as PKA phosphorylation, titin-actin interaction or Ca^{2+} binding on titin {Granzier, 2004 #1004} that may directly and/or indirectly affect the properties of the contractile and/or regulatory proteins.

β-MHC is characterized by lower adenosine triphosphatase activity and lower filament sliding velocity, but can generate cross-bridge force with a higher economy of energy consumption than α-MHC {Holubarsch, 1985 #37}. In accordance with the present data, previous {Pagani, 1983 #50; Jin, 2000 #51; Hwang, 2005 #53} but not all {Farrar, 1988 #52; Diffee, 2003 #49} studies have shown increased α–MHC isoform expression after sea-level training. **Therefore, increased in α-MHC isoform expression could be at least in part responsible for the enhanced stretch-dependent Ca^{2+} sensitization of the myocytes in NT rats**. In the present study, we did find a correlation between the level β-MHC and the stretch-dependent mechanism at sea level. However, Endo cells from N and HT rats had similar β-MHC content but different ΔpCa_{50}. This suggests that other proteins are very likely responsible for the changes in Ca^{2+} sensitivity observed in our study. In a previous publication {Cazorla, 2005 #1} we observed in normoxic sedentary rats that Endo cells had a higher ΔpCa_{50} than Epi cells due to a higher phosphorylation level of myosin-light chain 2 (MLC2*) after stretch. In post-myocardial infarcted rats, the MLC2* was no longer phosphorylated after stretching

and was associated with reduced ΔpCa_{50} in Endo cells. It is thus possible that the level of phosphorylation of MLC-2 had been increased in NT rats and decreased in HT rats, contributing thus to the Ca^{2+} sensitivity results of the present study. The slight decrease in passive tension and the rightward shift of the tension-pCa curves in HT rats may be due to an increase in PKA-dependent stimulation {Cazorla, 2006 #56}. The levels of phosphorylation of MLC2 and other regulatory proteins such as TnI and cMyBP-C before and after stretch have to be explored.

In conclusion, nonuniformity is a major characteristic of the normal adult left ventricle that is partly due to heterogeneous contractile properties of the myocytes across the wall. The major mechanical adaptations to **sea-level training improved the transmural gradient** of stretch-dependent Ca^{2+} sensitization of the myofilaments. This cellular mechanism should improve the Frank-Starling relationship **solicited particularly** during exercise. High-altitude training induced a reduction of Ca^{2+} sensitivity of the myofilaments and stretch-dependent Ca^{2+} sensitization mostly in the Endo layer, which might have contributed to the lack of improvement of the ventricular function observed in vivo.

Acknowledgments

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References

Figures and Legends

Fig. 1. Training features and left ventricular morphological characteristics of sedentary and trained rats maintained in normoxic or hypoxic conditions. (A) Maximal aerobic velocity (MAV) is expressed as the relative variation between pre- and post-protocol (left). Citrate syntase activity is expressed as $IU.g^{-1}$ wet weight (right). (B) Morphological parameters were measured by echocardiography and data are expressed as the relative variation between pre- and post-protocol for the LV end-diastolic diameter (LVEDd), end-diastolic anterior wall thickness (AWT), and relative wall thickness (RWT). (* $P<0.05$)

Fig. 2. Effects of training in normoxia or hypoxia on length-dependency of the active tension in isolated skinned cardiomyocytes**.** Tension (normalized to maximal tension)-pCa curves of left ventricular myocytes isolated from sup-epicardium (close symbols, left) or sub-endocardium (open symbols, right) were measured at 1.9 (continuous line) and 2.3 (dashed line) µm sarcomere length, in sedentary (N, H) and trained (NT, HT) rats maintained for 5-weeks in normoxic (A) or hypoxic (B) environments. (N: ■□, NT: ●○, H: ▲Δ, HT: ★✳, with Epi (close) and Endo (open)) (C) Ca^{2+} sensitivities determined at 1.9 (gray bar) and 2.3 (open bar) µm SL by the measurement of pCa_{50} (pCa for half-maximal activation) in individual Epi (left) and Endo (right) cells. The number of cells in each condition is indicated in Table 1. (* $P<0.05$)

Fig. 3 Stretch-dependent Ca^{2+} sensitization (ΔpCa_{50}) in cells isolated from sub-epicardium (left) or sub-endocardium (right) in normoxic (N) or hypoxic (H) sedentary and trained (NT-HT hatched pattern) rats. (3-way ANOVA,* $P<0.05$)

Fig. 4. Myosin heavy chain expression in cardiomyocytes isolated from sedentary and trained rats maintained in normoxic (open bar) or hypoxic (grey bar) conditions. (A) SDS-PAGE for α- and β-MHC of myocytes isolated from the left ventricle. β-MHC content was expressed relative to the total MHC in

Epi and Endo in each group (n= 4 hearts per group analysed in duplicate). * $P<0.05$. (B) Correlation between the ΔpCa_{50} and the relative amount of β-MHC. Linear regressions were calculated on a scatter plot including all raw data points from Epi (line, ■) and Endo (dash line, ○) cells isolated from N, NT, H, and HT rats. A significant negative correlation was found in Epi ($y=0.23-0.0020x$, $r^2=-0.44$, $P=0.005$) and Endo ($y=0.21-0.0025x$, $r^2=-0.53$, $P=0.01$) cells for the overall population. (C) The relationship between ΔpCa_{50} and β-MHC content was analysed in populations of cells obtained from rats exposed and/or trained either under normoxic (left) or hypoxic (right) environments. (N: ■□, NT: ●○, H: ▲Δ, HT: ★✲,, Epi (close symbols, continuous line) and Endo (open symbols, dashed line)). A significant negative correlation was found in normoxic Epi ($y=0.22-0.0045x$, $r=-0.48$, $P=0.03$), in normoxic Endo ($y=0.25-0.0036x$, $r=-0.46$, $P=0.03$), and in hypoxic Epi ($y=0.25-0.0038x$, $r=-0.58$, $P=0.007$) cells. No correlation was found in hypoxic Endo cells ($y=0.14-0.0003x$, $r=0.06$, $P=0.79$).

Fig. 5. Correlation between stretch-induced Ca^{2+} sensitization (ΔpCa_{50}) and passive tension across the wall. ΔpCa_{50} was plotted against mean passive tension for each tissue area. Symbols and error bars correspond to each group of cell (Endo: open symbols, Epi: close symbols) in sedentary (square for N and triangle for H) and trained (circle for NT and star for HT) rats living in normoxic (A) or hypoxic (B) conditions. Linear regressions were calculated on a scatter plot including all **raw data points** from both myocardial layers in each condition (line for sedentary, dash line for trained). Exercise increased ΔpCa_{50} and passive tension but values are distributed over the same linear relationship in N ($y=0.036+0.017x$, $r=0.67$ $p=0.001$) and NT rats ($y=0.069+0.016x$, $r=0.78$ $p=0.001$). Stretch-dependent sensitization was decreased by chronic hypoxia exposure ($y=0.071+0.010x$, $r=0.47$ $p=0.02$). No correlation was found in HT cells ($y=0.120+0.003x$, $r=0.21$ $p=0.38$).

Figure 1

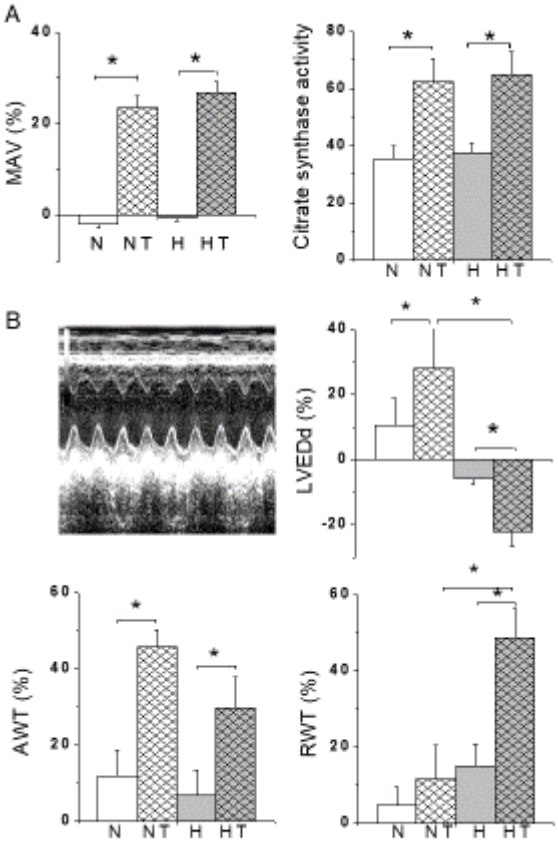
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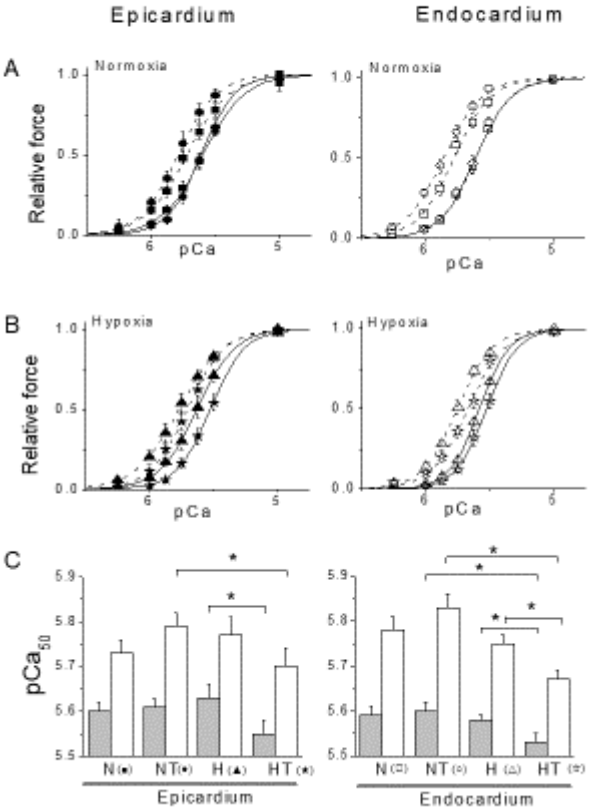
Figure 2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HT(☆)

Figure 3

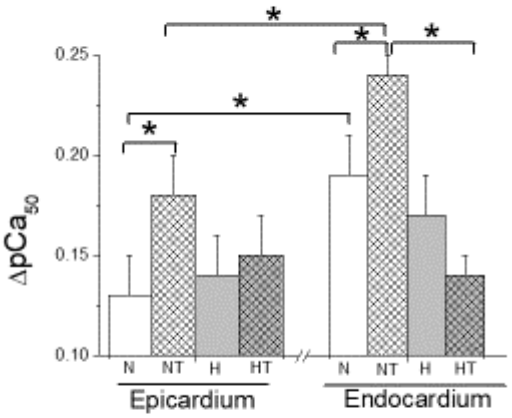

*
*
*
*
*
0.25
0.20
0.15
0.10
ΔpCa50
N
NT
H
HT
N
NT
H
HT
Epicardium
Endocardium

Figure 4

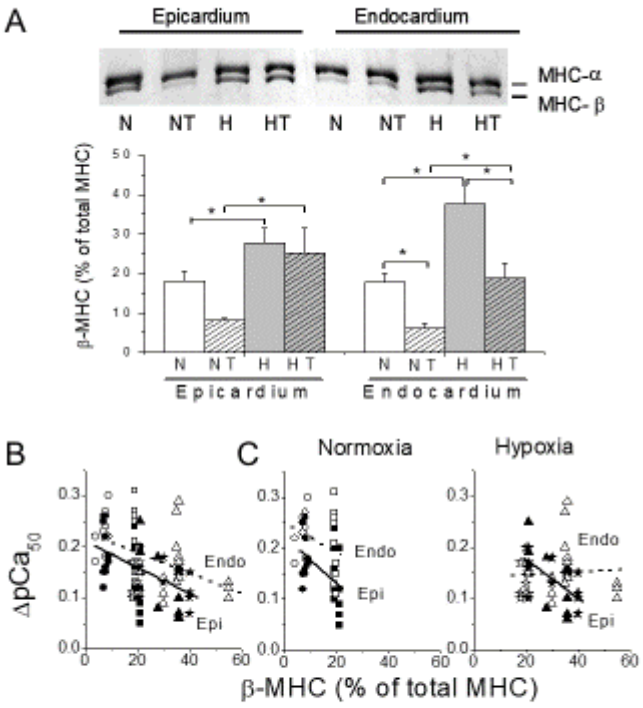
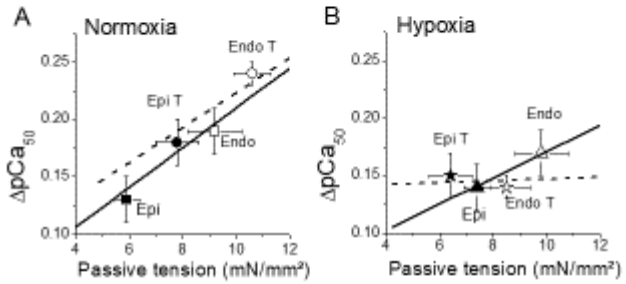

A
Epicardium
Endocardium
MHC-α
MHC-β
N NT H HT N NT H HT
β-MHC (% of total MHC)
N NT H HT N NT H HT
Epicardium
Endocardium
B
C
Normoxia
Hypoxia
ΔpCa50
Endo
Epi
β-MHC (% of total MHC)

Figure 5


A
Normoxia
Endo T
Epi T
Endo
Epi
ΔpCa50
0.25
0.20
0.15
0.10
4
6
8
10
12
Passive tension (mN/mm²)
B
Hypoxia
Endo
Epi T
Epi
Endo T
ΔpCa50
0.25
0.20
0.15
0.10
4
6
8
10
12
Passive tension (mN/mm²)