International Journal of Cardiology 253 (2018) 113–119

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Chronic exercise induces pathological left ventricular hypertrophy in adrenaline-deficient mice

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ARTICLE INFO

Article history:
Received 6 February 2017
Received in revised form 28 August 2017
Accepted 2 October 2017

Keywords:
Adrenaline
Blood pressure
Left ventricular hypertrophy
Chronic exercise, adrenaline-deficient mice
Phenylethanolamine-*N*-methyltransferase-knockout mice

ABSTRACT

Adrenaline-deficient phenylethanolamine-*N*-methyltransferase-knockout mice (Pnmt-KO) have concentric heart remodeling and though their resting blood pressure is normal, it becomes higher during acute exercise. The aim of this study was to evaluate cardiac morphological, functional and molecular alterations after chronic exercise in adrenaline-deficient mice.

Genotypes at the Pnmt locus were verified by polymerase chain reaction (PCR) of ear samples of Pnmt-KO and wild-type (WT) mice. These mice were submitted to chronic exercise training during 6 weeks. Blood pressure was determined by a photoelectric pulse detector. Mice were anesthetized and cardiac morphology and function were evaluated by echocardiography and hemodynamics. IGF-1, IGF-1R, ANP and BNP mRNA were quantified by real-time PCR in left ventricle (LV) samples.

Pnmt-KO mice showed increased systolic blood pressure compared with WT mice. A significant increase was found in LV mass, and LV posterior wall thickness in trained Pnmt-KO compared to trained WT mice, without significant differences in LV volumes. Acute β_1-adrenergic stimulation with dobutamine increased systolic function indexes in WT mice, but not in Pnmt-KO mice. LV expression of IGF-1 and ANP was increased in trained Pnmt-KO mice when compared to trained WT mice.

In conclusion, in response to chronic exercise adrenaline-deficient Pnmt-KO mice show concentric LV hypertrophy and impaired response to dobutamine, suggesting an initial stage of pathological cardiac hypertrophic remodeling. These results support the need for an efficient partial conversion of noradrenaline into adrenaline for prevention of blood pressure overshoot and thus pathological cardiac hypertrophic remodeling in chronic exercise.

1. Introduction

Mass activation of sympathetic neurons, with adrenaline and noradrenaline release, is the physiological basis of the fight-or-flight response. This response enables adaption to a stressful event, culminating in increased heart rate and blood pressure, enhanced energy mobilization, and neural reflexes [1]. A hyperactive sympathetic system is associated with various pathological conditions, in particular cardiovascular diseases [2–4].

Noradrenaline from post-ganglionic nerve terminals, and noradrenaline and adrenaline from the adrenal medulla, are the main endogenous catecholamines acting on adrenoceptors in the blood vessels to adjust arteriolar resistance and venous capacitance, and in the heart to regulate cardiac function [5]. The final step in catecholamine biosynthesis is the conversion of noradrenaline to adrenaline by phenylethanolamine-*N*-methyltransferase (Pnmt), a cytoplasmic enzyme [6,7]. Adrenaline synthesis (and Pnmt) is also present in some tissues outside of the adrenal medulla, such as the heart [8,9].

It has been difficult to decipher the role of adrenaline. Adrenal medullectomy can damage the adrenal cortex, altering the release of corticosteroids, and it also impairs the release of other adrenal amines and peptides, such as noradrenaline, chromogranin A, catestatin and

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https://doi.org/10.1016/j.ijcard.2017.10.014
0167-5273/

neuropeptide Y [10]. An alternative methodology is the use of Pnmt inhibitors to prevent adrenaline synthesis *in vivo* [11], but most of them also block monoamine oxidase [12] and α-adrenoceptors [13].

Nowadays, loss-of-function studies are widely used to clarify the role of a specific gene or protein. The Pnmt knockout (Pnmt-KO) mice ($Pnmt^{-/-}$) is an adrenaline-deficient mice model that allows to clarify the role of adrenaline [8,14]. These animals are viable and fertile, and they do not display gross abnormalities [8]. They show absent adrenaline in the adrenal medulla, heart, and plasma [14–16]. On simple echocardiographic evaluation, Pnmt-KO mice seem to present lower filling volumes and cardiac output, as well as increased wall thickness, suggesting a pattern of cardiac remodeling [14]. However, a precise pressure-volume hemodynamic evaluation with gold standard load-independent indexes of contractility and diastolic function is lacking.

In response to acute stress induced by restraint or treadmill exercise, as well as after denervation by ganglionic blockade, Pnmt-KO mice show impaired vasodilator response, which could be due both to lack of adrenaline [17] and abrogated β_2-adrenoceptor signaling [16]. Although blood pressure was normal at rest in Pnmt-KO mice, an exaggerated blood pressure response was demonstrated during acute treadmill exercise [14]. However, the role of adrenaline in response to chronic exercise training has also not been elucidated. The aim of this study is to evaluate cardiac morphological, functional and molecular alterations after chronic exercise training in adrenaline-deficient mice.

2. Methods

2.1. Animals

All animal care and experimental procedures were performed in accordance with the European Directive 63/2010/EU, transposed to the Portuguese legislation by the Directive Law 113/2013. Ten week-old wild type (WT, $Pnmt^{+/+}$, n = 20) and Pnmt-KO ($Pnmt^{-/-}$, n = 17) male mice (129 × 1/SvJ) were kept in cages under controlled environmental conditions (12 h light/dark cycle, room temperature 23 ± 1 °C, humidity 50%, autoclaved drinking water, and mice breeding diet 4RF25/I; Ultragene, Porto, Portugal), and housed with the respective litter. Pnmt-KO mice were produced by disruption of Pnmt locus by insertion of Cre-recombinase in exon 1 [8]. A couple of Pnmt-KO mice were kindly provided by Steven N. Ebert and animals were bred in our conventional vivarium. Genotyping was performed by PCR amplification analysis of the Pnmt gene in ear samples.

2.2. Chronic exercise training

WT and Pnmt-KO mice were randomly allocated to chronic exercise training or their regular physical activity. Before each training session, animals were allowed to adapt to the treadmill for 15 min. Exercise training was performed on a motor treadmill (Panlab Harvard Apparatus, Barcelona, Spain) at the same time of the day, 5 days/week for 6 weeks. Exercise started at 6 cm/s for 10 min, with an increase in speed of 3 cm/s every 2 min until 20 m/min, for 55 min [18]. Training was interrupted if exhaustion occurred, as defined by permanence in the shock grid (0.2 mA) for >5 consecutive seconds or the third time that the animal stays 2 or more seconds in the shock grid (without any attempt to return to the treadmill).

2.3. Blood pressure and heart rate measurements

Systolic and diastolic blood pressure, and heart rate were measured in conscious restrained animals, in a temperature control box at 37–38 °C, using a photoelectric tail-cuff pulse detector (Kent Scientific, CT, USA), as previously described [19]. Four determinations were made and averaged.

2.4. Echocardiographic and hemodynamic evaluation

Upon sedation and analgesia with fentanyl (50 μg/kg, i.p.) and midazolam (5 mg/kg, i.p.), mice were anesthetized by inhalation of 8% sevoflurane in vented containers, orotracheally intubated (20G) and mechanically ventilated using a MouseVent™ Automatic Ventilator (Physiosuite, Kent Scientific, CT, USA). Anesthesia was maintained with sevoflurane (2.5–3.5%) titrated according to the toe pinch reflex. Mice were placed in left-lateral decubitus on a heating pad, the electrocardiogram was monitored (Animal Bio Amp, FE136, ADInstruments, Dunedin, New Zealand) and their temperature was automatically kept at 38 °C (RightTemp™ Temperature Monitor & Homeothermic Controller, Physiosuite, Kent Scientific, CT, USA). The peripheral oximetry (MouseSTAT™ - Pulse Oximeter & Heart Rate Monitor, Physiosuite, Kent Scientific, CT, USA), capnography, respiratory rate and minute ventilation (CapnoScan™ - End-Tidal CO_2 Monitor, Physiosuite, Kent Scientific, CT, USA) were continuously motorized. Cardiac ultrasound was performed using a 15 MHz linear probe (15 MHz ACUSON™ Sequoia 15L8W, Siemens Medical Solutions, CA, USA) on an ultrasound system (ACUSON Sequoia™ C512, Siemens Medical Solutions, CA, USA). Before applying pre-warmed echocardiographic gel, the chest was shaved and depilated. LV end-systolic and end-diastolic cavity dimensions and wall thickness were measured using M-mode tracings and 2-D echocardiography, just above the papillary muscles in parasternal short-axis view. LV volumes, ejection fraction (EF) and fractional shortening (FS) were calculated according to Teichholz formula, and LV mass was determined in diastole through an uncorrected cube method [20]. The parasternal long axis view was used to measure LV long axis, from the mitral annulus to LV endocardial surface at the apex, and to record aortic root dimensions in M-mode. Pulsed-wave Doppler velocity tracings in the 4-chamber view allowed an assessment of LV filling with the peak early (E) and late (A) wave velocities of mitral inflow. Peak early diastolic (E′) and systolic (S′) mitral annular velocities were measured with tissue Doppler (TDI) at the lateral mitral annulus, and the E/E′ ratio was calculated. All the recordings were averaged from three consecutive heartbeats.

For hemodynamic evaluation, the right jugular vein was catheterized (24G) under surgical microscopy for fluid replacement with warm Ringer's lactate solution at 64 ml/Kg/h (NE-1000, New Era Pump Systems, NY, USA). After left thoracotomy, a pressure volume (PV) catheter (PV-1035, Millar Instruments, TX, USA), was inserted through the apex in left ventricle (LV) and signals were continuously acquired (MPVS 3000, Millar Instruments) and digitized at 1000 Hz (ML880 PowerLab 16/30, ADInstruments, Dunedin, New Zealand). Parallel conductance was assessed by injection of 10 μl of 10% hypertonic saline and slope factor α was derived by the measurement of cardiac output (CO) with echocardiography, immediately before. Baseline recordings were obtained after a stabilization period of 30 min and inferior vena cava occlusion with a 5–0 silk lace was also obtained to derive load-independent indexes of contractility and compliance by linear and exponential fitting of the end-systolic and end-diastolic PV relationships (ESPVR and EDPVR). After an intravenous infusion of dobutamine (β_1-adrenoceptor agonist, 5 μg/Kg/min), inferior vena cava occlusion recordings were repeated upon obtaining a stable effect of at least 10 min. All acquisitions were performed with ventilation suspended at end-expiration. Echocardiographic and hemodynamic stroke volume (SV) and CO were defined as SV = LVEDV − LVESV (LVEDV, LV end-diastolic volume; LVESV, LV end-systolic volume) and CO = HR × SV. EF was defined as EF = (LVEDV − LVESV)/LVEDV × 100 [14]. To account for large differences in body weight between groups, some parameters were indexed for body surface area (BSA) (LVM_i, $LVEDV_i$, SV_i and cardiac index), as estimated by 9.82 * body $weight^{2/3}$ in grams [21]. Indexed systemic vascular resistances (SVRI) were calculated by dividing end-systolic pressure (used as surrogate of mean blood pressure [22]) and cardiac index, neglecting right atrial pressure. Upon completion of experiments, animals were euthanized by exsanguination under anesthesia and hearts were removed and dissected. LV samples were collected and snap frozen in liquid nitrogen and stored at −80 °C.

2.5. RNA isolation and relative quantification of mRNA expression

Real-time PCR was performed in LV samples, as previously detailed [23]. In brief, samples were homogenized using the MagNA Lyser Instrument homogenizer (Roche Diagnostics, Basel, Switzerland). Total RNA isolation was carried out with the SV Total RNA Isolation System kit (Promega, WI, USA). Concentration and purity of the isolated RNA were measured using the NanoDrop 2000 spectrophotometer (Thermo Scientific, MA, USA). Reverse transcription was performed in a T100™ Thermal Cycler (Bio-Rad, CA, USA). A StepOne™ real-time PCR System (Applied BioSystems, MA, USA) was used in real-time PCR experiments. For each studied mRNA molecule, standard curves were generated from the correlation between the amount of starting total mRNA and PCR threshold cycle of graded dilutions from a pool of all samples. Maxima SYBR Green qPCR Master Mix (2×) (Thermo Scientific, MA, USA), Nuclease-free H_2O (Thermo Scientific, MA, USA) and gene specific primers (5 μM) were mixed before adding cDNA (1:20). Instead of cDNA, Nuclease-free H_2O (Thermo Scientific, MA, USA) was added as negative control. Gene specific primers were as follows: Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) FWD, CCATCACCATCTTCCAGGAG; GAPDH REV, GCATGGACTGTGGTCATGAG; type-A natriuretic peptide (ANP) FWD, AGGCAGTCGATTCTGCTTGA; ANP REV, CGTGATAGATGAAGGCAGGAAG; type-B natriuretic peptide (BNP) FWD, TAGCCAGTCTCCAGAGCAATTC; BNP REV, TTGGTCCTTCAAGAGCTGTCTC; insulin-like growth factor-1 (IGF-1) FWD, GAAGTCCCCGTCCCTATCGA; IGF-1 REV, CCTTCTCCTTTGCAGCTTCG; IGF-1 receptor (IGF-1R) FWD, AGTGACTCGGATGGCTTCGTT; IGF-1R REV, TTTCACAGGAAGCTCGCTCTC. GAPDH mRNA levels were similar in all experimental groups, which enabled the use of this gene as an internal control. Results of mRNA quantification are expressed in an arbitrary unit (AU) after normalization for GAPDH.

2.6. Statistics

All results are expressed as mean ± standard error of the means (SEM) for the indicated number of determinations. Student's unpaired *t*-test was applied to data regarding distance and total time run during chronic exercise training. Other results were assessed by two-way ANOVA and two-way repeated measures ANOVA. Residuals normality was checked by Shapiro-Wilk's test, homogeneity of variances by Levene's or Box's M test. *Post hoc* analysis was performed using Newman-Keuls' test. For the ESPVR and EDPVR analysis, volume intercept and scaling constant were included as covariates, respectively. Log transformation of variables was applied whenever necessary to overcome violation of assumptions. Comparisons were made using *STATISTICA* (StatSoft, Inc., OK, USA) and

GraphPad Prism statistics (GraphPad Software Inc., CA, USA) software. Statistical significant was set at $P < 0.05$.

3. Results

3.1. Exercise performance was normal in Pnmt-KO mice

No differences were found in total running distance (11,440 ± 2620 *vs* 13,166 ± 991 cm) or time (1031 ± 157 *vs* 1135 ± 57 min), during 6 weeks, between chronic exercise-trained Pnmt-KO and WT mice.

3.2. Systolic and diastolic blood pressure increased in Pnmt-KO mice

Pnmt-KO mice showed increased systolic and diastolic blood pressure compared with WT mice (main effect of genetic background, Table 1).

3.3. LV hypertrophy induced by chronic exercise in Pnmt-KO mice was the most remarkable

A main effect of chronic exercise was observed in body weight (Table 1). Both indexed LV mass (LVM_i and also LVM, Table 1) and the ratio between LV mass and LV end-diastolic volume (LVM/LVEDV, Table 1) increased in both chronically trained groups showing LV hypertrophy. In addition, LV hypertrophy in trained Pnmt-KO mice was higher than in trained WT mice (interaction between genetic background and chronic exercise in LVM_i, LVM, LVM/LVEDV and LVPW; Table 1). Moreover, LV end-diastolic posterior wall thickness (LVPW, Table 1), interventricular end-diastolic septal thickness (IVS, Table 1) and indexed LV end-diastolic volume ($LVEDV_i$ and also LVEDV, Table 2) increased with chronic exercise.

3.4. Pnmt-KO mice's heart works at lower volumes and cardiac index

On echocardiographic parameters, no gross differences in systolic (EF, S′) or diastolic (E/E′) function was observed in either trained or untrained Pnmt-KO and WT mice (Table 1). On hemodynamic evaluation, where load-independent gold standard left ventricular function indexes can be derived, in untrained groups we also did not find differences in contractility, as assessed by end-systolic elastance (E_{ES} and $E_{ES}i$) or preload-recruitable stroke work (PRSW, Table 2), or relaxation, as assessed by time constant of isovolumetric relaxation τ (Table 2). Nevertheless, we did observe decreased indexed LV end-diastolic volume ($LVEDV_i$ and also LVEDV), and upward-shifted end-diastolic pressure volume relationships (β_i) in Pnmt-KO, compared with WT mice (main effect of genetic background, Table 2). Moreover, systemic vascular resistance index (SVRI and also SVR) was higher in untrained Pnmt-KO mice, while indexed stroke volume (SV_i and also SV) and cardiac index (and also CO) were lower compared with WT mice (Table 2).

3.5. Chronic exercise training restores volumes and cardiac index in Pnmt-KO mice

Chronic exercise training increased both heart rate and left ventricular maximum developed pressure (P_{max}), while it enhanced relaxation (time constant of isovolumetric relaxation τ) (main effect of chronic exercise, Table 2). After chronic exercise, left ventricular compliance was also improved, as denoted by higher indexed LV end-diastolic volume ($LVEDV_i$ and also LVEDV), lower end-diastolic pressure (EDP) and downward-shifted end-diastolic pressure-volume relationship (β_i) (main effect of chronic exercise, Table 2). Most importantly, chronic exercise training remarkably increased indexed stroke volume (SV_i and also SV) and cardiac index (and also CO) in Pnmt-KO mice (interaction between genetic background and chronic exercise, Table 2).

3.6. Pnmt-KO mice respond worse to dobutamine stimulation

Compared to basal parameters, acute β_1-adrenergic stimulation with dobutamine increased cardiac index (Fig. 1A) and ventricular-vascular coupling index (VVC_i, Fig. 1B) only in WT mice. It was observed in both parameters an interaction between genetic background and dobutamine ($P < 0.05$).

3.7. LV IGF-1 and ANP mRNA expression increase in Pnmt-KO mice after chronic exercise

In the LV, mRNA expression of IGF-1 (Fig. 2A) and ANP (Fig. 2C) was significantly increased in trained Pnmt-KO mice, when compared to trained WT, and to untrained Pnmt-KO mice. An interaction between genetic background and chronic exercise was observed for these genes ($P < 0.05$). No differences were observed in IGF-1R (Fig. 2B) and BNP (Fig. 2D) mRNA expression.

4. Discussion

The insertion of Cre-recombinase into the Pnmt locus created Pnmt-KO mice, which cannot produce adrenaline [8,15,16]. We originally report the adaptions of Pnmt-KO mice to chronic exercise training using invasive pressure-volume hemodynamics and load-independent

Table 1
Body weight, blood pressure and echocardiography data in chronic exercise trained and untrained phenylethanolamine-*N*-methyltransferase-knockout (Pnmt-KO) and wild type (WT) mice.

	Untrained		Trained		Main effects		Interaction
	WT	Pnmt-KO	WT	Pnmt-KO	Genetic background	Chronic exercise	GB * CE
BW, g	29.0 ± 0.5	27.1 ± 0.3	29.9 ± 0.9	30.5 ± 1.4	*NS*	<0.01	*NS*
SBP, mmHg	126.8 ± 4.4	135.6 ± 7.0	112.1 ± 6.4	135.1 ± 5.1	<0.01	*NS*	*NS*
DBP, mmHg	99.6 ± 4.6	113.2 ± 8.0	85.8 ± 6.6	107.8 ± 5.3	<0.01	*NS*	*NS*
LVM, mg	33 ± 3	24 ± 1	50 ± 6*	71 ± 11*†	*NS*	<0.0001	<0.01
LVM_i, mg/cm^2	0.35 ± 0.03	0.27 ± 0.01	0.53 ± 0.06*	0.73 ± 0.13*†	*NS*	<0.0001	<0.01
LVM/LVEDV, mg/μl	0.39 ± 0.03	0.37 ± 0.03	0.61 ± 0.07*	0.97 ± 0.10*†	<0.01	<0.0001	<0.001
LVPW, mm	0.46 ± 0.02	0.48 ± 0.01	0.60 ± 0.03*	0.72 ± 0.03*†	<0.01	<0.0001	<0.05
IVS, mm	0.46 ± 0.02	0.51 ± 0.03	0.59 ± 0.03	0.69 ± 0.10	*NS*	<0.001	*NS*
EF, %	84.4 ± 2.3	87.8 ± 1.4	86.5 ± 2.6	80.0 ± 2.1	*NS*	*NS*	*NS*
S′, cm/s	5.0 ± 0.3	4.9 ± 0.3	5.0 ± 0.3	4.2 ± 0.7	*NS*	*NS*	*NS*
E/E′	15.2 ± 1.2	16.3 ± 1.4	17.0 ± 1.0	20.1 ± 3.3	*NS*	*NS*	*NS*

BW, body weight; SBP, systolic blood pressure; DBP, diastolic blood pressure; LVM, left ventricular mass; LVM_i, left ventricular mass indexed for body surface area; LVEDV, left ventricular end-diastolic volume; LVPW, left ventricular end-diastolic posterior wall thickness; IVS, interventricular end-diastolic septal thickness; EF, ejection fraction; S′, maximum systolic tissue Doppler velocity at the lateral mitral annulus; E/E′, ratio between peak velocity of early filling in transmitral flow pulsed-wave Doppler and maximum velocity of early diastolic myocardial motion at the lateral mitral annulus; NS, non-significant. Values are means ± SEM of n = 5–13 animals per group; * *vs* untrained mice with similar genetic background ($P < 0.05$); † *vs* WT mice with similar chronic exercise ($P < 0.05$).

Table 2
Hemodynamic parameters in chronic exercise trained and untrained phenylethanolamine-*N*-methyltransferase-knockout (Pnmt-KO) and wild type (WT) mice.

	Untrained		Trained		Main effects		Interaction
	WT	Pnmt-KO	WT	Pnmt-KO	Genetic background	Chronic exercise	GB * CE
LVEDV, μl	74.1 ± 4.6	49.1 ± 5.8	85.6 ± 7.5	79.5 ± 4.4	<0.05	<0.05	*NS*
$LVEDV_i$, μl/cm^2	0.79 ± 0.04	0.56 ± 0.07	0.88 ± 0.07	0.84 ± 0.06	<0.05	<0.05	*NS*
E_{ES}, mmHg/μl	1.4 ± 0.1	1.9 ± 0.3	1.5 ± 0.1	1.7 ± 0.2	*NS*	*NS*	*NS*
$E_{ES}i$, mmHg/μl/cm^2	133 ± 11	168 ± 23	149 ± 13	160 ± 18	*NS*	*NS*	*NS*
PRSW, mmHg	59 ± 7	62 ± 7	48 ± 4	59 ± 6	*NS*	*NS*	*NS*
τ, ms	5.8 ± 0.5	6.0 ± 0.6	4.8 ± 0.2	4.2 ± 0.1	*NS*	<0.05	*NS*
β_I, μl/cm^2	6.1 ± 0.8	8.5 ± 0.5	4.2 ± 0.3	6.1 ± 1.4	<0.05	<0.05	*NS*
SVR, mmHg/μl.min	0.0033 ± 0.0002	0.0052 ± 0.0007†	0.0038 ± 0.0001	0.0036 ± 0.0002	*NS*	*NS*	<0.05
SVRI, mmHg/μl.min/cm^2	0.31 ± 0.02	0.47 ± 0.06†	0.37 ± 0.01	0.35 ± 0.02	*NS*	*NS*	<0.05
SV, μl	48.3 ± 3.5	33.6 ± 3.4†	43.2 ± 1.6	45.4 ± 2.1*	*NS*	*NS*	<0.05
SV_i, μl/cm^2	0.51 ± 0.04	0.38 ± 0.01†	0.45 ± 0.01	0.48 ± 0.03*	*NS*	*NS*	<0.05
CO, μl/min	24,700 ± 1600	17,400 ± 1700†	25,500 ± 700	27,100 ± 1100*	*NS*	<0.05	<0.05
Cardiac index, μl/min/cm^2	262.5 ± 15.4	197.1 ± 20.1†	264.1 ± 6.6	284.4 ± 23.5*	*NS*	<0.05	<0.05
Heart rate,/min	518 ± 20	522 ± 17	592 ± 11	600 ± 17	*NS*	<0.01	*NS*
$P_{max,}$ mmHg	90 ± 3	90 ± 3	103 ± 1	104 ± 3	*NS*	<0.001	*NS*
EDP, mmHg	6.3 ± 0.8	7.2 ± 0.6	5.4 ± 0.9	4.2 ± 0.3	*NS*	<0.05	*NS*

Values of β_I were adjusted for the scaling constant covariate. i, indexed; LVEDV, left ventricular end-diastolic volume; E_{ES}, end-systolic elastance derived from end-systolic pressure-volume relationship; PRSW, preload recruitable stroke work; τ, time constant of isovolumetric relaxation derived by the monoexponential method; β_I, left ventricular chamber stiffness constant derived from end-diastolic pressure-volume relationship; SVR, systemic vascular resistance; SV, stroke volume; CO, cardiac output; P_{max}, maximum pressure; EDP, end-diastolic pressure; NS, non-significant. Values are means ± SEM of 5–10 animals per group. * *vs* untrained mice with similar genetic background ($P < 0.05$); † *vs* WT mice with similar chronic exercise ($P < 0.05$).

indexes of cardiac performance. Additionally, we report acute *in vivo* responses to β_1-adrenergic stimulation. We show that lack of adrenaline in Pnmt-KO mice associates with decreased compliance and lower filling volumes which jeopardize cardiac output, despite of preserved contractility indexes. Chronic exercise training restores filling volumes and cardiac index in Pnmt-KO mice at the expense of marked LV hypertrophy, with overexpression of markers of both physiological and pathological hypertrophy, IGF-1 and ANP, respectively. Moreover, Pnmt-KO mice revealed poor response to improve cardiac index and ventricular-vascular coupling (VVC_i) to acute *in vivo* stimulation with a β_1-adrenergic agonist (dobutamine), contrary to WT mice.

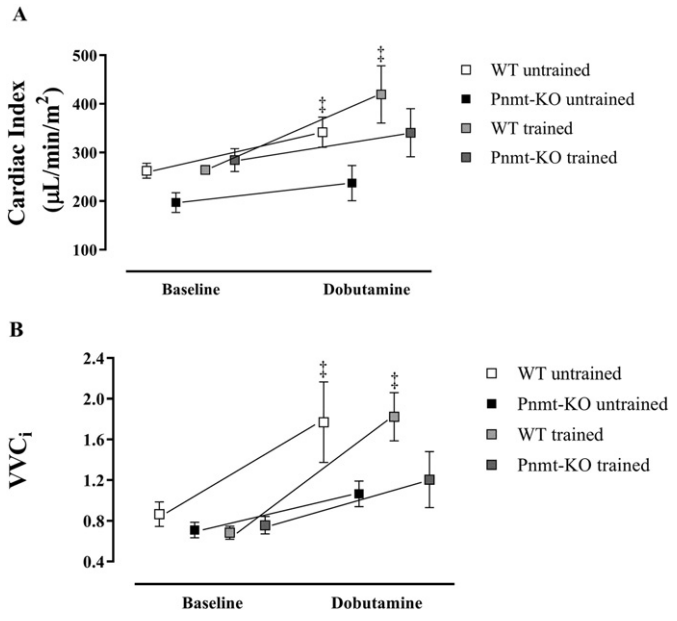

Fig. 1. Cardiac index (A) and left ventricular-vascular coupling for indexed volumes (VVC_i; B) in chronic exercise trained and untrained phenylethanolamine-*N*-methyltransferase-knockout (Pnmt-KO) and wild type (WT) mice after dobutamine perfusion and at baseline. Values are means ± SEM of 5–10 animals per group. ‡ *vs* baseline values ($P < 0.05$).

Similarly to Bao et al., we also did not find gross changes on LV functional evaluation by echocardiography in Pnmt-KO mice [14]. Furthermore, load-independent indexes of contractility were also unchanged in Pnmt-KO mice. Nevertheless, Pnmt-KO mice showed decreased filling volumes and lower cardiac index, which suggest a pattern of concentric remodeling, which has also been reported [14]. These changes may be partly due to decreased venous return, as a consequence of the lack of adrenaline action on α_1 and β-adrenoceptors, which constrict veins and enhance cardiac output, respectively [24]. However, since we newly describe an upward-shift in the left ventricular end-diastolic pressure volume relationship, which is preload-independent, at least part of these changes appear to be a consequence of lack of direct myocardial effects of adrenaline [25]. Part of the effect could also be explained by lack of β_2-adrenoceptor stimulation by adrenaline. Indeed, although β_2-adrenoceptor density is low in the myocardium (15 to 23% compared with 75 to 80% β_1-adrenoceptor density) [26], β_2-adrenoceptors have protective effects, including improved cardiac function and decreased apoptosis [27,28].

The main aim of the current study was to assess the role of adrenaline in chronic adaption to exercise training. Chronic treadmill [29], voluntary wheel [30] and swimming [31,32] exercise training are thought to induce physiological LV hypertrophy, as a compensatory mechanism, to meet increased demands of pressure or volume, while maintaining normal function [33]. The degree of physiological LV hypertrophy is typically mild in trained athletes and is generally non-pathologic [34]. Accordingly, after chronic exercise WT mice showed physiological concentric hypertrophy, as an attempt to limit LV systolic wall stress [33]. Overall functional indexes were preserved and no changes were observed in markers of pathological hypertrophy, such as ANP [35].

In classic classification, concentric LV hypertrophy is characterized by increased LV mass, increased wall thickness and normal LV end-diastolic volume. On the other hand, eccentric LV hypertrophy is characterized by an increased LV mass, increased LV end-diastolic volume and normal LV wall thickness. Our results showed increased LV mass (LVM), increased wall thickness (LVPW and IVS) and increased LV end-diastolic volume (LVEDV) with chronic exercise. These results are consistent with a recent proposal of a 4-tiered classification of left ventricular hypertrophy: increased concentricity without increased LVEDV ("thick hypertrophy"); increased LVEDV without increased concentricity ("dilated hypertrophy"); increased concentricity with increased

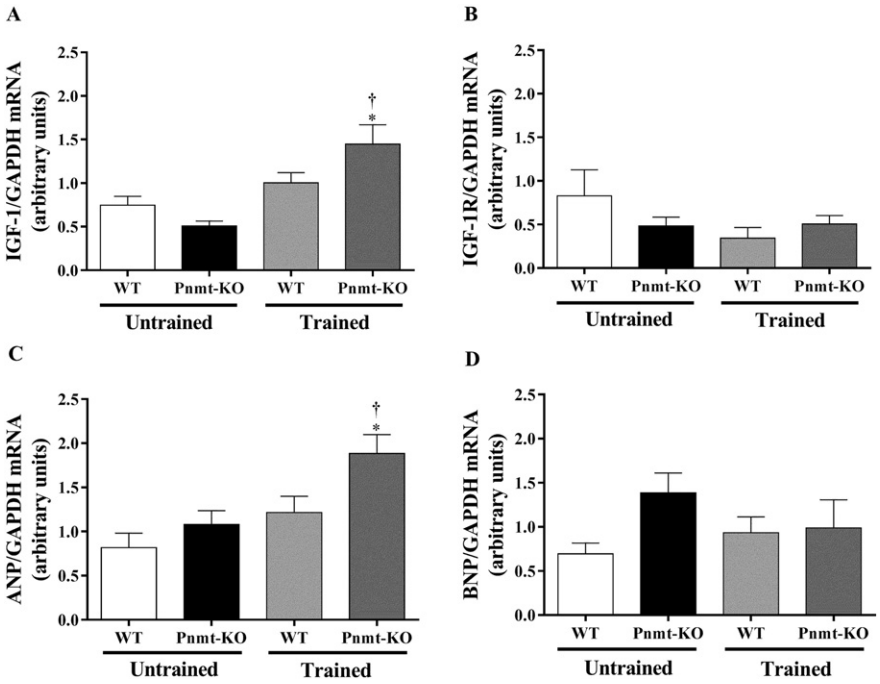

Fig. 2. Left ventricle (LV) mRNA expression of insulin-like growth factor-1 (IGF-1; A), insulin-like growth factor 1 receptor (IGF-1R, B), atrial natriuretic peptide (ANP; C), and brain natriuretic peptide (BNP; D) in chronic exercise trained and untrained phenylethanolamine-*N*-methyltransferase-knockout (Pnmt-KO) and wild type (WT) mice. Results are expressed as arbitrary units after normalization for GAPDH. Values are means ± SEM of 5–8 animals per group. * *vs* untrained mice with similar genetic background ($P < 0.05$); † *vs* WT mice with similar chronic exercise ($P < 0.05$).

LVEDV ("both thick and dilated hypertrophy"); and neither increased concentricity nor increased LVEDV ("indeterminate hypertrophy") [36]. Attending to this proposal our results show concentric hypertrophy (both thick and dilated) after chronic exercise.

As previously reported by Bao et al. [14], Pnmt-KO mice showed a normal ability to run on a treadmill. Thus, adrenaline does not seem essential to exercise performance with these protocols, and chronic exercise training was similar in both groups. In both groups, the increase in filling volume with chronic exercise may be ascribed to increased venous return, as a result of a combination of an increase in sympathetic activity (noradrenaline) to veins and the use of skeletal muscle and respiratory muscle pumps in exercise [37]. Indeed, in Pnmt-KO mice filling volumes increased possibly to the point of restoring cardiac index to levels comparable to WT mice. However, this happened at the expense of pronounced concentric LV hypertrophy and mixed overexpression of markers of physiological and pathological hypertrophy, such as IGF-1 [38,39] and ANP [35,40], respectively. Overexpression of these markers was not observed in trained WT mice, suggesting that trained Pnmt-KO mice underwent additional hypertrophic remodeling.

The IGF-1 pathway (IGF-1-phosphoinositide 3-kinase (PI3K)-protein kinase B (Akt) pathway) is associated with physiological cardiac growth in response to endurance training [39]. The natriuretic peptide ANP is produced under the influence of pro-hypertrophic neuroendocrine mediators, and the key transcriptional effector GATA4, as part of the pathological hypertrophy remodeling pathways [41]. The fact that both IGF-1 and ANP pathways are upregulated insinuates that remodeling in trained Pnmt-KO mice might be at an early stage of balance. These adaptations may protect against the development of full pathological cardiac hypertrophy [42], and prevent a further increase in blood pressure *via* the vasodilatory, natriuretic, and diuretic effects of ANP [43] in trained Pnmt-KO mice. However, a longer period of chronic exercise training in Pnmt-KO mice might lead to an imbalance, favoring pathological remodeling and heart failure.

Pnmt-KO mice develop higher blood pressure than control animals during acute exercise, suggesting that adrenaline may be required to prevent blood pressure overshoot during exercise [14]. Chronic bouts of training might have repeatedly exposed trained Pnmt-KO mice to higher afterload and thus may have contributed to more severe heart hypertrophy. The increase in blood pressure during acute exercise in Pnmt-KO mice [14] can be due both to less vasodilation, since noradrenaline poorly stimulates β_2-adrenoceptors compared with adrenaline [44], and to decreased β_2-adrenoceptor activity in chronically adrenaline-deprived Pnmt-KO mice [16]. Indeed, we have recently shown that β_2-adrenoceptor protein density is decreased in aorta cell membranes of Pnmt-KO mice and hinders the response to β_2-adrenergic agonists [16].

Finally, we assessed the *in vivo* response to acute β_1-adrenoceptor stimulation by dobutamine. Dobutamine increases myocardial contractility and reduces the reflex increase in sympathetic tone, leading to a decrease in peripheral vascular resistance [45–47]. We observed an increase in cardiac index and an improvement in ventricular-vascular coupling (VVC_i) after dobutamine infusion in WT mice, and not in Pnmt-KO mice. This further corroborated an inability to vasodilation under adrenergic stimulation in Pnmt-KO mice. Interestingly, *in vitro* we did not find differences in β_1-adrenoceptor-mediated aortic relaxation to dobutamine in Pnmt-KO mice, in a previous work [16]. However, in the current work, at the doses we used *in vivo*, β_2-adrenoceptor effects are also at play. Therefore, impaired β_2-adrenergic signaling in Pnmt-KO mice, as previously reported [16], might have contributed to impaired vasodilation and worse ventricular-vascular coupling (VVC_i) after dobutamine infusion.

In Pnmt-KO mice, although noradrenaline was significantly increased in adrenal glands, it was not found a compensatory increase in plasma [14,16]. The increase of noradrenaline in adrenal glands appears to be due to an upstream accumulation that would normally be methylated to adrenaline by Pnmt. However, even if higher noradrenaline levels played a role, the Pnmt-KO poor dobutamine response and previous findings of defective β_2-adrenoceptors [16] suggest that the lack of adrenaline has an important role in the pathophysiology of Pnmt-KO mice.

Regular physical activity is associated with lower basal blood pressure, being often used as a strategy to decrease blood pressure in

hypertension [48,49]. Exercise exerts its beneficial effects through reducing cardiovascular risk factors, and directly affecting the cellular and molecular remodeling of the heart [39]. During exercise the conversion of noradrenaline into adrenaline increases [17], and may explain these beneficial effects. We show that adrenaline appears to be essential, during chronic exercise, for maintaining a normal blood pressure, probably through β_2-adrenoceptor induced vasodilation, thus preventing LV pathological hypertrophy.

In conclusion, the increased blood pressure in Pnmt-KO mice appears to be associated with an increase in LV wall thickness and mass, suggesting concentric LV hypertrophy in adrenaline-deficient mice in response to chronic exercise. In addition, dobutamine induces an acute hemodynamic stress, and this stress increased the systolic function in WT mice, contrary to Pnmt-KO, suggesting a possible initial stage of pathological cardiac hypertrophy after chronic exercise in Pnmt-KO mice. Therefore, these results are in agreement with the need for a partial conversion of noradrenaline into adrenaline for prevention of blood pressure overshoot and thus pathological cardiac hypertrophy during chronic exercise.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Funding

This work was supported by grant awards from Professor Ernesto Morais Foundation (2012, Porto, Portugal), University of Porto (IJUP 2011-219) and Portuguese Foundation for Science and Technology (Grant SFRH/BD/133860/2017 to Raquel Martinho and SFRH/BD/110404/2015 to Sara Leite).

Acknowledgments

The authors thank António Carlos Ferreira and Teresa Silva for technical support.

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