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SHORT COMMUNICATION

Effects of homoarginine supplementation on heart and skeletal muscle of rats with heart failure with preserved ejection fraction

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Abstract

Aim Heart failure with preserved ejection fraction (HFpEF) is associated with left ventricular stiffness, impaired diastolic relaxation, and severe exercise intolerance. Decreased homoarginine (hArg) levels are an independent predictor of mortality in cardiovascular disease and correlate with impaired exercise performance. We recently reported alterations in arginine, hArg, and related amino acids in obese ZSF1 rats (O-ZSF1), with a HFpEF phenotype. Although low hArg is associated with diastolic dysfunction in humans, potential effects of hArg supplementation were not tested yet.

Methods and results At an age of 6 weeks, 12 O-ZSF1 were randomized into two groups: (1) O-ZSF1 rats supplemented with hArg in their drinking water (sO-ZSF1) or (2) O-ZSF1 rats receiving no hArg supplementation (O-ZSF1). At an age of 32 weeks, effects of primary prevention by hArg supplementation on echocardiographic, histological, and functional parameters of heart and skeletal muscle were determined. Lean ZSF1 rats (L-ZSF1) served as controls. hArg supplementation did not prevent impairment of diastolic relaxation (E/e': O-ZSF1 21 ± 3 vs. sO-ZSF1 22 ± 3, $P = 0.954$, L-ZSF1 18 ± 5) but resulted in more cardiac fibrosis (histological collagen staining: +57% in sO-ZSF1 vs. O-ZSF1, $P = 0.027$) and increased collagen gene expression (Col1a1: +48% in sO-ZSF1 vs. O-ZSF1, $P = 0.026$). In contrary, right ventricular function was preserved by hArg supplementation (TAPSE (mm): O-ZSF1 1.2 ± 0.3 vs. sO-ZSF1 1.7 ± 0.3, $P = 0.020$, L-ZSF1 1.8 ± 0.4). Musculus soleus maximal specific muscle force (N/cm^2) in O-ZSF1 (30.4 ± 0.8) and sO-ZSF1 (31.9 ± 0.9) was comparable but significantly reduced compared with L-ZSF1 (36.4 ± 0.7; both $P < 0.05$). Maximal absolute muscle force (g) (O-ZSF1: 177.6 ± 7.8, sO-ZSF1: 187.8 ± 5.0, L-ZSF1: 181.5 ± 7.9, all $P > 0.05$) and cross-sectional fibre area (arbitrary units) (O-ZSF1: 1697 ± 57, sO-ZSF1: 1965 ± 121, L-ZSF1: 1691 ± 104, all $P > 0.05$) were not altered.

Conclusions Preservation of physiological hArg level in HFpEF may not be suited to prevent alterations in left ventricular and skeletal muscle function and structure. However, hArg supplementation may be beneficial for right ventricular function especially in pulmonary hypertension in HFpEF. We may speculate that clinically observed decreased hArg level are not the cause but the consequence of a yet unrecognized pathomechanism that underpins HFpEF.

Keywords ZSF1 rat; HFpEF; Homoarginine; Skeletal muscle

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Background

Heart failure with preserved ejection fraction (HFpEF) is characterized by diastolic dysfunction, reduced endothelial function, increased arterial stiffness, and exercise intolerance with skeletal muscle fibre atrophy and contractile dysfunction.[1,2] These characteristics are closely related to nitric oxide (NO) metabolism[3] and NO synthase, with arginine

as a substrate and asymmetric dimethylarginine (ADMA) as an endogenous inhibitor. We recently reported that obese ZSF1 rats (O-ZSF1), an accepted HFpEF animal model,[4] exhibit severe alterations in arginine metabolism with the most prominent decrease in homoarginine (hArg).[5] Importantly, low hArg is an independent predictor of all-cause mortality, as reported for over 3000 Framingham Offspring Study participants,[6] and low hArg is associated with diastolic dysfunction in HFpEF patients.[7]

Aims

hArg supplementation may be therapeutic and prevent the detrimental characteristics of HFpEF including diastolic dysfunction. The aim of the present study was to examine effects of hArg supplementation in a primary intervention HFpEF rat model with a focus on characteristics and function of the heart and skeletal muscle.

Methods

Twelve female O-ZSF1 hybrid rats (ZSF1-Lepr^{fa} Lepr^{cp}/Crl, Charles River, Indianapolis, USA) and five lean ZSF1 rats (L-ZSF1) were included. At an age of 6 weeks, the O-ZSF1 animals were randomly grouped into (1) O-ZSF1 animals receiving no hArg supplementation (n = 6) and (2) O-ZSF1 animals receiving hArg supplementation (n = 6; drinking water enriched with hArg 70 mg/L) (sO-ZSF1). Water and chow was provided ad libitum. At an age of 32 weeks, echocardiography was performed under isoflurane anaesthesia, and afterwards, animals were sacrificed in deep anaesthesia as recently described.[5] Blood was collected from the beating heart, the right soleus muscle was dissected for functional measurements, and the other organs were weighted and snap-frozen or fixed in 4% paraformaldehyde for subsequent analyses. All experiments are in line with the ARRIVE guidelines and were approved by the local Animal Research Council, University of Leipzig, and the Landesbehörde Sachsen (TVV 40/19).

Echocardiography to evaluate cardiac function was performed as recently described.[5] Middle sections of the heart were fixed in 4% paraformaldehyde, embedded into paraffin, and 5 µm sections were stained for macrophages (CD68 antibody, MCA341R, Biorad, Hercules, USA, representative figure published recently[5]). Fibrotic tissue was visualized by Sirius red staining as recently described.[8] Fibrotic areas and number of CD68 positive cells were normalized to total area. Quantitative real-time PCR (qRT-PCR) was performed as recently described with Hprt1 as housekeeper[5] (Col1a1 primer: forward CATCTTGAGGTCACGGCATG/reverse TCAAGATGGTGGCCGTTACT).

For skeletal muscle function measurements, the right soleus muscle was mounted vertically in a Krebs–Henseleit buffer-filled organ bath between a hook and a force transducer. Force measurements were performed as described earlier.[1,4]

Arginine, hArg, ADMA, and symmetric dimethylarginine (SDMA) were determined from 25 µl serum spiked with stable isotope labelled internal standards using liquid chromatography–tandem mass spectrometry as described earlier.[5]

Data are presented as mean ± standard error of the mean (SEM). Group-wise comparisons were done using two-sided t-test. A P-value of <0.05 was considered as statistically significant.

Results

At an age of 32 weeks, O-ZSF1 animals presented with morphologic, functional, and echocardiographic features of a pronounced HFpEF compared with age-matched L-ZSF1 (*Table 1*). This was evident by a preserved left ventricular (LV) ejection fraction, enhanced myocardial dimension, and reduced diastolic function (enhanced E/e') and skeletal muscle atrophy (most evident in the tibialis anterior muscle) and dysfunction in musculus soleus (reduced maximal specific force). Furthermore, O-ZSF1 presented with obesity, enhanced serum glucose levels, reduced hArg levels, and enhanced ADMA concentrations (*Table 1*).

Weight gain, food/water consumption, glucose serum concentrations, arginine, and SDMA of O-ZSF1 and sO-ZSF1 were comparable under hArg supplementation. In sO-ZSF1, hArg supplementation stabilized hArg level compared with L-ZSF1 and mitigated ADMA compared with O-ZSF1 (*Table 1*). Analysing cardiac function by echocardiography revealed that hArg supplementation had no effect on functional and structural cardiac parameters. The only improvement due to hArg supplementation was observed for right ventricular (RV) function as evident by preserved tricuspid annular plane systolic excursion (TAPSE). Other RV measures were numerically improved in sO-ZSF1 compared with O-ZSF1 but, likely due to small sample number, did not reach significance.

Histological analysis revealed increased fibrosis in hearts of sO-ZSF1 compared with O-ZSF1, whereas no significant differences were detected between O-ZSF1 and L-ZSF1. This finding was supported by Col1a1 gene expression. Col1a1 is coding for collagen, type I, and alpha 1 and was significantly increased in O-ZSF1 compared with L-ZSF1 (+67%, P = 0.019) and was further increased in sO-ZSF1 compared with O-ZSF1 (+48%, P = 0.026). Increased inflammatory infiltration with CD68 positive macrophages in the heart of O-ZSF1 and sO-ZSF1 compared with L-ZSF1 was found without any effects of hArg supplementation.

With respect to skeletal muscle function and cross-sectional area, hArg supplementation had no beneficial

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Table 1 Animal characteristics at an age of 32 weeks

		L-ZSF1	O-ZSF1	sO-ZSF1	L-ZSF1 vs. O-ZSF1	O-ZSF1 vs. sO-ZSF1
Weight at sacrifice	Body weight (g)	283 ± 9	529 ± 23	533 ± 24	<0.001	0.760
	Heart (g)	1.16 ± 0.12	1.56 ± 0.15	1.71 ± 0.10	0.002	0.098
	Liver (g)	9.49 ± 0.30	23.71 ± 3.76	26.05 ± 1.28	<0.001	0.197
	Soleus/TL (mg/mm)	4.17 ± 0.11	4.39 ± 0.12	4.84 ± 0.11	0.152	0.011
	EDL/TL (mg/mm)	3.82 ± 0.08	3.81 ± 0.12	4.07 ± 0.11	0.890	0.123
	TA/TL (mg/mm)	16.4 ± 0.3	14.5 ± 0.3	15.7 ± 0.2	0.0012	0.01
	Lung ratio wet/dry	4.4 ± 0.1	4.2 ± 0.2	4.3 ± 0.5	0.061	0.739
Echocardiography	End-diastolic LV volume (μl)	600 ± 62	923 ± 94	1,032 ± 111	0.002	0.527
	End-systolic LV volume (μl)	250 ± 36	422 ± 102	466 ± 116	<0.001	0.560
	LV length diastolic (mm)	14.9 ± 1.7	15.8 ± 1.3	16.6 ± 0.4	0.498	0.778
	LV length systolic (mm)	12.7 ± 1.5	13.5 ± 1.2	14.5 ± 1.4	0.312	0.791
	LVEF (%)	58 ± 6	53 ± 6	55 ± 8	0.219	0.703
	Stroke volume (μl)	350 ± 68	501 ± 59	566 ± 122	0.023	0.724
	Fractional shortening (%)	30 ± 4.2	27.5 ± 5	28.5 ± 7.6	0.264	0.861
	LV inner diameter, diastolic	6.6 ± 0.4	7.8 ± 0.6	7.9 ± 0.5	0.006	0.294
	LV inner diameter, systolic (mm)	3.2 ± 0.6	4.5 ± 0.7	4.3 ± 0.4	0.044	0.161
	LV mass (mg)	478 ± 67	747 ± 130	804 ± 58	0.047	0.897
	E/e' septal	18 ± 5	21 ± 3	22 ± 3	0.256	0.954
	RV inner diameter, diastolic (mm)	3.2 ± 0.8	4.3 ± 1.0	3.7 ± 0.3	0.090	0.205
	RV inner diameter, systolic (mm)	2.1 ± 0.5	3.0 ± 0.8	2.5 ± 0.5	0.043	0.231
	Fractional area change (%)	30.12 ± 3.8	42.7 ± 7.4	37.8 ± 12.3	0.007	0.423
	TAPSE (mm)	1.8 ± 0.4	1.2 ± 0.3	1.7 ± 0.3	0.064	0.020
Histology	$CD68^+$ heart total	23 ± 20	105 ± 49	89 ± 48	0.017	0.615
	$CD68^+$ left ventricle	12 ± 11	56 ± 24	48 ± 24	0.012	0.577
	$CD68^+$ septum	7 ± 7	29 ± 17	26 ± 16	0.040	0.808
	$CD68^+$ right ventricle	4 ± 4	20 ± 16	15 ± 14	0.095	0.643
	Fibrosis %	1.11 ± 0.39	0.96 ± 0.13	1.51 ± 0.49	0.447	0.027
Muscle	Cross sectional area (arb. unit)	1,691 ± 104	1,697 ± 57	1965 ± 121	0.956	0.094
	Max. absolute muscle force (g)	181.5 ± 7.9	177.6 ± 7.8	187.8 ± 5.0	0.734	0.299
	Max. specific muscle force (N/cm^2)	36.4 ± 0.7	30.4 ± 0.8	31.9 ± 0.9	<0.001	0.259
Serum	Arginine (μmol/L)	132 ± 25	127 ± 48	164 ± 37	0.830	0.185
	Homoarginine (μmol/L)	1.79 ± 0.20	1.15 ± 0.27	1.54 ± 0.21	0.003	0.034
	SDMA (μmol/L)	0.25 ± 0.02	0.22 ± 0.03	0.21 ± 0	0.075	0.372
	ADMA (μmol/L)	0.53 ± 0.03	0.63 ± 0.05	0.56 ± 0.03	0.006	0.031
	Glucose (μmol/L)	19 ± 3	28 ± 5	26 ± 3	0.012	0.500

Abbreviations: ADMA, asymmetric dimethyl arginine; EDL, musculus extensor digitorum longus; E/e', ratio between early mitral inflow velocity and mitral annular early diastolic velocity; LV, left ventricle; RV, right ventricle; SDMA, symmetric dimethyl arginine; Soleus, musculus soleus; TAPSE, tricuspid annular plane systolic excursion; TA, musculus tibialis anterior; TL, tibia length.
Note: P-values were calculated using two-sided *t*-test.

effect despite a small but significant increase in muscle mass of the soleus and tibialis anterior muscle (*Table 1*).

Conclusion

In contrast to an earlier mouse study of post-myocardial infarction heart failure,[9] hArg supplementation in our present study in HFpEF rats did not preserve LV or skeletal muscle function. Importantly, fibrotic cardiac remodelling was even pronounced as determined by histological staining and analysis of collagen expression.

However, low levels of hArg are an independent risk factor for all-cause mortality,[6] predict outcomes in patients with cardiovascular disease,[10,11] and are associated with dilatation and decreased function of the LV in the general population.[12] Also, diastolic dysfunction in humans is associated with low hArg,[7] and a relation between hArg levels and changes in exercise capacity after a training intervention was observed.[2] In our HFpEF animal model, hArg levels are significantly reduced (*Table 1* and Büttner *et al.*[5]), and supplementation with hArg did not improve LV function. Clinical trials already aimed to regulate NO metabolisms in HFpEF directly by inhaled inorganic nitrite[13] or by inhibiting the downstream mediator phosphodiesterase-5.[14] These trials also showed no beneficial effects in terms of improved peak oxygen consumption,[14] exercise capacity,[13] or diastolic function.[13]

Importantly, we found evidence that RV function was preserved in supplemented HFpEF rats. These conflicting results for LV and RV were observed before. High-fat fed male O-ZSF1 with HFpEF and pulmonary hypertension were treated with a prostacyclin analogue and metformin resulting in RV function improvement but not prevent worsening LV function.[15] In line with our observation, hypoxic mice with RV hypertrophy show a down-regulation of arginine : glycine amidinotransferase (AGAT), the enzyme catalysing the first step in creatine synthesis and hArg, and the RV phenotype could not be rescued by creatine supplementation.[16] In HFpEF patients, administration of L-arginine and citrulline im-

proved RV function,[17] so it could be considered that hArg might also be beneficial, at least for certain subgroups of HFpEF patients.

Limitations that may have led to overlooking effects of homoarginine supplementation are small experimental groups (n = 6), only one time point in HFpEF progression, only female rats, no analysis of endothelial function.

In conclusion, the decrease of hArg in HFpEF may not be the cause but the consequence of a yet unrecognized pathomechanism, possibly immunometabolism, as proposed before.[18]

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Conflicts of interest

All authors declare that they have no conflicts of interest.

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