

DIETARY SUPPLEMENTATION WITH HOMOARGININE PRESERVES CARDIAC FUNCTION IN A MURINE MODEL OF POST-MYOCARDIAL INFARCTION HEART FAILURE

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Citation: Atzler D, McAndrew DJ, Cordts K, Schneider JE, Zervou S, Schwedhelm E, Neubauer S and Lygate CA. Dietary Supplementation with Homoarginine Preserves Cardiac Function in a Murine Model of Post-Myocardial Infarction Heart Failure. *Circulation*. 2017;135:400-402.

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Low plasma homoarginine (HA) is an emerging biomarker for cardiovascular disease and an independent predictor of mortality in patients with heart failure.¹ Plasma levels appear to reflect cardiac dysfunction, positively correlating with ejection fraction and inversely with circulating brain natriuretic peptide.² However, whether this is a bystander or cause-and-effect has yet to be established. Within the context of stroke, a direct causal relationship has been inferred since normal mice pre-treated with 14 mg/L HA had a smaller stroke size.³ In the present study we show for the first time that dietary supplementation with HA improves cardiac function in the setting of chronic heart failure, suggesting a novel preventive strategy and inferring that low HA levels may be inherently detrimental due to a loss of this effect.

We first confirmed that oral supplementation of C57BL/6J mice (Harwell, UK) with 14 mg/L L-homoarginine hydrochloride (HA; Sigma-Aldrich) in the drinking water for 4 weeks increased HA concentrations in both plasma (0.29 ± 0.03 vs. 0.89 ± 0.07 $\mu\text{mol/L}$) and myocardial tissue (17.6 ± 2.7 vs. 48.8 ± 6.8 nmol/g protein; $n=5-10$; $P<0.01$ for both), demonstrating a strong correlation between levels in plasma and myocardium ($r=0.74$, $P<0.01$). This dose did not significantly alter cardiac haemodynamic parameters or body weight and was chosen to match the dosing strategy previously shown to be cerebro-protective.³

To investigate the influence of HA-supplementation on heart failure development, adult female C57BL/6J mice were given drinking water with or without 14 mg/L HA for 4 weeks prior to myocardial infarction (MI) surgery and throughout the remaining 6 weeks follow-up. Echocardiography was performed at 4 weeks post-surgery to exclude infarcts $<25\%$, since these mice do not develop heart failure. High-resolution cine-MRI under isoflurane anaesthesia was applied *in vivo* at 5.5 weeks post-surgery to assess infarct size, left ventricular (LV) structure, mass and volumes. After 6 weeks, haemodynamic measurements were obtained by LV catheterisation and contractile reserve assessed under maximal dobutamine infusion (16ng/g body weight/minute) via the jugular vein. All surgery and *in vivo* phenotyping was as previously described.⁴ Cardiac blood, lungs and scar-free LV tissue were collected and stored at -80°C . Myocardial and plasma HA concentrations were measured using tandem-mass spectrometry.³ This investigation was approved by the Ethical Review Committee at the University of Oxford and conforms to the UK Animals (Scientific Procedures) Act, 1986, incorporating Directive 2010/63/EU.

There was no difference in overall survival between groups (control MI 64% vs. HA-supplementation 68%, $P=0.69$). For all subsequent analysis, experimental groups were retrospectively matched for infarct size, which is necessary to determine the effect of HA on heart failure development independent of effects on myocardial injury⁴. Cine-MRI revealed profound LV remodelling indicative of heart failure (i.e. dilatation and hypertrophy), but to a similar extent in both control and HA-supplemented animals (**Table**). Similarly, global function assessed by MRI (e.g. ejection fraction) was severely

impaired, but did not differ between groups, most likely because MRI could only be performed under basal (non-stimulated) conditions. In contrast, haemodynamic measures of isovolumetric function were better preserved with HA-supplementation, as evidenced by higher LV contractility (dP/dt_{max}) at baseline and upon β -adrenergic stimulation, manifesting as preserved contractile reserve ($\Delta dP/dt_{max}$). Furthermore, diastolic indices (dP/dt_{min} , tau) were also improved under stimulated conditions in the HA-supplemented animals (**Table**). These findings are unlikely to reflect altered loading conditions since markers for afterload (LVSP) and preload (LVEDP) did not differ significantly between groups and tau is relatively load-insensitive.

Conceivably, our results will stimulate further research. For example, the precise molecular mechanism remains elusive and it has yet to be established whether pre-supplementation is necessary or if HA has an additional effect on infarct size. Maintaining functional reserve would clearly be desirable for patient quality of life, but whether this is truly beneficial in the long-term will need to be tested in the clinical setting. In this context, it is notable that a beneficial effect was obtained from a 3-fold increase in plasma HA using a human dose equivalent of ~250 mg daily. Recently we investigated kinetic and dynamic properties of oral HA-supplementation in healthy humans demonstrating a 7-fold elevation in plasma HA levels when 125 mg HA was given daily for 4 weeks,⁵ suggesting that a relatively low dose would be sufficient to elevate plasma HA into the therapeutic range for at-risk patients. This contrasts with the known pharmacokinetic profile for L-arginine-supplementation, suggesting that HA is not a straightforward substitution for L-arginine metabolism.⁵ Nevertheless, future studies should determine the effect of HA on nitric oxide bioavailability.

Our study asked the question, whether, for the same extent of myocardial injury, dietary HA-supplementation modifies the subsequent development of chronic heart failure. Specifically we can conclude that HA did not alter structural LV remodelling post-MI, but that dietary HA-supplementation preserved contractile reserve, with treated hearts maintaining higher responses to β -adrenergic stimulation. This suggests that HA is more than a bystander biomarker, since higher levels directly influence heart failure pathophysiology. As it is safe, cheap and easily administered,⁵ dietary supplementation with homoarginine represents a promising approach for clinical translation.

Sources of Funding

D.A. acknowledges the support of the European Community under a Marie Curie Intra-European Fellowship for Career Development (623127). This work was funded by LMU Munich's Institutional Strategy LMUexcellent within the framework of the German Excellence Initiative (to D.A.) and by a British Heart Foundation Programme Grant (RG/13/8/30266 to C.L., J.E.S. and S.N.). JES is a Senior BHF Basic Science Research Fellow (FS/11/50/29038). The authors acknowledge a Wellcome Trust Core Award; Grant number: 090532/Z/09/Z.

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Conflict of interest: none declared.

Table: *In vivo* cardiac function six weeks after myocardial infarction (MI) in female C57BL/6J mice with (+HA) and without (-HA) dietary homoarginine supplementation (14 mg/L drinking water).

	MI		P value
	- HA	+ HA	
<i>Cine-MRI</i>	(n=19)	(n=23)	
Infarct size (%)	39.3 ± 9.1	39.8 ± 7.0	0.84
Heart rate (bpm)	461 ± 34	454 ± 34	0.54
Ejection fraction (%)	17 ± 5	15 ± 5	0.39 [#]
End diastolic volume (μL)	140 ± 35	147 ± 28	0.40 [#]
End systolic volume (μL)	118 ± 36	125 ± 29	0.50
Stroke volume (μL)	22 ± 5	22 ± 5	0.90
Cardiac output (mL/min)	10130 ± 2517	9893 ± 2653	0.67 [#]
<i>Haemodynamics (Baseline)</i>	(n=19)	(n=23)	
LV systolic pressure (mmHg)	84 ± 6	88 ± 6	0.06
LV end-diastolic pressure (mmHg)	14.6 ± 5.3	13.3 ± 4.2	0.36
Tau (ms)	15.0 ± 2.8	13.4 ± 3.4	0.11
dP/dt _{max} (mmHg/s)	4753 ± 1073	5515 ± 1292	0.03^{##}
dP/dt _{min} (mmHg/s)	-3308 ± 998	-4029 ± 1447	0.051 [#]
<i>Haemodynamics (Stimulated[§])</i>	(n=19)	(n=23)	
LV systolic pressure (mmHg)	84 ± 6	88 ± 7	0.06
LV end-diastolic pressure (mmHg)	13.5 ± 5.3	10.8 ± 3.9	0.069
Tau (ms)	11.8 ± 2.4	9.9 ± 2.5	0.014[*]
dP/dt _{max} (mmHg/s)	5710 ± 1791	7421 ± 2643	0.006^{##}
dP/dt _{min} (mmHg/s)	-4048 ± 1358	-5357 ± 2038	0.009^{##}
Δ dP/dt _{max} (mmHg/s)	956 ± 881	1906 ± 1570	0.014^{##}
<i>Post-mortem morphology</i>	(n=19)	(n=23)	
Tibial length (mm)	18.2 ± 0.3	18.3 ± 0.2	0.17
Lung/tibial length (mg/mm)	9.2 ± 2.9	9.6 ± 2.5	0.28 [#]
LV/tibial length (mg/mm)	7.1 ± 0.8	7.7 ± 1.0	0.06

All values are presented as mean ± SD. *indicates $P < 0.05$ for - HA vs. + HA using student's t-test when normally distributed or [#]Mann-Whitney-U test when normality assumption is violated. Haemodynamic measurements assessed at baseline and [§]after stimulation with dobutamine (16 ng/g body weight / minute). LV indicates left ventricle; dP/dt_{max}, contractility; dP/dt_{min}, relaxation; Δ dP/dt_{max}, contractile reserve.