Dietary Supplementation with Homoarginine Preserves Cardiac Function in a Murine Model of Post-Myocardial Infarction Heart Failure

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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 outcome is a bystander or cause-and-effect has yet to be established. Within the context of stroke, a direct causal relationship has been inferred because normal mice pretreated 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 because of a loss of this effect.

We first confirmed that oral supplementation of C57BL/6J mice (Harwell, UK) with 14 mg/L L-HA hydrochloride (Sigma-Aldrich) in the drinking water for 4 weeks increased HA concentrations in both plasma (0.29±0.03 vs 0.89±0.07 μmol/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 cerebroprotective.[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 before myocardial infarction surgery and throughout the remaining 6 weeks follow-up. Echocardiography was performed 4 weeks after surgery to exclude infarcts <25% because these mice do not develop heart failure. High-resolution cine-magnetic resonance imaging under isoflurane anesthesia was applied in vivo 5.5 weeks after surgery to assess infarct size, left ventricular (LV) structure, mass, and volumes. After 6 weeks, haemodynamic measurements were obtained by LV catheterization and contractile reserve assessed under maximal dobutamine infusion (16ng/g body weight/min) 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.

No difference was found in the overall survival between groups (control myocardial infarction 64% vs HA supplementation 68%, *P=0.69*). For all subsequent analyses, 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 injury.[4] Cine-magnetic resonance imaging revealed profound LV remodeling, which is indicative of heart failure (ie, dilatation and hypertrophy), but to a similar extent in both control and HA-supplemented animals (Table). Similarly, global function assessed by magnetic resonance imaging

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(eg, ejection fraction) was severely impaired but did not differ between groups most likely because magnetic resonance imaging could only be performed under basal (nonstimulated) 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 because markers for afterload (LV systolic pressure) and preload (LV end-diastolic pressure) 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 presupplementation is necessary or 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

Table. In Vivo Cardiac Function 6 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	
Cine-Magnetic Resonance Imaging	(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†
Haemodynamics (baseline)	(n=19)	(n=23)	
LV systolic pressure (mm Hg)	84±6	88±6	0.06
LV end-diastolic pressure (mm Hg)	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} (mm Hg/s)	4753±1073	5515±1292	0.03†*
dP/dt_{min} (mm Hg/s)	–3308±998	–4029±1447	0.051†
Haemodynamics (stimulated‡)	(n=19)	(n=23)	
LV systolic pressure (mm Hg)	84±6	88±7	0.06
LV end-diastolic pressure (mm Hg)	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} (mm Hg/s)	5710±1791	7421±2643	0.006†*
dP/dt_{min} (mm Hg/s)	-4048±1358	-5357±2038	0.009†*
Δ dP/dt_{max} (mm Hg/s)	956±881	1906±1570	0.014†*
Postmortem morphology	(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. *$P<0.05$ for –HA versus +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/min). dP/dt_{max} indicates contractility; dP/dt_{min}, relaxation; Δ dP/dt_{max}, contractile reserve; and LV, left ventricle.

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 questioned 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 remodeling after myocardial infarction but that dietary HA supplementation preserved contractile reserve, with treated hearts maintaining higher responses to β–adrenergic stimulation. This finding suggests that HA is more than a bystander biomarker because higher levels directly influence heart failure pathophysiology. Because it is safe, cheap, and easily administered,[5] dietary supplementation with homoarginine represents a promising approach for clinical translation.

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DISCLOSURES

None.

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FOOTNOTES

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