Alpha-lipoic acid does not acutely affect resistance and conduit artery function or oxidative stress in healthy men

James E. Sharman, Prasad Gunaruwan, Wade L. Knez, Matthias Schmitt, Susan A. Marsh, Gary R. Wilson, John R. Cockcroft & Jeff S. Coombes
The University of Queensland, Exercise and Oxidative Stress Research Group, School of Human Movement Studies, Australasian Centre on Ageing, St Lucia, Brisbane 4072 and University of Queensland Department of Medicine, Princess Alexandra Hospital, Brisbane, Queensland 4102, Australia, and Department of Cardiology, Wales Heart Research Institute, University Hospital of Wales College of Medicine, Heath Park, Cardiff CF14 4XN, UK

Aims
Alpha-lipoic acid (ALA) is a thiol compound with antioxidant properties used in the treatment of diabetic polyneuropathy. ALA may also improve arterial function, but there have been scant human trials examining this notion. This project aimed to investigate the effects of oral and intra-arterial ALA on changes in systemic and regional haemodynamics, respectively.

Methods
In study 1, 16 healthy older men aged 58 ± 7 years (mean ± SD) received 600 mg of ALA or placebo, on two occasions 1 week apart, in a randomized cross-over design. Repeated measures of peripheral and central haemodynamics were then obtained for 90 min. Central blood pressure and indices of arterial stiffness [augmentation index (AIx) and estimated aortic pulse wave velocity] were recorded non-invasively using pulse wave analysis. Blood samples obtained pre- and post-treatments were analysed for erythrocyte antioxidant enzyme activity, plasma nitrite and malondialdehyde. In study 2 the effects of incremental cumulative doses (0.5, 1.0, 1.5 and 2.0 mg ml⁻¹ min⁻¹) of intra-arterial ALA on forearm blood flow (FBF) were assessed in eight healthy subjects (aged 31 ± 5 years) by conventional venous occlusion plethysmography.

Results
There were no significant changes on any of the central or peripheral haemodynamic measures after either oral or direct arterial administration of ALA. Plasma ALA was detected after oral supplementation (95% confidence intervals 463, 761 ng ml⁻¹), but did not alter cellular or plasma measures of oxidative stress.

Conclusions
Neither oral nor intra-arterial ALA had any effect on regional and systemic haemodynamics or measures of oxidative stress in healthy men.

Introduction
Alpha-lipoic acid (ALA) is an endogenous disulphide-containing compound which functions as an essential cofactor in the oxidative decarboxylation of α-keto acids [1]. In Germany, intravenous ALA is used in the treatment of diabetic neuropathy. It has been shown to enhance insulin-stimulated glucose disposal [2], improve peripheral microcirculation [3] and reduce neu-
ropathic symptoms [4], possibly through attenuated oxidative stress [5]. Both ALA and its reduced form, dihydrolipoic acid (DHLA), act as intra and extracellular antioxidants that support the recycling of vitamin C and E [6]. The antioxidant potency of ALA and DHLA is also due to the capability of one, or both, compounds to scavenge several reactive oxygen species, including those suspected to contribute to endothelial dysfunction in people with risk factors for cardiovascular disease [7, 8].

Several lines of evidence indicate that ALA may improve arterial haemodynamics by beneficial changes to vascular structure and function [9–12]. Indeed, recently ALA was shown to increase nitric oxide (NO)-mediated vasodilatation in patients with diabetes, via a mechanism possibly linked to reduced oxidative stress [13]. Despite the potential vascular benefits of ALA, there have been no human trials investigating its acute effect on prognostic indicators of cardiovascular mortality, such as systemic arterial stiffness (AIx) and aortic pulse wave velocity [14]. Nor have the forearm blood flow (FBF) responses to clinically applicable doses of ALA been reported. The first aim of the present study was to examine the effect of an acute oral dose of ALA (600 mg) on arterial stiffness and oxidative stress in older men. We chose to investigate an older healthy subject population because antioxidant defence mechanisms decline and arterial stiffness increases with age, and we were interested in the effect of ALA without the confounding factors associated with disease or risk factors for cardiovascular disease. Second, in a healthy younger population, we examined the effect of intra-arterially administered ALA on FBF as an indicator of peripheral vascular tone.

Methods

Study population

Sixteen healthy older males (aged 58 ± 7 years; mean ± SD) participated in study 1 and eight healthy young men (aged 31 ± 5 years) in study 2. Volunteers were excluded if they were smokers, taking antioxidant supplements or receiving medication. Additionally, those with a clinical history of cardiovascular disease, diabetes mellitus, gastrointestinal disease, hypertension (>150/90 mmHg), hypercholesterolaemia (total cholesterol >6.5 mmol l⁻¹), a history of abdominal or thyroid surgery, acute illness or chronic inflammatory condition were also excluded. The Local Medical Research Ethics Committee approved procedures and participants gave their written informed consent. The studies conformed to the Declaration of Helsinki.

Study 1: acute effect of 600 mg orally administered ALA on large artery haemodynamics and oxidative stress

Subjects were studied in the morning after an overnight fast (8–10 h) on two occasions within 7 days. Subjects lay supine in a temperature-controlled room (21 ± 2 °C) for 30 min, following which baseline haemodynamics were recorded and a 20-ml sample of blood was obtained. Subjects then orally ingested six tablets of placebo or ALA (total= 600 mg; a dose known to reach maximum plasma concentrations in 15 min to 1 h) [15]. Haemodynamic measures were made 15 min after placebo/ALA ingestion, and thereafter at 5-min intervals for 75 min. A second blood sample was then taken. At the second visit the same protocol was observed with the opposite supplement (ALA/placebo) provided.

Haemodynamics

A validated [16] oscillometric device (HEM-705CP; Omron Corporation, Tokyo, Japan) was used to record brachial blood pressures at the dominant arm (triplicate). The AIx, a measure of systemic arterial stiffness, and central (aortic) blood pressure were determined by pulse wave analysis using SphygmoCor version 6.1 software (AtCor Medical, Sydney, Australia). This technique utilizes a validated generalized transfer function [17] to obtain central pressure and markers of arterial stiffness by radial artery applanation tonometry. The AIx is the difference between the first and second systolic peaks of the central pressure waveform expressed as a percentage of pulse pressure. Estimated aortic pulse wave velocity $v_{ak}$ was the time between the foot of the pressure wave and the inflection point [18]. The subendocardial viability ratio (SEVR) was derived as the ratio of the diastolic to systolic area under the curve of the central pressure waveform, expressed as a percentage [19]. A previous study, utilizing the same technique as the current study, only required eight subjects to detect a significant change ($P < 0.01$) from baseline values in AIx over time after oral administration of an antioxidant [20].

Blood samples

Plasma ALA was determined by reverse-phase high-performance liquid chromatography (HPLC; Shimadzu SLC-10A, Kyoto, Japan) using an adapted method [21]. In our hands the within-run coefficient of variation (% CV) was 5% and the sensitivity of the assay was 1 ng ml⁻¹. Plasma malondialdehyde (MDA) was measured as a marker of oxidative stress according to the method of Sim [22], and the % CV was 3%. To determine total NO production, plasma nitrite was measured using a commercially available kit (cat. no. 482655;
Calbiochem, Darmstadt, Germany). Erythrocyte glutathione peroxidase (GPX) was measured by continuous spectrophotometric rate determination via a method adapted from [23]. Superoxide dismutase (SOD) was measured by microtiter plate-based colorimetric detection with a modified method [24] and catalase was determined by the method of Slaughter and O’Brien [25]. Enzyme activities were expressed in units per gram (or milligram) of haemoglobin and the % CV for the GPX, SOD and catalase assays were 5%, 7% and 2%, respectively.

Study 2: effect of intra-arterial ALA on FBF
All studies were performed in a quiet temperature-controlled room (21 ± 2 °C) with subjects in the supine position. A 27-G unmounted steel needle, connected to an epidural catheter, sealed with dental wax, was inserted into the brachial artery of the nondominant arm. Twenty minutes after the commencement of 0.9% saline infusion, ALA was delivered by syringe pump (MS 2000; Graseby Medical, Watford, UK) at 1 ml min⁻¹. ALA was infused for 15 min at each incremental dose followed by 15 min of 0.9% saline after the highest dose of ALA. The forearm resistance vasculature was assessed at 5-min intervals using conventional venous occlusion plethysmography as described elsewhere [26].Changes in FBF (in ml 100 ml⁻¹ 1 min⁻¹) were assessed in the infused and non-infused arms, and changes in the former were expressed as a ratio of those in the latter [26]. Blood pressure was continuously monitored by finger photoplethysmography (TNO-TPD Biomedical Instrumentation, Amsterdam, the Netherlands). With a mean (± SD) resting FBF of 2.69 ± 0.19 ml min⁻¹, seven subjects were needed to detect a 10% change in FBF (with α = 0.05 and β = 0.9) [27].

Statistical analyses
All data are presented as mean ± SD unless otherwise stated and P < 0.05 was considered statistically significant. Two-way repeated measures analysis of variance (ANOVA) was used to analyse the response to treatments over time. Paired t-tests were used at individual time points as specified. Pearson correlations were used for associations between variables. The area under the AIx–time curve (by trapezoidal rule) was determined from zero and the AIx baseline.

Results
Study 1
The baseline subject characteristics are shown in Table 1. When compared by t-test, there were no significant differences in any measured baseline variable between ALA and placebo visits. When compared by repeated measures ANOVA, there was no significant change over time between treatments for AIx (P = 0.97), TR (P = 0.87), SEVR (P = 0.9), heart rate (P = 0.74), brachial systolic (P = 0.87) and diastolic (P = 0.93) blood pressure, or aortic systolic (P = 0.94) and diastolic (P = 0.92) blood pressure. Figure 1 shows the comparison in AIx over time after oral administration of ALA and placebo. The area under the AIx–time curve was not significantly different between treatments when analysed from zero (P = 0.50) or the AIx baseline (P = 0.37).

Biochemical data are presented in Table 2. Plasma ALA was not detectable at baseline or after placebo, but post-ALA supplementation was 612 ± 279 ng ml⁻¹ (95% confidence intervals 463, 761; P < 0.001). After treatment there were no significant changes in oxidative stress (MDA), NO production (nitrite) or red cell antioxidant enzyme activity (GPX, SOD and catalase). No significant relationships were observed between plasma ALA and peripheral blood pressures (P > 0.05), AIx (r = 0.14; P = 0.44), TR (r = −0.06; P = 0.74) or central blood pressures (P > 0.05).

Study 2
Characteristics of study 2 participants are shown in Table 1. There was no significant difference in the FBF

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**Table 1**
Baseline characteristics of subject population for studies 1 and 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study 1 (n = 16)</th>
<th>Study 2 (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>58 ± 7</td>
<td>31 ± 5</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>175 ± 0.1</td>
<td>178 ± 0.1</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>80.7 ± 8.7</td>
<td>76.2 ± 6.9</td>
</tr>
<tr>
<td>Brachial systolic blood pressure (mmHg)</td>
<td>128 ± 10</td>
<td>117 ± 16</td>
</tr>
<tr>
<td>Brachial diastolic blood pressure (mmHg)</td>
<td>79 ± 6</td>
<td>61 ± 9</td>
</tr>
<tr>
<td>Augmentation index (%)</td>
<td>27 ± 7</td>
<td>–</td>
</tr>
<tr>
<td>Central (aortic) systolic blood pressure (mmHg)</td>
<td>120 ± 10</td>
<td>–</td>
</tr>
<tr>
<td>Central (aortic) diastolic blood pressure (mmHg)</td>
<td>80 ± 7</td>
<td>–</td>
</tr>
<tr>
<td>Glucose (mmol l⁻¹)</td>
<td>5.1 ± 0.6</td>
<td>5.3 ± 0.8</td>
</tr>
<tr>
<td>Total cholesterol (mmol l⁻¹)</td>
<td>5.0 ± 0.8</td>
<td>4.3 ± 0.8</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol l⁻¹)</td>
<td>3.6 ± 0.8</td>
<td>2.2 ± 1.0</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol l⁻¹)</td>
<td>1.2 ± 0.4</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td>Triglycerides (mmol l⁻¹)</td>
<td>1.1 ± 0.6</td>
<td>1.7 ± 0.8</td>
</tr>
</tbody>
</table>
ratio ($P = 0.76$) throughout the study, which is illustrated in Figure 2. When assessed by $t$-test, there was no significant change in the FBF ratio from baseline to the highest dose of ALA (2.0 mg ml$^{-1}$; $P = 0.73$). Blood pressure did not change significantly throughout the study (data not shown; $P > 0.05$).

**Discussion**

The first purpose of this study was to assess the systemic haemodynamic and oxidative stress response to an acute 600-mg oral dose of ALA in healthy older men. Second, we measured FBF following therapeutic equivalent doses of ALA infused into the brachial artery of healthy younger men. Our results showed that an acute oral dose of ALA had no detectable effect on indices of arterial stiffness, central (aortic) or peripheral (brachial) blood pressure. Equally, oral ALA did not modify any of the markers of oxidative stress chosen in this study, and local ALA administration failed to change forearm resistance vessel tone.

Previous work has suggested that ALA may acutely increase arterial vasodilatory capacity by attenuating oxidative stress leading to increased activity of the L-arginine–NO pathway [13]. The healthy subjects in the current study had normal blood levels of lipid peroxida-

### Table 2

<table>
<thead>
<tr>
<th>Blood variable</th>
<th>Placebo Pre</th>
<th>Placebo Post</th>
<th>ALA Pre</th>
<th>ALA Post</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALA (ng ml$^{-1}$)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>612 ± 279*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma MDA ($\mu$M)</td>
<td>11.2 ± 3.4</td>
<td>11.9 ± 4.1</td>
<td>11.1 ± 3.0</td>
<td>11.7 ± 3.2</td>
<td>0.88</td>
</tr>
<tr>
<td>Plasma nitrite ($\mu$M)</td>
<td>6.9 ± 2.8</td>
<td>6.5 ± 2.4</td>
<td>7.7 ± 2.7</td>
<td>7.0 ± 2.1</td>
<td>0.44</td>
</tr>
<tr>
<td>Erythrocyte GPX activity (U g Hb$^{-1}$)</td>
<td>31.8 ± 6.1</td>
<td>31.5 ± 6.6</td>
<td>32.0 ± 6.3</td>
<td>31.1 ± 6.8</td>
<td>0.97</td>
</tr>
<tr>
<td>Erythrocyte SOD activity (U mg Hb$^{-1}$)</td>
<td>7.7 ± 2.3</td>
<td>7.0 ± 1.1</td>
<td>6.9 ± 1.9</td>
<td>7.1 ± 1.5</td>
<td>0.56</td>
</tr>
<tr>
<td>Erythrocyte catalase activity (U g Hb$^{-1}$)</td>
<td>8.5 ± 1.2</td>
<td>8.4 ± 1.2</td>
<td>8.3 ± 1.2</td>
<td>8.5 ± 1.1</td>
<td>0.87</td>
</tr>
</tbody>
</table>

GPX, Glutathione peroxidase; MDA, malondialdehyde; ND, not detectable; SOD, superoxide dismutase. Data were analysed by repeated measures ANOVA. *Indicates significant change over time.
tion metabolites indicative of oxidative stress (MDA), whereas in the study by Heitzer et al. [13], the acute improvement in endothelium-dependent vasodilatation from ALA was positively correlated with plasma MDA and negatively with the antioxidant ubiquinol-10. These findings may indicate that favourable vascular effects of ALA are more likely to occur in people with oxidative stress-associated pathologies (i.e. diabetes mellitus, hypertension and hyper-cholesterolaemia) rather than in healthy individuals as were examined in the current study.

Although ALA may act through several biochemical pathways to alter endothelial function (e.g. by affecting pyruvate dehydrogenase function and improving insulin-mediated glucose disposal), the intention of the present study was to determine if acute vascular changes may be mediated via free radical scavenging. A 4-year international trial (NATHAN I Study) is underway, aimed at examining the chronic effect of orally administered ALA in diabetic polyneuropathy. It is possible that ALA may improve haemodynamics in these patients by long-term protection against oxidative stress [10], vascular hypertrophy [28], the formation of advanced glycation end products [11] and the expression of atherogenic proteins (e.g. tissue factor and endothelin-1) via inhibition of the inflammatory cytokine nuclear factor κB [29].

We used sensitive and validated techniques to assess haemodynamics in the present study, but we cannot definitively exclude the possibility of a Type I error. This chance, however, is unlikely given the consistency of negative findings with oral and intra-arterial administration of ALA in both the large and small arteries, in addition to the lack of effect on oxidative stress markers. Furthermore, the selection of sample sizes was based on previous investigations using the same techniques that were able to detect significant changes from baseline using pharmacological doses of compounds affecting the vasculature [20, 27]. Most importantly, we assessed a wide dose range of ALA in order to achieve plasma ALA levels similar to therapeutic doses [15, 30] known to reduce symptoms of neuropathy in diabetics [4] and, therefore, expected to be clinically meaningful. Nevertheless, it is also possible that the ALA diluent (ethyl-diamin) blocked a direct FBF action of ALA. Lastly, ALA may have had a delayed effect that went undetected in the first study because the time to achieve maximal plasma concentrations of ALA for a 600-mg oral dose has considerable individual variation, ranging from 15 min [15] to 4 h [31]. Indeed, the plasma concentrations of ALA at 90 min post ingestion in the present study ranged from 255 to 1253 ng ml\(^{-1}\), suggesting differences in ALA uptake.

In summary, whilst there is evidence to show that ALA may produce an acute positive effect on the function of large and small arteries in disease states, the present study showed no beneficial effect in healthy subjects. Therapeutic doses were administered orally as well as by direct application to peripheral vascular beds, but no significant changes in arterial stiffness indices, blood pressure or biochemical responses (related to oxidative stress) were observed. Thus, neither oral nor intra-arterial ALA acutely affects regional and systemic haemodynamics or measures of oxidative stress in healthy men.

We thank Herron Pharmaceuticals for their generous donation of ALA and placebo supplements. This project was made possible by The University of Queensland Graduate School who granted a GSRT Award to J.E.S.

References


