

# Tetrahydrobiopterin Supplementation Improves Endothelial Function But Does Not Alter Aortic Stiffness in Patients With Rheumatoid Arthritis

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**Background**—Rheumatoid arthritis is a systemic inflammatory condition associated with increased cardiovascular risk that may be due to underlying endothelial dysfunction and subsequent aortic stiffening. We hypothesized that supplementation with tetrahydrobiopterin (BH<sub>4</sub>) would recouple endothelial nitric oxide synthase and thus improve endothelial function and consequently reduce aortic stiffness.

**Methods and Results**—We conducted 2 randomized, double-blinded, placebo-controlled crossover studies examining 2 separate regimens: an acute regimen, with a single dose of BH<sub>4</sub> 400 mg versus placebo (n=18), and a short-term regimen, composed of a 1-week treatment with BH<sub>4</sub> 400 mg once daily versus placebo (n=15). Flow-mediated dilatation and aortic pulse wave velocity were studied 4 times, before and after each treatment phase. Acute BH<sub>4</sub> supplementation led to an improvement of flow-mediated dilatation, whereas placebo had no effect (mean±SD of effect difference 2.56±4.79%; *P*=0.03). Similarly, 1-week treatment with BH<sub>4</sub> improved endothelial function, but there was no change with placebo (mean±SD of effect difference 3.50±5.05%; *P*=0.02). There was no change in aortic pulse wave velocity following acute or short-term BH<sub>4</sub> supplementation or placebo (mean±SD of effect difference: acute 0.09±0.67 m/s, *P*=0.6; short-term 0.03±1.46 m/s, *P*=0.9).

**Conclusion**—Both acute and short-term supplementation with oral BH<sub>4</sub> improved endothelial function but not aortic stiffness. This result suggests that BH<sub>4</sub> supplementation may be beneficial for patients with rheumatoid arthritis by improving endothelial dysfunction and potentially reducing risk of cardiovascular disease. There appears to be no causal relationship between endothelial function and aortic stiffness, suggesting that they occur in parallel, although they may share common risk factors such as inflammation. (*J Am Heart Assoc.* 2016;5:e002762 doi: 10.1161/JAHA.115.002762)

**Key Words:** arteriosclerosis • endothelial function • inflammation • rheumatoid arthritis • tetrahydrobiopterin

Rheumatoid arthritis (RA) and other chronic inflammatory diseases are associated with increased premature mortality, mostly due to an excess of cardiovascular disease (CVD).<sup>1,2</sup> A number of underlying mechanisms have been proposed, including endothelial dysfunction. Reduced nitric oxide (NO) bioavailability or endothelial dysfunction is thought to be a key early step in the initiation of atherosclerosis and

predicts future CVD-related events in a variety of populations, improving risk classification beyond the Framingham Heart Study scores.<sup>3,4</sup> We and others have demonstrated that RA patients have endothelial dysfunction,<sup>5–9</sup> which can be improved with anti-inflammatory therapies.<sup>10–12</sup> Aortic stiffening, an independent risk factor for CVD,<sup>13</sup> has also been implicated. Several groups have reported increased aortic pulse wave velocity (aPWV) in RA and suggested that this could be a consequence of endothelial dysfunction.<sup>10,14–16</sup> Previous studies suggested a link between endothelial function and stiffness of muscular arteries,<sup>17,18</sup> but whether endothelial function is causally related to aortic stiffness is unknown.

The mechanism of endothelial dysfunction in RA is incompletely understood, but reduced tetrahydrobiopterin (BH<sub>4</sub>) availability or stability<sup>19</sup> and increased production of reactive oxygen species have been suggested.<sup>20</sup> Under basal conditions, endothelial nitric oxide synthase (eNOS) is responsible for NO production, but during inflammation, inducible NOS is also expressed in arterial endothelial cells.

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Received November 18, 2015; accepted December 16, 2015.

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Inducible NOS activation impairs vasorelaxation in vitro, at least in part, by limiting availability of BH<sub>4</sub> to eNOS,<sup>21</sup> and we have demonstrated in vivo that inducible NOS activation in RA is independently related to endothelial function.<sup>9</sup> BH<sub>4</sub> deficiency is thought to lead to eNOS uncoupling, in which electrons flowing from the reductase domain to the oxygenase domain are diverted to molecular oxygen rather than L-arginine, leading to production of superoxide (O<sub>2</sub><sup>-</sup>) rather than NO.<sup>22</sup> Increased release of myeloperoxidase from activated neutrophils may also play an important role in endothelial dysfunction in RA. Myeloperoxidase catalytically consumes NO and increases the production of reactive oxygen species, subsequently leading to uncoupling of eNOS<sup>20</sup> and oxidation of BH<sub>4</sub> to inactive BH<sub>2</sub>.<sup>19,23,24</sup> We and others have shown that circulating myeloperoxidase levels are elevated in patients with RA<sup>9,25</sup> and independently predict endothelial dysfunction in patients with unstable coronary disease<sup>26</sup> and RA.<sup>9</sup> Inflammation appears to reduce BH<sub>4</sub> bioavailability, which may in part mediate the endothelial dysfunction present in RA; therefore, supplementation with BH<sub>4</sub> may be a reasonable therapeutic option to restore normal eNOS function and thus endothelial function in RA. Previous studies have demonstrated that intra-arterial administration of BH<sub>4</sub> improves endothelium-dependent dilation in patients with hypercholesterolemia,<sup>27</sup> coronary artery disease,<sup>28</sup> and type II diabetes.<sup>29</sup> Oral BH<sub>4</sub> supplementation improves endothelial function in elderly participants,<sup>30</sup> in long-term smokers,<sup>31</sup> and in patients with hypercholesterolemia<sup>32</sup> and hypertension.<sup>33</sup> No studies to date, however, have investigated the effects of BH<sub>4</sub> in patients with RA. Moreover, it is unclear if improvement of endothelial function would lead to a reduction of aortic stiffness or if other mechanisms are responsible for increased aortic stiffness.

Our aim was to test the hypotheses (1) that oral BH<sub>4</sub> supplementation would improve endothelial function in patients with RA and (2) that an improvement of endothelial function would lead to subsequent amelioration of aortic stiffening, providing evidence about the causal relationship between endothelial function and aortic stiffness; BH<sub>4</sub> is thought to have little direct effect on inflammation per se.

## Methods

### Study Population

Overall, 33 patients with active RA (disease activity score [DAS28] >3.5) who met the 1987 American Rheumatism Association criteria were recruited from the rheumatology clinics at Addenbrooke's Hospital, Cambridge, United Kingdom. Patients with manifest CVD, untreated hypertension (blood pressure ≥140/90 mm Hg), diabetes, hypercholesterolemia

(total cholesterol ≥6.5 mmol/L), or renal disease and current smokers were excluded because these conditions are associated with endothelial dysfunction and arterial stiffening.<sup>34</sup> To determine whether baseline endothelial function and aortic stiffness were abnormal in RA patients, 33 age- and sex-matched healthy control participants were randomly selected from our database to compare baseline endothelial function and aortic stiffness. A favorable ethics opinion was obtained from the National Research Ethics Service, and written informed consent was obtained from each participant prior any study procedures.

### Pilot Proof-of-Concept Study

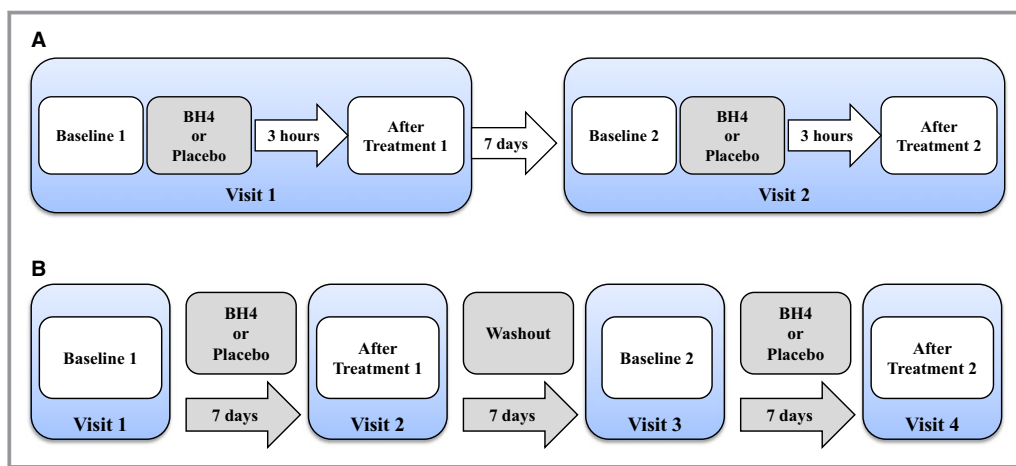
We conducted a small pilot study in 5 healthy nonsmoking control participants (mean age 27±6 years) to test whether a single dose of BH<sub>4</sub> 400 mg would improve endothelial function. We found that flow-mediated dilatation (FMD) response increased (from 5.79±1.77% to 11.37±4.05%; *P*=0.009), and there was a significant change in the baseline diameter (from 3.77±.52 to 4.10±0.62 mm; *P*=0.008); however, there was no change in mean arterial pressure (*P*=0.1) or aPWV (*P*=0.2). Data are presented as mean±SD, and significance was determined using the paired Student *t* test. Based on these pilot data, the 400 mg once-daily dose was deemed sufficient for the main study in RA patients and was similar to doses used previously in other conditions.<sup>19,33</sup>

### Experimental Protocol

The present study was conducted in a randomized, double-blind, crossover manner. To determine whether acute changes were sustained, patients were allocated to 1 of 2 groups: acute or short-term BH<sub>4</sub> supplementation.

In the acute study, participants made two 6-hour visits to the vascular laboratory (Figure 1A). On day 1, following the baseline measurement of endothelial function, aortic stiffness, DAS28, and venous blood sampling, participants received a single oral dose of BH<sub>4</sub> 400 mg or placebo (random assignment). At 3 hours after the administration of BH<sub>4</sub> or placebo, the hemodynamic measurements were repeated. A 3-hour time point was chosen based on published pharmacokinetic data.<sup>35</sup> One week later, the same protocol was followed, and participants again received either BH<sub>4</sub> or placebo.

In the short-term supplementation study, participants made 4 visits, each separated by 1 week. Participants received BH<sub>4</sub> 400 mg once daily or placebo (random order), each for 1 week, with 1-week washout between treatments (Figure 1B). All hemodynamic measurements were assessed at baseline and at the end of each 7-day treatment period (treatment 1, washout, and treatment 2). Blood was drawn at



**Figure 1.** Schema of the study design. A, All participants made 2 visits, separated by 1 week. At each time point, blood pressure, arterial stiffness, and endothelial function were assessed, and a blood sample was taken. B, All participants made 4 visits to the unit on days 0, 7, 14, and 21. At each visit, blood pressure, arterial stiffness, endothelial function, and disease activity were assessed, and a blood sample was taken. BH<sub>4</sub> indicates tetrahydrobiopterin.

each time point for the measurement of biochemical markers, and DAS28 was calculated.

### BH<sub>4</sub> Tablets

BH<sub>4</sub> was given in a form of sapropterin dihydrochloride (6R-BH<sub>4</sub>). 6R-BH<sub>4</sub> is the synthetic form of naturally occurring BH<sub>4</sub> (BioMarin Pharmaceutical Inc) and is approved for treatment of BH<sub>4</sub>-responsive phenylketonuria.

### Hemodynamic Measurements

All studies were conducted in a quiet, temperature-controlled room. Blood pressure was recorded in the brachial artery using a validated oscillometric technique (HEM-705CP; Omron Corp). Radial artery waveforms were obtained with a high-fidelity micromanometer (SPC-301; Millar Instruments) from the wrist, and a corresponding central waveform was generated using a validated transfer function (Sphygmocor; AtCor Medical). Augmentation index (AIx), a composite measure of wave reflection, mean arterial pressure and heart rate were determined using the integrated software. Aortic and brachial pulse wave velocities were measured, as previously described.<sup>36</sup>

Endothelial function was assessed in the brachial artery using the FMD technique.<sup>37</sup> Vessel diameter was measured using high-resolution vascular ultrasound (Acuson Aspen; Siemens AG) with a 10.0-MHz linear-array transducer. Brachial artery diameter was measured continuously for 1 minute at baseline and for a further 5 minutes following cuff deflation. The cuff was placed below (distal to) the ultrasound transducer and inflated to 200 mm Hg for 5 min-

utes. After return to baseline, vessel diameter was again measured continuously for 5 minutes following administration of 25 µg of sublingual glyceryl trinitrate. FMD was defined as the maximum percentage increase in vessel diameter during reactive hyperemia; glyceryl trinitrate-mediated dilatation was defined as the maximum percentage increase in vessel diameter after sublingual glyceryl trinitrate. FMD recordings were analyzed offline by a blinded operator using Cardiovascular Suite Software (Quipu).

### Laboratory Measurements and DAS28

Fasting lipid profile, high-sensitivity C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) were determined using standard methodology at the Biochemistry Laboratory at Addenbrooke's Hospital.

DAS28 is a validated composite disease activity score. The components of DAS28 include the number of swollen and tender joints from 28 assessed joints, ESR, and patient-assessed visual analog score of overall well-being (scale 0–100). DAS28 was calculated as described previously.<sup>38</sup>

### Data Analysis

The primary outcome of the study was the difference in FMD response between BH<sub>4</sub> and placebo treatments. Secondary outcomes were differences in aPWV between treatments, the relationship between changes in aPWV and FMD, and the relationship between CRP and disease activity and change in FMD and aPWV.

Based on our pilot data and published data on BH<sub>4</sub> supplementation, we calculated that a total of 15 patients (per

study) were needed to enter the 2-treatment crossover study to provide >80% power at  $P<0.05$  to detect a 4.0-U difference in FMD response between treatments with an SD of 5.0. Data were analyzed using SPSS software (version 21.0; IBM Corp). Two-way repeated-measures ANOVA was used to investigate the effect of the treatment. Custom hypothesis testing (simple) of within-participant contrasts was performed for comparison of BH<sub>4</sub> versus placebo, in which treatment order was entered as a between-participant factor to investigate whether the order in which BH<sub>4</sub> and placebo were received influenced the outcome. In post hoc tests, the effect of individual treatments was determined using paired Student *t* tests with Bonferroni adjustment for 2 comparisons. In addition, in post hoc tests, the use of methotrexate (MTX) was entered as a between-participant factor to establish whether dihydrofolate reductase inhibitor use influenced the BH<sub>4</sub> response. For the skewed variables (CRP and ESR), log-transformed values were used for the analyses. Differences between the RA and control groups were determined using the unpaired Student *t* test. A probability of <0.05 was considered significant. Data are given as mean±SD.

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agreed to the manuscript as written.

## Results

Thirty-three patients with active RA (mean DAS28 4.21±1.06) were studied before and after oral BH<sub>4</sub> and placebo. The mean age of the participants was 57±12 years, and 27 were female. Overall, 18 and 15 participants concluded the acute and short-term supplementation studies, respectively. There were no differences in age, BMI, DAS28, CRP, ESR, or blood pressure between groups; however, total cholesterol was slightly higher in the participants allocated to the short-term treatment group (4.4±0.9 versus 5.4±1.1 mmol/L;  $P=0.01$ ). Baseline characteristics are detailed by group in Table 1.

### Acute BH<sub>4</sub> Supplementation

The effect of BH<sub>4</sub> supplementation on hemodynamics and vascular function are detailed in Table 2. Following a single dose of BH<sub>4</sub>, there was a significant increase in the FMD response, whereas placebo had no effect (+3.57±4.14% versus +1.01±3.12%, between treatments;  $P=0.03$ ) (Figure 2A). The order in which the treatments were given did not affect the outcome ( $P=0.8$ ). Baseline diameter of brachial artery or response to sublingual glyceryl trinitrate did not change following the treatment. There were no significant differences between the changes in blood pressure, heart rate, AIx, brachial pulse wave velocity, or aPWV (Figure 2B) following BH<sub>4</sub> or placebo.

### Short-Term BH<sub>4</sub> Supplementation

The 1-week treatment with BH<sub>4</sub> led to a significant increase in FMD compared with placebo (+3.69±4.90% versus +0.19±2.51%, between treatments;  $P=0.02$ ) (Figure 2C). The order in which the treatments were given did not affect the outcome ( $P=0.7$ ). Again, there was no change in blood pressure or aPWV (Figure 2D).

Seventeen of the 33 RA patients were receiving MTX, a dihydrofolate reductase inhibitor, at the time of the study. Post hoc testing demonstrated that MTX had no impact on the effect of BH<sub>4</sub> on endothelial function ( $P=0.34$ ) for the drug×visit×MTX interaction. If anything, there was a non-significant trend demonstrating that those on MTX had greater improvement of FMD following BH<sub>4</sub> in comparison to those not on MTX (+4.6±5.0% versus +2.49±3.40%;  $P=0.18$ ). This was despite no difference in baseline FMD ( $P=0.9$ ) or brachial artery diameter ( $P=0.98$ ).

### The Effect of BH<sub>4</sub> on Inflammatory Markers and Disease Activity

Following short-term supplementation with BH<sub>4</sub> and placebo, there were no changes in CRP (−0.79±2.6 versus −0.93±3.17 mg/L;  $P=0.9$ ), ESR (0±7 versus 1±5 mm/h;  $P=0.6$ ), or DAS28 (0.02±0.46 versus −0.09±0.43;  $P=0.4$ ) (Table 2).

There was no correlation between the change in FMD and change in aPWV following BH<sub>4</sub> ( $R=0.27$ ;  $P=0.20$ ); however, the change in FMD after BH<sub>4</sub> correlated negatively with age ( $R=-0.44$ ;  $P=0.01$ ). Baseline aPWV was significantly ( $P<0.05$ ) correlated with mean arterial pressure ( $R=0.52$ ), age ( $R=0.52$ ), CRP ( $R=0.39$ ), and ESR ( $R=0.66$ ).

### Baseline Comparison With Healthy Controls

The mean age of the healthy control group was 56±11 years. At baseline, RA patients had reduced endothelial function in comparison to control participants (FMD 3.39±2.36% versus 6.16±3.63%;  $P=0.001$ ). After treatment with BH<sub>4</sub>, the FMD response was no longer different between RA patients and controls (6.82±4.83% versus 6.16±3.63%;  $P=0.5$ ). Baseline aPWV was higher in the RA group compared with controls (7.83±1.80 versus 6.8±1.03 m/s;  $P=0.006$ ), whereas blood pressure did not differ between the groups (Table 1).

## Discussion

The main findings of the present study are that both acute and short-term therapy with BH<sub>4</sub> significantly improves endothelial function, as assessed by vasodilatory response to reactive

**Table 1.** Baseline Characteristics and Demographics

	Acute	Short Term	Controls	P Value*
n	18	15	33	
Sex, female/male, n/n	14/4	13/2	26/7	0.5
Age, y	55±13	59±11	56±11	0.3
RA years	12±10	14±9	—	0.7
Weight, kg	71.4±14.3	70.9±10.1	71.8±16.2	0.9
BH <sub>4</sub> dose, mg/kg	5.8±1.1	5.8±0.8	—	0.9
BMI, kg/m <sup>2</sup>	25.8±4.5	26.7±4.0	26.2±5.2	0.6
Systolic BP, mm Hg	126±17	125±13	121±9	0.7
Diastolic BP, mm Hg	73±9	77±9	77±7	0.3
MAP, mm Hg	92±12	94±11	91±7	0.5
Heart rate, bpm	74±12	68±12	66±9 <sup>†</sup>	0.1
aPWV, m/s	7.87±2.24	7.79±1.15	6.8±1.03 <sup>†</sup>	0.9
Augmentation index, %	26±12	31±12	23±12	0.2
Brachial diameter, mm	3.98±0.72	3.91±0.51	3.62±0.61 <sup>†</sup>	0.8
FMD, %	3.17±2.68	3.67±1.92	6.16±3.63 <sup>†</sup>	0.6
GTN, %	9.39±4.38	7.64±3.04	9.42±5.41	0.4
DAS28	4.34±0.92	4.05±1.22	—	0.5
CRP, mg/L	6.50±5.90	4.80±4.07	—	0.3
ESR, mm/h	25±25	16±14	—	0.2
TC, mmol/L	4.4±0.9	5.4±1.1	5.2±1.0	0.01
Statin, n	5	5	0	0.7
Anti-TNF- $\alpha$ , n	5	6	0	0.5
Methotrexate, n	8	9	0	0.4
Other DMARDs, n	9	6	0	0.6
NSAIDs, n	9	8	0	0.6
Prednisolone, n (mg/day)	7 (4.7±1.5)	2 (7.50±3.5)	0	0.1 (0.1)

Values represent mean±SD. Significance was determined using an unpaired Student *t* test. BMI indicates body mass index; BP, blood pressure; bpm, beats per minute; CRP, C-reactive protein; DAS28, disease activity score; DMARDs, disease-modifying antirheumatic drugs; ESR, erythrocyte sedimentation rate; NSAIDs, nonsteroidal anti-inflammatory drugs; TC, total cholesterol; TNF- $\alpha$ , tumor necrosis factor  $\alpha$ .

\*P value for the comparison between acute and short-term study participants.

<sup>†</sup>P<0.05 between RA patients and control participants.

hyperemia in patients with active RA, but does not affect blood pressure, Alx, aPWV, or brachial pulse wave velocity. These findings suggest that modulation of endothelial function does not ameliorate arterial stiffness in RA.

The current study demonstrates, for the first time, that oral BH<sub>4</sub> supplementation improves endothelial function in patients with RA by  $\approx$ 3.5%, restoring it to levels comparable to those of healthy controls. The improvement of FMD was inversely associated with age, suggesting that endothelial dysfunction in younger RA patients may be more reversible than in older patients. FMD has been validated recently as a surrogate marker of future cardiovascular risk. Yeboah et al demonstrated that FMD is a predictor of incident cardiovascular events and that it improves the classification of

participants as low, intermediate, and high CVD risk compared with the Framingham risk score in a large population-based cohort.<sup>4</sup> The mean FMD in their whole cohort was 4.4±2.8% versus 3.4±2.5% in incident cases, suggesting that the improvement seen in our study is likely to have a clinical impact on cardiovascular risk reduction in patients with RA.

Previous studies have demonstrated that intra-arterial administration of BH<sub>4</sub> improves endothelium-dependent dilatation in patients with hypercholesterolemia,<sup>27</sup> coronary artery disease,<sup>28</sup> and type II diabetes.<sup>29</sup> Oral BH<sub>4</sub> supplementation also improves endothelial function in elderly patients,<sup>30</sup> in long-term smokers,<sup>31</sup> and in patients with hypercholesterolemia<sup>32</sup> and hypertension.<sup>33</sup> Conversely, Cunningham et al showed that although oral BH<sub>4</sub> led to an  $\approx$ 2.5-fold increase of

**Table 2.** Change in Disease Activity, Inflammation, and Hemodynamic Parameters Following BH<sub>4</sub>

	Acute			Short Term		
	BH <sub>4</sub>	Placebo	ANOVA P Value	BH <sub>4</sub>	Placebo	ANOVA P Value
DAS28				0.02±0.46	-0.09±.43	0.4
CRP, mg/L				-0.79±2.6	-0.93±3.17	0.9
ESR, mm/h				0±7	1±5	0.6
SBP, mm Hg	-2±13	-6±10	0.1	-4±11	-4±8	0.9
DBP, mm Hg	-3±7	-2±6	0.6	0±5	-1±7	0.6
MAP, mm Hg	-3±9	-5±8	0.2	-2±7	-3±7	0.8
HR, bpm	-6±7	-6±7	0.8	0±8	0±7	0.8
Alx, %	-1±7	0±6	0.3	-2±7	-1±5	0.9
bPWV, m/s	-0.14±0.74	0.09±0.77	0.6	0.07±1.14	-0.25±0.52	0.6
aPWV, m/s	-0.13±0.58	-0.22±0.43	0.6	-0.22±1.33	-0.25±0.51	0.9
Adjusted aPWV, m/s	0.15±0.82	0.31±0.80	0.4	-0.22±1.27	0.28±0.72	0.3
Brachial diameter, mm	0.14±0.37	-0.02±0.40	0.2	-0.17±0.40	0.04±0.39	0.3
FMD, %	3.57±4.14*	1.01±3.12	0.03	3.69±4.90**	0.19±2.51	0.02
GTN, %	-2.8±5.1	-1.8±2.7	0.8	0.36±2.02	1.33±2.07	0.3

Values represent mean change±SD. Significance was determined using 2-way repeated-measures ANOVA. The effect of individual treatments was determined in post hoc tests with Bonferroni correction for 2 comparisons when overall significance in ANOVA was  $P<0.05$ . Results of these comparisons are indicated by asterisks. aPWV indicates aortic pulse wave velocity; bpm, beats per minute; bPWV, brachial pulse wave velocity; CRP, C-reactive protein; DAS28, disease activity score; DBP, diastolic blood pressure; ESR, erythrocyte sedimentation rate; FMD, flow-mediated dilatation; GTN, glyceryl trinitrate; HR, heart rate; MAP, mean arterial pressure; SBP, systolic blood pressure.

\* $P=0.015$ .

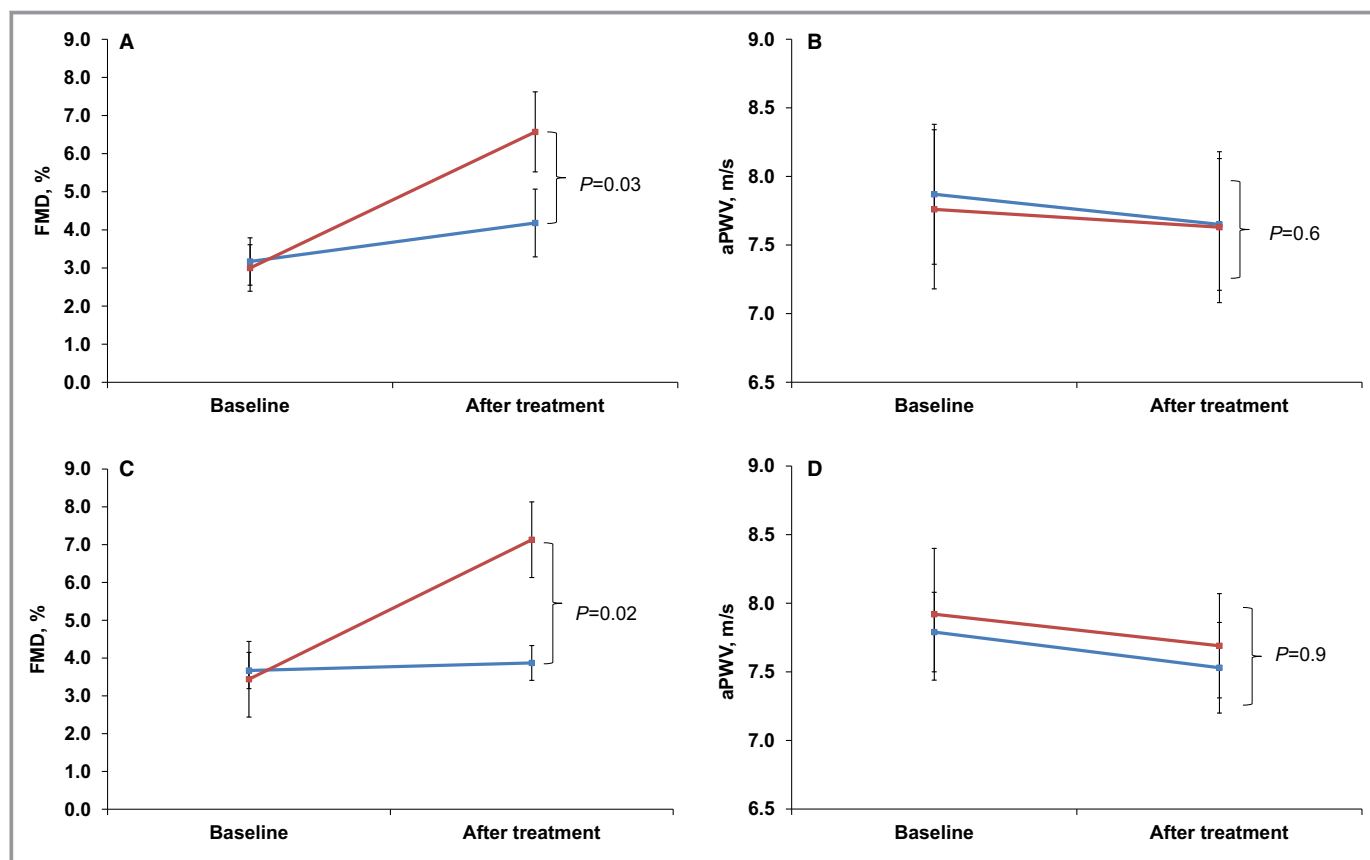
\*\* $P=0.002$ .

plasma BH<sub>4</sub> levels in patients with established coronary artery disease, it had no effect on vascular function or superoxide production because of systemic oxidation of BH<sub>4</sub> to BH<sub>2</sub>.<sup>19</sup> The impact of BH<sub>4</sub> on endothelial function has not been assessed previously in RA; however, other interventions (eg, individually designed exercise program) have been shown to improve endothelial function in RA patients.<sup>39</sup> Because intracellular BH<sub>4</sub> levels are regulated by de novo synthesis of BH<sub>4</sub> from guanosine triphosphate and through recycling of BH<sub>2</sub> to BH<sub>4</sub> via dihydrofolate reductase,<sup>40</sup> use of dihydrofolate reductase inhibitors (eg, disease-modifying antirheumatic drugs, MTX) may inhibit the bioavailability of BH<sub>4</sub> in the endothelium. Nevertheless, we found no difference in the effect of BH<sub>4</sub> supplementation on endothelial function between those RA patients on MTX and those not receiving MTX. A trend suggested that those patients on MTX had a greater increase in FMD following BH<sub>4</sub> supplementation, perhaps because of reduced levels of inflammation. Notably, our study was not powered to look at this difference.

We and others have demonstrated that patients with RA have increased aortic stiffening in comparison to healthy participants,<sup>10,14–16</sup> and that can be reversed with immunomodulatory therapy.<sup>10,12,41</sup> Our previous studies suggested a link between arterial stiffness and endothelial function,<sup>17,18</sup> but it is unclear if this relationship is causal or

if they occur in parallel in response to common drivers such as increased inflammation. Interestingly, in the present study, neither a single dose nor short-term supplementation with oral BH<sub>4</sub> led to a change in aPWV, brachial pulse wave velocity, Alx, or blood pressure in comparison to placebo. Park et al recently demonstrated in patients with chronic kidney disease that 6R-BH<sub>4</sub> significantly improved Alx<sup>42</sup>; however, there was also a drop in blood pressure, and it appears that the change in Alx was not corrected for change in mean arterial pressure, only for heart rate. Similarly, Porkert et al showed a 15-mm Hg reduction in systolic blood pressure following 8-week treatment with BH<sub>4</sub> 400 mg in hypertensive patients, but this study was not placebo controlled.<sup>33</sup> In healthy older men, BH<sub>4</sub> was shown to reduce carotid artery compliance, but there was a confounding fall in systolic blood pressure.<sup>43</sup> In postmenopausal women, however, Moreau et al showed an improvement in carotid artery compliance and FMD response following a single dose of oral BH<sub>4</sub> without change in mean arterial pressure.<sup>44</sup>

Our study also provided evidence about causality for endothelial function and aortic stiffness. We demonstrated previously that under resting conditions, local NO production modulates distensibility of muscular iliac artery in humans and that exogenous NO increases arterial distensibility<sup>18</sup>; however, data relating to elastic aorta are more controversial.



**Figure 2.** The effect of BH<sub>4</sub> 400 mg. Data represent means and SEM. Significance was determined using 2-way repeated-measures ANOVA and Bonferroni-corrected post hoc tests. Red represents BH<sub>4</sub>; blue represents placebo. A, Endothelial function was measured before and 3 hours after BH<sub>4</sub> and placebo in random order. ANOVA:  $P=0.03$  between groups; post hoc BH<sub>4</sub>:  $P=0.015$ ; placebo:  $P=0.5$ . B, Aortic stiffness was measured before and 3 hours after BH<sub>4</sub> and placebo in random order. ANOVA between groups:  $P=0.6$ ; post hoc BH<sub>4</sub>:  $P=0.5$ ; placebo:  $P=0.6$ . C, Endothelial function was measured before and 7 days after BH<sub>4</sub> and placebo in random order. ANOVA between groups:  $P=0.02$ ; post hoc BH<sub>4</sub>:  $P=0.002$ ; placebo:  $P=0.4$ . D, Aortic stiffness was measured before and 7 days after BH<sub>4</sub> and placebo in random order. ANOVA between groups:  $P=0.9$ ; post hoc BH<sub>4</sub>:  $P=0.5$ ; placebo:  $P=0.5$ . aPWV indicates aortic pulse wave velocity; BH<sub>4</sub>, tetrahydrobiopterin; FMD, flow-mediated dilatation.

A recent study by Butlin et al showed that exogenous NO donor sodium nitroprusside does not alter aortic stiffness in vivo in rats.<sup>45</sup> Nevertheless, numerous studies,<sup>10,11,46</sup> including ours, have demonstrated a reduction in aortic stiffness, which mirrors the changes in endothelial function, following anti-inflammatory or cholesterol-lowering therapies. The current study clearly demonstrated that improvement of endothelial function, without change in inflammation or lipids, does not lead to reduction of aortic stiffness, strongly suggesting that there is no direct causal link between aortic stiffness and endothelial function. We cannot exclude the possibility that this finding is specific to RA because previous studies have demonstrated changes in aPWV in healthy participants in acute models of induced inflammation.<sup>47,48</sup>

## Limitations

We did not assess serum BH<sub>4</sub> and BH<sub>2</sub> levels in our study; however, numerous studies have demonstrated that

supplementation with 6R-BH<sub>4</sub> leads to increase in serum BH<sub>4</sub> levels.<sup>19,32</sup> Moreover, we chose the time points based on published findings. Fiege et al demonstrated that the maximal plasma biopterin levels peaked between 1 and 4 hours, with median peak of 3 hours and maximum concentration of 258.7 to 295.0 nmol/L for 10 mg/kg; even 24 hours after administration, 6R-BH<sub>4</sub> levels were still 4–5 times the basal values.<sup>35</sup> Moreover, our treatment period of 1-week was very short, and we can neither extrapolate these results to what may be the chronic effects of BH<sub>4</sub> supplementation in patients with RA nor predict whether it may have beneficial effects on future cardiovascular events.

## Conclusion

This study shows for the first time in patients with RA that oral BH<sub>4</sub> improves endothelial function as measured by a response to reactive hyperemia. These findings suggest that recoupling of eNOS using cofactor BH<sub>4</sub> may provide a rational therapeutic

approach to prevent CVD in patients with chronic inflammatory diseases. BH<sub>4</sub> supplementation did not appear to reduce aortic stiffness, suggesting that endothelial dysfunction is not a pathophysiology behind aortic stiffening in RA and that these conditions may just exist in parallel, both influenced by common risk factors such as inflammation and increased reactive oxygen species production.

## Sources of Funding

Mäki-Petäjä and Wilkinson were funded by British Heart Foundation. Wilkinson, Cheriyan and Shenker received funding from the Comprehensive Local Research Network and Wilkinson and Cheriyan from the National Institute for Health Research: Cambridge Biomedical Research Centre.

## Disclosures

None.

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*J Am Heart Assoc.* 2016;5:e002762; originally published February 19, 2016;

doi: 10.1161/JAHA.115.002762

The *Journal of the American Heart Association* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231  
Online ISSN: 2047-9980

The online version of this article, along with updated information and services, is located on the World Wide Web at:

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