Folic Acid Ingestion Improves Skeletal Muscle Blood Flow during Graded Handgrip and Plantar Flexion Exercise in Aged Humans

Steven A. Romero, Daniel Gagnon, Amy N. Adams, Gilbert Moralez, Ken Kouda, Manall F. Jaffery, Matthew N. Cramer, and Craig G. Crandall

1University of Texas Southwestern Medical Center and Institute for Exercise and Environmental Medicine, Texas Health Presbyterian Hospital, USA
2Montreal Heart Institute, Université de Montréal, Montréal, Canada
3Département de pharmacologie et physiologie, Faculté de médecine, Université de Montréal, Montréal, QC, Canada.
4Wakayama Medical University, Wakayama, Japan

*Corresponding author

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Address for correspondence:
Dr. Craig G. Crandall
Institute for Exercise and Environmental Medicine
Texas Health Presbyterian Hospital Dallas
7232 Greenville Ave
Dallas, TX 75231
craigcrandall@texashealth.org
214-345-4623

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Abstract

Skeletal muscle blood flow is attenuated in aged humans performing dynamic exercise, which is due, in part, to impaired local vasodilatory mechanisms. Recent evidence suggests that folic acid improves cutaneous vasodilation during localized and whole-body heating through nitric oxide-dependent mechanisms. However, it is unclear if folic acid improves vasodilation in other vascular beds during conditions of increased metabolism (i.e. exercise). The purpose of this study was to test the hypothesis that folic acid ingestion improves skeletal muscle blood flow in aged adults performing graded handgrip and plantar flexion exercise via increased vascular conductance. Nine healthy aged adults (2 males; age 68 ± 5 years) performed graded handgrip and plantar flexion exercise before (control), two hours after (acute, 5 mg), and following 6 weeks (chronic, 5 mg/day) folic acid ingestion. Forearm (brachial artery) and leg (superficial femoral artery) blood velocity and diameter were measured via Duplex ultrasonography and used to calculate blood flow. Acute and chronic folic acid ingestion increased serum folate (both P < 0.05 vs. control). During handgrip exercise, acute and chronic folic acid ingestion increased forearm blood flow (both conditions P < 0.05 vs. control) and vascular conductance (both P < 0.05 vs. control). During plantar flexion exercise, acute and chronic folic acid ingestion increased leg blood flow (both P < 0.05 vs. control), but only acute folic acid ingestion increased vascular conductance (P < 0.05 vs. control). Taken together, folic acid ingestion increases blood flow to active skeletal muscle primarily via improved local vasodilation in aged adults.

New and Noteworthy

Our findings demonstrate that folic acid ingestion improves blood flow via enhanced vascular conductance in the exercising skeletal muscle of aged humans. These findings provide evidence for the therapeutic use of folic acid to improve skeletal muscle blood flow, and perhaps exercise and functional capacity, in human primary aging.
Abbreviations list

BH₄, Tetrahydrobiopterin
5-MTHF, 5-methyltetrahydrofolate
Introduction

The arterial vasculature undergoes profound changes across the human lifespan. This “vascular aging” includes structural remodeling, in addition to alterations in local vascular control mechanisms (21, 34, 50, 71, 72). Ultimately, these vascular changes contribute to malperfusion of active skeletal muscle (e.g. limited exercise hyperemia) which reduces functional and exercise capacity, independent of changes in cardiac function (21). Importantly, attenuated functional and exercise capacities directly increase the risk for cardiovascular morbidity and mortality (1, 4, 5, 17, 26, 33).

Augmented sympathetic vasoconstriction (i.e. impaired functional sympatholysis) and an altered release of endothelial-derived local vasodilator and vasoconstrictor substances are the primary factors contributing to malperfusion of skeletal muscle associated with human primary aging (21, 29). These changes not only influence regional or bulk blood flow (31, 43, 46), but can also impair the distribution of oxygenated blood within exercising skeletal muscle (39). Reduced availability of endothelial-derived nitric oxide is one mechanism by which exercise hyperemia is attenuated with primary aging. In a seminal study, Schrage et al. (58) reported that the contribution of nitric oxide to exercise hyperemia is reduced by ~45% with primary aging, an effect that likely occurs secondary to increased vascular oxidative stress, inflammation, and the reduced availability of essential substrates and co-factors necessary for optimal endothelial nitric oxide synthase activity (12, 32, 67). Interestingly, synthetic analogues of tetrahydrobiopterin (BH₄), a critical co-factor necessary for optimal endothelial nitric oxide synthase activity, improve nitric oxide-mediated vasodilation in the microcirculation of middle-aged and aged adults (23, 60, 62). However, the extraordinary expense and limited availability of synthetic BH₄ analogues available for human use precludes its mainstream therapeutic use. Recent evidence suggests that nitric oxide-dependent vasodilation in the cutaneous microvasculature of aged adults is improved through local administration (via microdialysis) and chronic ingestion of folic acid (61), an essential vitamin that may improve the vasodilatory response through a direct interaction with endothelial nitric oxide synthase and/or an indirect restoration of BH₄ availability (28, 65). Additionally, chronic folic acid ingestion improved macrovascular dilator function (assessed via flow-mediated dilation) in young female athletes with vascular dysfunction (24, 25). Folic acid supplementation could therefore represent an alternative therapy to improve nitric oxide-dependent vasodilation and increase skeletal muscle blood flow in aged adults. However, it remains unclear if the vascular benefits of folic acid
extend to non-cutaneous vascular beds during conditions of elevated metabolism, such as exercise.

Therefore, the purpose of this study was to test the hypothesis that acute and chronic folic acid ingestion improves skeletal muscle blood flow in aged adults performing graded handgrip and plantar flexion exercise via heightened vasodilation (i.e., improved vascular conductance in active skeletal muscle). Furthermore, we hypothesized that the exercise-induced dilation of the brachial artery, an indirect measure of nitric oxide availability, would be increased with acute and chronic folic acid ingestion. The dilator response during plantar flexion exercise was not assessed given the negligible conduit vessel dilation observed during isolated leg exercise (52, 71). To determine the systemic vasodilatory effects of folic acid, we employed isolated arm and leg exercise models given the known heterogeneities in vascular control and function between limbs (40, 42, 45, 48, 49, 54).

Methods

Subjects

Subject physical characteristics and fasting blood data are shown in Table 1. Written informed consent was obtained from all subjects subsequent to a verbal and written briefing of all experimental procedures. This study and consent were approved by the Institutional Review Boards at the University of Texas Southwestern Medical Center and Texas Health Presbyterian Hospital Dallas and was performed in accordance with the principles outlined in the Declaration of Helsinki. Nine (2 males) recreationally active adults, aged 60 - 80 years, participated in this study. All subjects were free from known cardio-metabolic disease and were deemed healthy following the completion of an in-depth medical history questionnaire and a resting 12-lead electrocardiogram. Subjects were required to abstain from caffeine, supplements (i.e., multivitamin and vitamin D), alcohol, and exercise for 24 h before the study. Subjects were required to abstain from over-the-counter (e.g., NSAID, antihistamine, etc.) or prescription medications (i.e. levothyroxine and pravastatin) at the time of the study. Additionally, subjects reported to the laboratory after an overnight fast. All aged females were post-menopausal (18 ± 4 y from cessation of menstruation) and not currently on hormone replacement therapy.

Experimental Approach
Subjects reported to the laboratory at 9:00 AM. Subsequent to obtaining a fasting blood sample, subjects consumed a standardized breakfast (oatmeal raisin walnut Clif Bar® and a Boost® nutritional beverage containing 90 µg folic acid). Dietary time control trials indicate that this breakfast does not increase serum folate measured 1 h after consumption. Maximal voluntary handgrip contraction was then determined as the average of three maximal contractions using a handgrip dynamometer (Stoelting, Chicago, IL, USA). Following instrumentation and a ~25 min rest period, subjects performed 3 stages of graded handgrip exercise. Subjects then rested for ~20 min before performing 3 stages of graded plantar flexion exercise. Following a brief recovery period, subjects ingested 5 mg of folic acid (Bio-tech Pharmacal Inc., Fayetteville, AR, USA) and then rested quietly for 2 h before repeating handgrip and plantar flexion exercise protocols (acute folic acid ingestion trial). A second sample of venous blood was obtained immediately prior to the second bout of handgrip and plantar flexion exercise for determination of serum folate. Folic acid is absorbed largely in the proximal portion of the small intestine and has a time-to-peak concentration of 1-2 h and a half-life of 2-5 h (7, 41, 64, 69). A hemodynamic time-control trial was not performed as Richards et al. (53) previously demonstrated, using a nearly identical experimental approach, that central and peripheral exercising hemodynamics are similar across this time frame in aged adults. Room temperature was maintained at ~23 °C.

Subsequent to the completion of the acute folic acid ingestion trial, subjects ingested 5 mg of folic acid daily, for the next 6 weeks (chronic folic acid ingestion trial). After this period, subjects returned to the laboratory and completed the same handgrip and plantar flexion exercise protocols. A final sample of venous blood was obtained prior to the consumption of the standardized breakfast and prior to exercise for determination of serum folate. The final dose of folic acid was taken at least 12 h prior to blood sampling and completion of the final exercise trial. The dose and duration of folic acid ingestion was chosen based on prior reports suggesting that this pharmacological approach increases circulating folate and improves endothelial function in primary aging, hyperhomocysteinemia, and coronary artery disease (2, 14, 15, 61).

**Exercise Protocols and Measurements**

**Handgrip and plantar flexion exercise.** Graded (3 kg, 6 kg, 9 kg) intermittent isometric handgrip exercise was performed using a handgrip dynamometer and graded (5 kg, 9 kg, 14 kg) dynamic plantar flexion exercise was performed using a custom-built ergometer. Exercise stages were 4 min, with 1 min rest separating each stage in order to prevent fatigue, though this
was not confirmed experimentally. Handgrip and plantar flexion exercise was performed using a duty cycle of 1 s contraction and 2 s relaxation (20 contractions min$^{-1}$). Subjects were provided with real-time visual (hand grip only) and audio feedback to ensure correct contraction timing.

**Central hemodynamics.** Arterial blood pressure was measured from the left arm using an automated sphygmomanometer (Tango+, SunTech Medical, Raleigh, NC, USA). Beat-by-beat arterial blood pressure was measured non-invasively by finger photoplethysmography and stroke volume was estimated via Modelflow (Nexfin, Edwards Life Sciences, Irvine, CA, USA). Heart rate was monitored via electrocardiogram (Solar 8000i, GE Healthcare, Milwaukee, WI, USA) interfaced with a cardiotachometer (CWE, Ardmore, PA, USA).

**Blood flow.** Duplex ultrasonography was used to measure simultaneously blood velocity and diameter in the brachial and superficial femoral arteries. Velocity and diameter measurements were made proximal to the brachial artery bifurcation and distal to the common femoral artery bifurcation. All velocity measurements were made using a linear-array transducer (11 MHz, Phillips iE33, Andover, MA, USA) operating with an insonation angle of 60° and a Doppler sample volume that encompassed the entire vessel lumen. The ultrasound system was interfaced with a computer running custom audio-recording software (DUC$^2$) to capture blood velocity (8, 16, 36, 37, 55, 56). Throughout each exercise trial, the ultrasound transducer was manually held in place to ensure consistent measurements of velocity and diameter. All ultrasound measurements were performed by the same sonographer (SAR). Additionally, an outline of the ultrasound transducer was made on the skin to ensure consistent placement across time (acute folic acid ingestion trial only).

**Serum folate.** Venous blood was sampled prior to exercise for the three conditions of control, acute folic acid ingestion, and chronic folic acid ingestion. Venous blood was collected into a Vacutainer® and centrifuged within 10 min at 1950xG. Serum was kept at 4°C until analysis, which was performed within 24 h. Serum folate was quantified using a chemiluminescent microparticle folate binding protein assay (ARCHITECT Folate Assay, Abbott Laboratories, Abbott Park, IL, USA).

**Data and Statistical Analyses**

Central hemodynamics were collected at 50 Hz and were averaged across the final min of each stage of exercise using commercially available software (Biopac MP150, Santa Barbara, CA, USA). Blood velocities were determined from the Doppler ultrasound audio
recordings using a custom intensity-weighted algorithm (DUC$^2$), subsequent to demodulation of forward and reverse Doppler frequencies (9, 56). Brachial artery diameter was determined using custom edge-detection and wall-tracking software (3, 70). Leg movement associated with plantar flexion exercise precluded the use of wall-tracking software for determination of vessel diameter. Therefore, ultrasound calipers were used to determine manually superficial femoral artery diameter. All (arm and leg) blood velocities and brachial artery diameter were averaged across the final min of each stage of exercise. Superficial femoral artery end-diastolic diameter was measured in triplicate across several (3-5) cardiac cycles in the final min of each stage of exercise. Blood flow was calculated as the cross-sectional area of the imaged artery multiplied by mean blood velocity and reported in ml min$^{-1}$. Vascular conductance was calculated by dividing blood flow by mean arterial pressure then multiplying by 100 and expressed as ml min$^{-1}$ 100 mmHg$^{-1}$. Shear rate was calculated by multiplying 8 by the quotient of blood velocity and vessel diameter and expressed as s$^{-1}$. Recent evidence suggests that exercise-induced dilation of the brachial artery is highly dependent on nitric oxide (68, 73). We therefore examined exercise-induced brachial artery dilation during handgrip exercise as an indirect measure of nitric oxide availability. This response was not examined given the negligible conduit vessel dilation observed during isolated leg exercise (52, 71).

Our primary outcome variables were analyzed using a mixed model analysis of variance with repeated measures (JMP Pro 12; SAS Institute Inc. Cary, NC, USA). Planned comparisons were used to examine specific condition-exercise stage interactions. General interactions were examined using Tukey’s post hoc procedure. For the relative assessment of exercise-induced brachial artery dilation, shear rate was entered into the model as a covariate to account for this dilatory stimulus. Significance was set at $P \leq 0.05$. Data are reported as mean ± SEM unless stated otherwise (e.g., SD in Table 1).

**Results**

**Serum Folate**

Serum folate is shown in Figure 1. Compared with control, serum folate increased with acute and chronic folic acid ingestion (both $P < 0.05$). Relative to acute ingestion, serum folate was lower following chronic ingestion ($P < 0.05$).

[Insert Figure 1 here]

**Resting Hemodynamics**
Resting hemodynamics are shown in Table 2. Heart rate was greater for chronic folic acid ingestion when compared with acute folic acid ingestion ($P = 0.05$). Cardiac output tended to be greater for control ($P = 0.08$) and chronic folic acid ingestion ($P = 0.06$) when compared with acute folic acid ingestion. Leg blood flow tended to be greater with chronic folic acid ingestion when compared with acute folic acid ingestion ($P = 0.08$). Likewise, leg vascular conductance did not differ between control and acute folic acid ingestion ($P = 0.1$), but tended to be greater with chronic folic acid ingestion ($P = 0.08$ vs. acute folic acid ingestion).

[Insert Table 2 here]

**Exercising Hemodynamics**

**Handgrip Exercise.** Central hemodynamic responses to hand grip exercise are shown in Table 3, whereas forearm hemodynamics are shown in Figures 2 and 3. As expected, forearm blood flow (main effect of exercise, $P < 0.05$) and vascular conductance (main effect of exercise, $P < 0.05$) progressively increased during exercise for all three conditions. The increase in forearm blood flow was greater during exercise with acute and chronic folic acid ingestion (both $P < 0.05$ vs. control). Likewise, the increase in forearm vascular conductance was greater during exercise with acute and chronic folic acid ingestion (both $P < 0.05$ vs. control). Compared with the control condition (3 kg: $102 \pm 10$ ml min$^{-1}$, 6 kg: $130 \pm 13$ ml min$^{-1}$, 9 kg: $164 \pm 14$ ml min$^{-1}$), absolute blood flow was greater during handgrip exercise with acute (3 kg: $115 \pm 9$ ml min$^{-1}$, 6 kg: $146 \pm 9$ ml min$^{-1}$, 9 kg: $195 \pm 12$ ml min$^{-1}$; $P < 0.05$ vs. control) and chronic (3 kg: $120 \pm 11$ ml min$^{-1}$, 6 kg: $156 \pm 12$ ml min$^{-1}$, 9 kg: $198 \pm 12$ ml min$^{-1}$; $P < 0.05$ vs. control) folic acid ingestion.

[Insert Table 3 and Figure 2 here]

Shear rate increased progressively throughout exercise for all conditions (main effect of exercise, $P < 0.05$), which induced a progressive dilation of the brachial artery (main effect of exercise, $P < 0.05$). Compared with control ($317 \pm 26$ s$^{-1}$), the average increase in shear rate during exercise was greater with acute ($359 \pm 30$ s$^{-1}$) and chronic folic acid ingestion ($355 \pm 27$ s$^{-1}$; both $P < 0.05$ vs. control). The increase in brachial artery diameter during exercise was greater with acute and chronic folic acid ingestion (both $P < 0.05$ vs. control, Figure 3). When expressed as a relative change (i.e. flow-mediated dilation) and after adjusting (via ANCOVA) for shear rate stimulus, the brachial artery dilatory response was increased with acute and chronic folic acid ingestion (both $P < 0.05$ vs. control, Figure 3).
Plantar Flexion Exercise. Central hemodynamic responses to plantar flexion exercise are shown in Table 4, whereas leg hemodynamics are shown in Figure 4. Leg blood flow and vascular conductance increased with workload (main effect of exercise, $P < 0.05$). The magnitude of the increase in leg blood flow ($P < 0.05$ vs. control) and vascular conductance ($P < 0.05$ vs. control) was greater during exercise with acute folic acid ingestion. A greater increase in leg blood flow during exercise persisted following chronic folic acid ingestion ($P < 0.05$ vs. control). However, in contrast to acute folic acid ingestion, this response was not mediated by improved vascular conductance ($P = 0.3$). Compared with the control condition (5 kg: 262 ± 22 ml min$^{-1}$, 9 kg: 352 ± 22 ml min$^{-1}$, 14 kg: 434 ± 33 ml min$^{-1}$), absolute blood flow was greater during plantar flexion exercise with acute (5 kg: 282 ± 20 ml min$^{-1}$, 9 kg: 394 ± 19 ml min$^{-1}$, 14 kg: 488 ± 33 ml min$^{-1}$; $P < 0.05$ vs. control) and chronic (5 kg: 304 ± 24 ml min$^{-1}$, 9 kg: 401 ± 25 ml min$^{-1}$, 14 kg: 466 ± 12 ml min$^{-1}$; $P < 0.05$ vs. control) folic acid ingestion.

Discussion

The purpose of this study was to test the hypothesis that acute and chronic folic acid ingestion improves skeletal muscle blood flow in aged adults performing graded handgrip and plantar flexion exercise. In agreement with our hypothesis, we found that acute and chronic folic acid ingestion improved forearm blood flow during graded handgrip exercise, a response mediated by improved vasodilation within exercising skeletal muscle (i.e. augmented vascular conductance). The mechanism responsible for this improvement appears to be improved nitric oxide availability given that exercise-induced dilation of the brachial artery was similarly improved with folic acid ingestion. We also found that acute and chronic folic acid ingestion improved leg blood flow during graded plantar flexion exercise. Elevated leg blood flow with acute folic acid ingestion was mediated by improved vascular conductance, whereas an increase in perfusion pressure was responsible for the improvement associated with chronic folic acid ingestion, given an absence of an effect of chronic folic acid on vascular conductance.

Mechanism/s of Improved Skeletal Muscle Blood Flow

Prior work has demonstrated that that exercise-induced dilation of the brachial artery is nitric oxide-dependent and attenuated with primary aging (68, 73). We found that folic acid induces an upward shift of this response (absolute and relative change, Figure 3) throughout
handgrip exercise, which suggests that folic acid increased nitric oxide availability. Along these lines, we speculate that folic acid functions as a proxy for naturally occurring BH₄, thereby improving nitric oxide availability. Folic acid or more specifically its active metabolite, 5-methyltetrahydrofolate (5-MTHF), can directly bind (at the BH₄ docking site) to endothelial nitric oxide synthase in a manner analogous to that of naturally occurring BH₄ (28). Thus, with folic acid ingestion, endothelial nitric oxide synthase transitions from its monomeric uncoupled form, which produces superoxide, to its dimeric coupled form, which catalyzes the formation of nitric oxide from L-arginine and molecular oxygen (18, 74, 75).

Several indirect pathways exist by which folic acid could improve nitric oxide availability (35, 63). First, 5-MTHF can augment the synthesis of BH₄ from its oxidized form (dihydrobiopterin, BH₂) by upregulating activity of the enzyme dihydrofolate reductase (10, 11). Second, 5-MTHF mediated stabilization of BH₄ and/or improved efficacy of BH₄ to prevent nitric oxide synthase uncoupling, could improve nitric oxide availability (38, 65). Third, 5-MTHF scavenging of superoxide could attenuate endothelial nitric oxide synthase uncoupling. However, the capacity of 5-MTHF is far below that which is considered efficacious in scavenging superoxide in vivo (65). Interestingly, Trinity et al. (67) recently demonstrated that the intra-arterial infusion of ascorbate improved brachial artery dilation and skeletal muscle blood flow in aged humans performing handgrip exercise. However, they found that direct and indirect measures of oxidative stress were not reduced during exercise despite a high dose antioxidant infusion, leading them to conclude that their results can be explained, in part, through improved BH₄-dependent signaling (22, 27). Finally, previous findings suggest that, in patients with hyperhomocysteinemia and vascular disease, folic acid ingestion can reduce circulating concentrations of the potent vasoconstrictor, endothelin-1 (19). Given the recent observations that endothelin-1 mediated vasoconstriction is augmented in aged humans performing exercise, we speculate that it is possible that folic acid ingestion can directly improve vasodilatory signaling in aging by attenuating circulating endothelin-1 or indirectly through an endothelin-1 nitric oxide interaction (6).

**Folic Acid Ingestion: Do Duration and Dose Matter?**

The vast majority of investigations examining vascular responses to folic acid ingestion have utilized experimental paradigms employing a chronic intervention. However, our results and others (61, 66) suggest that acute folic acid administration is efficacious, provided that the administration route is direct (e.g. intradermal) and/or the dose is high. With respect to the latter,
the dose employed in our study (5 mg) is well above the daily intake of 350 µg day\(^{-1}\) recommended for healthy adults (59). However, it should be noted that folic acid ingestion, even in high doses such as that employed in our study, is considered safe and poses no toxicity risk (38). It is unclear at present if a lower dose of folic acid or if increased consumption of food-derived folates would be equally advantageous.

**Arm vs. Leg Exercise**

We found that acute and chronic folic acid ingestion improved forearm and leg blood flow during graded exercise. However, the improvement in leg blood flow observed with chronic folic acid ingestion was not mediated by improved vasodilation. It is unclear at present why this response differs from the arm, and why it differs (within the leg) from acute folic acid ingestion. This discrepancy may be related to the amount of circulating folate and the threshold concentration necessary to improve vasodilatory signaling within a given limb. Relative to baseline, serum folate increased with chronic ingestion, but was approximately half of the concentration attained with acute ingestion. Despite this difference, forearm vascular conductance remained elevated (vs. control) to a similar extent between conditions. Given the known heterogeneities in vascular control and function between limbs (13, 40, 71), it is possible that the threshold concentration necessary to improve vasodilation in the leg is greater than that in the arm.

**Experimental Considerations**

Several experimental considerations warrant discussion. First, our study did not include a young cohort. However, our intention was not to determine if folic acid ingestion will “rescue” skeletal muscle blood flow, thereby minimizing age-related differences during exercise, but rather to examine if folic acid ingestion improves skeletal muscle blood flow in aged adults. Second, we lack a more direct measure of improved nitric oxide availability, such as a venous metabolite draining the active skeletal muscle or via the pharmacological inhibition of nitric oxide production. Further, our indirect measure of nitric oxide availability was assessed in large conducting vessel, the results of which may not extend to the resistance vasculature. Third, it is well documented that age-related decrements in exercise hyperemia are more pronounced in women (44, 47, 50, 51). Thus, given the unbalanced \(N\) between sexes, it is possible that our observations are driven predominantly by women with little contribution from men. Fourth, it is possible that the small amount of folic acid contained in the standardized breakfast could have influenced our results, particularly those for the control exercise trial. However, dietary time
control trials indicate that serum folate does not increase 1 h post consumption of the
standardized breakfast. Fully controlling for folic acid intake is further complicated given that we
did not track dietary history prior to the initial visit or throughout 8 weeks of daily folic acid
ingestion. While we recognize that we cannot fully account for folic acid intake, based on normal
baseline serum folate concentrations measured for the control condition, we can reason that
folic acid intake was normal and likely within daily requirements. Fifth, without inclusion of a
blinded placebo condition, it is possible that the observed findings, though unlikely, could have
been influenced by the participants and not as a direct effect of folic acid ingestion. Finally, it is
possible that our findings, regarding chronic folic acid ingestion, could merely be an extension of
the acute response, despite evaluating participants at least 12 hours following ingestion of the
final folic acid tablet. However, our findings of a lack of improved vasodilation in the leg after
chronic folic acid ingestion would argue against this possibility. Nevertheless, this does not
diminish our conclusions regarding the efficacy of folic acid. Indeed, it could be reasoned that if
the primary goal is to improve skeletal muscle blood flow in aged adults performing exercise,
then the present data would suggest that the timing in which folic acid is ingested is irrelevant
and chronic use unnecessary.

Perspectives

Human primary aging is associated with a significant decline in physical activity and
exercise capacity, which increases the risk for cardiovascular disease and mortality (1, 4, 5, 26,
33, 57). Aerobic capacity is highly dependent on the adequate delivery and distribution of
oxygen and nutrients to the active skeletal muscle. As such, it stands to reason that the folic
acid-mediated improvement in skeletal muscle blood flow could improve oxygen utilization and
exercise tolerance in aged humans; an effect that could ultimately reduce the cardiovascular
disease risk by increasing functional and aerobic capacity. Moreover, preserving and/or
improving endothelial health/function through folic acid ingestion could decrease cardiovascular
disease risk independent of its effect on issues related to exercise tolerance (20, 30). Taken
together, various mechanisms exist by which folic acid could improve cardiovascular health and
improve quality of life in human primary aging. These remain exciting avenues for future
research.

Summary

Vascular changes associated with human primary aging contribute to malperfusion of
skeletal muscle during dynamic exercise. In this study, we examined the efficacy of folic acid
ingestion to improve skeletal muscle blood flow via improved vascular conductance (i.e. vasodilation) in aged adults performing exercise. Our findings demonstrate that folic acid ingestion improves blood flow primarily via enhanced vascular conductance in the aging skeletal muscle vasculature. These findings provide evidence for the therapeutic use of folic acid to improve skeletal muscle blood flow in human primary aging.
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Author contributions

SAR, DG, and CGC contributed to conception or design of the work. SAR, DG, ANA, GM, KK, MJ, MNC, and CGC performed acquisition, analysis or interpretation of data for the work. SAR drafted the manuscript and DG, ANA, GM, KK MJ, MNC, and CGC revised it critically for important intellectual content.

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Disclosures

The authors have no competing interests to declare.
### Table 1. Subject Characteristics

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<tr>
<td>Age (yrs)</td>
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<td>Height (cm)</td>
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<td>Weight (kg)</td>
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<td>Body mass index (kg m(^{-2}))</td>
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<td>Mean arterial pressure (mmHg)</td>
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<td>MVC (kg)</td>
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<td>Glucose (mg dl(^{-1}))</td>
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Values are mean ± SD. MVC, maximal voluntary handgrip contraction; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
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<th>Control</th>
<th>Acute Folic Acid Ingestion</th>
<th>Chronic Folic Acid Ingestion</th>
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<tr>
<td>Heart Rate (beats min(^{-1}))</td>
<td>66 ± 2</td>
<td>62 ± 2</td>
<td>68 ± 3(^\dagger)</td>
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<td>Mean Arterial Pressure (mmHg)</td>
<td>84 ± 2</td>
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<td>Forearm Blood Flow (ml min(^{-1}))</td>
<td>44 ± 5</td>
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<td>Forearm Vascular Conductance (ml min(^{-1}) 100mmHg(^{-1}))</td>
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<td>Leg Blood Flow (ml min(^{-1}))</td>
<td>125 ± 9</td>
<td>113 ± 11</td>
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<tr>
<td>Leg Vascular Conductance (ml min(^{-1}) 100mmHg(^{-1}))</td>
<td>147 ± 13</td>
<td>126 ± 12</td>
<td>149 ± 15</td>
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Values are mean ± SEM. \(^\dagger\) P = 0.05 vs. acute folic acid ingestion.
### Table 3. Central Hemodynamics during Handgrip Exercise

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<tr>
<td></td>
<td>3 kg</td>
<td>6 kg</td>
<td>9 kg</td>
</tr>
<tr>
<td>Δ Heart Rate (beats min⁻¹)</td>
<td>3 ± 1</td>
<td>5 ± 1</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>Δ Mean Arterial Pressure (mmHg)</td>
<td>4 ± 1</td>
<td>5 ± 1</td>
<td>7 ± 1*</td>
</tr>
<tr>
<td>Δ Cardiac Output (l min⁻¹)</td>
<td>0.4 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Δ Stroke Volume (ml)</td>
<td>1 ± 1‡</td>
<td>-1 ± 2‡</td>
<td>0 ± 1‡</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.* main effect of exercise intensity, \( P < 0.05 \) vs. 3 kg; ‡ main effect of condition, \( P \leq 0.05 \) vs. acute folic acid ingestion.
### Table 4. Central Hemodynamics during Plantar Flexion Exercise

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Acute Folic Acid Ingestion</th>
<th>Chronic Folic Acid Ingestion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 kg</td>
<td>9 kg</td>
<td>14 kg</td>
</tr>
<tr>
<td>Δ Heart Rate (beats min⁻¹)</td>
<td>3 ± 1</td>
<td>5 ± 1*</td>
<td>9 ± 1†</td>
</tr>
<tr>
<td>Δ Mean Arterial Pressure (mmHg)</td>
<td>2 ± 1</td>
<td>3 ± 1</td>
<td>7 ± 1*†</td>
</tr>
<tr>
<td>Δ Cardiac Output (l min⁻¹)</td>
<td>0.4 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.6 ± 0.1*</td>
</tr>
<tr>
<td>Δ Stroke Volume (ml)</td>
<td>2 ± 1</td>
<td>1 ± 1</td>
<td>0 ± 1</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. * main effect of exercise intensity, *P < 0.05 vs. 5 kg; † main effect of exercise intensity, *P < 0.05 vs. 9 kg; # main effect of condition, *P ≤ 0.05 vs. control; ‡ main effect of condition, *P ≤ 0.05 vs. acute folic acid ingestion.
Figure Legends

Figure 1. Serum folate concentrations at baseline and following acute and chronic folic acid ingestion. Open bar, baseline control; black bar, acute folic acid ingestion; gray bar, chronic folic acid ingestion. * P < 0.05 vs. baseline; # P < 0.05 vs. acute folic acid ingestion.

Figure 2. The change from baseline in forearm blood flow (top panel) and the change in vascular conductance (bottom panel) during graded handgrip exercise. Open circles, control; black circles, acute folic acid ingestion; gray circles, chronic folic acid ingestion. * P < 0.05 for main effect of acute folic acid ingestion vs. control; † P < 0.05 for main effect of chronic folic acid ingestion vs. control; ‡ P < 0.05 for main effect of exercise intensity.

Figure 3. The absolute change from baseline in exercise-induced brachial artery diameter (top panel) and the relative flow-mediated dilatory response after correcting for shear stimulus via ANCOVA (bottom panel). Open circles, control; black circles, acute folic acid ingestion; gray circles, chronic folic acid ingestion. * P < 0.05 for main effect of acute folic acid ingestion vs. control; † P < 0.05 for main effect of chronic folic acid ingestion vs. control; ‡ P < 0.05 for main effect of exercise intensity.

Figure 4. The change from baseline in leg blood flow (top panel) and the change in vascular conductance (bottom panel) during graded plantar flexion exercise. Open circles, control; black circles, acute folic acid ingestion; gray circles, chronic folic acid ingestion. * P < 0.05 for main effect of acute folic acid ingestion vs. control; † P < 0.05 for main effect of chronic folic acid ingestion vs. control; ‡ P < 0.05 for main effect of exercise intensity.