INTRODUCTION

Little is known regarding the long-term health consequences of contracting the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing the novel coronavirus disease 2019 (COVID-19) (1). The etiology of COVID-19 progression involves interaction between SARS-CoV-2 and the angiotensin-converting enzyme 2 (ACE2) receptor (2), which is present in nearly every human tissue including lung, heart, kidneys, and intestine, suggesting vast consequences for physiological function (3). Further, the systemic cytokine-induced inflammatory response (4, 5) caused by viral detection and propagation can have prolonged, deleterious effects downstream of the initial viral parasitism in the lung (6), quite possibly causing severe physiological impairments to the vasculature (7).

Early investigations have revealed SARS-CoV-2 is able to infect endothelial cells, which are primarily responsible for regulating vascular tone (8). The ACE2 receptor, which the SARS-CoV-2 virus binds to, can be found on these endothelial cells (2), potentially jeopardizing vascular function among COVID-19-positive individuals. Indeed, previous investigations in animal models (9) and humans (10) of viral mimetic activation of Toll-like receptor 3 have reported an increase in inflammation mediated by innate and adaptive immunity, which may provoke vascular dysfunction. Furthermore, the association between SARS-CoV-2 transmission and stroke (11) as well as myocardial infarction (12) risk suggests a link between blood flow delivery impairments and acute cardiovascular risk. While proposals have suggested that endothelial biomarkers and tests of vascular function should be evaluated (13), we have yet to identify any assessments of
vascular impairments among those who have recently contracted SARS-CoV-2.

Thus, the purpose of this initial investigation was to determine if contracting SARS-CoV-2 may have prolonged effects on the systemic vasculature among otherwise healthy young adults. Using a cross-sectional comparison with young healthy adults, we hypothesized vascular function, as assessed by flow-mediated dilation (FMD) (14) and reactive hyperemia (RH) (15) in the arm as well as the single passive limb movement (sPLM) (16), would be reduced several weeks after testing positive for SARS-CoV-2. Further, we hypothesized these functional decrements would be accompanied by heightened vascular stiffness, as determined by carotid-femoral pulse wave velocity (PWVcf) (17).

METHODS

Subjects

Subjects were relatively healthy, as evidenced by the lack of chronic cardiovascular, pulmonary, or metabolic diseases as well as based on a subjective physical activity questionnaire; were not pregnant or trying to become pregnant; were premenopausal for female subjects; were nonsmokers; and were not taking any medications known to alter vascular function, including sympathetic adrenergic agonists or antagonists, cholinergic agonists or antagonists, β-blockers, diuretics, statins, or ACE inhibitors. Subjects were included in the SARS-CoV-2 group if they tested positive for SARS-CoV-2 using nasopharyngeal swab polymerase chain reaction assay 3–4 wk before study testing. Control subjects were studied February 4–6, 2020, before the first confirmed case of COVID-19 in North Carolina, United States, on March 3, 2020 (18), and before the World Health Organization declaring COVID-19 a pandemic on March 11, 2020. Control subjects had not experienced flu-like symptoms. All procedures were approved by the Appalachian State University Institutional Review Board, and the measurements were performed in a thermoneutral environment. The subjects provided written informed consent in accordance with the standards outlined by the Declaration of Helsinki.

Study Procedures

Subjects were tested in a fasted state, having abstained from food and caffeine for at least 12 h and alcohol or exercise for at least 24 h before testing procedures. All subjects had their health history recorded, including physical activity and any current medications. Subjects were tested in a quiet, thermoneutral environment (barometric pressure: 692–736 mmHg, temperature: 22–23°C, relative humidity: 33–50%). All procedures were performed using similar Doppler ultrasound system settings for the brachial artery FMD procedure. Immediately after baseline measurements, a blood pressure cuff, placed distal to the elbow, was rapidly inflated to 250 mmHg for 5 min. The blood pressure cuff was rapidly deflated, and brachial artery diameter and velocity were recorded for 2 min. Brachial artery diameter, blood velocity, blood flow, shear rate from cuff deflation to peak diameter, and 2-min RH were analyzed offline for continuous second-by-second measurements (Cardiovascular Suite v. 4.0, Quipu, Pisa, Italy). Blood flow was determined as: 

\[ \text{Blood flow} = \pi \left( \frac{\text{arterial diameter}}{2} \right)^2 \times \text{blood velocity, where blood velocity was obtained as the time average on the Doppler ultrasound.} \]

RH was determined as the area under the curve for the blood flow response following cuff occlusion, providing an index of microvascular function, which is inversely related to cardiovascular disease risk (20) and is predictive of future cardiovascular events in healthy and diseased populations (15).

Experimental Measurements

COVID-19 symptom severity survey.

Subjects who tested positive for SARS-CoV-2 were asked to rank their COVID-19 symptoms on the day of study testing. On a scale of 0–100 of increasing severity, subjects subjectively ranked their symptoms of chest pain, chills, diarrhea, dizziness or vertigo, dry cough, dry eyes, dry mouth, fatigue, fever over 37.9°C, headache, lack of appetite, loss of smell or taste (anosmia), muscle or body aches, nasal congestion or runny nose, nausea or vomiting, shortness of breath, difficulty breathing, dyspnea, sore joints, or sore throat. The values for each symptom were totaled and averaged for each symptom severity.

Brachial artery flow-mediated dilated and reactive hyperemia.

Brachial artery FMD measurements were obtained from the right brachial artery using current guidelines as a functional, upper limb marker of vascular function and cardiovascular risk (14). Baseline measurements of the right brachial artery diameter and blood velocity were taken for 1 min using a Doppler ultrasound system (GE Logiq eR7 and L4-12T-RS transducer, GE Medical Systems, Milwaukee, WI). Sample volume was optimized in relation to vessel diameter and centered within the vessel for each subject. Measurements of brachial artery diameter and velocity were obtained with the Doppler ultrasound in duplex mode with B-mode imaging frequency of 12 MHz and Doppler frequency of 4 MHz. An angle of insonation of <60° (19) was achieved for all measurements. Immediately after baseline measurements, a blood pressure cuff, placed distal to the elbow, was rapidly inflated to 250 mmHg for 5 min. The blood pressure cuff was rapidly deflated, and brachial artery diameter and velocity were recorded for 2 min. Brachial artery diameter, blood velocity, blood flow, shear rate from cuff deflation to peak diameter, and 2-min RH were analyzed offline for continuous second-by-second measurements (Cardiovascular Suite v. 4.0, Quipu, Pisa, Italy). Blood flow was determined as:

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RH was determined as the area under the curve for the blood flow response following cuff occlusion, providing an index of microvascular function, which is inversely related to cardiovascular disease risk (20) and is predictive of future cardiovascular events in healthy and diseased populations (15).

Femoral artery single passive leg movement.

Femoral artery sPLM measurements were obtained from the right femoral artery using current guidelines as a functional, lower-limb marker of vascular function (16). While in the supine position with the subject’s left leg supported on a stool and right leg supported by a research team member at heart level, baseline measurements of the common femoral artery diameter and blood velocity, at least 3 cm proximal the femoral artery bifurcation, were recorded for 1 min before passive limb movement using similar Doppler ultrasound system settings used for the brachial artery FMD procedure. Immediately following baseline measurements, the research team member supporting the right thigh and ankle manually moved the knee joint one time through 90° range of motion, flexion-extension, at 1 Hz while common femoral artery diameter and blood velocity were recorded for 1 min after the movement.

Carotid-femoral pulse wave velocity.

Ultrasound Doppler measurements were taken at the carotid and femoral arteries to assess peripheral arterial stiffness.
RESULTS

Subject Characteristics

Subject characteristics of 15 female and five male subjects who did not test positive for SARS-CoV-2 (control group) as well as four male and seven female subjects who tested positive for SARS-CoV-2 (SARS-CoV-2 group) are presented in Table 1. Subjects who tested positive for SARS-CoV-2 were studied 25 ± 5 days since symptom onset (n = 10) and 24 ± 6 days after their positive testing date (n = 11). One female subject who tested positive for SARS-CoV-2 was asymptomatic, although most had mild, lingering symptoms. Subjects were devoid of any medication usage other than oral contraceptives for most of the female subjects in each group.

Brachial Artery Flow-Mediated Dilation

Measurements of brachial artery FMD are presented in Fig. 1. Baseline brachial artery diameters were similar between groups (total control: 3.69 ± 0.49 mm, total SARS-CoV-2: 3.85 ± 0.38 mm; male control: 4.23 ± 0.19 mm, male SARS-CoV-2: 4.11 ± 0.34 mm; female control: 3.49 ± 0.41 mm, female SARS-CoV-2: 3.71 ± 0.34 mm). Time to peak vasodilation was similar between groups (total control: 58 ± 23 s, total SARS-CoV-2: 59 ± 26 s; male control: 64 ± 28 s, male SARS-CoV-2: 55 ± 19 s; female control: 55 ± 22 s, female SARS-CoV-2: 61 ± 31 s). Absolute change in brachial artery diameter from baseline to peak vasodilation was different between groups (total control: 0.30 ± 0.12 mm, total SARS-CoV-2: 0.12 ± 0.07 mm, P < 0.01; male control: 0.37 ± 0.08 mm, male SARS-CoV-2: 0.14 ± 0.12 mm, P < 0.01; female control: 0.28 ± 0.12 mm, female SARS-CoV-2: 0.12 ± 0.03 mm, P < 0.01). Sum of shear at peak vasodilation was not different between groups (total control: 79,077 ± 29,859 AU, total SARS-CoV-2: 78,279 ± 28,528 AU; male control: 65,262 ± 24,192 AU, male SARS-CoV-2: 70,987 ± 18,613 AU; female control: 83,682 ± 30,852 AU, female SARS-CoV-2: 82,445 ± 33,579 AU). The FMD response was different between groups when expressed as a percentage (total control: 8.81 ± 2.96%, total SARS-CoV-2: 2.71 ± 1.21%, P < 0.01; male control: 8.70 ± 1.75%, male SARS-CoV-2: 1.87 ± 1.45%, P < 0.01; female control: 8.85 ± 3.31%, female SARS-CoV-2: 3.20 ± 0.81%, P < 0.01) and when made relative to the shear stimulus (total control: 0.13 ± 0.06 AU, total SARS-CoV-2: 0.04 ± 0.02 AU, P < 0.01; male control: 0.14 ± 0.05 AU, male SARS-CoV-2: 0.03 ± 0.02 AU, P < 0.01; female control: 0.12 ± 0.06 AU, female SARS-CoV-2: 0.04 ± 0.01 AU, P < 0.01).

Reactive Hyperemia

Baseline brachial artery blood flow was similar between groups (total control: 125 ± 63 mL/min, total SARS-CoV-2: 150 ± 60 mL/min; male control: 157 ± 64 mL/min, male SARS-CoV-2: 186 ± 48 mL/min; female control: 115 ± 60 mL/min, female SARS-CoV-2: 130 ± 59 mL/min). Likewise, the blood flow response to the 5-min cuff occlusion, as assessed by area under the curve (AUC), between groups was similar (total control: 570 ± 210 mL/min, total SARS-CoV-2: 613 ± 175 mL/min; male control: 730 ± 265 mL/min, male SARS-CoV-2: 711 ± 200 mL/min; female control: 559 ± 277 mL/min, female SARS-CoV-2: 557 ± 145 mL/min).

Femoral Artery Single Passive Limb Movement

Measurements of femoral artery sPLM are presented in Fig. 2. Baseline femoral artery blood flow was similar between

Table 1. Subject characteristics

|                      | Age, yr | Height, cm | Weight, kg | BMI, kg/m² | Supine systolic arterial pressure, mmHg | Supine diastolic arterial pressure, mmHg | Supine mean arterial pressure, mmHg | Physical activity frequency, day/wk | Physical activity duration, min/day | Oral contraceptive use, % females | Number of symptoms | Average symptom severity, 0–100 |
|----------------------|---------|------------|------------|------------|----------------------------------------|----------------------------------------|------------------------------------|-------------------------------------|------------------------------------|-----------------------------------|-------------------|--------------------------|
| Control (n = 5 M/15 F) | 23.0 ± 1.3 | 167.4 ± 9.3 | 63.0 ± 7.4 | 22.5 ± 2.2 | 118.8 ± 13.4 | 77.7 ± 7.7 | 89.9 ± 7.5 | 4.1 ± 1.5 | 44.3 ± 14.4 | 2.9 ± 2.3 | 15.0 ± 12.2 |
| SARS-CoV-2 (n = 4 M/7 F) | 20.1 ± 1.1 | 171.5 ± 11.9 | 69.5 ± 12.4 | 23.5 ± 2.9 | 121.3 ± 12.3 | 71.8 ± 7.1 | 88.3 ± 8.2 | 3.6 ± 1.2 | 38.9 ± 12.5 | 2.8 ± 1.0 | 13.0 ± 6.9 |
| Control (n = 5 M) | 22.6 ± 1.1 | 179.3 ± 7.4 | 66.8 ± 7.0 | 20.8 ± 1.7 | 114.2 ± 11.2 | 79.0 ± 12.0 | 90.8 ± 9.6 | 5.4 ± 1.8 | 51.0 ± 12.3 | 2.8 ± 1.0 | 13.0 ± 6.9 |
| SARS-CoV-2 (n = 4 M) | 20.8 ± 0.5 | 182.7 ± 7.0 | 75.1 ± 8.1 | 22.5 ± 1.1 | 122.0 ± 13.5 | 86.8 ± 6.5 | 86.4 ± 8.4 | 3.0 ± 0.8 | 37.5 ± 12.3 | 3.0 ± 2.9 | 16.1 ± 14.9 |
| Control (n = 15 F) | 23.2 ± 1.3 | 163.4 ± 7.3 | 61.8 ± 7.3 | 23.1 ± 2.1 | 110.9 ± 14.5 | 77.2 ± 6.0 | 91.4 ± 9.5 | 3.7 ± 1.1 | 42.0 ± 14.7 | 3.0 ± 2.9 | 16.1 ± 14.9 |
| SARS-CoV-2 (n = 7 F) | 19.9 ± 1.2 | 165.4 ± 9.4 | 66.3 ± 13.8 | 24.1 ± 3.5 | 120.9 ± 12.7 | 73.6 ± 7.3 | 89.4 ± 6.6 | 3.9 ± 1.4 | 39.6 ± 13.5 | 7.1 |

Values are means ± SD; two-tailed Student’s t tests for two samples of equal variance were performed between control (n = 5 M/15 F) and SARS-CoV-2 (n = 4 M/7 F) groups. BMI: body mass index. *P < 0.01, between groups.
groups (total control: 563 ± 159 mL/min, total SARS-CoV-2: 743 ± 358 mL/min; male control: 592 ± 221 mL/min, male SARS-CoV-2: 673 ± 118 mL/min; female control: 553 ± 141 mL/min, female SARS-CoV-2: 784 ± 448 mL/min). Peak blood flow following the sPLM was similar between groups (total control: 914 ± 298 mL/min, total SARS-CoV-2: 1,053 ± 474 mL/min; male control: 908 ± 535 mL/min, male SARS-CoV-2: 975 ± 179 mL/min; female control: 916 ± 199 mL/min, female SARS-CoV-2: 1,098 ± 592 mL/min). Area under the curve, as an indication of microvascular hyperemic response to the sPLM, was different between groups (total control: 118 ± 114 mL, total SARS-CoV-2: 25 ± 11 mL, P < 0.01) but not when separated by sex (male control: 85 ± 113 mL, male SARS-CoV-2: 39 ± 24 mL, P > 0.01; female control: 129 ± 117 mL, female SARS-CoV-2: 25 ± 71 mL, P > 0.01).

**DISCUSSION**

Our data indicate SARS-CoV-2 may have detrimental effects on the systemic vasculature in young adults. In support of our hypothesis, we observed a significantly lower brachial artery FMD among subjects who, 3–4 wk before

**Figure 1.** Brachial artery flow-mediated dilation (FMD) expressed as percentage change (A) and normalized to shear (B). Two-tailed Student’s t tests for two samples of equal variance were performed between control (n = 5 M/15 F) and SARS-CoV-2 (n = 4 M/7 F) groups. *P < 0.01, between groups. Data are means ± SD.

**Figure 2.** Single passive limb movement. Common femoral artery blood flow change from baseline following a single passive limb movement (A) with the 60-s area under the curve (B). Two-tailed Student’s t tests for two samples of equal variance were performed between control (n = 5 M/15 F) and SARS-CoV-2 (n = 4 M/7 F) groups. *P < 0.01, between groups. Data are means ± SD.

**Carotid-Femoral Pulse Wave Velocity**

Measurements of PWVcf are presented in Fig. 3. PWVcf was different between groups (total control: 5.17 ± 0.66 m/s, total SARS-CoV-2: 5.83 ± 0.62 m/s, P < 0.01) but not when separated by sex (male control: 5.09 ± 0.28 m/s, male SARS-CoV-2: 5.79 ± 0.58 m/s, P > 0.01; female control: 5.20 ± 0.74 m/s, female SARS-CoV-2: 5.85 ± 0.68 m/s, P > 0.01).
while rather alternative pathways such as inwardly rectifying K⁺ channels and Na⁺/K⁺ ATPase (27). Therefore, the discrepancy in the observed reduction in FMD and lack of a change in RH should not be too surprising if NO is to be primarily affected by SARS-CoV-2 directly or by a subsequent cytokine storm, oxidative stress, or inflammation (4, 5).

While SARS-CoV-2 may induce systemic inflammatory response, previous investigations have provided evidence of direct inflammatory response in endothelial cells, which may provoke vascular dysfunction (9). Several mechanisms, including cytokines, Toll-like receptors, immune cell activation, and NADPH oxidase 2, may underlie endothelial and vascular dysfunction in SARS-CoV-2 (28). However, more work should surely discern whether the observed functional decrements are caused by an oxidative stress-induced decrease in NO or other vascular regulators.

**Passive Limb Movement and SARS-CoV-2**

The sPLM test is a lower-limb assessment of microvascular function, as the movement provokes NO-dependent microvascular vasodilation (14). The low hyperemic response to the sPLM test in the current investigation may indicate a diminished ability of the small arterioles to dilate when necessary. Most notably, the quick restoration of femoral artery blood flow following the movement may be an indication of diminished NO bioavailability, as previously observed when NO synthesis is blocked using Nω-monomethyl-L-arginine acetate (L-NMMA) (29). Ultimately, the results from the FMD and sPLM tests provide evidence for lower vascular function, which warrants further review to determine if NO or other vascular regulators are responsible for these observed functional decrements.

**Pulse Wave Velocity and SARS-CoV-2**

An ∼1-m/s elevation in PWV is associated with a 15% higher risk of cardiovascular events, mortality, and all-cause death (30). While we observed a 0.75-m/s higher PWVcf with the SARS-CoV-2 group, which may suggest higher arterial stiffness and cardiovascular disease risk, this elevated level is still within the expected range for this age-group and may not be clinically relevant.

**Limitations**

We recognize SARS-CoV-2 was in the United States before the COVID-19 pandemic declaration in March 2020. However, all control subjects were healthy at the time of testing in a US region lacking any positive SARS-CoV-2 cases (18). While the current investigation utilized noninvasive, functional biomarkers of vascular function, we recognize the current assessments cannot determine endothelial-dependent vasodilation. Certainly, future experiments could provoke endothelial-independent vasodilation using sublingual nitroglycerine or examining circulating or urinary nitrate/nitrite levels to determine NO bioavailability among individuals with SARS-CoV-2. While sPLM repeated measures may improve the precision of this measure, we believe these results corroborate the observed vascular function decrements regardless.

**Conclusion**

These results suggest numerous systemic vascular consequences among young adults that should not be overlooked, especially among those at higher risk of cardiovascular complications from contracting SARS-CoV-2. Remarkably, these decrements occurred in young, relatively healthy individuals devoid of any chronic diseases. Certainly, more work is...
needed to discern if these observations persist beyond the first 3–4 wk of contracting SARS-CoV-2 in male and female, symptomatic and asymptomatic individuals. We recognize the limitations of a cross-sectional comparison such as this and encourage future investigations to track individuals with SARS-CoV-2 longitudinally to determine the vascular recovery period. However, compared with healthy individuals, the effects of SARS-CoV-2 on the vasculature appear striking and imperative for scientific and medical community to understand the impact of this disease on human health.

GRANTS
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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS
S.M.R., J.L.S., and A.S.L.S. conceived and designed research; S.M.R., J.L.S., V.M.P., M.A.A., L.K.K., L.K.B., and A.S.L.S. performed experiments; S.M.R., J.L.S., V.M.P., M.A.A., L.K.K., L.K.B., and A.S.L.S. analyzed data; S.M.R., J.L.S., V.M.P., M.A.A., L.K.K., L.K.B., and A.S.L.S. interpreted results of experiments; S.M.R. and V.M.P. prepared figures; S.M.R., J.L.S., V.M.P., and A.S.L.S. drafted manuscript; S.M.R., J.L.S., V.M.P., M.A.A., L.K.K., L.K.B., and A.S.L.S. edited and revised manuscript; S.M.R., J.L.S., V.M.P., M.A.A., L.K.K., L.K.B., and A.S.L.S. approved final version of manuscript.

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