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RESEARCH ARTICLE

Experimental intermittent ischemia augments exercise-induced inflammatory cytokine production

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[1]*Department of Kinesiology, University of Georgia, Athens, Georgia;* [2]*Department of Foods and Nutrition, University of Georgia, Athens, Georgia;* [3]*Regenerative Bioscience Center, University of Georgia, Athens, Georgia; and* [4]*Augusta University-University of Georgia Medical Partnership, Athens, Georgia*

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Shill DD, Polley KR, Willingham TB, Call JA, Murrow JR, McCully KK, Jenkins NT. Experimental intermittent ischemia augments exercise-induced inflammatory cytokine production. *J Appl Physiol* 123: 434–441, 2017. First published June 1, 2017; doi: 10.1152/japplphysiol.01006.2016.—Acute exercise-induced inflammation is implicated in mediating the beneficial adaptations to regular exercise. Evidence suggests that reduced oxygen and/or blood flow to contracting muscle alters cytokine appearance. However, the acute inflammatory responses to hypoxic/ischemic exercise have been documented with inconsistent results and may not accurately reflect the ischemia produced during exercise in patients with ischemic cardiovascular diseases. Therefore, we determined the extent to which local inflammation is involved in the response to ischemic exercise. Fourteen healthy males performed unilateral isometric forearm contractions for 30 min with and without experimental ischemia. Blood was drawn at baseline, 5 and 10 min into exercise, at the end of exercise, and 30, 60, and 120 min after exercise. Oxygen saturation levels, as measured by near-infrared spectroscopy, were reduced by 10% and 41% during nonischemic and ischemic exercise, respectively. Nonischemic exercise did not affect cytokine values. Ischemia enhanced concentrations of basic fibroblast growth factor, interleukin (IL)-6, IL-10, tumor necrosis factor-alpha, and vascular endothelial growth factor during exercise, but IL-8 was not influenced by ischemic exercise. In conclusion, the present study demonstrates that ischemic, small-muscle endurance exercise elicits local inflammatory cytokine production compared with nonischemic exercise.

NEW & NOTEWORTHY We demonstrate that ischemic, small-muscle endurance exercise elicits local inflammatory cytokine production compared with nonischemic exercise. The present study advances our knowledge of the inflammatory response to exercise in a partial ischemic state, which may be relevant for understanding the therapeutic effects of exercise training for people with ischemic cardiovascular disease-associated comorbidities.

blood flow restriction; peripheral arterial disease; skeletal muscle; near-infrared spectroscopy

ACUTE EXERCISE-INDUCED increases in cytokines have been implicated in mediating some beneficial metabolic and vascular effects of chronic physical activity (32, 34, 43–46, 48). In addition to regulating local homeostasis, exercise releases skeletal muscle-derived cytokines into circulation that exert systemic effects (44, 51). Evidence indicates that secreted pro- and anti-inflammatory cytokines regulate angiogenesis, insulin sensitivity, and lipolysis (5, 9, 32, 44, 48, 61). Generally, cytokines are released from contracting skeletal muscle (34, 43–45, 51), but the studies are mixed on the consistency and magnitude of exercise-induced cytokine production.

Multiple factors including contraction intensity, exercise duration, the amount of muscle mass recruited, nutrition, and training status all influence the inflammatory response (22, 34–36, 46, 48). Additionally, evidence suggests that a lack of oxygen and/or blood flow to working muscle modifies cytokine appearance (20, 23–25, 28, 38, 42, 50, 60, 66). However, the acute inflammatory responses to hypoxic (i.e., reduced inspired oxygen) and ischemic (i.e., reduced blood flow to working muscle) exercise have been documented with inconsistent results (20, 23–25, 28, 38, 42, 50, 60, 66). Moreover, a constant oxygen reduction or suprasystolic occlusion to contracting muscle may not reflect the true nature of ischemia during exercise in patients with ischemic cardiovascular diseases (3, 4, 27, 29, 31, 58).

Therefore, the purpose of the present study was to determine the extent to which local inflammation is involved in the response to ischemic exercise. In light of evidence indicating that experimental ischemia and hypoxia alter inflammatory cytokine production during exercise (20, 23–25, 28, 38, 42, 50, 60, 66), it was hypothesized that experimentally induced ischemia designed to mimic intermittent claudication would enhance angiogenic and inflammatory cytokine production by working forearm muscle.

METHODS

Screening. Fourteen healthy 18- to 40-yr-old men who participated in 30 min of vigorous activity at least 1 day/wk or 30 min of moderate activity at least 2 days/wk were recruited. Exclusion criteria included any history of cardiovascular, inflammatory, autoimmune, pulmonary, or metabolic diseases and currently taking greater than or equal to three prescription or nonprescription pharmacological therapies including anti-inflammatory, cardiovascular, or metabolic drugs. The University of Georgia Institutional Review Board (Athens, GA) approved all study procedures, and all subjects provided written, informed consent before participation.

Protocol. Before experimental trials, subjects completed a baseline visit comprised of a maximal voluntary isometric wrist contraction (MVC) and protocol familiarization. Two randomly assigned experimental conditions, separated by 7 days, were completed with and without experimental ischemia. Subjects reported to the laboratory between 0600 and 0800, following an overnight fast (>10 h) and after

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abstaining from alcohol and exercise for the preceding 24 h, and from caffeine for 12 h. Additionally, dietary intake was recorded 24 h before the first visit and subjects were instructed to repeat the nutritional value of the first dietary log in the 24 h preceding their second visit. An indwelling catheter was inserted into an antecubital vein of the nondominant arm, and a near-infrared spectroscopy (NIRS) device was placed over the nondominant wrist-flexor musculature (i.e., palmaris longus, flexor carpi radialis, flexor carpi ulnaris). Subjects remained seated with both arms flat on a table and were instructed to limit arm movement for the entire duration of the protocol. After 5 min of rest, a baseline blood sample was obtained before the onset of exercise. Blood was drawn 5 and 10 min into exercise, at the end of exercise, and 30, 60, and 120 min after exercise. For the nonischemic exercise condition, forearm handgrip exercise was performed using a hand dynamometer (BIOPAC, BIOPAC Systems, Goleta, CA), and consisted of one unilateral isometric wrist contraction, in the nondominant arm, every 3 s for 30 min at 65% of maximal isometric force. Subjects received visual feedback on a computer screen indicating the amount of force applied during each contraction. Additionally, a metronome was used to maintain consistent contraction timing. Every 5 min during exercise, subjects reported their feelings of perceived pain, from 1 to 4, using the American College of Sports Medicine Claudication Pain Rating Scale (62), and 0 to 10 using a Subjective Pain Scale (8). After the last blood sample was collected, the venous catheter was removed and the NIRS device was calibrated (described below).

The aforementioned procedures were repeated during the ischemic exercise condition, with the following modifications. After 5 min of rest and baseline blood sampling, blood pressure was recorded (Omron, Omron Healthcare, Vernon Hills, IL) on the nonexercising arm. A blood pressure cuff (6.35-cm-wide polyurethane bladder encased by nylon), equipped with a rapid cuff-inflation system (Hokanson E20 cuff inflator, Hokanson, Bellevue, WA), was placed above the elbow joint, around the upper portion of the exercising arm and rapidly inflated to 95% of systolic blood pressure immediately before the start of exercise. Blood pressure was recorded on the nonexercising arm to avoid potential interference with local cytokine measurements. After 5 min of exercise with the blood pressure cuff inflated, subjects stopped exercising and the blood pressure cuff was rapidly reduced to 0 mmHg for 20 s. At the end of the 20-s rest period the blood pressure cuff was reinflated to 95% of systolic blood pressure and exercise resumed. This intermittent exercise-rest pattern continued for 30 min. At the end of exercise, the blood pressure cuff was fully deflated and the postexercise blood sample was obtained. The cuff was removed for the remainder of the protocol until the NIRS device was calibrated.

Pilot testing indicated that an exercise intensity of 65% MVC with a 95% systolic blood pressure occlusion during exercise was well tolerated and could be maintained for 5 min without failure. Additionally, pilot testing showed no changes in heart rate or blood pressure throughout the duration of either exercise protocols (data not shown).

Blood sample processing and cytokine analysis. EDTA collected plasma samples were prepared by centrifugation and stored at −80°C until assayed in duplicate for concentrations of basic fibroblast growth factor (bFGF), interleukin (IL)-6, IL-8, IL-10, tumor necrosis factor-alpha (TNF-α), and vascular endothelial growth factor (VEGF) using a multiplex cytokine assay (HCYTOMAG-60K Milliplex, EMD Millipore, Billerica, MA) on a MAGPIX instrument (Luminex Technologies, Luminex, Austin, TX) according to the manufacturer's instructions. The multiplex assay was custom ordered with all six cytokines of interest on the same plate. Cytokines were chosen based on their proposed roles in both ischemic cardiovascular diseases and the beneficial vascular effects of exercise.

Near-infrared spectroscopy. Oxygenated (HbO_2), deoxygenated (HHb), and total hemoglobin (tHb) were quantified using continuous-wave NIRS device (Portamon, Artinis Medical Systems, Elst, The Netherlands). The NIRS probe was placed over the contracting forearm muscles, and the device collected data at 10 Hz throughout both protocols. All NIRS signals were calibrated using an ischemic calibration performed following the last blood draw as previously described (52). Briefly, a blood pressure cuff (6.35-cm-wide polyurethane bladder encased by nylon) was placed proximal to the elbow joint of the exercising arm and inflated to 270 mmHg. Ischemia was maintained until 0% saturation in the muscle tissue was achieved (~5 min), and the subsequent cuff release produced a peak hyperemic response (100% oxygenation). The range of optical density units (ODU_{Range}) calculated from this calibration was used for normalization of NIRS signals. Furthermore, the ODU_{Range} was used to quantify blood volume at baseline (ODU_{BL}) and changes in blood volume that occurred during ischemic and nonischemic exercise (ODU_{Ex}). The model of ischemia in the present study employed a venous occlusion during exercise that resulted in a disproportionate increase of arterial inflow compared with venous outflow as measured by NIRS (see Fig. 3). Absolute hemoglobin concentrations were higher during ischemic exercise compared with nonischemic exercise (see Fig. 3), indicating increases in blood volume (65). Changes in blood volume were estimated using a previously described equation (13), that was modified based on a 350 cap/mm^2 microvascular density (49), a constant myoglobin concentration of 0.5 mM (63), a hemoglobin (Hb) concentration of 0.06 mM (11, 64), and to account for the relative light absorbing contributions of hemoglobin to the total NIRS signal (11). Dill and Costill's blood volume equation (13) has been implemented in recent blood flow restriction exercise studies examining circulating inflammatory markers (6, 26, 41). The observed increase in blood volume during ischemic exercise is likely the result of the venous occlusion employed during the ischemic exercise protocol (23, 65). These findings are consistent with previous studies that have shown increases in hemoglobin concentrations after 1 min of handgrip exercise with an arterial occlusion and 120 repetitions of knee extensions with a suprasystolic occlusion (23, 65). Furthermore, hemoglobin concentrations remained unchanged during 120 repetitions of knee extension exercise without an occlusion (23), in agreement with the current results. For determination of cytokine concentrations, the blood volume correction factor was calculated as follows and denotes an absolute increase in [Hb] from baseline (Hb_{BL}) to the specified exercise time point (Hb_{Ex}). No differences in [tHb] at 60, 90, or 150 min were observed; therefore the blood volume correction factor calculated for each individual was only applied to cytokine values at time points 0, 5, 10, and 30 for both conditions.

$$32\% \times ODU_{Range} = 0.06 \text{ mM Hb} \quad (1)$$

$$1 \text{ ODU} = \frac{0.06 \text{ mM Hb}}{32\% \times ODU_{Range}} \quad (2)$$

$$Hb_{Ex} = \left(ODU_{Ex} - ODU_{BL}\right) \times ODU_{Range} \quad (3)$$

$$\text{correction factor} = \text{measured cytokine value}_{Ex} \times \left(Hb_{Ex} \div Hb_{BL}\right) \quad (4)$$

Force. Force production was measured from handgrip dynamometry during all exercise bouts at a frequency of 100 Hz. Data were visualized and recorded in real time using AcqKnowledge software (BIOPAC, BIOPAC Systems, Goleta, CA) and analyzed using custom-written routines in MATLAB R2014b (The MathWorks, Natick, MA). Subjects were instructed to contract as quickly as possible to their predetermined 65% MVC value and relax. Force measured at the time of contraction was examined and reported as 5-min averages.

Statistics. A priori power analysis indicated a sample size of 14 subjects would provide 81% power to detect a 0.57 SD change between the protocols, based on previous findings of forearm exercise-induced cytokine production (33). Analysis was performed in SPSS, version 23.0 (SPSS, IBM, Armonk, NY). Data were analyzed using two-factor, condition (ischemia vs. nonischemia) × time, repeated-measures ANOVA and Fisher's least significant difference tests for post hoc comparisons, unless otherwise stated. Data are

presented as means ± SE. Statistical significance was accepted at $P < 0.05$. P values of ≤0.10 but >0.05 were considered to be approaching statistical significance.

RESULTS

Subject characteristics. Subject characteristics are reported in Table 1. All participants successfully completed both exercise protocols with no adverse events.

Claudication pain and pain intensity rating scales. A main effect of condition was observed during exercise for claudication pain ratings. Claudication pain ratings were higher during ischemic exercise compared with nonischemic ($P < 0.05$) (Fig. 1*A*). For pain intensity scales, we observed a main effect of condition. Pain intensity ratings were higher during ischemic exercise compared with nonischemic ($P < 0.05$). Additionally, a main effect of time was observed for pain intensity ratings ($P < 0.01$). Pain intensity ratings during exercise were higher than baseline (all $P < 0.001$) (Fig. 1*B*).

Force production. A main effect of time is reported for percent MVC achieved with no differences between conditions ($P < 0.05$) (Fig. 1*C*). The first 5-min averages were 63.0 ± 1.1% and 63.9 ± 2.2% of MVC for nonischemic and ischemic exercise, respectively. The final 5-min averages were 59.8 ± 1.9% and 61.4 ± 2.1% of MVC for nonischemic and ischemic exercise, respectively. The 5-min averages reported at 15, 20, and 25 min were lower compared with baseline ($P < 0.05$).

Oxygen saturation and hemoglobin concentrations. A condition × time interaction was observed in oxygen saturation during exercise ($P < 0.001$). During nonischemic exercise and ischemic exercise oxygen saturation levels were reduced by 10% and 41%, respectively ($P < 0.001$). Oxygen saturation was significantly lower during ischemic exercise vs. nonischemic exercise ($P < 0.001$) (Fig. 2). One-way ANOVA analysis of the increase of Hb concentrations from baseline revealed 155%, 139%, and 230% more Hb at 5, 10, and 30 min, respectively, during ischemic exercise compared with nonischemic exercise ($P < 0.05$) (Fig. 3). This indicated a greater blood volume shift during the ischemic exercise conditions compared with nonischemic exercise.

Cytokines. All cytokine concentrations returned to baseline values 30 min after exercise with no differences at 60, 90, or 150 min between ischemic and nonischemic exercise ($P > 0.05$).

Basic fibroblast growth factor. A condition × time interaction was observed during exercise ($P < 0.05$) (Fig. 4*A*). From baseline, ischemic exercise induced a 65% and 148% increase in bFGF at 5 and 30 min, respectively ($P < 0.05$). Nonischemic exercise resulted in a 35% decrease in bFGF at 30 min compared with baseline ($P < 0.05$). bFGF values at 5 and 30 min were significantly higher during ischemic exercise compared with nonischemic exercise ($P < 0.05$). Ten-minute concentrations approached a statistically significant difference between conditions ($P = 0.076$).

Interleukin-6. During exercise, a condition × time interaction was noted ($P < 0.05$) (Fig. 4*B*). From baseline, ischemic

Table 1. *Subject characteristics*

	Value
Subjects	14
Age, yr	21.8 ± 0.4
Height, m	1.8 ± 0.03
Mass, kg	79.1 ± 0.6
BMI, kg/m^2	24.5 ± 0.6
Resting blood pressure, mmHg	
Systolic	121.4 ± 1.9
Diastolic	66.4 ± 2.9
MVC, kg	41.3 ± 2.3
Exercising arm, left/right	12/2

Values are means ± SE. BMI, body mass index; MVC, maximal voluntary contraction.

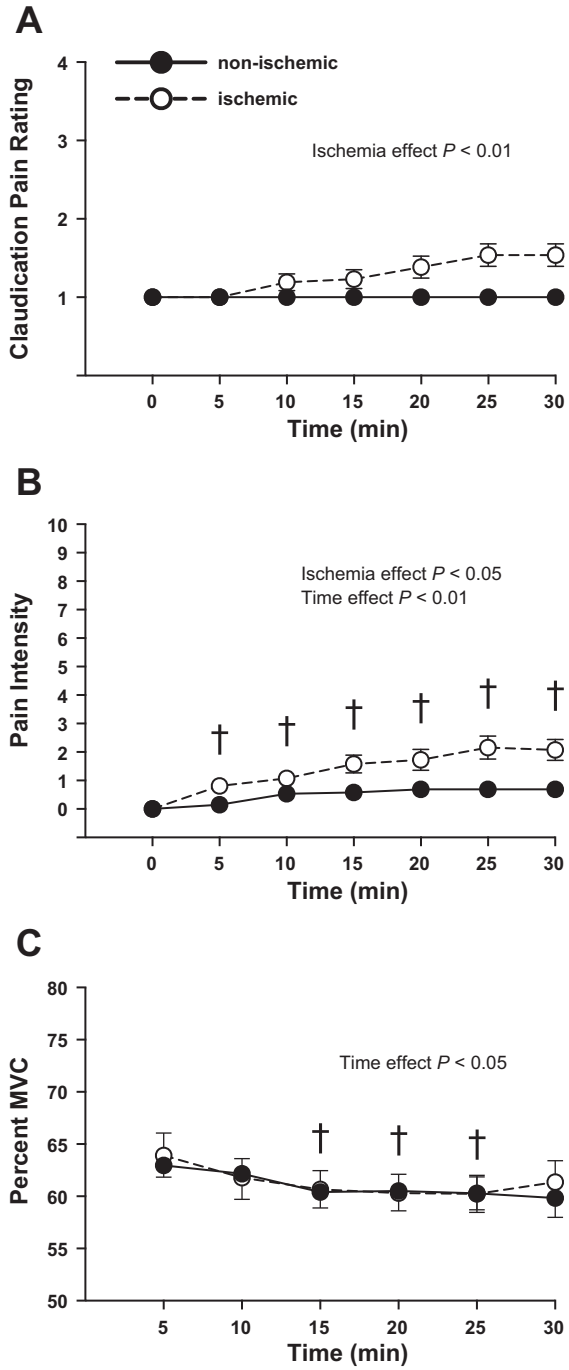

Fig. 1. Claudication pain ratings (*A*), pain intensity ratings (*B*), and force production (*C*) during nonischemic (black) and ischemic (white) exercise. †Statistically significant effect of time compared with baseline ($P < 0.05$).

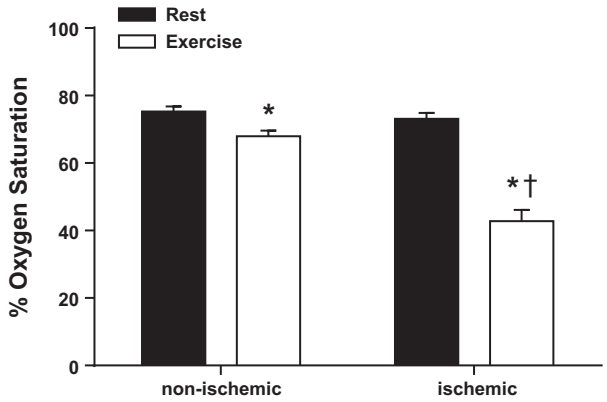

Fig. 2. Oxygen saturation levels at rest (black) and during exercise (white) during nonischemic and ischemic exercise. *Statistically significant effect of exercise within condition ($P < 0.001$). †Statistically significant difference between conditions at corresponding time point ($P < 0.001$).

exercise induced a 197% increase in IL-6 at 30 min ($P < 0.05$). Additionally, ischemic exercise resulted in a 60% increase at 5 min that approached a statistically significant increase ($P = 0.063$). Nonischemic exercise resulted in a 32% decrease at 30 min in IL-6 from baseline ($P < 0.05$). IL-6 concentrations at 30 min were significantly higher during ischemic exercise compared with nonischemic exercise ($P < 0.05$), and 5-min concentrations approached a statistically significant increase ($P = 0.067$).

Interleukin-8. No interactions or main effects were observed for IL-8 concentrations ($P > 0.05$) (Fig. 4*C*).

Interleukin-10. A condition × time interaction was noted for IL-10 values during exercise ($P < 0.05$) (Fig. 4*D*). During ischemic exercise, IL-10 increased 129% from baseline ($P < 0.05$), with a 53% increase approaching a statistically significant increase at 5 min ($P = 0.089$). At 30 min, IL-10 concentrations were significantly higher during ischemic exercise compared with nonischemic exercise ($P < 0.05$).

Tumor necrosis factor-α. During exercise, a condition × time interaction was observed for TNF-α concentrations ($P < 0.05$) (Fig. 4*E*). During ischemic exercise, TNF-α increased 154% from baseline to 30 min ($P < 0.05$) (Fig. 4*E*). Nonischemic exercise resulted in a 31% decrease at 30 min in TNF-α from baseline ($P < 0.05$). At 30 min, TNF-α concentrations were significantly higher during ischemic exercise compared with nonischemic exercise ($P < 0.05$). TNF-α values at 10 min approached a statistically significant difference between ischemic and nonischemic exercise ($P = 0.100$).

Vascular endothelial growth factor. A condition × time interaction was noted during exercise ($P < 0.01$) (Fig. 4*F*). During ischemic exercise VEGF increased 74% and 164% from baseline, at 5 and 30 min, respectively ($P < 0.05$), with a 67% increase approaching a statistically significant increase at 10 min ($P = 0.090$). Nonischemic exercise resulted in a 34% decrease at 30 min in VEGF from baseline ($P < 0.05$). At 30 min, VEGF values were significantly higher during ischemic exercise compared with nonischemic exercise ($P < 0.05$). VEGF concentrations at 10 min approached a statistically significant difference between ischemic and nonischemic exercise ($P = 0.058$).

DISCUSSION

The primary finding of the present study is that ischemic, small-muscle endurance exercise elicits local inflammatory cytokine production compared with nonischemic exercise in young healthy men. Moreover, we demonstrate increases in cytokines produced under ischemic conditions return to baseline values within 30 min of exercise cessation. Although there is precedent for working muscle to release cytokines under normal physiological conditions, our data suggest that local ischemia may enhance this response. These data might also have relevance for understanding the therapeutic effects of exercise training for people with ischemic cardiovascular diseases [e.g., peripheral arterial disease (PAD)]. Specifically, by using a model of intermittent ischemia during exercise, the present study advances our knowledge of the inflammatory response to exercise in a partial ischemic state, which may more closely resemble the disruption of blood flow and oxygen desaturation caused by intermittent claudication compared with models used in previous studies.

Hypoxic and ischemic exercise models used in previous studies may not effectively reproduce the true nature of ischemia experienced during exercise in patients with ischemic cardiovascular diseases. Reducing inspired oxygen decreases arterial saturation; however, healthy controls and patients with PAD do not experience declines in arterial saturation at rest or during exercise (3, 4). However, as a result of the arterial atherosclerotic occlusions in patients with PAD, exercise hyperemia is restricted which elicits a plateau in oxygen delivery, indicating that impaired hemodynamics contribute to PAD-associated reductions in exercise capacity (29, 58). Moreover, we observed reduced oxygen saturation during ischemic and nonischemic exercise, although oxygen saturation levels were significantly lower during ischemic exercise compared with nonischemic exercise. Our findings that oxygen saturation is reduced by 41% during ischemic exercise are in agreement with prior investigations demonstrating 10–80% lower oxygen saturation levels in people with ischemic PAD during exercise (16, 18, 27, 29, 31).

Endothelial cell damage, inflammation, and hypoxia stimulate the synthesis of bFGF (47), which contributes to angiogenesis via endothelial cell proliferation and stabilization of tubelike structures (59). During ischemic exercise, we demon-

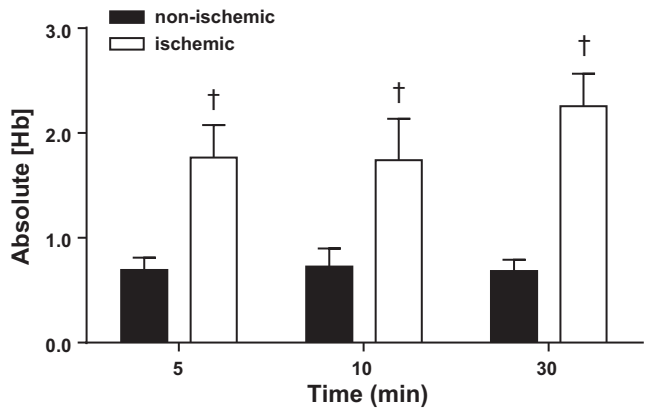

Fig. 3. Absolute increases in hemoglobin concentrations from baseline during nonischemic (black) and ischemic (white) exercise. †Statistically significant difference between conditions at corresponding time point ($P < 0.05$).

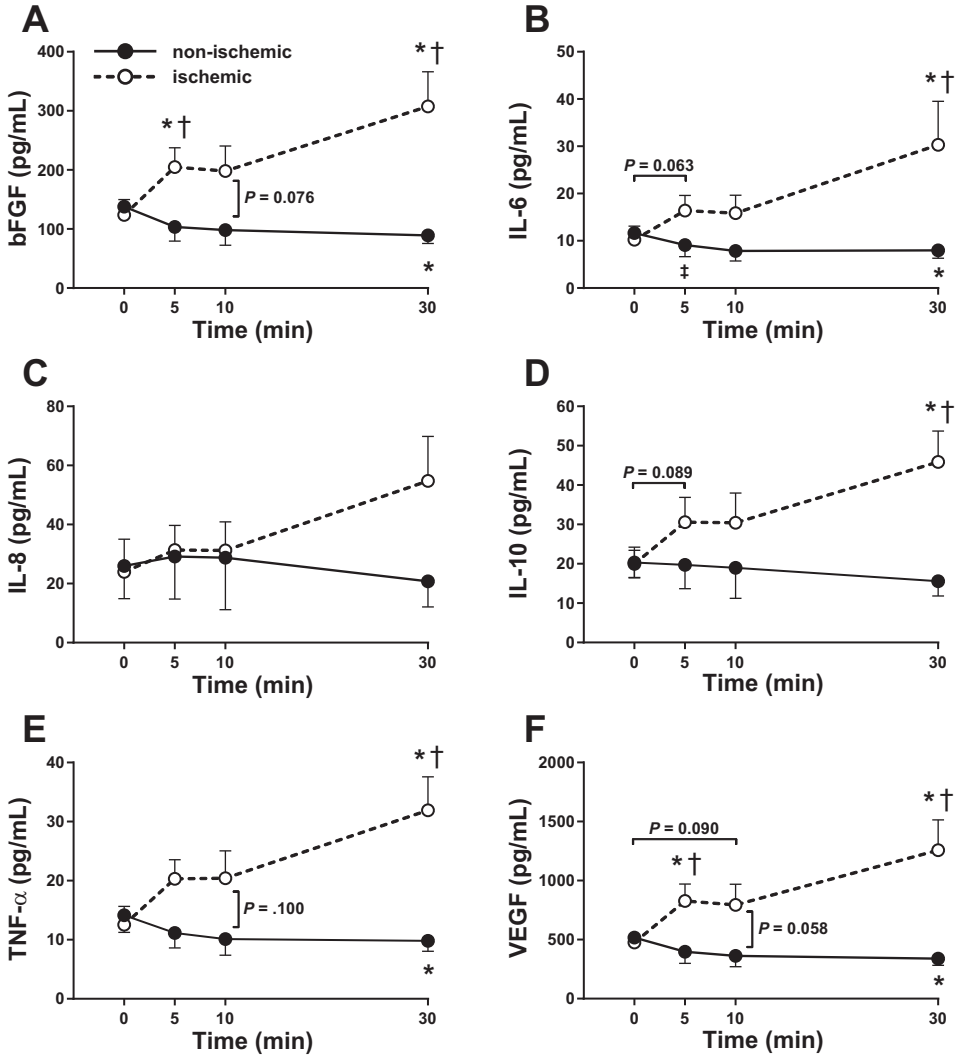

Fig. 4. bFGF (*A*), IL-6 (*B*), IL-8 (*C*), IL-10 (*D*), TNF-α (*E*), and VEGF (*F*) concentrations during nonischemic (black) and ischemic (white) exercise. *Statistically significant difference from baseline within condition ($P < 0.05$). †Statistically significant difference between conditions at corresponding time point ($P < 0.05$). ‡$P = 0.067$ between conditions at corresponding time point. Brackets and *P* values indicate data approaching statistical significance.

strate a transient increase in bFGF concentrations that return to baseline values 30 min postexercise. Although bFGF is not necessary for exercise-induced angiogenesis (12), evidence indicates that its appearance stimulates the release of VEGF (59), which was also found to follow a similar time course. Our findings that exercising muscle releases bFGF under ischemic conditions are in contrast with results of previous investigations on the influence of exercise on bFGF in people with ischemic PAD (39, 54).

Chronic low-grade inflammation, specifically IL-6, is regarded as proinflammatory which cause deleterious metabolic effects, facilitates atherosclerosis, and is related to the functional decline in patients with PAD (30, 44, 61). However, acute exercise briefly enhances circulating IL-6 exerting anti-inflammatory properties such as augmenting IL-10 production (34, 46). Interestingly, we found that ischemic exercise induced a significant increase in IL-6 concentrations at the end of exercise. Contrary to a previous study examining local IL-6 production during wrist flexion, we did not observe any changes over time during or following nonischemic exercise (33). Moreover, IL-6 concentrations did not change in hind limb-ligated mice, even after exercise (10). In patients with PAD, IL-6 has been documented to decrease after 30 min of submaximal treadmill exercise, but increase after maximal exercise, potentially due to ischemia-reperfusion injury (1, 15, 39, 56). The transient increase, and return to baseline, of IL-6 observed in the present study indicates an anti-inflammatory effect of ischemic exercise.

Fundamental to leukocyte transendothelial cell migration and atherosclerotic plaque destabilization, high levels of IL-8 correlate with an increased risk for cardiovascular disease (2, 55). In response to exercise, IL-8 is released from endothelial cells with slight systemic increases, indicating the angiogenic factor may locally stimulate exercise-induced angiogenesis (34, 44). Our data are in contrast to previous findings indicating that exercise enhances IL-8 production in patients with PAD and in animal models of ischemic cardiovascular disease (17, 21, 37).

Recognized as anti-inflammatory, high concentrations of IL-10 are correlated to atherosclerotic plaque stabilization and indicative of better prognosis in cardiovascular disease patients (55, 61). Moreover, IL-10 inhibits the synthesis of IL-6 and TNF-α, subsequently preventing the recruitment and activation of inflammatory cells to sites of inflammation (46, 55). The present study identified a condition × time interaction in IL-10 concentrations during exercise, with increased values apparent

5 min into ischemic exercise. However, other data indicate IL-10 typically appears in circulation postexercise (46). With previous evidence supporting an increase in IL-10 under ischemic conditions (57), it is plausible that ischemic exercise elicits an increase in IL-10 concentrations during exercise, rather than after exercise (46).

TNF-α, expressed in smooth muscles and leukocytes, perpetuates atherosclerosis through autocrine and paracrine mechanisms (61). However, low concentrations have been shown to facilitate angiogenesis (14, 53). During and after exercise in healthy populations, a slight increase in TNF-α is typically detected, but intensity and duration influence the magnitude of its appearance (43, 46). Conversely, most (15, 39, 54) but not all (56) studies indicate that exercise does not elicit changes in TNF-α among patients with exercise-induced ischemia. Moreover, neither a 15-min suprasystolic cuff nor 30 min of unilateral wrist flexion evoked changes in TNF-α levels among healthy individuals (33, 54). However, the observed condition × time interaction in our study demonstrates increased levels at 5 and 30 min into exercise during ischemic exercise.

VEGF, a robust angiogenic factor, contributes to beneficial and pathological vascular tree remodeling (40). Inconsistencies in basal VEGF concentrations exist between patients with PAD and healthy controls (17, 54), and in the response to exercise (39, 54, 67). However, in a healthy state VEGF concentrations increase 120 min after a 15-min suprasystolic cuff, 30 min of unilateral wrist flexion, and immediately after exercise in hypoxic conditions (33, 54, 66). Furthermore, VEGF concentrations increased in hindlimb-ligated mice subjected to exercise compared with sedentary ischemia and sham controls (10). The present study demonstrates an increase in VEGF concentrations during ischemic exercise, concurring with these previous studies.

Perspectives and limitations. A notable finding was the lack of cytokines produced during nonischemic exercise. In agreement with the present investigation, a similar study employing 10 min of unilateral wrist flexion exercise did not demonstrate increases in bFGF, IL-6, or VEGF after exercise (33). These results are likely due to the small muscle mass recruited. However, we demonstrate that the addition of ischemia during exercise increases inflammatory cytokine production, suggesting that local ischemia during exercise enhances the inflammatory response during exercise, even from a relatively small amount of contracting muscle. Our subjects were young, healthy, and free of PAD and its associated comorbidities (e.g., smoking, obesity, diabetes). Therefore, our findings raise the possibility that these comorbidities may suppress exercise-induced proangiogenic cytokine secretion from ischemic contracting muscle in patients with PAD. This hypothesis deserves testing in future studies.

Our study has limitations that warrant mention. First, we did not include women in our examination. Second, the implemented protocol serves as an exercise model to simulate the effect of exercise in patients with PAD. Accordingly, our findings should be interpreted with caution. Third, based on our use of a 10-cm-wide cuff to record resting blood pressure, it is possible the effective occlusion produced by the 6.35-cm-wide cuff employed during ischemic exercise was closer to 85% instead of 95% systolic blood pressure (19). Additionally, we did not include an ischemic condition without exercise and therefore cannot isolate the direct effect of ischemia on inflammatory cytokine production. Furthermore, we cannot exclude the possibility of venous congestion/distension influencing our results during ischemic exercise, given venous outflow was inhibited due to the 95% systolic blood pressure restriction, and 75 min of a vascular occlusion has been shown to increase IL-6 concentrations whereas a 15-min suprasystolic cuff enhanced VEGF levels in healthy subjects, despite no changes in TNF-α (7, 54). Future studies should address these limitations.

In conclusion, we demonstrate that submaximal ischemic exercise in small muscle mass elicits an inflammatory response. The influence of ischemic cardiovascular disease-associated comorbidities on the inflammatory response during and after exercise warrants further attention. Overall, using a model of intermittent claudication by effectively reducing oxygen saturation, our study provides the first evidence for an enhanced inflammatory response to exercise under a partial arterial occlusion with reduced oxygen tension.

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DISCLOSURES

J. R. Murrow is Chief Operating Officer and K. K. McCully is President of Infrared Rx. The other authors declare no conflicts of interest, financial or otherwise.

AUTHOR CONTRIBUTIONS

D.D.S., J.A.C., J.R.M., K.K.M., and N.T.J. conceived and designed research; D.D.S. and K.R.P. performed experiments; D.D.S., K.R.P., T.B.W., and N.T.J. analyzed data; D.D.S., K.R.P., T.B.W., J.A.C., J.R.M., K.K.M., and N.T.J. interpreted results of experiments; D.D.S. prepared figures; D.D.S. and N.T.J. drafted manuscript; D.D.S., K.R.P., T.B.W., J.A.C., J.R.M., K.K.M., and N.T.J. edited and revised manuscript; D.D.S., K.R.P., T.B.W., J.A.C., J.R.M., K.K.M., and N.T.J. approved final version of manuscript.

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