Human Enterovirus in the Gastrocnemius of Patients With Peripheral Arterial Disease

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Background—Peripheral arterial disease (PAD) is characterized by myofiber degeneration and loss of function in muscles of the lower limbs. Human enterovirus (HEV) infection has been implicated in the pathogenesis of a number of muscle diseases. However, its association with PAD has not been studied. In this study, we tested the hypothesis that infectious HEV is present in skeletal muscle of patients with PAD and is associated with severity of disease.

Methods and Results—Gastrocnemius biopsies from 37 patients with PAD and 14 controls were examined for the presence of HEV RNA, viral capsid protein, viral RNA copy number, and viral infectivity. HEV RNA was detected in 54% of the biopsies from patients with PAD but was not detected in muscle biopsies from control patients. This difference in prevalence among PAD and control patients was significant at P<0.001. Viral RNA copy numbers were increased significantly at the later stages of disease; Fontaine Stage IV (10^{5.50} copies/mg muscle wet weight, at P<0.005) and Stage III (10^{4.87} copies/mg, at P<0.010) compared to Stage II (10^{2.50} copies/mg). Viral replication was confirmed by the presence of the negative-strand of viral RNA in all specimens positive for HEV RNA. Cultures of HeLa and human skeletal muscle cells treated with muscle homogenates showed HEV replication and the presence of HEV capsid protein.

Conclusion—Our data identified infectious HEV in the gastrocnemius of PAD patients but not in controls. Viral copy number and prevalence of infection were higher in the later stages of disease. Our data point to the need for further studies to determine the contribution of HEV infection to the pathophysiology of PAD. (J Am Heart Assoc. 2013;2:e000082 doi: 10.1161/JAHA.113.000082)

Key Words: coxsackievirus • human enterovirus • peripheral arterial disease • skeletal muscle degeneration
myopathies.\textsuperscript{3,5} The presence of these similarities between PAD and other diseases of skeletal muscles, raises the question of whether HEV is present in the leg muscles of PAD patients and whether that is associated with the pathophysiology of the disease. In this study we tested the hypothesis that infectious HEV is present in skeletal muscle of patients with PAD and correlates with a stage of disease. We analyzed biopsy specimens of the gastrocnemius from PAD and control patients for HEV RNA, viral capsid protein, viral RNA copy number, and viral infectivity.

**Methods**

**Muscle and Serum Specimens**

The institutional review board at the University of Nebraska Medical Center (UNMC) and the VA Nebraska and Western Iowa Medical Center approved the experimental protocols and consent forms. After informed consent, muscle and serum samples were obtained from PAD and control patients. Gastrocnemius samples weighing \( \approx 250 \text{ mg} \) were obtained from the anteromedial aspect of the muscle belly, 10 cm distal to the tibial tuberosity. All biopsies were obtained with a 6 mm Bergstrom needle and were frozen for future analysis.

**PAD group**

We recruited 37 patients undergoing lower extremity operations for symptomatic PAD (Table 1). For every patient, the diagnosis of PAD was established on the basis of medical history, physical examination, significantly decreased ankle-brachial index (ABI < 0.9), and computerized or standard arteriography demonstrating stenosis and/or occlusions in the arteries supplying the lower extremities. PAD patients presenting with ischemic, nonhealing ulcers, and/or gangrene were categorized as stage IV PAD patients. PAD patients presenting with ischemic rest pain and no evidence of tissue loss were categorized as stage III PAD patients. Those presenting with intermittent claudication, who had no symptoms of ischemic rest pain and/or evidence of tissue loss were categorized as stage II PAD patients.

**Control group**

We recruited 14 patients with normal blood flow to their lower limbs undergoing lower extremity operations for indications other than PAD (Table 1). These patients had no history of PAD symptoms and all had normal lower extremity pulses at examination. All controls were selected to have normal ABIs at rest and after stress, and all led sedentary lifestyles.

**Cells and Virus Stocks**

HeLa cells (ATCC) were used for HEV propagation, HEV detection, and infectivity experiments.\textsuperscript{4,13,14} Primary human skeletal muscle (HSkm) cells, cultured in MyoTonic Growth Medium (Cook MyoSite), also were used to study virus infectivity. Control HEV strain, Coxsackievirus B1 (CVB1, Table 1. Demographics for Control and Peripheral Arterial Disease (PAD) Patients Recruited for this Study

|                      | Control Patients | PAD Patients |
|----------------------|------------------|--------------|
| Number of patients   | 14               | 37           |
| Gender male/female, %| 86/14            | 91/9         |
| Age, y\textsuperscript{\textsuperscript{2}}  | 63.4±2.0        | 61.6±2.3     |
| Race, w/h/a\textsuperscript{\textsuperscript{3}}  | 86/7/7          | 100/0/0      |
| Smoking, n/f/c\textsuperscript{\textsuperscript{4}}  | 43/21/36        | 9/18/73      |
| CAD \textsuperscript{\textsuperscript{5}}  | 35.7%            | 45.5%        |
| Obesity \textsuperscript{\textsuperscript{6}}  | 28.6%            | 27.3%        |
| DM \textsuperscript{\textsuperscript{7}}  | 21.4%            | 18.2%        |
| Dyslipidemia \textsuperscript{\textsuperscript{8}}  | 50.0%           | 63.6%        |
| HTN \textsuperscript{\textsuperscript{9}}  | 57.1%            | 81.8%        |
| Family history \textsuperscript{\textsuperscript{10}}  | 21.4%           | 9.1%         |
| ABI\textsuperscript{\textsuperscript{11}}  | 1.12±0.04       | 0.34±0.05    |

CAD indicates coronary artery disease; DM, diabetes mellitus; HTN, hypertension; ABI, ankle brachial index.

\textsuperscript{*}Fontaine Stage.

\textsuperscript{1}P values are for differences between Control and all PAD patients combined; Chi-Square or Fisher’s exact tests for categorical variables; Independent t Test for continuous variables.

\textsuperscript{2}Values are mean±SEM.

\textsuperscript{3}Percent of patients in each category; w, Caucasian; h, Hispanic; a, African American.

\textsuperscript{4}Percent of patients in each category; n, never; f, former; c, current.

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ATCC) was propagated in HeLa cells, and its stocks were titrated by an assay based on the 50% tissue culture infective dose (TCID₅₀). CVB1 was used for viral infectivity testing and subgroup-genotyping.

**Muscle Homogenates and Cell Lysates**

Muscle samples (~100 mg wet weight) were homogenized in tissue buffer (PBS/protease inhibitor) with a 2 mL-Dounce homogenizer (Wheaton), and stored in aliquots at −84°C. Cell lysates were prepared in RIPA buffer (Thermo Scientific) and stored in aliquots at −84°C as described elsewhere. Muscle homogenates were frozen/thawed 3-times and clarified by centrifugation. Tissue pellets were stored in RNA homogenates frozen/thawed 3-times and clarified by centrifugation. Tissue pellets were stored in RNA homogenates at −84°C for RNA isolation.

To determine virus infectivity, the homogenates prepared from all muscle biopsies or the supernatants of cell lysates were used to infect HeLa cell cultures. At 48 to 72 hours post inoculation (hpi) the treated cells were frozen/thawed and clarified by centrifugation, and supernatant was collected (VS1; viral supernatant “1”). Next, fresh HeLa cells were treated with a 1:2 dilution of the VS1. At 48 to 72 hpi, supernatants of the treated HeLa cells (VS2; viral supernatant “2”) were prepared as described for VS1. Treated cell cultures were inspected for cytopathic effect (CPE; an indication of HEV replication) by light microscopy. For the detection of CPE, HeLa cells were fixed and stained with crystal violet.

Prior to analysis, supernatants of muscle homogenates or cell lysates were treated with RNase A/T cocktail (Ambion) to digest exogenous free RNA in the sample, leaving encapsidated viral RNA intact, filtered through 0.45-µm filters to remove fine cell debris, and stored in aliquots at −84°C.

**Analysis of Muscle Homogenates, Sera, and Cell Lysates by RT-PCR, RT-qPCR and Western Blot**

RT-PCR analyses were performed to detect HEV RNA in the muscle homogenates, sera, or cell lysates. A primer pair, EV1 (5′-CGGCCCTGATGCGGC-3′) and EV2 (5′-CACCAGATGGCCAATCCA-3′) was used for HEV RNA detection as these primers are specific for sequences located within a highly conserved region of the 5′-NTR of HEV. Other primers used in this study are presented in Table S1. The numbering system is based on the published sequence for CVB1 (GeneBank Accession No. M16560.1).

By utilizing real-time quantitative PCR (RT-qPCR), positive-sense viral RNA copy numbers present in muscle homogenates or cell lysates were determined. A standard curve for the determination of viral RNA copy numbers was developed with a PCR-fragment (193 bp long), amplified by RT-PCR using a primer pair EV1/EV2, and cloned into a pGEM-T easy vector (Promega) (Figure S3A).

For the detection of the negative-strand of viral RNA, total RNAs were isolated from samples that were not treated with RNase and then mixed with biotinylated strand-specific primer EV1 (for the negative-strand). The annealing was carried out in an ABI 2720 thermal cycler (Applied Biosystems) by incubating the RNA/Primer mixture at 75°C for 5 minutes followed by gradual cooling to room temperature. Viral RNAs annealed with the biotinylated primer were mixed with Streptavidin MagneSphere Paramagnetic particles (Promega) and separated using the MagneSphere Magnetic Separation Stand (Promega) per the manufacturer’s instruction. The biotinylated primer/RNA complex was then washed with nuclease-free water. The separation/washing steps were performed 3 to 4 times. After suspending the complex, RT-qPCR was carried out as described above. Synthesized cDNA was then treated with RNase H to remove the RNA from the cDNA/RNA complex. PCR with a primer pair EV1/EVU2 (5′-CCCCTGAATGCGGCTAAT-3′) was carried out to amplify the negative-strand viral RNA.

HEV capsid protein VP1 was detected by Western blot with the primary monoclonal HEV VP1 antibody (5-D8/1, Dako). For a more extensive description of methods, please refer to the “Supplemental Materials.”

**Statistical Analysis**

Differences between demographic values of controls and all PAD patients combined were tested by Chi-square analysis for categorical variables and an independent t test for continuous variables. Fisher’s exact tests were used to analyze categorical variables yielding small cell counts by Chi-square analysis, and to compare the HEV prevalence in control and PAD-stage groups. Kruskal–Wallis nonparametric statistics were used to test the effect of PAD stage on HEV RNA copy number. All analyses were completed using SAS statistical software version 9.3 (SAS Institute Inc), with P<0.05 considered significant.

For a more extensive description of methods, please refer to the “Supplemental Materials.”

**Results**

**Study and Control Groups**

Thirty-seven patients with PAD and undergoing lower extremity operations (28 bypass procedures and 9 amputations) were recruited. Eleven were diagnosed as Fontaine Stage II (ABI; 0.34±0.05, Mean±SEM), 9 were diagnosed as Fontaine Stage III (ABI; 0.24±0.05), and 17 were diagnosed as Fontaine Stage IV (ABI; 0.17±0.02). For the control group, 14 patients undergoing lower extremity operations for the treatment of superficial varicose veins were recruited (ABI;
1.15±0.03). These patients had no history of PAD symptoms and all had absence of PAD based on physical exam and noninvasive testing. The demographics of PAD and control subjects are presented in Table 1. No significant differences were found between the control and the PAD groups except for ABI (P<0.001).

HEV RNA is Present in Biopsies of PAD Gastrocnemius

Total RNAs of the muscle biopsies were analyzed for the presence of HEV RNA by RT-PCR (Figure 1A), using the HEV detection specific primer pair, EV1 and EV2 (EV1/EV2, EVU2). Total RNAs of the muscle biopsies were analyzed for the presence of HEV RNA by RT-PCR (Figure 1A), using the HEV detection specific primer pair, EV1 and EV2 (EV1/EV2, EVU2).
HEV in PAD Muscle is Infectious but Lacks Cytolytic Activity

Next, we determined whether the HEV RNA, detected in the muscle biopsies by RT-PCR, represents infectious virus. HeLa cells (an indicator cell line for HEV replication) were treated with homogenates of all muscle biopsies. Cell lysates VS1 (HeLa cells treated with muscle homogenates) or VS2 (HeLa cells treated with VS1) appeared to have no CPE (upper panels in Figure 2A and 2B), showing no difference from untreated HeLa cells (TC, Figure 2C). In contrast, cell lysis was observed within 24 hours after treatment of HeLa cells with the control virus, CVB1, or supernatants from the cells infected with CVB1 (Figure 2C). These data led us to investigate whether HEV RNA was still present in cell cultures treated with muscle homogenates.

Viral RNA was readily detectable in both VS1 (data not shown) and VS2 (lower panel in Figure 2A and 2B), derived from PAD muscle homogenates previously determined as HEV RNA positive (Figure 1). However, no viral RNA was detected in VS1 or VS2, derived from PAD muscle homogenates determined as HEV RNA-negative. As previously noted in the methods section (also in Supplemental Materials), VS1 and VS2 were treated with RNase A/T1 cocktail to remove free RNA before analysis. Therefore, the detection of HEV RNA in VS1 and VS2 cell lysates indicates the presence of infectious encapsidated HEV rather than free, viral RNA.

Subsequently, HEV-positive muscle homogenates or cell lysates were analyzed for viral capsid protein VP1 by Western Blot analysis. Viral VP1 protein was detected at varying levels in the homogenates with nonspecific labeling of host proteins, possibly due to cross-reactivity of the anti-VP1 antibody (data not shown). On the other hand, HEV VP1 protein was readily detected in VS2 cell lysates as a single band, in the absence of the nonspecific labeling (Figure 3). Also, HEV RNA and VP1 were readily detected in primary human skeletal muscle cells treated with PAD muscle homogenates positive for HEV RNA (data not shown). Hence, these data, together with the data from RT-PCR, strongly suggest that infectious HEV is present in skeletal muscles of PAD patients. Such noncytolytic HEV has been reported for cardiac muscle diseases.14,17

HEV Copy Numbers were Higher in the Later Stages of Disease

HEV found in PAD muscles does not induce CPE in HeLa cells (upper panels in Figure 2A and 2B), consequently the virus could not be quantified on the basis of plaque forming or TCID50 units. Instead, HEV RNA copy number in PAD muscle was measured by RT-qPCR (Figure 4A). Viral RNA copy numbers varied with disease stage (Table 2). Overall, higher copy numbers were observed in Stage III and IV (median copy number 4.87 and 5.50 \[\log_{10} \text{copy number/mg muscle wet weight}\], respectively), compared to Stage II (median copy number 2.50 \[\log_{10} \text{copy number/mg muscle wet weight}\]).

PAD Muscle HEV Replicates in Vitro in Primary Human Skeletal Muscle Cells and in Situ

Replication of HEV present in PAD muscle was analyzed in a single step growth curve (Figure 4B).13,14 Primary HSkm cells

**Table 2. Prevalence of HEV Infection in PAD Gastrocnemius and Viral RNA Copy Number in the HEV Positive Specimens**

|                          | Control | Fontaine Stage |                |                |               | P Value |
|--------------------------|---------|----------------|----------------|----------------|---------------|---------|
|                          |         | Stage II       | Stage III      | Stage IV       |               |         |
| Total number             | 14      | 11             | 9              | 17             |               | —       |
| HEV positive*, count/%   | 0/0%    | 4/36%          | 6/66%          | 10/58%         | 0.001†        |         |
| HEV RNA copy number, \[\log_{10}/mg\] | N/A     | 2.5 (2.25 to 3.75) | 4.87 (3.25 to 5.75) | 5.5 (4.75 to 6.25) | 0.002§        |         |

HEV indicates human enterovirus; PAD, peripheral arterial disease.
* Detection of HEV RNA by RT-PCR and VP1 by Western blot.
† Fisher’s exact test: PAD patients grouped by Fontaine Stage differed significantly from the controls: Stage II \(P = 0.026\), Stage III \(P = 0.001\) and Stage IV \(P < 0.001\).
‡ HEV RNA copy number expressed as median (ranges).
§ Kruskal–Wallis test: Stage II differed significantly from Stage III \(P = 0.010\) and Stage IV \(P = 0.005\). A P value of 0.016 was set as significant in these post hoc comparisons.

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were treated either with supernatants of PAD muscle that were positive for HEV (PAD-HEV) or with control virus CVB1. No lysis (CPE) was observed in the HSkm cell cultures infected with PAD-HEV or CVB1 throughout the experimental period extending 24 hpi. Lysis was evident at 20 hpi in HeLa cell cultures infected with CVB1 (data not shown). Consequently, PAD-HEV could not be quantified on the basis of plaque forming or TCID50 units. Instead, the copy number of HEV RNA at various time points was determined by RT-qPCR. Overall the RNA copy numbers of both PAD-HEV and CVB1 were lower in HSkm cells compared to their numbers in HeLa cells (Figure 4B). By 24 hpi, the RNA copy number of PAD-HEV in the HSkm cell cultures was about 2 log-fold lower than that of CVB1. Although PAD-HEV generated fewer copy numbers and replicated slower than CVB1, it nevertheless replicated efficiently in primary HSkm cells. These data indicate persistent replication of PAD-HEV in skeletal muscle of patients with PAD.

Discussion

Persistent HEV infections have been implicated in the pathogenesis of a number of disorders of skeletal and cardiac muscle. Here we present, for the first time, data demonstrating persistent HEV in the leg muscles of PAD patients. HEV was present in 54% of skeletal muscle samples from PAD patients, but was not detected in the muscles of healthy controls. HEV detected in PAD muscle both replicates and is infectious. These findings are compelling and raise the possibility that HEV infection is associated with initiation and/or severity of PAD. Although our study design does not permit a determination of the mechanism by which HEV infection of PAD skeletal muscles may lead to myopathy, a review of the literature identified 3 likely pathways. First, infectious HEV may produce myofiber damage by competing with native mRNA for ribosomal elf4E, a translation initiation factor that directs mRNAs to the ribosomal start site, thus suppressing
Second, viral infection may cause an accumulation of unfolded proteins in the endoplasmic reticulum (ER), interfering with normal ER protein assembly and trafficking, a condition termed ER stress. Unresolved ER stress caused by persistent viral infection will lead to cell death. Finally, myofiber infection by HEV may lead to the release of skeletal muscle antigens that can induce an autoimmune response which may further damage the skeletal muscles. These mechanisms, based on infection of myofibers per se, are supported by the presence of virus-like particles in the sarcoplasm of patients with idiopathic dilated cardiomyopathy. In regard to PAD, it will be necessary to determine (eg, by in situ hybridization or viral capsid labeling) whether the virus is present in myofibers of infected muscle. However, it is possible that HEV simply coexists in patients with PAD and is not involved in the pathogenesis of PAD.

The absence of detectable virus in serum and the nonlytic phenotype of HEV in PAD gastrocnemius are consistent with chronic, persistent infection of PAD skeletal muscle. In patients with sporadic idiopathic dilated cardiomyopathy, skeletal muscle hosts persistent enteroviral infection. Consequently, myocardial damage may be caused by recurrent heart infection by virus harbored in skeletal muscle or by enterovirus-dependent myopathy that releases antigens shared by skeletal muscle and myocardium, inducing an autoimmune response against the heart. In another report, the investigators concluded from their study of persistent, limited heart infection with enterovirus that viral RNA persisted in the myocardium but was not detected in peripheral blood mononuclear cells. The authors point out that attempts to isolate virus from blood often fail because enterovirus viremias are short in duration and occur early in infection. Thus, the characteristics of persistent versus acute enterovirus infection include the presence of virus in skeletal muscle as host during persistent infection and the absence of a viremia (seen during acute infection). Spread of persistent enterovirus does not require a lytic phase that can produce

![Figure 3](image1.png)

**Figure 3.** Viral capsid protein (VP) was detected by Western blot analysis. VP1 was detected in VS2 from HeLa cells treated with HEV RNA-positive muscle homogenates from PAD patients (lanes 1 to 3, 5 and 7 to 14) but was not detected in VS2 from HeLa cells treated with HEV RNA-negative homogenates from PAD patients (lanes 4 and 6) or in VS2 from HeLa Cells treated with control homogenates (A, B). Arrows indicate VP1. CVB1 infected HeLa cell lysate (positive control), lane “CB1”; Uninfected HeLa cell lysate (negative control), lane “TC.” Patient samples: Controls, (lanes C1 and C2); PAD Stage II, (lanes 4 and 11 to 13); PAD Stage III, (lanes 2 and 8 to 10); PAD Stage IV, (lanes 1, 3, 5 to 7 and 14); VS indicates viral supernatant; HEV, human enterovirus; PAD, peripheral arterial disease; CVB1, coxsackievirus B1.

![Figure 4](image2.png)

**Figure 4.** Viral RNA copy numbers were determined by RT-qPCR. (A) Viral RNA copy numbers were compared among the Stage groups. (B) Single-step growth curves of PAD-HEV were examined at the various time points in the primary HSkm cells. Viral RNA copy numbers were determined using in vitro transcripts (see more detail in Supplemental Materials). PAD-HEV1 (Stage III), PAD-HEV2 (Stage II), PAD-HEV3 and 4 (Stage IV). RT-qPCR indicates reverse-transcription real-time quantitative polymerase chain reaction; PAD, peripheral arterial disease; HEV, human enterovirus; HSkm, primary human skeletal muscle; CVB1, coxsackievirus B1.
viremia. Nonlytic virus may be transmitted to other sites of infection by exploiting microvesiculation, a process by which cells produce and release microvesicles that mediate cell-to-cell communication. This process can be induced by many cellular events (e.g., cell death, hypoxia, stress, and viral infection) and would permit direct transmission of viral particles from infected cells to nearby uninfected cells (e.g., myofiber-to-myofiber, or to more distant uninfected cells) avoiding release of virions into the extracellular environment. Fiber-to-fiber transmission of microvesiculated virions may favor skeletal muscle as depot of a slowly expanding population of viral particles. Our study design does not permit determination of the clinical and functional impairment produced by persistent viral infection in the gastrocnemius of PAD patients. However, in a recent study of patients with prior viral myocarditis, Kuhl et al demonstrated persistence of entervoiral genome in almost one-third of the samples 1 year after acute infection. Persistence of viral RNA in the myocardium of patients was associated with a progressive impairment of heart function, whereas spontaneous viral elimination was associated with a significant improvement in the function of the left ventricle.

Our data indicate a higher prevalence of HEV infection in the gastrocnemius of PAD patients (54%) compared to the affected tissues of patients with other chronic diseases of skeletal and cardiac muscle, including chronic fatigue syndrome (20.8% to 26.4%), chronic inflammatory muscle diseases (20%), fibromyalgia (13%), dilated cardiomyopathy (37.5% to 46%), or idiopathic dilated cardiomyopathy. Again, our study design does not permit identification of a mechanism for these differences; however, chronic progression of end-organ (skeletal muscle) damage seen in PAD may provide an expanding tissue involvement over a longer period of time, producing increased accumulation of viral particles. Another possible mechanism is related to the presence of oxidative stress in PAD muscle. It is possible that the levels of oxidative stress in PAD muscle support increased viral replication. Oxidative stress has been shown to enhance replication of a number of viruses including HEV, HCV, and West Nile virus. A preliminary study in our laboratory has shown that HEV replicates at higher rates in HSkm cells with increased oxidative stress induced by tunicamycin than in normal cells (data not shown).

The prevalence of HEV infection of the gastrocnemius increased from 0% in control patients to 36%, 66% and 58% in PAD patients with disease stage II, III, and IV, respectively. Similarly, viral copy number increased from 2.5 to 4.87 and 5.5 (log_{10}/mg) in disease stage II, III, and IV, respectively. Again, a mechanism that involves increased oxidative stress may be responsible for the higher prevalence of HEV RNA-positive specimens and increased viral copy number per specimen among patients with more advanced PAD. Increased oxidative stress characteristic of PAD muscle may induce increased sensitivity to HEV infection and increased viral replication in the later stages of disease. It is also possible that a longer period of viral growth and expansion in patients with more advanced, and likely longer lasting, disease may account for these observations.

The principal limitation of this study is that the findings are based on correlational analyses of the association between HEV and PAD and, as such, do not identify cause and effect linkages. These correlations, nonetheless, establish the rationale for mechanistic studies that can determine whether HEV infection contributes to initiation and/or severity of PAD. Another concern is that the hemodynamic limitation of most patients in our study may be considered severe on the basis of their low ABIs and raises the possibility that our findings may not apply in the same manner to patients with milder hemodynamic limitation. We also note that biopsies were not taken from skeletal muscles other than the gastrocnemius. Consequently, we were not able to determine whether virus detected in PAD but not in control patients was localized to muscles of the affected leg, only. Finally, our study design identified control and PAD patients who had sedentary life styles, but did not allow precise quantification of differences in physical activity among our study groups. Different levels of inactivity among patients could produce differences in the prevalence of HEV infection in their gastrocnemius.

To the best of our knowledge, this is the first reported study of HEV infection of skeletal muscle in the ischemic limbs of PAD patients. HEV was found in the gastrocnemius of PAD patients but not in controls and both viral copy number and prevalence of infection increased with disease severity. Our data point to the need for further studies to determine the contribution of HEV infection to the pathophysiology of PAD.

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Disclosures
None.

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