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The effect of intermittent pneumatic compression of legs on the levels of nitric oxide related species in blood and on arterial function in the arm

Joseph M. Rifkind [b], Enika Nagababu [b], Devon A. Dobrosielski [c], Maria T. Salgado [b], Michael Lima [a], Pamela Ouyang [a], Harry A. Silber [a,*]

[a] Division of Cardiology, Department of Medicine, Johns Hopkins University School of Medicine, Suite 2400, 301 Building, 4940 Eastern Avenue, Baltimore, MD 21224, USA
[b] Molecular Dynamics Section, National Institute on Aging, National Institutes of Health, Biomedical Research Center, 251 Bayview Boulevard, Suite 100, Baltimore, MD 21224-6825, USA
[c] Department of Kinesiology, Towson University, 8000 York Road, Towson, MD 21252, USA

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ABSTRACT

Background: Intermittent pneumatic compression (IPC) of legs exerts beneficial local vascular effects, possibly through local release of nitric oxide (NO). However, studies demonstrating systemic transport of nitrogen oxide species and release of NO prompt the question of whether IPC could also exert nonlocal effects. We tested whether IPC (1) affects systemic levels of nitrite, S-nitrosothiols and red blood cell (RBC) NO, and (2) exerts vasoactive effects in the brachial artery (BA), although this hypothesis-generating pilot study did not investigate cause and effect relationship between (1) and (2).
Methods: In 10 healthy subjects, ages 24–39 years, we measured plasma nitrite, plasma S-nitrosothiols and RBC-NO from venous blood samples drawn before and after IPC treatment. We also measured BA responses to 5 min of upper arm occlusion at rest and during1 h of leg IPC.
Results: There was a significant decrease in plasma nitrite (112 ± 26 nM to 90 ± 15 nM, $p = 0.0008$) and RBC-NO (129 ± 72 nM to 102 ± 41 nM, $p = 0.02$). Plasma S-nitrosothiols were unchanged (5.79 ± 4.81 nM to 6.27 ± 5.79 nM, $p = 0.3$). BA occlusion-mediated constriction (OMC) was significantly attenuated with IPC treatment (−43 ± 13% to −33 ± 12%, $p = 0.003$). High-flow mediated BA dilation was unchanged (13.3 ± 9.4% to 11.5 ± 7.2%, $p = 0.2$).
Conclusion: Plasma nitrite, RBC-NO, and BA OMC decreased with leg IPC. We hypothesize that this decrease in circulatory pool of plasma nitrite and RBC-NO may result from the transfer of their NO-bioactivity from blood to the hypoxic arm tissue, to be stored and released under hypoxic stress and oppose OMC. Future studies should investigate whether IPC-induced decreases in brachial OMC are caused by the changes in systemic NO activity, and whether leg IPC could benefit distant arterial function in systemic cardiovascular disease.

1. Introduction

Intermittent pneumatic compression (IPC) has several therapeutic uses in the lower extremities, including reducing the risk of deep vein thrombosis [1,2], decreasing edema and helping to heal leg venous stasis ulcers [3], and improving walking distance in patients with claudication due to peripheral arterial disease [4,5]. These benefits are thought to be derived mostly from local production of nitric oxide [6,7], a vasculoprotective molecule [8] generated by increased arterial shear rate [9–11].

Although NO acts over a very short time and distance, recent studies suggest that NO can be transported in blood, bound to the beta-93 cysteine residue of hemoglobin and to plasma thiols – especially to 34-cysteine residue of albumin – or it may be oxidized to nitrite and transported either in plasma or by being taken up by red blood cells [12–19]. Thus, it is possible that NO that is released or delivered locally may have distant effects. Inhaled NO causes skeletal muscle arteriolar dilation [20], and infused nitrite causes forearm vasodilation and pulmonary vasodilation [12,21].

Abbreviations: IPC, intermittent pneumatic compression; NO, nitric oxide; OMC, occlusion-mediated constriction; FMD, flow mediated dilation.

* Corresponding author. Fax: +1 410 550 0020.
E-mail addresses: rifkindj@mail.nih.gov (J.M. Rifkind), enikan@grc.nia.nih.gov (E. Nagababu), ddobrosielski@towson.edu (D.A. Dobrosielski), salgadomt@yahoo.com (M.T. Salgado), michaeljdlima@gmail.com (M. Lima), pouyang@jhmi.edu (P. Ouyang), hsilber@jhmi.edu (H.A. Silber).

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Furthermore, evidence suggests that local hypoxia potentiates the reactions that release NO from its stored forms in blood [22,23]. Accordingly, it is possible that the local production of NO due to IPC could translate to increased global NO availability, yet this has not been empirically tested. Such an effect would likely have clinical implications for those with coronary artery disease and heart failure, in whom there is decreased NO bioavailability [24].

In this study, we sought to determine whether there are non-local effects of leg IPC. We investigated whether leg IPC changes systemic levels of the transportable forms of NO. We also investigated whether vasoactive properties of distant arteries are changed during IPC. In this exploratory and hypothesis-generating pilot study, however, we did not investigate any cause-and-effect relationship between changes in systemic NO forms and changes in distant arterial properties with IPC.

2. Material and methods

2.1. Study participants

Men and women free from overt cardiovascular disease or cardiovascular disease risk factors, including; hypertension, diabetes, hyperlipidemia, smoking, or obesity were recruited to participate in the study. Subjects abstained from eating or drinking except water for at least 6 h before the study. The protocol was approved by the Johns Hopkins Institutional Review Board.

2.2. Intermittent pneumatic compression system

An intermittent pneumatic compression system with high peak inflation pressure and high inflation rate was used (ArtAssist, ACI Medical, San Marcos, California) [25]. The subject was placed in a sitting position. Inflatable cuffs were placed on the foot and calf of both legs. Foot and calf placement together maximize the hemodynamic effects compared with foot or calf placement alone [9]. Settings were consistent with prior studies that have optimized settings for greatest hemodynamic effects [9,26]. Settings included peak inflation of 120 mmHg, minimum deflation pressure of 0 mmHg, inflation time of 4 s, and deflation time of 16 s. The foot cuff was inflated first, followed by the calf cuff 1 s later.

2.3. Experimental protocol

Subjects underwent testing prior to, and just before the end of, 1 h of leg IPC treatment. Testing consisted of brachial arterial ultrasound measurements, and measurements of RBC-NO and plasma nitrite in venous blood. Fig. 1 shows the protocol timeline.

Blood draws were performed to measure systemic venous red blood cell nitric oxide, plasma nitrite, and plasma S-nitrosothiol. Brachial flow was measured at 5 min into the IPC session to test whether mechanically increasing leg flow by IPC acutely changes brachial artery flow.

2.4. Measurement of red blood cell nitric oxide, plasma nitrite, and plasma S-nitrosothiols

Blood was collected from an arm vein contralateral to the arm undergoing reactivity testing. Blood was obtained just prior to the IPC session and at 5 min prior to the end of the first IPC session. Within 30 s of collection, the blood was centrifuged at 5800 RPM using a small portable centrifuge for 3 min. The plasma and buffy coat were removed. A volume of 1 ml of clear plasma free from hemolysis was transferred immediately to dark colored microtubes that contained 0.1 mM diethylenetriaminepentaacetate (DPTA) and 6.25 N-ethyl maleimide to protect S-nitrosothiols. The plasma S-nitrosothiols, nitrite and red blood cell NO were determined using improved techniques developed by two of the investigators [14,27,28]. The analyses were completed within 1 h of blood collection.

2.5. Brachial artery ultrasound testing

We measured hyperemia-induced arterial dilation, and low flow induced arterial constriction. Endothelial-dependent flow mediated dilation (FMD) was measured as the change in arterial diameter following reactive hyperemia. Our methods for obtaining FMD follow guidelines designed to maximize the validity and reliability of this widely used ultrasound technique [29]. Brachial artery images were obtained using an Agilent Sonos 5500 ultrasound unit with a 7.5-MHz linear array ultrasound transducer and recorded on videotape. Subjects were studied while seated in a quiet room. An ECG was also recorded during the study. The left brachial artery was visualized 2 cm above the antecubital fossa and the image was recorded for 30 s. A pressure cuff placed on the upper arm was rapidly inflated to exceed systolic blood pressure by 50 mmHg for 5 min, and then the cuff was rapidly deflated. The brachial artery image was then recorded for 3 min. The upper arm was occluded because it creates more ischemic downstream tissue than forearm occlusion, which is important in this study involving hypoxia-enhanced NO release. We measured the degree of distal brachial arterial constriction near the end of the 5 min of proximal brachial arterial occlusion (occlusion-mediated constriction, OMC). Low-flow mediated constriction of an artery proximal to an occlusion occurs due to low shear stress and is dependent on endothelially released factors [30–32]. However, because other mechanisms including passive collapse also contribute to constriction that occurs distal to an occlusion, we use the term "occlusion-mediated constriction" in this study.

2.6. Image analysis

Study personnel performed image analysis using Brachial Tools™ software package (Version 3.2.6, Medical Imaging Applications, Iowa City, Iowa. The images were digitized at 8 frames/s, which adequately represents the highest relevant frequency in

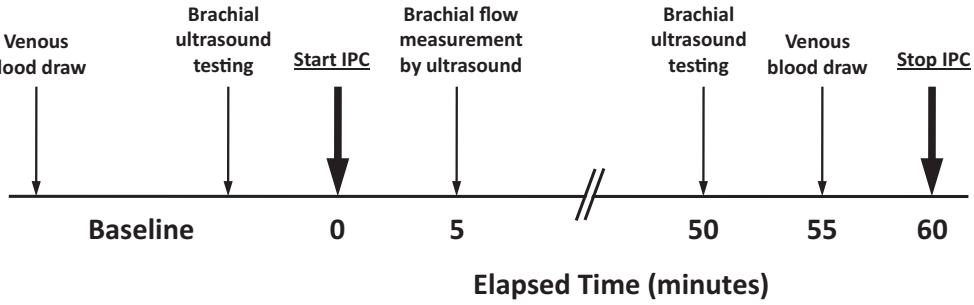

Fig. 1. Timeline of experimental protocol. IPC = intermittent pneumatic compression.