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Inorganic nitrate supplementation enhances functional capacity and lower-limb microvascular reactivity in patients with peripheral artery disease

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Abstract

Peripheral artery disease (PAD) is characterized by functional and vascular impairments as well as elevated levels of inflammation which are associated with reduced nitric oxide (NO) bioavailability. Inorganic nitrate supplementation boosts NO bioavailability potentially improving functional and vasodilatory capacities and may reduce inflammation. Twenty-one patients with PAD were randomly assigned to sodium nitrate ($NaNO_3$) or placebo supplementation groups for eight-weeks. Outcome measures included a six-minute walk test (6MWT), blood flow and vasodilator function in the forearm and calf, as well as plasma inflammatory and adhesion biomarker concentrations. $NaNO_3$ elevated plasma nitrate (32.3±20.0 to 379.8±204.6μM) and nitrite (192.2±51.8 to 353.1±134.2nM), improved 6MWT performance (387±90 to 425±82m), peak calf blood flow (BF_{Peak}; 11.6±4.9 to 14.1±5.1mL/dL tissue/min), and peak calf vascular conductance (VC_{Peak}; 11.1±4.3 to 14.2±4.9mL/dL tissue/min/mmHg) ($p<0.05$ for all). Improvements in calf BF_{Peak} (r=0.70, $p<0.05$) and VC_{Peak} (r=0.61, $p<0.05$) correlated with changes in 6MWT distance. Placebo supplementation did not change plasma nitrate or nitrite, 6MWT, calf BF_{Peak}, or calf VC_{Peak}. Forearm vascular function nor inflammatory and adhesion biomarker concentrations changed in either group. Eight-weeks of $NaNO_3$ supplementation improves vasodilatory capacity in the lower-limbs of patients with PAD, which correlated with improvement in functional capacity.

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Keywords

peripheral artery disease; vascular function; exercise capacity; inorganic nitrate; nitric oxide

1. Introduction

Peripheral artery disease (PAD) is characterized by atherosclerotic stenosis of arteries supplying the extremities (32), associated with an increased risk of fatal cardiovascular events (10), and affects over eight million Americans (43). Phenotypically, PAD manifests in many ways (e.g., intermittent claudication, critical limb ischemia) and is associated with poor functional capacity (32). Functional limitations in patients with PAD are rooted in the mismatch of oxygen supply and demand induced by atherosclerosis; however, revascularization fails to restore functional capacity (41). Thus, it appears as if functional limitations in patients with PAD are likely more closely related to microvascular impairments than the degree of stenosis.

Regulation of microvascular blood flow involves complex interactions between vasodilatory and vasoconstrictor signaling (25). To this point, patients with PAD have impaired endothelium-dependent vasodilation (endothelial dysfunction) (2); due to, in part, reduced production or activity of nitric oxide (NO). Byproducts of NO metabolism (nitrate and nitrite) have been implicated as NO reservoirs through the nitrate-nitritenitric oxide pathway (26). This system operates in parallel to the classic L-arginine pathway but is differentially regulated as the nitrate-nitrite-nitric oxide pathway becomes more prolific during ischemia (36). Given the prevalence of ischemia in patients with PAD (32) and the low NO bioavailability in this patient population (33), supplementation of inorganic nitrate has significant potential to improve functional outcomes in patients with PAD.

Inorganic nitrate supplementation improves various health-related outcomes namely blood pressure and exercise capacity (36). Specifically, a single dose of inorganic nitrate acutely improves exercise tolerance in patients with PAD (20); however, longitudinal data supporting these findings are lacking. A recent study investigated the effects of sodium nitrite on brachial artery endothelial function and exercise capacity in patients with PAD (31). Albeit a novel study, vascular function was exclusively assessed in an upper-extremity conduit artery thus ignoring vascular function in the lower-limb. Thus, our primary aims were to investigate whether inorganic nitrate supplementation enhances limb blood flow, specifically in the lower-limbs, as well as vasodilatory and functional capacities in patients with PAD. We hypothesized that eight-weeks of sodium nitrate ($NaNO_3$) supplementation would improve lower-limb blood flow and vasodilation, and increase total distance walked during a six-minute walk test (6MWT).

It is well known that patients with atherosclerotic disease express elevated levels of inflammation and adhesion biomarkers (22), which may in part explain the low bioavailability of NO in patients with PAD (8). Moreover, excessive inflammation and adhesion biomarkers are associated with poor functional capacity in patients with PAD (35). To this, NO has demonstrated an inhibitory-effect on several biomarkers of inflammation and adhesion (12, 18, 45). To our knowledge, translational work on this paradigm has yet

been conducted. Therefore, a secondary aim of this study was to investigate if $NaNO_3$ supplementation reduces circulating biomarkers of inflammation and adhesion in patients with PAD.

2. Materials and Methods

2.1. Subjects

Twenty-one patients (12 male/9 female, 72±10 years) with diagnosed PAD (classified as Fontaine Stage 1–2a, Rutherford 0–1) (34) were recruited from the University of Iowa's Vascular Clinic into the study. This cohort of 21 patients were enrolled into a clinical trial (clinicaltrials.gov/ct2/show/NCT01983826) with several outcome measures; some of which, have previously been published by our group (23). Exclusion criteria were non-atherosclerotic vascular disease, critical limb ischemia, active ischemic ulceration, recent (six months) revascularization, symptomatic coronary artery disease, heart failure, renal disease, hypotension (systolic blood pressure < 90 mmHg), smoking or history of smoking within one-year, or the use of phosphodiesterase five inhibitor drugs. All women enrolled in the study were postmenopausal and not receiving hormone therapy. All subjects provided written informed consent approved by the University of Iowa's Institutional Review Board.

2.2. Experimental design

A randomized, double-blind, placebo-controlled study design was employed using block randomization (block sizes of six with a 4:2 randomization). Subject randomization and supplement manufacturing were handled by a compounding pharmacy to ensure a true double-blinded study. Subjects were assigned to $NaNO_3$ (1g/day, 8.5mM) or placebo (microcrystalline cellulose) groups for eight-weeks similar to previous works using sodium nitrite (15, 16, 31). Pre- and post-supplementation assessment protocols spanned two-days and were identical on both visits (described below). Subjects followed a low-nitrate diet 72 hours prior to all visits and abstained from exercise, alcohol use, as well as caffeine consumption 24 hours prior to visiting the laboratory. Subjects refrained from prescription medication use on the morning of all visits and arrived following an overnight fast. Furthermore, subjects did not take their respective supplement the morning of post-assessment to account for potential confounding influence of acute supplementation. Visit one consisted of venipuncture, ankle-brachial index assessment, and 6MWT. Vasodilator function was assessed on study day two as to avoid any confounding effects of acute exercise on blood flow.

2.3. Venous blood sampling

Venous blood samples were obtained from an antecubital vein for determination of plasma [nitrate], [nitrite], as well as inflammatory and adhesion biomarkers (listed below). Blood was collected in tubes containing ethylenediaminetetraacetic acid and immediately underwent centrifugation at 3,000 rpm for 15 minutes. Plasma was then aliquoted into Eppendorf tubes and frozen at −80°C for later analysis. Quantification of [nitrate] and [nitrite] were made by their addition to vanadium III chloride in hydrochloric acid at 90°C and to potassium iodide in acetic acid at room temperature, respectively, within 30 minutes

of thawing using a Sievers chemiluminescence NO analyzer (NOA 280i; Sievers Instruments, Boulder, CO) as previously reported (6).

2.4. Inflammation and adhesion biomarkers

Inflammatory and adhesion biomarker concentrations were quantified using commercially available enzyme-linked immunosorbent assays (RnD Systems, Minneapolis, MN). Plasma samples were analyzed in triplicate for interleukin-six (IL-6), soluble vascular adhesion molecule-one (sICAM-1), soluble vascular adhesion molecule-one (sVCAM-1), and monocyte chemoattractant protein-one (MCP-1). Plate well optical densities were measured using a microplate reader and were wavelength-corrected per assay protocol. Values were compared to a standard curve created on the same plate. Intra- and inter-assay coefficients of variation were: 8.5 and 9.9% (IL-6), 2.1% and 5.5% (sICAM-1); 4.0% and 7.6% (sVCAM-1), 3.1% and 3.0% (MCP-1), respectively.

2.5. Functional capacity assessment

Functional capacity was assessed using a self-paced 6MWT per the American Thoracic Society (1) on a 30.5-meter long course with start and end points indicated by cones. Subjects walked from one cone to the other pivoting immediately after passing each one with total distance walked serving as the outcome measurement.

2.6. Venous occlusion plethysmography

Venous occlusion plethysmography (EC-6, D.E. Hokanson Inc. Indianapolis, IN) was used to determine endothelium-dependent reactivity in the left forearm and left calf (14). Baseline blood flow was measured by inflating the distal cuff (wrist or ankle) to a supra-systolic pressure (220 mmHg) then cyclically inflating the respective proximal cuff (upper arm or thigh) to 60 mmHg every 15 seconds for two minutes. Baseline blood flow was the average of all eight cycles. Following baseline, circulation to the experimental limb was arrested for five minutes by rapidly inflating the proximal cuffs to 240 mmHg inducing ischemia. Peak blood flow (BF_{Peak}) was the highest measurement observed following deflation (reactive hyperemia). Total blood flow (BF_{Total}) for three minutes after cuff release was recorded and was defined as the area under the curve using the trapezium rule (28). Vascular conductance served as a surrogate of vasodilatory capacity and calculated as blood flow/mean arterial pressure (Nexfin, Edwards Lifesciences Irvine, CA). Peak and total vascular conductance (VC_{Peak} and VC_{Total}, respectively) were determined in the same fashion as BF_{Peak} and BF_{Total}, respectively. Blood pressure and plethysmography data were collected at 250 Hz and analyzed offline (WinDaq, DATAQ Instruments Akron, OH).

2.7. Statistical analyses

Data are expressed as mean±standard deviation, unless otherwise noted. Subject characteristics were assessed between groups via independent samples t-tests or a chi-squared test where appropriate. A two-way repeated measures analysis of variance was used to compare outcome variables between groups pre- and post-supplementation. When significant F-ratios were detected, pairwise comparisons were made using Tukey's *post hoc* analysis with effect sizes calculated using Cohen's D (d). Additionally, Pearson correlations

were used to quantify the relationship between improvements in 6MWT distance and calf vascular function (BF_{Peak} and VC_{Peak}). All statistical analyses were deemed significant *a priori* at $\alpha<0.05$ and completed using SigmaPlot version 11.0 (Systat Software Inc., San Jose, CA).

3. Results

3.1. Subjects' Demographical Data

Subjects' demographical data are displayed in Table 1 and may also be referenced in our previous works (23). No differences were observed between groups for any data. All subjects whom started the study completed pre- and post-supplementation visits consuming 98.7% of their respective supplement based upon pill bottles collected on the final study visit. None of the subjects reported experiencing adverse effects from supplementation, medication changes, or surgical procedures during post-supplementation study visits. Systolic blood pressure was reduced following $NaNO_3$ (136±15 to 129±17 mmHg, $p<0.05$, $d=0.21$) but not placebo (132±13 to 132±12 mmHg, $p=0.97$) supplementation. Diastolic blood pressure was unchanged in $NaNO_3$ (72±9 to 70±10 mmHg) and placebo (77±10 to 75±9 mmHg) groups (group-by-time interaction $p=0.71$). Additionally, ankle-brachial index was unchanged following supplementation of $NaNO_3$ (0.76±0.21 to 0.86±0.21) and placebo (0.81±0.14 to 0.85±0.15, group-by-time interaction $p=0.24$)

3.2. Plasma nitrate and nitrite

$NaNO_3$ supplementation increased plasma [nitrate] (32.3±20.0 to 379.8±204.6 μmol/L, $p<0.05$, $d=2.36$) and [nitrite] (192.2±51.8 to 353.1±134.2 nmol/L, $p<0.05$, $d=1.52$). Placebo supplementation did not change plasma [nitrate] (34.3±18.7 to 77.5±69.9 μmol/L, $p=0.23$) or [nitrite] (249.7±33.2 to 230.3±76.5 nmol/L, $p=0.47$). Subsequently, plasma [nitrate] and [nitrite] were higher following eight-weeks of $NaNO_3$ than placebo ($p<0.05$ for both).

3.3. Blood flow and conductance

Forearm blood flow and vascular conductance results are displayed in Figure 1. No group-by-time interactions were observed in forearm BF_{Peak} ($p=0.22$) or BF_{Total} ($p=0.99$). Likewise, forearm VC_{Peak} and VC_{Total} were also unchanged following $NaNO_3$ and placebo supplementation (group-by-time interactions $p=0.45$ and 0.90, respectively). Calf blood flow and vascular conductance are illustrated in Figure 2. Due to discomfort during thigh cuff inflation, testing was prematurely stopped for three subjects. Subsequently, data collected on the calf reflects 11 and seven subjects from the $NaNO_3$ and placebo groups, respectively. Calf BF_{Peak} increased following $NaNO_3$ supplementation (11.6±4.9 to 14.1±5.1 mL/dL tissue/min, $p<0.05$, $d=0.50$) but was unchanged following placebo (13.1±3.5 to 11.9±3.7 mL/dL tissue/min, $p=0.32$). However, calf BF_{Total} was unchanged after $NaNO_3$ and placebo supplementation (group-by-time interaction $p=0.11$). Similarly, calf VC_{Peak} was increased following $NaNO_3$ (11.1±4.3 to 14.2±4.9 mL/dL tissue/min/mmHg, $p<0.05$, $d=2.21$) but not placebo (12.1±3.2 to 12.0±4.6 mL/dL tissue/min/mmHg, $p=0.92$) supplementation whereas calf VC_{Total} was unchanged in both groups (group-by-time interaction $p=0.18$).

3.4. Functional capacity

As shown in Figure 3A, patients in the $NaNO_3$ group increased their 6MWT distance ($p<0.01$) whereas patients in the placebo group demonstrated no change ($p=0.74$). Moreover, improvements in 6MWT performance following $NaNO_3$ supplementation correlated with calf BF_{Peak} (n=11, r=0.70, $p<0.05$) and calf VC_{Peak} (Figure 3B, r=0.61, $p<0.05$).

3.5. Inflammatory and adhesion biomarkers

Table 2 presents plasma inflammatory and adhesion biomarker data prior to, and following supplementation in both groups. No group-by-time interactions were found for any investigated biomarkers.

4. Discussion

This is the first study investigating the effects of long-term inorganic nitrate supplementation on functional and vasodilatory capacities in patients with PAD. We demonstrate that inorganic nitrate elevates circulating [nitrate] and [nitrite] while improving functional and vasodilator capacities of patients with PAD. Specifically, eight-weeks of daily $NaNO_3$ supplementation increases 6MWT distance as well as calf blood flow and vasodilation in response to reactive hyperemia. Additionally, patients who demonstrated the greatest improvement in calf BF_{Peak} and VC_{Peak} had the largest increases in 6MWT distance. Importantly, none of the patients enrolled in this study experienced adverse responses to sodium nitrate supplementation contrasting similar works using sodium nitrite (31). Collectively, these findings provide novel insight into the safe therapeutic potential of inorganic nitrate in patients with PAD.

Recently, a single dose of inorganic nitrate was shown to enhance functional capacity in patients with PAD (20). While this study provided proof-of-concept, longitudinal investigations were lacking. Data from the present study extend the findings of Kenjale et al. (20) as we demonstrated prolonged inorganic nitrate supplementation increases functional capacity (6MWT, Figure 3A). Although criteria for a clinically-meaningful change in the 6MWT has yet been established for patients with PAD, several studies in aged populations with and without chronic diseases suggest a change of 20 meters is small yet meaningful, whereas large meaningful change is defined as 50 meters (39). In the present study, 6MWT distance increased nearly 40 meters which is comparable to improvements following exercise training in patients with PAD (38). This finding yields strong clinical significance as 6MWT performance (total distance walked) is predictive of all-cause as well as cardiovascular mortality (29). The authors do not propose inorganic nitrate supplementation is an alternative to exercise in patients with PAD as there are immense benefits associated with regular physical activity (24); rather, we suggest the benefits of inorganic nitrate supplementation and exercise training may be synergistic. Indeed, this concept was investigated by Woessner et al. (48) who demonstrated that exercise combined with beetroot juice (source of inorganic nitrate) improved exercise tolerance and hyperemic blood flow in patients with PAD compared to exercise alone. Nonetheless, our data suggest that daily inorganic nitrate supplementation over eight-weeks is sufficient to improve functional capacity in a population with well documented exercise intolerance.

Vascular function was assessed in the present study using reactive hyperemia allowing us to index microvascular function in the forearm and calf. Given that our intervention was effective in boosting NO bioavailability, our study provides insight into the relationship between NO and microvascular function in patients with PAD. Although factors such as endothelium-derived hyperpolarizing factor (37) and potassium channels (9) contribute to reactive hyperemia, NO is also considered influential (30, 44, 47). We have previously shown the reliance on NO for vasodilation during exercise becomes greater in experimentally hypoperfused contracting skeletal muscle (7). In addition to chronic hypoperfusion, a hallmark of PAD is endothelial dysfunction characterized by abnormal vascular reactivity, mediated in part by reduced levels of endothelium-derived NO (2). Interestingly, the degree of endothelial dysfunction in patients with PAD is indicative of exercise capacity (2). Specifically, peak hyperemic blood flow in the calf of patients with PAD is associated with functional impairments (42). To this, pathological reductions of blood flow and vasodilation during exercise impair functional capacity as patients with PAD have a well-documented attenuation in functional capacity compared to healthy adults (32). Within the current study, inorganic nitrate supplementation improved both calf blood flow and vasodilation which were associated with greater functional capacity (Figure 3B). Collectively, our results demonstrate a beneficial effect of longer-term inorganic nitrate supplementation on microvascular health in the lower-leg and functional capacity of patients with PAD.

Despite PAD being viewed as exclusive to the lower extremities, endothelial dysfunction has also been found in the upper limbs of patients with PAD (31). Subjects in our study corroborate this notion as their forearm BF_{Peak} and VC_{Peak} appear to be lower than previously reported (46). Despite lower forearm blood flow responses in patients with PAD, there were no improvements in forearm vascular function following inorganic nitrate supplementation (Figure 1). These results support the recent findings of Mohler et al. (31) who also did not observe improvements in brachial artery reactivity following 10 weeks of sodium nitrite supplementation. Collectively, the limb-specific benefits of NO-boosting therapies indicate that vascular dysfunction may be more pronounced and more amendable to interventions in lower- compared to upper-extremities of patients with PAD.

Patients with PAD exhibit elevated levels of inflammatory and adhesion biomarkers (22); some of which have been directly associated with limitations in functional capacity (35). Subjects from the present study align with these notions as their plasma levels of inflammation and cellular adhesion exceeded those reported in healthy subjects as well as patients with claudication and critical limb ischemia (11). In the present study, prolonged inorganic nitrate supplementation did not affect circulating biomarkers of inflammation or adhesion and is the first study to examine the anti-inflammatory properties of inorganic nitrate in humans. Our findings support recent preclinical data suggesting prolonged inorganic nitrate supplementation does not influence systemic inflammation (27), yet contradict other preclinical studies demonstrating anti-inflammatory effects of inorganic nitrate (21). Despite demonstrating no statistical improvements in inflammation, subjects in the present study increased their functional capacity with concomitant improvement in vascular reactivity following inorganic nitrate supplementation. Thus, functional and vasodilator capacities can be enhanced without change in inflammation or adhesion

biomarkers; however, these results should be interpreted with caution as our investigation of inflammation was not comprehensive.

While novel, our study contains a few limitations that should be considered. First, we only examined a single dose of inorganic nitrate with outcome assessments occurring exclusively before and after supplementation. While a study containing incremental doses and multiple measurement points is indeed more comprehensive, it would reduce feasibility and complicate statistical analysis. However, it should be noted that the increases in plasma [nitrate] and [nitrite] observed in the current study (eight weeks, 8.5mM) are similar to other studies utilizing shorter supplementation periods (3–14 days) and similar doses (8.0–8.4mM) of inorganic nitrate in various populations (3, 4, 13, 19). Second, the smaller sample size of our study is also worth noting given our *a priori* power analysis proposing 15 subjects per group. While our findings were adequately powered to detect differences in functional and vasodilatory capacities, a sample of 21 subjects is not sufficient to make conclusive statements on the efficacy of inorganic nitrate in this population. Along these lines, a larger sample size may have powered our study enough to observe a significant group-by-time interaction for the total calf blood flow and vascular conductance. Despite the relatively small sample size included in the $NaNO_3$ group, none of the subjects reported adverse events on study visit two and thus it appears that inorganic nitrate supplementation can be considered safe in patients with PAD. It was recently shown that females have greater nitrate-reducing bacteria in their saliva (17); thus, the potential for sex-related differences in the present study is also worth noting where 54% of the $NaNO_3$ group and 25% of the placebo group were female. Additionally, differences in sex breakdown between groups, in concert with our smaller sample size, may have contributed to the differences in baseline 6MWT distances. Lastly, a collective four biomarkers of inflammation and adhesion were investigated and that a more comprehensive analysis may yield different results as an array of circulating inflammation and adhesion markers are implicated in this population; specifically, high-sensitivity C-reactive protein (22). However, biomarkers assessed in the present study are known to be inhibited by NO (12, 18, 45). Despite these considerations, we demonstrate that vasodilatory and functional capacities can be improved in patients with PAD following eight-weeks of a nutraceutical intervention aimed at boosting NO levels.

Our findings demonstrate novel insight into the relationship between inorganic nitrate supplementation, functional capacity, and vasodilatory capacity in patients with PAD. Additionally, the present study supports the notion that diets rich in nitrate are strongly associated with attenuation in adjusted all-cause mortality in patients with atherosclerosis (5). Moreover, we suggest that therapies aimed at increasing NO bioavailability may exert beneficial effects in other patient populations as evidenced by ongoing clinical trials (40).

5. Conclusion

The present study demonstrates that eight-weeks of daily $NaNO_3$ supplementation improved exercise tolerance and vasodilator capacity in patients with PAD. Our findings are of clinical relevance as these patients are known to have exercise intolerance (32) which is partially attributable to poor vascular health (2). Moreover, improvements in exercise tolerance exceeded the minimal important difference (39) and are similar to habitual exercise in this

patient population (29). Collectively, our results carry implications for NO-boosting therapies in patients with systemic atherosclerosis.

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Highlights to “Inorganic nitrate supplementation enhances functional capacity and lowerlimb microvascular reactivity in patients with peripheral artery disease”

- Sodium nitrate increases functional capacity in patients with PAD
- Improvements in functional capacity were related to greater calf vasodilation
- Biomarkers of inflammation and adhesion were unchanged with sodium nitrate use
- No adverse events were reported with 8-weeks of sodium nitrate supplementation

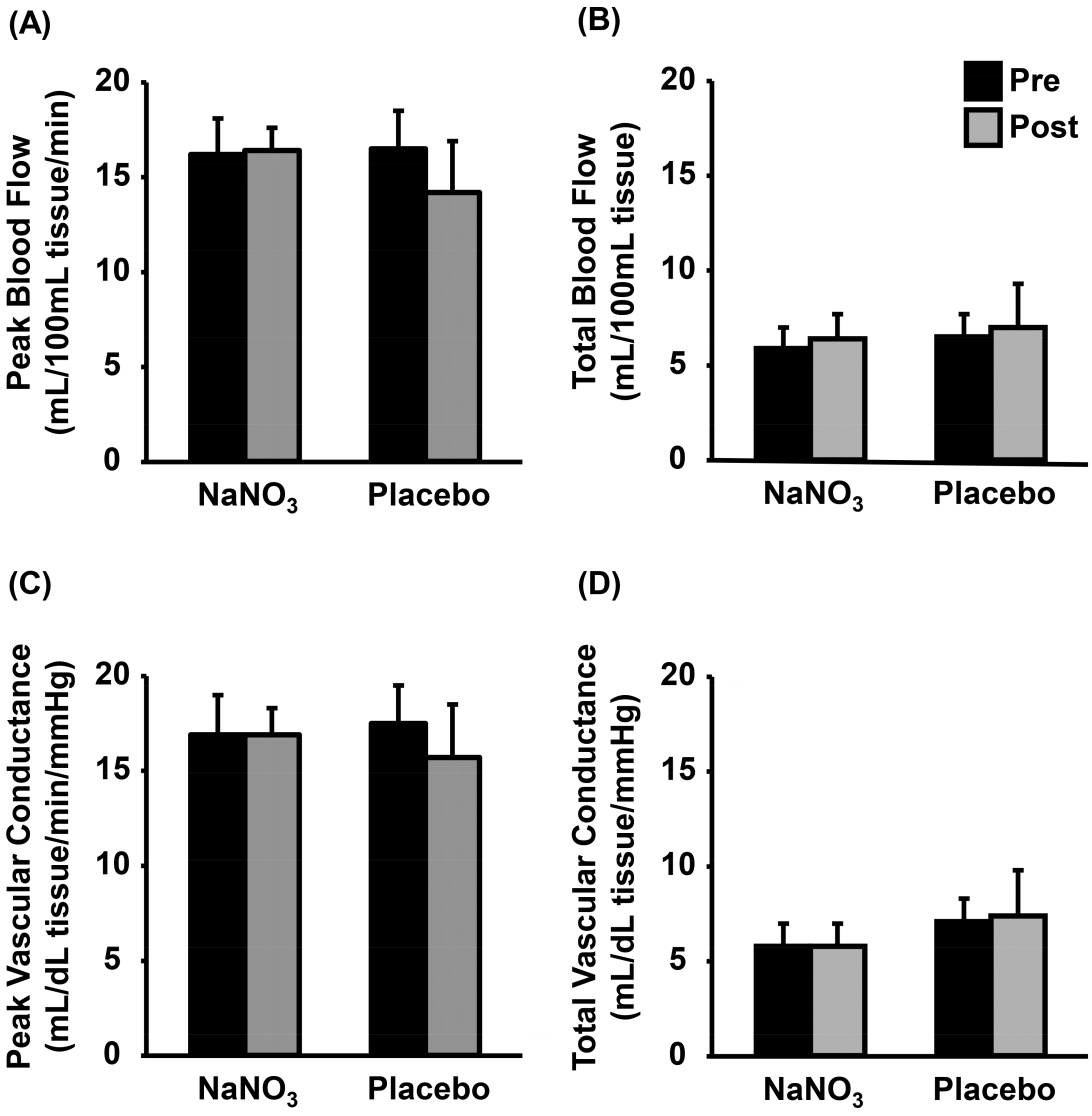

Figure 1.

Forearm blood flow (Peak [A] and Total [B]) and conductance (Peak [C] and Total [D]) obtained by venous occlusion plethysmography pre- (black) and postsupplementation (grey) of sodium nitrate ($NaNO_3$) or placebo. Data presented as mean±standard error.

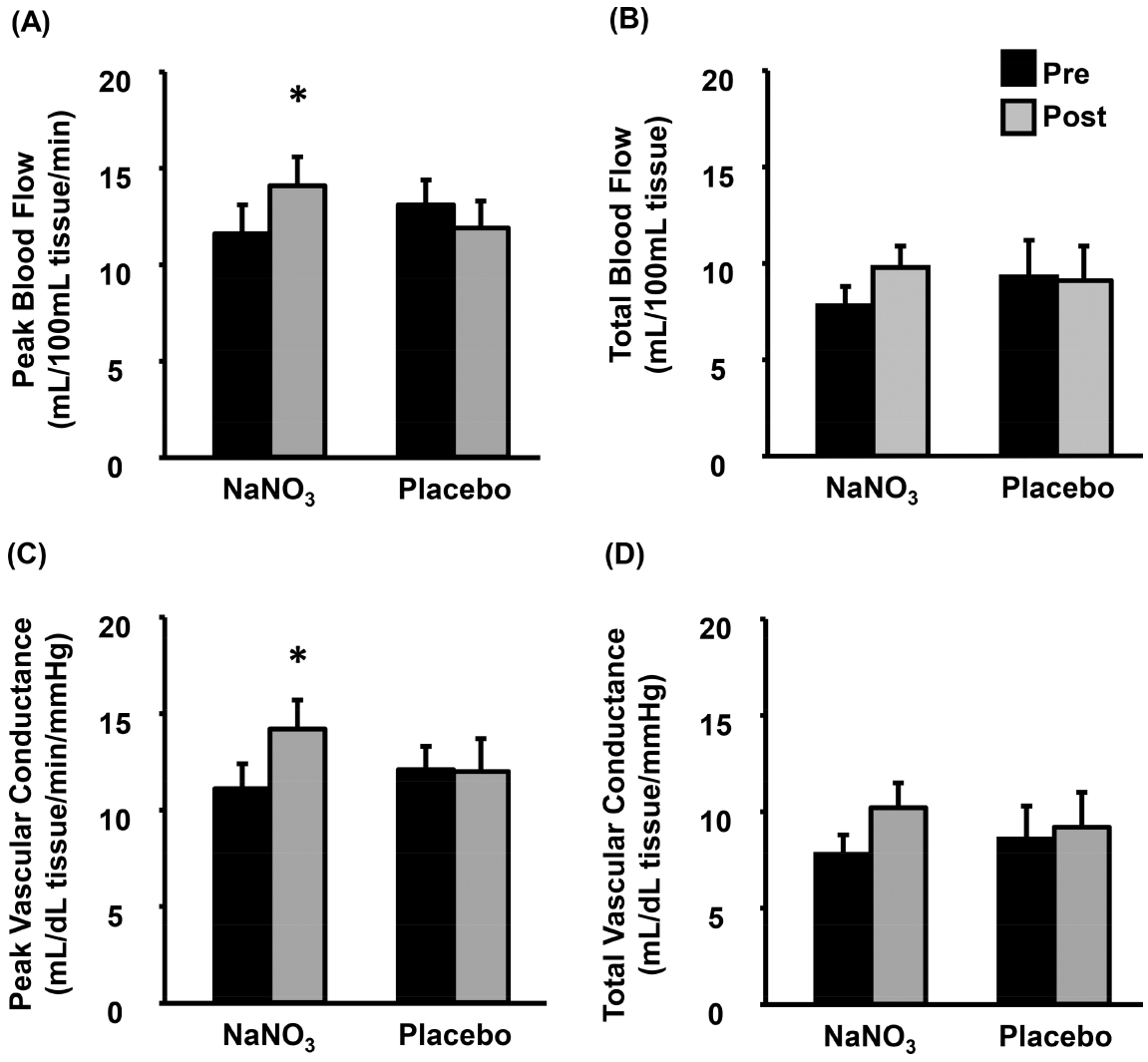

Figure 2.

Calf blood flow (Peak [A] and Total [B]) and conductance (Peak [C] and [D]) obtained by venous occlusion plethysmography pre- (black) and post-supplementation (grey) of sodium nitrate ($NaNO_3$) or placebo. Data presented as mean±standard error. *$p<0.05$ vs. pre-supplementation.

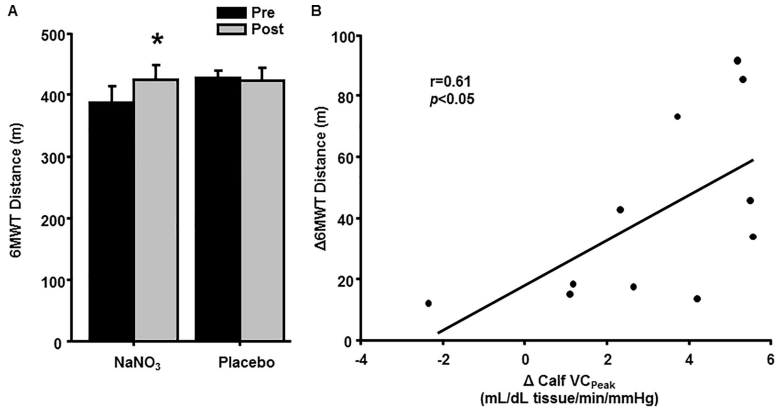

Figure 3.

Six-minute walk test (6MWT) distance before (Pre) and after (Post) eight weeks of $NaNO_3$ and placebo supplementation (A). The relationship between individual changes (Δ) in 6MWT distance and peak calf vascular conductance (VC_{Peak}) from the $NaNO_3$ group (B). Data in (A) are presented as mean±standard error. Data in (B) reflect the 11 subjects with calf data from the $NaNO_3$ group. * $p<0.05$ compared to Pre.

Table 1.

Demographic and clinical characteristics of the PAD cohort.

	$NaNO_3$ (n=13)	Placebo (n=8)	*p*-value
Age (years)	73±9	69±10	0.32
Sex (Male/Female)	6/7	6/2	0.20
Body mass index (kg/m^2)	29.2±5.9	28.1±3.6	0.63
Systolic blood pressure (mmHg)	136±15	132±13	0.46
Diastolic blood pressure (mmHg)	72±9	77±10	0.30
Ankle-brachial index	0.76±0.21	0.81±0.14	0.57
Previous revascularization	12 (92)	6 (75)	0.34
Coronary artery disease	3 (23)	0 (0)	0.65
Type II diabetes mellitus	4 (31)	2 (25)	0.89
Use of a statin	12 (92)	7 (88)	0.78
Use of ACE inhibitors or ARBs	6 (46)	2 (25)	0.62
Use of beta-blocker	6 (46)	3 (38)	0.83
Use of calcium channel blocker	5 (38)	2 (25)	0.76
Use of anticoagulants	6 (46)	2 (25)	0.62
Use of insulin	2 (15)	1 (13)	0.97

Data are mean±standard deviation or n (%). NaNO3=sodium nitrate group; ACE=angiotensin converting enzyme; ARB=angiotensin receptor blocker. Groups were compared using an independent samples t-test or chi-squared analysis when appropriate.

Table 2.

Plasma concentrations of inflammatory and adhesion biomarkers pre- and postsupplementation.

	$NaNO_3$		Placebo		
	Pre	**Post**	**Pre**	**Post**	**Interaction**
IL-6 (pg/ml)	4.0±1.8	3.4±1.4	2.9±0.8	3.1±1.2	$p = 0.11$
sICAM-1 (ng/ml)	338±116	325±99	265±39	263±35	$p = 0.72$
sVCAM-1 (ng/ml)	908±302	820±197	703±208	687±185	$p = 0.47$
MCP-1 (pg/ml)	212±56	220±61	203±42	198±34	$p = 0.71$

Data are mean±standard deviation. NaNO3=sodium nitrate; IL-6=interleukin-six; sICAM1=soluble vascular adhesion molecule-one; sVCAM-1=soluble vascular adhesion molecule-one; MCP-1=monocyte chemoattractant protein-one. Data were compared using a two-way repeated measures analysis of variance.