The Effect of 12-Week Treatment with Intermittent Negative Pressure on Blood Flow Velocity and Flowmotion, Measured with a Novel Doppler Device (Earlybird). Secondary Outcomes from a Randomized Sham-Controlled Trial in Patients with Peripheral Arterial Disease

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Background: Treatment with intermittent negative pressure (INP) is proposed as an adjunct to standard care in patients with peripheral arterial disease (PAD). The aims of this study were to evaluate the applicability of a novel ultrasound Dopplerdevice (earlybird) to assess blood flow characteristics in patients with PAD during a treatment session with INP, and whether certain flowproperties could determine who could benefit from INP treatment.

Methods: Secondary outcomes of data from a randomized sham-controlled trial were explored. Patients were randomized to 12 weeks of treatment with 40 mm Hg or 10 mm Hg INP, for one hour twice daily. Earlybird blood flow velocity recordings were made before and after the 12-week treatmentperiod and consists of a 5-min recording in rest, 3-min during INP treatment and 5-min recording after ended INP test-treatment. Mean blood flow velocity (v_{mean}), relative changes in flow and frequency spectrum by Fourier-transform of the respective bandwidths of endothelial, sympathetic, and myogenic functions, were analyzed for the different series of blood flow measurements.

Results: In total, 62 patients were eligible for analysis, where 32 patients were treated with 40 mm Hg INP. The acquired recordings were of good quality and were used for descriptive analyses of flow characteristics. An immediate increase in v_{mean} during the negative pressure

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periods of the INP test-treatment was observed in the 40 mm Hg INP treatment group at both pre- and post-test. There was a significant difference between the treatment groups, with a difference between the medians of 13.7 ($P < 0.001$) at pre-test and 10.7 ($P < 0.001$) at posttest. This finding was confirmed with spectrum analysis by Fourier-transform of the bandwidth corresponding to INP treatment. The change in amplitude corresponding to myogenic function after 12-weeks of treatment, was significantly different in favor of the 40 mm Hg INP treatment group. We were not able to detect the specific flow characteristics indicating who would benefit INP-treatment.

Conclusions: Earlybird is an applicable tool for assessing blood flow velocity in patients with PAD. Analysis of the flow velocity recordings shows that INP induces an immediate increase in blood flow velocities during INP. The positive effects of INP may be attributed to recruitment of arterioles, and thereby, increasing blood flow. In these analyses, no flow characteristics were determined which could predict who would benefit INP treatment.

INTRODUCTION

Standard of care for patients with peripheral arterial disease (PAD) includes optimizing lifestyle risk factors and medical treatment, as well as exercise treatment, and preferably supervised exercise treatment.[1] A proportion of the patients are not suitable for exercise treatment,[2–4] and a wide range of noninvasive nonpharmaceutical treatment options for PAD have emerged, such as intermittent pneumatic compression and electronic nerve and muscle stimulators.[5] Intermittent negative pressure (INP) applied to the extremities was already introduced in the early 20th century as a noninvasive method to improve peripheral blood circulation.[6] Several studies have suggested additional effect of INP-treatment to the standard care for patients with PAD on walking capacity,[7,8] although 2 recent randomized controlled studies found no additional beneficial effect.[9,10]

Our study group has previously shown that INP causes an acute increase in arterial blood flow.[6,11,12] The mechanism-of-action is proposed to be an increase in the arteriovenous pressure gradient promoting arterial flow.[6] Opposed to constant negative pressure, INP avoids vasoconstriction of the arterioles caused by the venoarteriolar reflex. Holder et al.[13] proposed that fluctuations in shear pattern, induced by INP-treatment, promotes endothelial function. The most effective INP-treatment protocol to induce an acute increase in arterial flow was found to be 10 sec of 40 mm Hg negative pressure and 7 sec of atmospheric pressure.[11,12,14] With this treatment protocol, hard-to-heal wounds showed a tendency to heal after INP-treatment.[15,16]

We have earlier published results from a randomized sham-controlled trial, including 63 patients with intermittent claudication randomized to 12 weeks of 40 mm Hg INP-treatment or sham treatment with 10 mm Hg INP for 1 hr, twice everyday, in a home-based setting.[17] The main finding was that INP-treatment increased pain-free walking distance (PWD) in the 40 mm Hg INP treatment groups, with an estimated treatment effect of 50 meters (95% CI 11, 89), $P = 0.014$, compared to the 10 mm Hg INP treatment group.[17] In a follow-up study of 10 patients who were treated for 24 weeks, with INP, a mean increase in PWD and maximal walking distance (MWD) was found.[18] However, in both studies a proportion of the patients did not show an improvement in walking capacity. It may therefore be clinically relevant to find criteria that could select whom would benefit from INP-treatment, and to rather focus on other efforts to increase peripheral blood circulation in patients most likely not to benefit from INP-treatment.

Earlybird is a novel ultrasound Doppler monitoring device, with a single element highly sensitive transducer.[19,20] The large aperture area of 80 mm^2, makes it easy to place over the vessels of interest. Earlybird correlates well with laser Doppler flowmetry and pulsed wave Doppler to assess microcirculatory function in healthy subjects[20] and it measures volume flow-rate in arteriovenous fistulas for hemodialysis with comparable accuracy as duplex ultrasound.[21] The underlying technology has been used to monitor cerebral blood flow velocities in neonates.[22,23]

The aim of this study was to evaluate the applicability of a novel Doppler ultrasound device (earlybird), to assess blood flow characteristics in patients with PAD during a treatment session with INP and whether certain flow-properties could determine who could benefit INP-treatment.

MATERIALS AND METHODS

This is an exploratory study of secondary outcomes from an earlier published randomized sham-controlled multicenter trial on the effect of 12-

weeks treatment with INP on walking distance in patients with intermittent claudication.[17]

Patients

Between January and September 2019, patients were enrolled from 3 vascular surgery departments in Norway (St. Olavs Hospital, Trondheim, Oslo University Hospital, Oslo, and Sørlandet Hospital, Kristiansand). Data collection was completed in 2019. A total of 72 patients were included and randomized, in a 1:1 ratio, to either a 10 mm Hg or 40 mm Hg INP treatment. Of these, 63 patients completed the 12-week intervention. One patient was excluded due to software failure during earlybird recordings, as described in the Consort Flow Diagram, Figure 1. Labeling of the treatment devices was performed by the producer (Otivio AS) by a person not involved in patient recruitment or data collection. Patients and participating personnel were blinded to the group allocation.

Intermittent Negative Pressure

INP was applied to the treated leg using a custom-built boot with a proximal sealing zone, Figure 2. A hose connected the boot to a control unit (FlowOx 2.0, Otivio AS, Oslo, Norway), which provided periods of alternating 7 sec of atmospheric and 10 sec of 10 mm Hg or 40 mm Hg negative pressure. After randomization, the patients were given and trained in their personal device. The patients' most limiting leg was decided based on a treadmill test. They were instructed to treat that leg for 1 hr in the morning and 1 hr in the evening at home for 12-weeks.

Treadmill Test

PWD and MWD were measured on a treadmill using a ramp protocol at a constant speed of 3.2 km/h starting at 0% slope and increasing by 2% every 2 min.[24]

Blood Flow Measurement

Blood flow velocity measurements were recorded with earlybird, a novel unfocused ultrasound Doppler device, which we have validated in an earlier study.[20] Earlybird consist of a highly sensitive single element Doppler transducer,[19] scanner and inhouse produced interface (MATLAB, MathWorks R2018a), Figure 2.

The ultrasound Doppler probe was attached to the posterior tibial or dorsal pedal artery at the treated leg. The Doppler probe was mounted to provide an angle to the skin of approximately 60°, however the exact insonation angle related to the vessel of interest is not known. All equipment was attached before the leg was lowered into the INP-boot. The patients were seated comfortable and asked to relax without moving. They were left undisturbed while the measurements were recorded.

Earlybird measurements were done both before (pre-test) and after the 12-week intervention period (post-test). We recorded a 5-min earlybird-recording at rest (baseline). After a short break we performed a 9-min recording consisting of 1-min recording before INP test-treatment was started (pre INP-period), a 3-min recording during INP-treatment sequence (INP-period), and a 5-min recording after the INP-period was stopped (post INP-period), Figure 3. All recordings were saved for post processing.

Data Analysis and Statistics

All recorded Doppler flow curves were optimized regarding sample volume, gain and signal tracing to improve signal quality. The Doppler traces were analyzed in the MATLAB and exported to excel with mean blood flow velocities (v_{mean}) for each of the pre-defined recorded periods for both pre- and post-test and includes baseline, pre INP-periods, INP-period, and post INP-period. Within the INP-period, the Doppler velocity curves of the negative and atmospheric pressure-periods were analyzed separately. Although measurement for peak systolic, mean and end-diastolic flow velocity for all relevant time periods were acquired, only v_{mean}, were used for further analyses. Flow velocities are reported in the arbitrary units due to unknown exact insonation angle to the vessel measured, Figure 3.

Power spectrum analysis by Fourier-transform was applied to the Doppler blood flow velocity-curves, presenting frequency specter in a logarithmic scale. Spectrum analyses were performed for baseline, INP-period, and post INP-period. We calculated the area under the power spectrum curve of each frequency band corresponding to specific flowmotion characteristics; 0.007 to 0.02 Hz, 0.02 to 0.06 Hz, 0.06 to 0.2 Hz, 0.2 to 0.6 Hz and 0.6 to 1.8 Hz associated to endothelial, sympathetic, vascular smooth muscle, respiratory and heart activity respectively,[25–27] Figure 4. Amplitude was normalized to v_{mean}.

Descriptive statistics is presented as median, with its 25th and 75th percentile, or mean, with its 95% confidence intervals for continuous variables, and the number with its percentage for categorical variables. Normality was assessed with Shapiro-Wilk

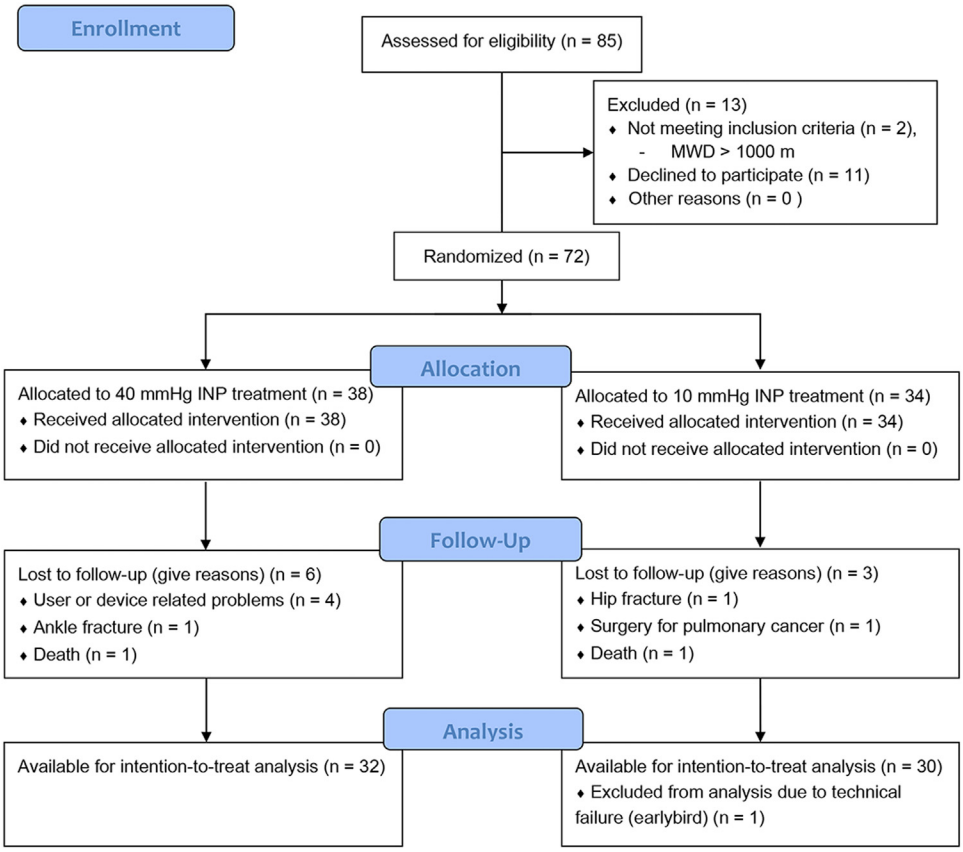


Fig. 1. CONSORT (consolidated standards of reporting trials) flow diagram. The flow diagram is showing inclusion, exclusion, treatment allocation, and outcomes. INP, Intermittent negative pressure; MWD, maximal walking distance.

test. One-Sample Wilcoxon Signed Rank for differences within the groups, Mann-Whitney *U* test for difference between the groups and Kruskal-Wallis H for difference between categories were used. For post-hoc analysis Mann-Whitney *U* for pairwise comparison with Holm-Bonferroni correction was used. Spearman's rank correlation (r_s) was used for correlation analysis. Statistical analyses were done in the SPSS (IBM Corp. Released 2017. The IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp).

Ethics

The study was approved by the Regional Committee for Medical and Health Research Ethics in Norway (reference no 2018/748) and registered in Clinical trials.gov (NCT03640676). The use of earlybird was approved by the Norwegian Government of Health, June 12th, 2017. All study participants gave their written informed consent before inclusion.

RESULTS

In total, 62 patients were eligible for analysis, of these 30 were randomized to 10 mm Hg and 32 to 40 mm Hg INP-treatment. One posttest baseline-measurement in the 10 mm Hg INP treatment-group, and one complete posttest measurement for 1 patient treated with 40 mm Hg INP, were not recorded and not available for analyses. There were no demographic differences between the groups, except a larger proportion of patients with diabetes in the 40 mm Hg INP treatment group, Table I.

Flow Velocity Recordings and Characteristics

The acquired earlybird-recordings were of good quality and eligible for further analyses of changes in v_{mean}, example of flow velocity curve is given in Figure 3. Treatment with 40 mm Hg INP, induce an immediate increase in v_{mean} for the negative pressure period during the 3-min INP-period (19.4% (3.1, 41.2), $P < 0.001$ for pre-test and

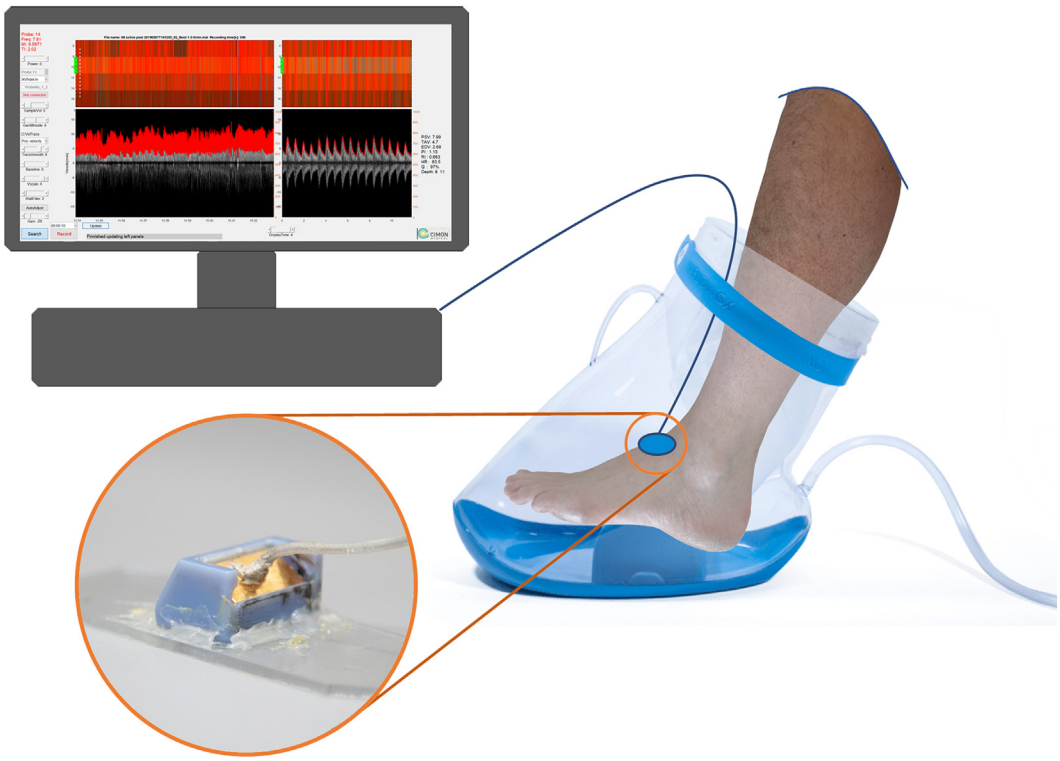


Fig. 2. Illustration of the treated leg placed in an INP-treatment boot (FlowOx 2.0). There is a proximal sealing zone around the calf. The external pump is not visualized. The earlybird probe (highlighted picture) is connected to a scanner and monitor with the interface. (Illustration: Erik Mulder Pettersen/NTNU, photo: Karl Jørgen Marthinsen/NTNU)

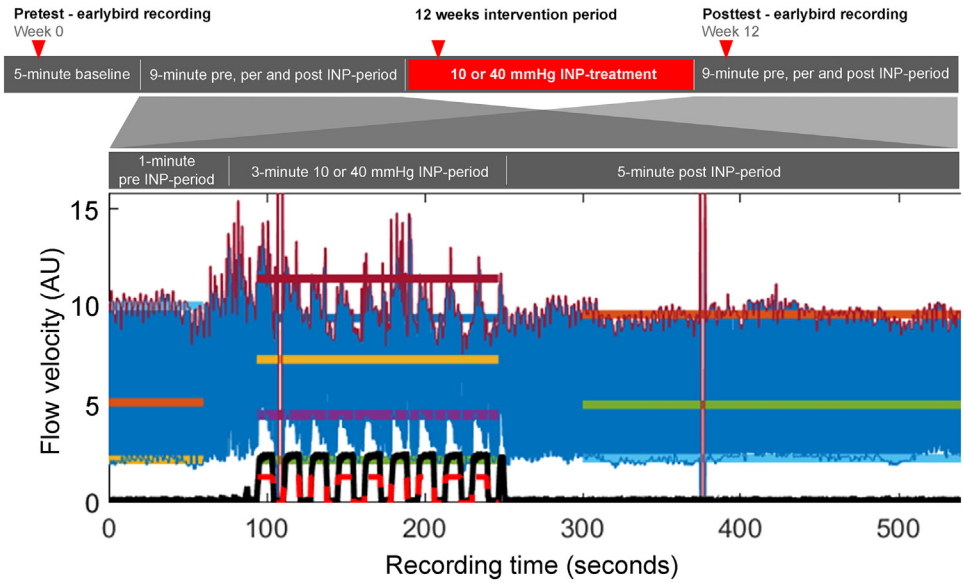


Fig. 3. Overview of pre- and post-test earlybird recordings. Mean blood flow velocity (v_{mean}) was calculated for baseline (not visualized), pre INP-period (orange), the negative-pressure periods of the INP-period (yellow), atmospheric-pressure periods of the INP-period (purple) and post INP-period (green) respectively. The black line indicates inverted negative-pressure periods.

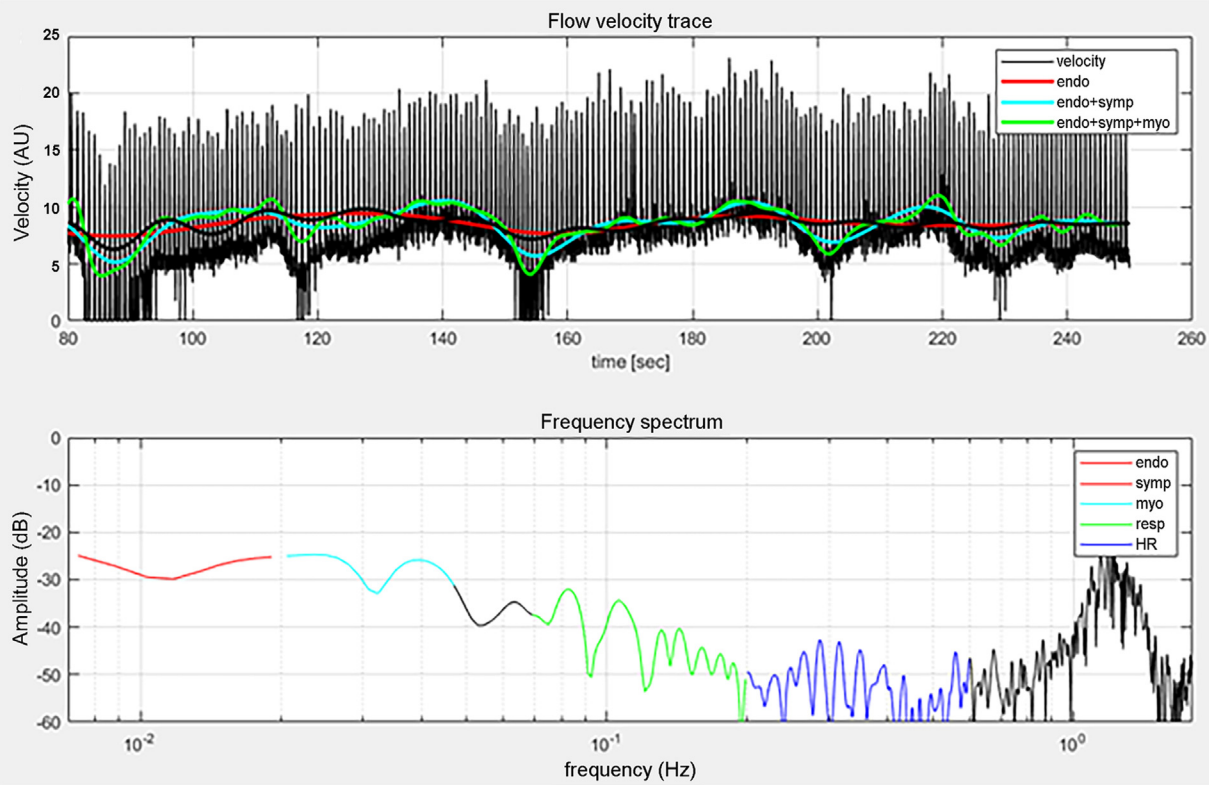


Fig. 4. Example of frequency specter analysis for 5-min baseline velocity trace. Bandwidths were set for endothelial (0.007–0.02 Hz), sympathetic (0.02–0.06 Hz), myogenic 0.06–0.2 Hz), respiratory (0.2–0.6 Hz) and heart rate (0.6–1.8 Hz) distribution frequency respectively.

9.4% (2.2, 24.9), $P < 0.001$ for the post-test). There is a significant difference between the treatment groups, in favor of 40 mm Hg INP, difference between the medians 13.7 ($P < 0.001$), and 10.7 ($P < 0.001$), pre- and post-test respectively, Table II.

There is a significant relative increase in v_{mean}, from the pre-to the post INP-period in the 40 mm Hg INP treatment group (median increase 10.5% (−0.7, 19.2), $P < 0.001$), but only at the pre-test. For the post-test there was no significant relative increase (median increase 2.1% (−5.9, 19.9), $P = 0.124$). Neither there was a significant relative increase in v_{mean} from pre-to post INP-period for the 10 mm Hg INP treatment-group ($P = 0.102$ and $P = 0.360$), nor a difference between the two treatment groups for pre- and post-test ($P = 0.260$ and $P = 0.492$, respectively), Table II.

Fourier-Transformation and Analysis of INP-Response

INP-treatment increased the normalized amplitude of the power density of area under the curve for 1/17 Hz bandwidth, corresponding to the frequency of the INP treatment-cycle. Both at pre- and post-test there was a significant difference between the treatment groups, in favor of 40 mm Hg INP-treatment (difference between medians 4.9, $P < 0.001$ and 2.3, $P < 0.001$, respectively), Table III and Figure 5. No change in normalized amplitude for 1/17 Hz bandwidth was seen at the post-test, compared to the pre-test (median −0.47 (−2.4, 1.3), $P = 0.271$ and −0.6 (−3.7, 1.1), $P = 0.171$, for 10 mm Hg and 40 mm Hg INP treatment groups, respectively) Table IV and Figure 5.

Fourier-Transformation and Analysis of Flowmotion

We analyzed the Fourier-transformation for the 5-min baseline recordings of the respective bandwidths for endothelial, sympathetic, and myogenic function (0.007–0.02, 0.02–0.06 and 0.06–0.2 Hz respectively), Figure 4.

There was a difference between the 10 and 40 mm Hg INP treatment-groups for the change,

Table I. Baseline characteristics of patients eligible for analyses

Variable	40 mm Hg INP $n = 32$	10 mm Hg INP $n = 30$
Age (years)	72 (67, 77)	73 (69, 78)
Male sex	19 (59)	23 (72)
Body mass index (kg/m^2)	26.7 (24.7, 30.7)	27.1 (23.9, 30.6)
Ankle-brachial-index (ABI), at rest	0.50 (0.42, 0.67)	0.57 (0.47, 0.64)
Pain-free walking distance (PWD) (m)	92 (47, 150)	86 (50, 152)
Maximal walking distance (MWD) (m)	233 (139, 313)	250 (103, 379)
Smoking		
Current	11 (34%)	10 (31%)
Previous	18 (56%)	17 (53%)
Never	3 (9%)	3 (9%)
Diabetes mellitus	16 (50%)	6 (19%)
Chronic renal failure	2 (6%)	4 (13%)
Hypertension	26 (81%)	25 (78%)
Hypercholesterolemia	18 (56%)	25 (78%)
Coronary artery disease	14 (44%)	17 (53%)
Cerebrovascular disease	3 (9%)	8 (25%)
Antiplatelet agent	27 (84%)	24 (75%)
Anticoagulation	5 (16%)	6 (19%)
Statin	26 (81%)	28 (88%)
Antihypertensiva	28 (88%)	28 (88%)

INP, intermittent negative pressure; continuous data presented as median with its 25th and 75th percentile.

from pre-to post-test, in the normalized amplitude corresponding to myogenic function (0.06–0.2 Hz), with a difference between the medians of 0.86, $P = 0.002$. At pre-test, the 10 mm Hg INP treatment-group presented a higher normalized amplitude corresponding to myogenic function (6.9 (4.7, 9.5)) compared to the 40 mm Hg INP treatment group (4.9 (2.8, 7.7)), difference between the medians of 2.0, $P = 0.015$, Table III. Within the 10 mm Hg INP treatment-group, a reduction in normalized amplitude corresponding to myogenic function was seen from the pre-to the post-test, median change −0.99 (−4.9, 0.01), $P = 0.003$. No change was seen within the 40 mm Hg INP treatment-group at post-test, median change 0.85 (−1.3, 2.6), $P = 0.167$, Table IV and Figure 6.

No difference was found for the normalized amplitude of the respective flowmotion bandwidths and categories for PWD (<50, 50–100, 100–150, and >150 meters) or for MWD (<100, 100–200, 200–300 and > 300 meters), except for endothelial function categorized for PWD at pre-test ($P = 0.041$) and sympathetic function for the compiled amplitude for both treatment groups at the post-test ($P = 0.046$), Supplementary Material I. However, post-hoc analyses showed no significant difference between the identified categories ($P = 0.096$ and 0.342, respectively). Between the 10 and 40 mm Hg INP treatment-groups for the respective flowmotion bandwidths, no significant change in normalized amplitude from pre-to post-test was found, except for myogenic function as mentioned above. No differences between the categories for improvement in PWD or MWD, was found, Supplementary Material II.

Flow Characteristics at Pretest to Predict Outcome after 12 weeks of 40 mm Hg INP-Treatment

In the 40 mm Hg INP treatment-group, we found no correlation between v_{mean} at the pre INP-period, relative increase in v_{mean} during INP-period or post INP-period at pre-test, and improvement in PWD or MWD after 12-weeks of INP-treatment, Table V. There was no significant difference in the relative increase in v_{mean} for the different categories in improvement in PWD or MWD, Figure 7. Neither there was a correlation between improved walking distance and normalized amplitude for flowmotion bandwidths, nor for the normalized amplitude for bandwidth corresponding to INP-treatment Table V and Supplementary Material II.

DISCUSSION

The main finding in this study of secondary outcomes of a randomized sham-controlled trial, was that earlybird is a feasible tool to measure blood flow velocity during ongoing treatment with INP in patients with PAD. Treatment with 40 mm Hg INP elicits an immediate increase in blood flow

Table II. Acute effects on flow velocity of INP-treatment

	10 mm Hg INP ($n = 30$)	40 mm Hg INP ($n = 32$)	
	Median[a]	Median[a]	
	(25th, 75th percentile)	(25th, 75th percentile)	Absolute difference between medians[b]
Pretest			
Pre INP-period (AU)	6.13 (3.86, 9.13)	6.52 (3.89, 9.52)	0.39, $P = 1.000$
Relative increase in v_{mean} during negative-pressure-periods in the INP-period, compared to pre INP-period	5.7 % (−6.8, 12.2) $P = 0.106$	19.4 % (3.7, 41.2) $P < 0.001$	13.7, $P = 0.001$
Relative increase in v_{mean} post INP-period, compared to pre INP-period.	4.9 % (−4.4, 15.7) $P = 0.102$	10.5 % (−0.7, 19.2) $P < 0.001$	5.6, $P = 0.260$
Posttest (after 12-week INP-treatment)			
Pre INP-period (AU)	7.62 (4.60, 11.86)	6.21 (4.02, 10.20)	1.41, $P = 0.379$
Relative increase in v_{mean} during negative-pressure-periods in the INP-period, compared to pre INP-period	−0.3 % (−5.4, 9.1) $P = 0.355$	9.4 % (2.2, 24.9) $P < 0.001$	10.7, $P = 0.003$
Relative increase in v_{mean} post INP-period, compared to pre INP-period.	3.0 % (−7.9, 12.9) $P = 0.360$	2.1 % (−5.9, 19.9) $P = 0.124$	−0.9, $P = 0.493$

INP, intermittent negative pressure; AU, arbitrary units; v_{mean}, mean blood flow velocity.
[a]One-Sample Wilcoxon Signed Rank test.
[b]Mann-Whitney *U*.

Table III. Normalized amplitude for flowmotion-bandwidths (Fourier-transform) at pre- and post-test

	Pretest (before 12-week INP treatment)			Posttest (after 12-week INP treatment)		
	10 mm Hg INP	40 mm Hg INP		10 mm Hg INP	40 mm Hg INP	
	Median	Median		Median	Median	
	(25th, 75th)	(25th, 75th)	*P* between groups[a]	(25th, 75th)	(25th, 75th)	*P* between groups[a]
INP-treatment (1/17 Hz)	3.9 (2.7, 5.0)	8.8 (5.1, 10.5)	<0.001	3.4 (2.3, 4.7)	5.7 (4.6, 10.2)	<0.001
Flowmotion						
Endothelial function (0.007−0.02 Hz)	7.3 (4.4, 16.5)	7.7 (3.8, 15.9)	0.345	7.0 (5.1, 10.4)	7.5 (4.5, 13.3)	0.807
Sympathetic function (0.02−0.06 Hz)	7.8 (5.2, 12.3)	6.9 (3.4, 11.4)	0.115	7.4 (5.7, 19.4)	7.2 (5.7, 10.1)	0.801
Myogenic function (0.06−0.2 Hz)	6.9 (4.7, 9.5)	4.9 (2.8, 7.7)	0.015	5.6 (4.6, 7.0)	5.8 (4.0, 8.2)	0.668

INP, intermittent negative pressure.
[a]Mann-Whitney *U*.

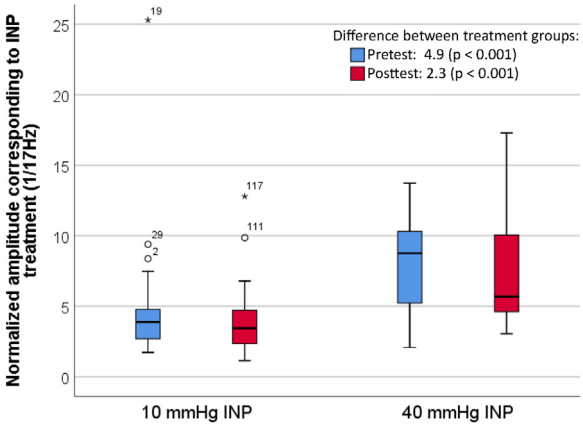


Fig. 5. Normalized amplitude of the power density under the curve corresponding to INP treatment (1/17 Hz), for both treatment groups at pre- and post-test. The absolute difference between the medians of the pre are post-test are presented, see also Table III. For change within the treatment groups at post-test, compared to pre-test, see also Table IV.

velocity. This finding is confirmed in the spectrum analyses by Fourier-transformation of the normalized amplitude of the bandwidth corresponding to the INP-response (1/17 Hz), and are in line with recent studies on INP and the effects on micro- and macro-circulation.[11,12,14] Evaluation of flowmotion at the post-test, showed a difference between the treatment groups of the change in normalized amplitude corresponding to myogenic function (0.06–0.2 Hz), in favor of 40 mm Hg INP treatment-group. No differences were found for endothelial or sympathetic function.

Changes in amplitude of the bandwidth associated to myogenic function has been proposed to be caused by activity of the vasomotor cells in the arterioles.[28] The observed difference in change in myogenic function between the groups, after 12-weeks of INP-treatment, may indicate that the mechanically induced flowmotion pattern by INP has a positive impact on the myogenic regulation of arterioles, and thereby, improve peripheral blood circulation and increase walking ability. Results from previous studies have demonstrated inhibited flowmotion patterns in patients with lower extremity atherosclerotic disease, especially endothelial activity.[26,27,29,30] In the present study, however, we did not observe any association between the degree of reduced walking ability and the amplitude of the different flowmotion bandwidths, neither at the pre-nor post-test. The lack of findings may be due to limitations by the study, see chapter 4.1 Limitations.

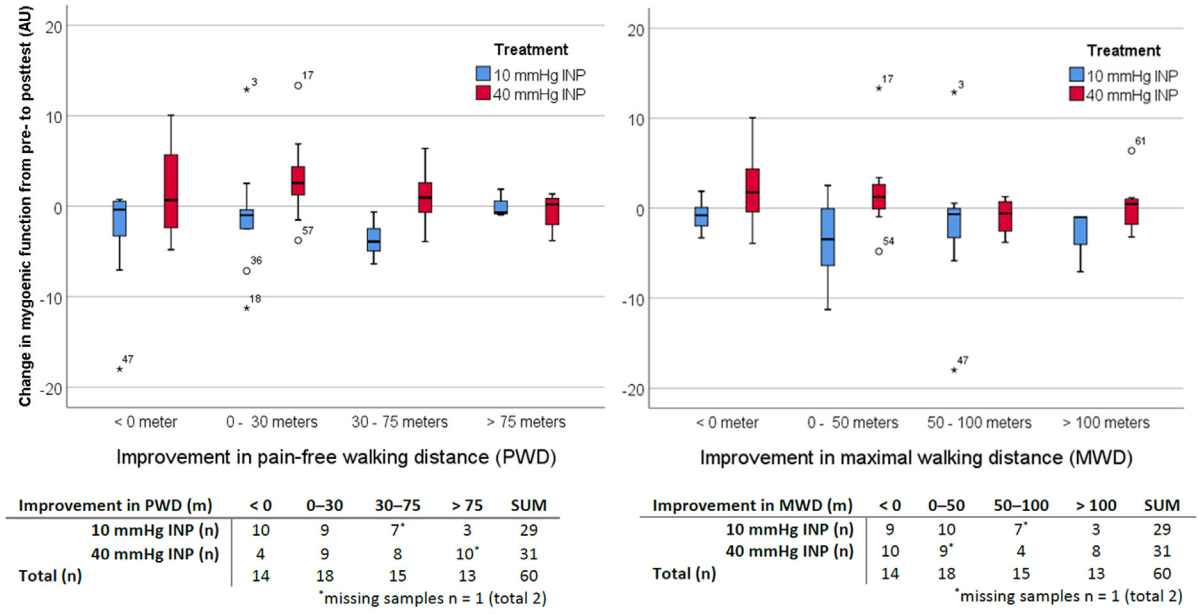
Sundby[11] and Hoel,[14] with colleagues, determined that INP-treatment of ten seconds of 40 mm Hg negative pressure and 7 sec of atmospheric pressure was the optimal protocol to increase blood flow velocity. A constant negative pressure of 40 mm Hg mimics the gravitational force in upright position,[31] and leads to an increase in the arteriovenous pressure gradient and a temporary increase in blood flow.[32] Increased in-flow and reduced emptying of the venous reservoir increase the venous pressure and evens out the pressure gradient. The venoarteriolar reflex elicits vasoconstriction of arterioles which again reduces blood flow.[32] Oscillations with negative pressure, in contrast, extinguish the venoarteriolar reflex[6] and increases arterial and cutaneous blood flow in healthy individuals and in patients with peripheral arterial disease.[11,12] Direct suction on the arteriolar vascular bed or increased emptying of the venous reservoir may be two mechanisms of action. It is suggested that INP-treatment stimulates and promotes vascular health through mimicking blood flow fluctuations,[6] and that this could be the etiology for the observed positive effect of INP-treatment. The fluctuations and increase in arterial flow increase shear stress, release vasoactive substances, and may lead to flow mediated dilation.[13,33,34] The main stimulus for improving vascular health may be the fluctuations in blood flow, more than increase in mean flow velocity.[12,13,15,35] The difference between the treatment groups in change in myogenic function, found in this study, may contribute to further elucidate the mechanisms of effect of INP treatment, with the possible role of arterioles.

Within group analysis for 40 mm Hg INP treatment-groups at pretest, showed a relative increase in v_{mean} post INP-period when compared to pre INP-period. This was not present in the 10 mm Hg INP-treatment-group, and no significant difference was seen between the groups. Neither this was confirmed in the post-test, nor by analysis among groups difference. A relative increase in v_{mean} after a 3-min INP test-treatment could be explained as flow mediated dilation.[13] Patients with a change in PWD $\leq$0 meters after 12 weeks of INP-treatment showed no increase in mean flow velocity after a 3-min INP-period, Figure 7. It would be intriguing to pursuit the thought that patients who do not produce flow mediated dilation after 3 min with 40 mm Hg INP are more likely to not benefit from INP-treatment. Future, well-powered, studies could clarify if certain flow characteristics could be used to select those who do not respond to INP-treatment. The use of a provocation test with a well-known vascular response associated with the

Table IV. Absolute change in normalized amplitude for flowmotion-bandwidths at posttest (after 12 weeks of INP treatment), compared to pretest

	Absolute change in flowmotion from pre-test to post-test				
	10 mm Hg INP		40 mm Hg INP		
	Median (25th, 75th)	*P* within groups[a]	Median (25th, 75th)	*P* within groups[a]	*P* between groups[b]
INP-treatment (1/17 Hz)					
	−0.47 (−2.4, 1.3)	0.271	−0.6 (−3.7, 1.1)	0.171	0.690
Flowmotion					
Endothelial function (0.007–0.02 Hz)					
	−0.44 (−8.9, 1.7)	0.304	−0.18 (−6.2, 5.0)	0.624	0.569
Sympathetic function (0.02–0.06 Hz)					
	0.20 (−5.6, 10.4)	0.699	0.73 (−2.6, 3.0)	0.537	0.391
Myogenic function (0.06–0.2 Hz)					
	−0.99 (−4.0, 0.01)	0.003	0.85 (−1.3, 2.6)	0.167	0.002

INP, intermittent negative pressure.
[a](One-Sample) Wilcoxon Signed Rank test.
[b]Mann-Whitney *U*.



Improvement in PWD (m)	< 0	0–30	30–75	> 75	SUM
10 mmHg INP (n)	10	9	7*	3	29
40 mmHg INP (n)	4	9	8	10*	31
Total (n)	14	18	15	13	60

*missing samples n = 1 (total 2)

Improvement in MWD (m)	< 0	0–50	50–100	> 100	SUM
10 mmHg INP (n)	9	10	7*	3	29
40 mmHg INP (n)	10	9*	4	8	31
Total (n)	14	18	15	13	60

*missing samples n = 1 (total 2)

Fig. 6. Absolute change in normalized amplitude for flowmotion-bandwidths for improvement in pain-free (PWD) and maximal walking distance (MWD) at post-test (after 12 weeks of INP-treatment), compared to pre-test. Difference for the 10 and 40 mm Hg INP-treatment-groups, between the categories of improvement in PWD: $P = 0.234$ and 40 mm Hg INP: $P = 0.182$, respectively, and MWD: $P = 0.443$ and 0.551, respectively. Difference between treatment groups: $P = 0.002$.

degree of peripheral arterial disease, for example, post-occlusive reactive hyperemia ,[36–38] could increase the sensitivity.

Intermittent pneumatic compression (IPC) to the foot and calf, or thigh, in patients with PAD, has been demonstrated to increase walking distance and wound healing.[5,39–41] IPC cause a post-compression hyperemia, seen as an increase in arterial blood flow and velocity measured by laser Doppler or ultrasound Doppler devices.[39] The mechanism is suggested to be a combination of stimulation of vasoactive substances, increase

Table V. Correlation between flow characteristics for v_{mean} at pretest, and improvement in pain-free (PWD) and maximal walking distance (MWD) for the 40 mm Hg INP treatment group after 12 weeks of INP-treatment

Change in walking distance from pre- to post-test for	Blood flow velocity (v_{mean})			Fourier-transform			
	Pre INP-period	Relative increase INP-period	Relative increase (post INP-teriod)	Endothelial function (normalized)	Sympathetic function (normalized)	Myogenic function (normalized)	Amplitude INP (1/17 Hz)
PWD							
r_s	−0.118	−0.110	0.178	0.241	0.310	0.264	0.317
P	0.521	0.550	0.329	0.184	0.084	0.144	0.077
MWD							
r_s	−0.239	0.176	−0.134	0.176	0.171	0.235	0.007
P	0.187	0.335	0.465	0.335	0.350	0.196	0.970

r_s, spearman's rank correlation coefficient; *P*, significance level (2-tailed); INP, intermittent negative pressure; Hz, hertz, v_{mean}, mean blood flow velocity; $n = 32$ for all groups.

in the arterio-venous pressure gradient, reduction in venous-arterial reflex, and stimulation of arterial collateralization.[40,41] The mechanism of action of IPC is similar to those proposed for INP-treatment. The common feature being a manipulation of the venous reservoir, while INP may in addition perform an active suction on the capillary and arteriole vascular network.

In this study all blood flow velocity recordings were done with earlybird. Earlybird has a highly sensitive transducer and monitors blood flow in a large area, making it stable and easy-to-use. One of the greatest limitations with earlybird is that the insonation angle to the underlying vessel is not known. In this study, it is therefore not possible to evaluate changes over time in absolute flow values, only relative change can be assessed. Information of change in absolute flow velocities may be lost. The recordings made were of good quality, making them eligible for further analysis. In other studies, examining the effects of INP-treatment, pulsed-wave Doppler and laser Doppler flowmetry have been used.[12] We find similar results as in these studies indicating that earlybird is a valid method for assessing and monitoring blood flow velocity.

Pedal acceleration time (PAT) measured distal for eventual arterial stenosis has been used to determine the severity of stenosis,[42] and is associated with ankle-brachial-index, toe-brachial index and toe-pressure,[42,43] as well as the degree of critical limb-threatening ischemia.[44,45] Earlybird provides Doppler velocity-curves eligible for analysis of acceleration time. However, these analyses were not conducted in this study.

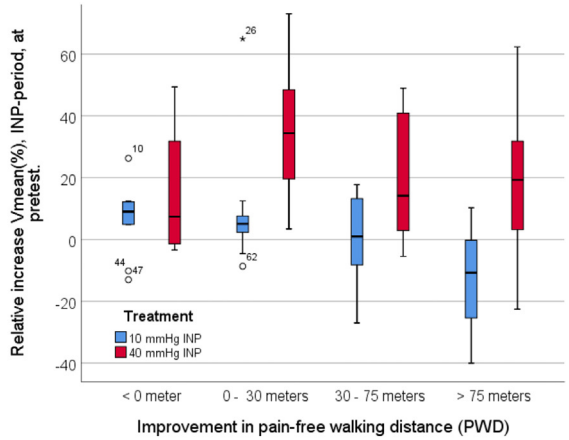
Limitations

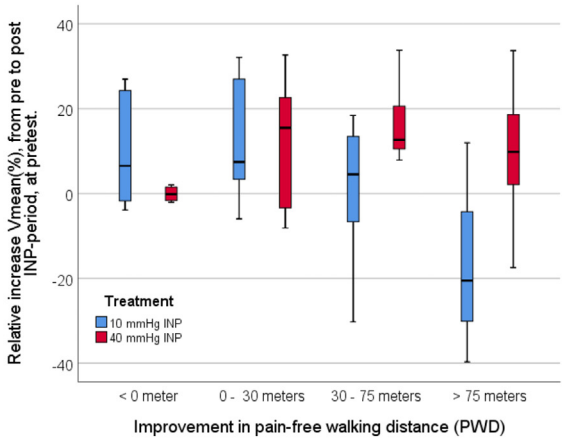
Flowmotion characteristics may vary between skin localizations and time.[26,46] The analysis of flowmotion, especially the low frequency specter of endothelial function (0.007−0.02 Hz), is complicated by the short recording-time in this study.[46] A 5-min recording only capture one to two cycles of endothelial flowmotion, while more cycles are measured for higher frequencies. Longer recordings are therefore needed to fully assess flowmotion as response to INP-treatment. This may be a potential explanation of the lack of association between flowmotion and walking distance.

Tibial and pedal arterial anatomy and degree of stenosis was not known for the patients. The degree of collateral circulation may influence the measured arterial velocity measurements[45] and may limit the ability to detect potential circulatory effects of INP-treatment. In our study a larger proportion of

A

Improvement in PWD (m)	< 0	0 – 30	30 – 75	> 75	SUM
10 mmHg INP (n)	10	9	8	3	30
40 mmHg INP (n)	4	9	8	11	32
Total (n)	14	18	16	14	62

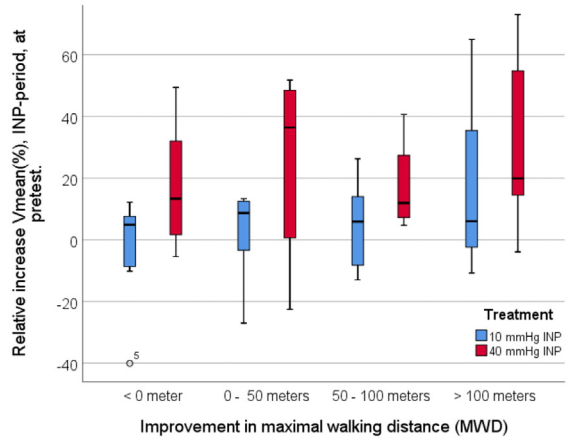


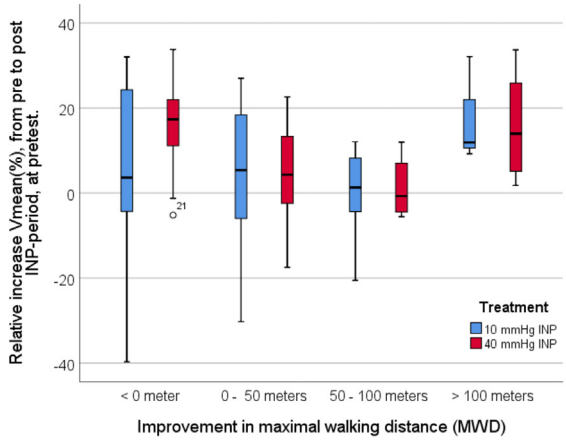


For improvement in PWD	10 mmhg	40 mmhg
relative increase in v_{mean} in INP-period	p = 0.467[a]	p = 0.362[a]
relative increase in v_{mean} from pre to post INP-period	p = 0.451[a]	p = 0.195[a]

B

Improvement in MWD (m)	< 0	0 – 50	50 – 100	> 100	SUM
10 mmHg INP (n)	9	10	8	3	30
40 mmHg INP (n)	10	10	4	8	32
Total (n)	19	20	12	11	62





For improvement in MWD	10 mmhg	40 mmhg
relative increase in v_{mean} in INP-period	p = 0.730[a]	p = 0.521[a]
relative increase in v_{mean} from pre to post INP-period	P = 0.376[a]	p = 0.059[a]

Fig. 7. (A) Relative increase (%) in mean velocity (v_{mean}) at INP-period, and **(B)** from pre to post INP-period, for categories in improved PWD and MWD. INP, intermittent negative pressure; PWD, pain-free walking distance; MWD, maximal walking distance; *n*, number. Kruskal-Wallis H.

patients with diabetes were randomized to the 40 mm Hg INP-treatment group. We recorded Doppler flow velocity curves eligible for further analyses in all patients. But the difference in proportion of patients with diabetes may influence the results, due to possible inhibited detection of blood flow Doppler signals due to heavily peripheral calcified vessels and mediasclerosis.

The main results from the randomized trial showed only a modest clinical effect regarding PWD, while no significant effect for MWD.[17] This study was conducted without implementing a validated physiological test which could elicit a vasomotor response but used a 3-min INP test-treatment. The moderate effect of INP treatment, small sample size, and the fact that only 32 of the 62 study participants was tested with 40 mm Hg INP test-treatment, due to the double-blinded nature of the study, may mask flow characteristics that could determine whom would benefit INP-treatment.

Lack of association between flow characteristics, walking distance and effect of INP treatment may be due to large variation in measurements and low sample size.

CONCLUSION

The novel ultrasound Doppler device, earlybird, is an applicable tool for assessing blood flow velocity in patients during treatment with INP in patients with intermittent claudication. Analysis of the acquired flow velocity recordings shows that arterial blood flow velocities immediate increase during INP-treatment. There is a difference between the groups in favor of 40 mm Hg INP, in vasomotor activity corresponding to an increase in myogenic function. This may indicate an involvement of vascular smooth muscle cells of the arterioles and may contribute to the understanding of the mechanism of action of INP treatment. In this study, we did not find specific flow characteristics that are able to determine who would benefit INP-treatment. Additional well-designed studies examining the effect and selection criteria of INP-treatment are needed to fully understand the underlying mechanism of action and to reliably propose clinical decision criteria for indications for INP-treatment.

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SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.avsg.2022.04.025.

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