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Increased leg blood flow and improved femoral artery shear patterns in metabolic syndrome after a diet and exercise program

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Abstract

Background: Altered vascular shear profiles may contribute to the development of atherosclerosis. Physical activity promotes anti-atherogenic shear patterns, resulting in reduced cardiovascular disease risk. Adults with metabolic syndrome (MetSyn) are at increased risk of developing atherosclerosis and cardiovascular disease. Thus, we hypothesized conduit artery antegrade shear rate (ASR) would increase and retrograde shear rate (RSR) and oscillatory shear indices (OSI) would decrease in MetSyn patients (n=16, 51±2 years) after participation in a diet and exercise program (DEP).

Methods: Blood flow (Doppler ultrasound, brachial and femoral arteries) was measured and shear rates were calculated in MetSyn patients before and after 12-weeks of DEP participation. In addition, plasma samples collected to measure atherogenic markers.

Results: DEP participation increased resting leg blood flow and femoral artery ASR (p = 0.05), and tended to decrease OSI (p=0.09); RSR did not change (p>0.05). No changes in resting arm blood flow or ASR were observed (p>0.05) and both RSR and OSI increased after participation (p = 0.05). DEP participation reduced plasma VCAM-1 (p=0.03), with a trend for reduced ICAM-1 (p=0.09) (i.e. atherogenic markers).

Conclusion: Modest changes in diet and physical activity result in limb-specific improvements in vascular shear profiles and reduced systemic markers of atherosclerotic risk in MetSyn patients. These data provide novel physiologic insight into adaptations that may limit the progression of atherosclerosis in patients with MetSyn.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

Keywords

pre-diabetes; atherosclerosis; diabetes prevention; forearm; obesity

INTRODUCTION

Nearly one-third of the United States population has metabolic syndrome (MetSyn). Adults with MetSyn are at increased risk of developing cardiovascular disease, atherosclerosis, and type 2 diabetes (1, 2). To address this risk, individuals with MetSyn are encouraged to participate in regular physical activity (1, 3). Diabetes prevention programs have been successful in introducing lifestyle changes to patients with MetSyn including increases in activity and diet modification (4, 5). Participation in such programs can improve body composition, lipid profile, blood pressure, and have been shown to reduce the incidence of type 2 diabetes by nearly 60% (4, 5). Curiously, improvements in traditional risk factors (i.e. blood lipids, glucose, blood pressure) can account for only 50-60% of reduced cardiovascular disease risk (Reviewed in (6, 7)). Further physiologic testing is warranted to determine whether a portion of the remaining risk reduction can be attributed to vascular adaptations in response to lifestyle changes. Taken together, it is reasonable to speculate participation in a diabetes prevention program may lead to improvements in blood flow and vascular shear profiles.

During an acute bout of exercise, healthy adults exhibit increased antegrade (ASR) and reduced retrograde (RSR) and oscillatory shear rates (OSI)(8). Both *in vitro* and *in vivo* research suggest such flow patterns may favor anti-atherogenic endothelial cell phenotypes (9-11). Thus, acute changes in blood flow and shear stress during exercise may evoke vascular adaptations which aid in limiting atherosclerosis and cardiovascular disease development. However the clinical and/or translational applications of acute exercise studies are limited given their focus on a short-term assessment of primarily younger, healthy humans (8, 12-14). Whether long-term increases in physical activity result in chronic positive changes in resting vascular shear patterns (i.e. increased ASR or decreased RSR and OSI) in a clinical population was previously unexamined. It is reasonable to propose improved shear profiles may slow and/or reverse the atherosclerotic processes present in diseased patients, resulting in meaningful long-term improvements in cardiovascular disease risk not otherwise explained by changes in traditional clinical measures.

Taken together, improvements in resting skeletal muscle blood flow and conduit artery shear patterns may play an important role in altered cardiovascular disease risk after participation in a diet and exercise program. Surprisingly, very little is known about potential alterations in limb hemodynamics and vascular shear profiles after modest lifestyle changes in adults with MetSyn. The aim of the current study was to test the novel idea that lifestyle improvements can have a significant impact on resting skeletal muscle perfusion and hemodynamic shear profiles in MetSyn. We tested the hypothesis that skeletal muscle blood flow and conduit artery ASR would increase in both the arm and the leg after 12-weeks of participation in a DEP in patients with MetSyn. In addition, we hypothesized DEP participation would result in decreased RSR and oscillatory shear indices (OSI).

METHODS

Subjects

Adults with clinically diagnosed MetSyn (National Cholesterol Education Program Adult Treatment Panel III criteria as modified by the American Diabetes Association) participated in the present study (n=16, 41-61 years old). All subjects were recruited from an ongoing DEP organized and conducted by Preventive Cardiology at the University of Wisconsin School of Medicine and Public Health (5). Written, informed consent was obtained from all subjects. All procedures followed were in accordance with ethical standards, were approved by the Institutional Review Board at the University of Wisconsin – Madison, and conformed to the standards set by the Declaration of Helsinki.

Diet and Exercise Program (DEP)

The DEP was developed after a well-established diabetes prevention program(4) and specifics of the entire program were published previously (5). Briefly, subjects completed 1 hour of education and 1 hour of supervised physical activity each week for a total of 12 weeks. Sessions were supervised by a registered dietician and exercise physiologist. Weekly physical activity and dietary logs were completed, reviewed by DEP personnel, and returned with feedback. Subjects signed a commitment agreement to work toward a 7% weight loss goal, 150 minutes of moderate intensity physical activity per week, and healthy eating habits (5). Subjects were required to be compliant with the program—defined as missing no more than two weekly sessions (83% compliance) (5).

Measurements

Weight, height, and waist circumference measurements were performed, and body mass index (BMI) was calculated ($\text{kg}\cdot\text{m}^{-2}$). Venous whole blood was collected and fasting levels of HDL cholesterol, triglycerides and glucose were measured immediately. Additional plasma samples were collected, frozen at -80°C , and analyzed at a later time for measures of insulin (Millipore; Billerica, MA, USA), vascular cell adhesion molecule (VCAM)-1, intercellular cell adhesion molecule (ICAM)-1, endothelial (E)-selectin, and platelet (P)-selectin (R&D Systems; Minneapolis, MN, USA). Physical activity was assessed using the Paffenbarger physical activity questionnaire (PAQ, (15)) and medical history was collected via self-report. All measurements were performed at each study visit.

Baseline blood pressure was measured in the supine position by electronic sphygmomanometer with the cuff on the upper arm of the dominant limb (Datex-Ohmeda; Helsinki, Finland). Heart rate was monitored by electrocardiography (Datex-Ohmeda; Helsinki, Finland). Blood flow (artery diameter, blood velocity) was measured using Doppler ultrasound (Vivid 7, General Electric; Milwaukee, WI, USA). A 12-MHz linear array probe was placed at an insonation angle of 60° with the sample volume adjusted to cover the width of the artery (16, 17). A commercial interface unit (Multigon Industries; Yonkers, NY) processed the angle-corrected, intensity-weighted Doppler audio information from the GE Vivid ultrasound system as two separate flow velocity signals (antegrade and retrograde velocities). Velocities were sampled in real time with signal-processing software (PowerLab, ADInstruments; Colorado Springs, CO). All hemodynamic data were digitized,

stored on a computer at 400 Hz, and analyzed off-line using PowerLab's Chart application package.

For non-dominant arm measurements, the ultrasound probe was placed approximately midway between the antecubital and axillary regions, medial to the *biceps brachii* muscle. For leg measurements, the probe was placed below the inguinal ligament over the right common femoral artery, at least 2 cm upstream from its bifurcation into superficial and profunda branches (16). Due to the unlikelihood for training effects in the leg of recreationally active subjects (18), we chose to study blood flow in the right leg for all subjects. Repeatability of the arterial measurement site was controlled by measuring the distance from the antecubital fossa (arm) and femoral bifurcation (leg) to the ultrasound probe at each visit.

Study Protocol

As part of the current project, subjects completed two research study visits: 1) At the introduction of the DEP (Weeks 1-2 of participation), 2) At the end of the DEP (Weeks 12-13 of participation). Subjects were instructed to refrain from exercise, alcohol, and caffeine for 12-hours prior to the study visit. Visits were completed at the same time of day within subjects. Prescription medications were not withheld and subjects were instructed to continue with their usual medication regimen.

Resting arm blood flow measurements required subjects to lay supine with non-dominant arm extended to the side ($\sim 90^\circ$) at heart level. Subjects laid quietly for 15 minutes before collection of blood flow measures. Beat-to-beat brachial artery blood velocity measurements were obtained continuously for 2 minutes during quiet rest, and diameter measurements were taken at the conclusion of 2 minutes.

Resting leg blood flow measurements required subjects to sit in a semi-supine position ($\sim 20^\circ$ incline from supine). Subjects rested quietly in a specialized chair with the upper leg parallel to the floor and the lower leg hanging perpendicular to the floor for 15 minutes before collection of blood flow measures. Given over two-thirds of the day is often spent in an upright position (19, 20), this posture allowed us to examine leg flow and pressure environments commonly occurring throughout the day (21). Beat-to-beat femoral artery blood velocity measurements were obtained continuously for 2 minutes, and diameter measurements were taken at the conclusion of 2 minutes.

Data Acquisition and Analysis

Artery diameters were obtained from B-mode video images and are reported as the median of five measurements taken in late diastole. Arterial diameter was measured in the part of the artery running perpendicular to the ultrasound beam, identified by strong wall signals in a longitudinal section of the artery (measuring the distance between near and far wall intima-media interface). All measurements were made off-line by a trained operator. Similar procedures have been used previously (16, 17).

Blood flow was analyzed from the last 30 seconds of quiet rest and was determined as the product of mean blood velocity ($\text{cm}\cdot\text{s}^{-1}$) and vessel cross-sectional area (radius in cm^2),

multiplied by 60 to convert from mL.s^{-1} to mL.min^{-1} (16, 17). Clinically significant changes in blood pressure are commonly observed with participation in a DEP (5, 22). To assess vascular tone in the face of changing blood pressure, blood flow measures were normalized for perfusion pressure (measured during the last 30 seconds of quiet rest) and are reported as vascular conductance ($\text{Blood flow} \div \text{Mean Arterial Blood Pressure}$; $\text{mL.min}^{-1}.100\text{mmHg}^{-1}$).

Both RSR and ASR (s^{-1}) were calculated using the following equation where V is blood velocity (cm.s^{-1}) and D is conduit artery diameter (cm): $[(4 \times V) \div D]$ (8, 23). OSI was calculated as: $[|\text{Retrograde shear}| \div (|\text{Antegrade shear}| + |\text{Retrograde shear}|)]$ (8, 23); a value of zero for OSI corresponds to a unidirectional shear and a value of 0.5 represents pure oscillation with a time-average shear equal to zero. Relative changes (%) in primary outcome variables were calculated as: $[(\text{Visit 2}-\text{Visit 1}) \div (\text{Visit 1})] \times 100\%$. This analysis allowed us to examine vascular adaptations independent of individual differences in body size, baseline blood flow, and lean mass and is thus the most appropriate index of change. In further support of reporting relative changes, baseline measurements (Visit 1) of primary outcome variables were well-correlated with final measurements (Visit 2) (R-value Range: 0.56-0.66).

All data are presented as mean $\pm$ standard error and significance was determined a priori at $p = 0.05$ with trends discussed at $p = 0.10$. Subject demographics, blood flow, and shear rates were compared between visits using paired, one-tail t-tests. In addition, one-sample, one-tail t-tests were used to assess relative changes between visits (with μ equal to zero). Statistical analysis was completed using MiniTab Version 15 (Minitab Inc., State College, PA, USA).

RESULTS

Subject Characteristics

We studied 15 females and 1 male ranging from 41-61 years of age (mean 51 ± 2 years) participating in the DEP at the University of Wisconsin - Madison. Female subjects were postmenopausal ($n=5$), sterile (hysterectomy, $n=3$), taking birth control ($n=4$), or naturally cycling ($n=3$). Five participants had a self-reported family history of cardiovascular disease and none reported having diagnosed coronary artery disease. Even when medicated, subjects were obese, hyperglycemic, and hypertensive (Table 2). The majority of subjects were taking prescription medication at the introduction of the DEP (Table 1). Under the supervision of their physician, some prescriptions changed over the 12-week period. Specifically, 1 subject stopped taking a statin, 1 was prescribed a β -blocker and 1 stopped taking hormonal birth control (Table 1).

Efficacy of DEP

Changes in subject demographics can be found in Table 2. After 12-weeks of participation in the DEP, there was a relative increase in physical activity (change in PAQ $238 \pm 123\%$, $p=0.04$). Participation in the DEP also resulted in a significant reduction in weight, BMI, waist circumference, heart rate, and mean arterial blood pressure ($p<0.05$). Supine systolic blood pressures decreased an average of 5 mmHg ($p=0.01$) to a level no longer considered

clinical hypertension. There was a trend for lower glucose levels ($p=0.06$) and lower diastolic blood pressure ($p=0.06$) after participation in the DEP.

Impact of DEP on Leg Hemodynamics

Figure 1A and Table 3 summarize the impact of 12-weeks of participation in the DEP on leg hemodynamics in 15 subjects (1 subject did not complete leg trials for both visits). Participation resulted in a relative increase in leg blood flow ($38\pm22\%$, $p=0.05$), vascular conductance ($39\pm19\%$, $p=0.03$), and ASR ($12\pm6\%$, $p=0.04$). There was a trend for decreased OSI ($p=0.09$), but no changes were seen in RSR ($p=0.30$) after participation in the DEP.

Impact of DEP on Arm Hemodynamics

Figure 1B and Table 3 summarize the impact of 12-weeks of participation in the DEP on arm hemodynamics in 15 subjects (data from 1 subject was excluded due to an error in data acquisition). Participation did not alter arm blood flow, conductance, or ASR ($p>0.05$). Contrary to our hypothesis, participation in the DEP resulted in a significant relative increase in RSR ($32\pm15\%$, $p=0.02$) and OSI ($52\pm30\%$, $p=0.05$).

Impact of DEP on markers of inflammation and atherosclerosis

To test systemic effects of DEP participation, we measured circulating levels of adhesion molecules in a subset of participants ($n=12$ due to difficulty obtaining a large volume of plasma from each participant). E-selectin and P-selectin did not change with DEP participation ($p=0.13$ and $p=0.45$, respectively). In contrast, there was a significant relative decrease in VCAM-1 levels ($p=0.03$) and a trend for reduced ICAM-1 ($p=0.09$) with participation in the DEP (Table 2, Figure 1C).

DISCUSSION

The present study aimed to examine the potential for vascular improvements after DEP participation in MetSyn patients. We hypothesized 12 weeks of DEP participation would increase resting blood flow and ASR, and decrease RSR and OSI in both arm and leg circulations. This study produced several novel findings. After 12-weeks participation in the DEP, adults with MetSyn exhibited: 1) increased resting leg blood flow, 2) increased ASR in the femoral artery, 3) maintained arm blood flow, 4) increased RSR in the brachial artery, 5) reduced systemic VCAM-1. Despite only small changes in traditional clinical markers of cardiovascular disease risk (Table 2), these data are the first to indicate DEP participation can improve femoral artery blood flow, shear profiles, and atherosclerotic markers. These chronic adaptations may counteract proatherogenic signals present in MetSyn and may be a key physiologic component behind reduced cardiovascular disease risk.

Leg Hemodynamics

Results from the current study show 12-weeks of DEP participation increases blood flow and ASR in the leg of MetSyn patients. Changes in leg hemodynamics were assessed with the subject in the upright position. Given humans spend over two-thirds of the day in an upright position (19, 20), this posture allowed us to examine leg flow and pressure environments commonly occurring throughout the day (21). Potential clinical implications

for improved blood flow include improved glucose disposal and blood pressure regulation (24). We can only speculate on the mechanisms involved, however muscle sympathetic nerve activity (MSNA) has been shown to be reduced after DEP participation (22). High levels of MSNA—common in adults with MS—can lead to reduced blood flow and increased conduit artery OSI (25). Thus a reduction in MSNA may explain the observed increases in leg blood flow and improved conduit artery shear profiles (Figure 1A, Table 3), in addition to reductions in blood pressure (Table 1) after DEP participation. This theory is partially supported in the current study in the form of significantly lower heart rates after 12-weeks (Table 1).

The chronic vascular adaptations presently observed in the leg circulation may be crucial to slowing or preventing thrombus formation and atherosclerosis in this susceptible clinical population. Specifically, increases in unidirectional high shear stress have been shown to induce expression of anti-atherogenic proteins (eNOS, SOD) and inhibit expression of atherogenic factors (VCAM-1, ICAM-1, E-selectin, MCP-1) (9-11, 26). A significant reduction in plasma VCAM-1 and a trend for reduced ICAM-1 levels in the current study support this concept (Table 2). Taken together, positive changes in ASR and OSI in the leg combined with systemic reductions in pro-atherogenic mediators may contribute to reduced atherosclerotic risk after 12-weeks of DEP participation.

Arm Hemodynamics

In contrast to femoral artery adaptations, arm blood flow and ASR were maintained after participation in the DEP. This finding was contrary to our hypothesis, however is consistent with the concept that lower limb conduit artery function is not predictive of upper limb function (27, 28). Such discrepancies may be due, in part, to limb-specific pressures and shear stress and/or differences in posture (supine vs upright) during testing (21). Additionally, taking into consideration atherosclerosis and peripheral artery disease are more prevalent in the leg circulation (29, 30), significant changes in shear patterns may not play as important of a role in the arm circulation.

Despite the lack of significant changes in arm blood flow and ASR after DEP participation, changes in RSR and OSI may actually suggest deleterious adaptations; this was an unexpected finding. One explanation for the current findings may be impaired cutaneous vasodilation during exercise in the non-exercising limb (the majority of exercise in this patient group was lower limb-specific, with primarily treadmill walking and stationary cycling). Cutaneous vasodilation contributes to reductions in retrograde shear profiles during steady-state exercise (8). If cutaneous vasodilation does not occur, this may lead to greater brachial artery retrograde shear during prolonged exercise and potential chronic adaptations that can be observed at rest. Consistent with this concept, adults with type 2 diabetes demonstrate reduced capacity to increase skin blood flow during thermal stress (31). This is an interesting area of research that has not yet been explored in human MetSyn.

Experimental Considerations

Each subject served as his/her own control and a healthy control group and/or cross-sectional design was not employed. This limitation is minor given previous research using

control groups has shown blood flow and endothelial function do not change over time in similar populations [4 weeks, (32); 12 weeks, (33)]. In addition, given the heterogeneous nature of a clinical population, a healthy control group would provide limited insight. For example, female subjects were sterile, taking birth control, or naturally cycling. Female hormone levels have been shown to alter the control of blood flow, blood pressure, and arterial compliance; thus by each female subject serving as her own control, the effect of such factors was limited. Altogether, the lack of a control group does not diminish the clinical and physiological significance of current novel findings.

Because this research study was combined with an ongoing clinical intervention, we did not have access to full medical records and are unaware of the length of time each subject was taking medication prior to participation. Whereas potentially interesting, this is a minor limitation given the study was designed to examine the impact of diet and exercise on vascular shear profiles, rather than interactions between long-term pharmacological treatment and diet and exercise. Furthermore, patients with clinically diagnosed MetSyn are encouraged to participate in lifestyle interventions while taking medications; thus, this study design has high clinical relevance.

Results from the current study cannot identify whether significant changes in vascular shear profiles after 12-weeks of DEP participation are primarily driven by changes in physical activity and/or weight loss. However, increased physical activity—independent of weight loss—is known to improve vascular function (34). In addition, results from the current study support limb-specific differences in vascular adaptations predictive of an exercise-mediated response. Despite this support, future research will be necessary to examine the independent effect of physical activity and/or dietary modification on vascular shear profiles, in addition to differential effects of resistance versus aerobic exercise training. Furthermore, responses are likely time-dependent such that a program of longer duration may result in further improvements in leg hemodynamics and a possible reversal of changes in arm hemodynamics. Importantly, present novel findings support chronic improvements in shear patterns after participation in a DEP, which may be a key physiologic component behind reduced atherogenic markers and cardiovascular disease risk that may otherwise be unaccounted for by traditional clinical markers.

Conclusion

Increases in physical activity and dietary modification are known to lower cardiovascular disease risk. After 12-weeks of DEP participation, we observed minor reductions in traditional cardiovascular disease risk factors including waist circumference and blood pressure which is consistent with the literature, however these changes only partially explain improvements in cardiovascular disease risk. We hypothesized additional risk-reduction may be the result of improved shear profiles. Novel findings from the current study indicate 12-weeks of DEP participation result in increased leg blood flow and femoral artery shear profiles, in addition to reductions in systemic markers of atherosclerosis. These results add quantitative physiologic support for the emerging concept that even *modest* lifestyle changes can protect against cardiovascular disease. In addition, current data clearly demonstrate adults with MS maintain the capacity for limb-specific vascular adaptations in response to a

diet and exercise program. These data provide novel physiologic insight into vascular adaptations that may contribute to improvements in cardiovascular disease risk in patients with MetSyn.

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ABBREVIATIONS AND ACRONYMS

ABF	Arm blood flow
ASR	Antegrade shear rate
BMI	Body mass index
DEP	Diet and exercise program
E-sel	Endothelial-selectin
FMD	Flow-mediated dilation
ICAM-1	Intercellular cell adhesion molecule-1
LBF	Leg blood flow
MS	Metabolic syndrome
MSNA	Muscle sympathetic nerve activity
OSI	Oscillatory shear index
P-sel	Platelet-selectin
PAQ	Physical activity questionnaire
RSR	Retrograde shear rate
VCAM-1	Vascular cell adhesion molecule-1

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Figure 1. Relative Changes in Limb Hemodynamics and Systemic Markers with DEP Participation

Data are presented as Mean $\pm$ SE, n=15. LBF = Leg Blood Flow, ABF = Arm Blood Flow, ASR = Antegrade Shear Rate, RSR = Retrograde Shear Rate, OSI = Oscillatory Shear Index.

A) Leg: A relative increase in resting leg blood flow and ASR were observed, whereas OSI tended to be lower after 12 weeks.

B) Arm: A relative increase in RSR and OSI were observed in the arm after 12 weeks.

C) Adhesion Molecules: DEP participation did not change E-selectin (E-Sel) or P-selectin (P-Sel) plasma concentrations, but decreased VCAM-1 levels, with a trend for reduced ICAM-1.

†p 0.05 vs No change from Visit 1, # p=0.09 vs No change from Visit 1.

Table 1

Medications

	Visit 1	Visit 2
Statin	10	9
Aspirin	8	8
Beta Blocker	4	5
ACE Inhibitor	7	7
Hormone	4	3
Vitamin	13	13

Table 2**Demographics**

	Visit 1	Visit 2	Relative Change (%)
Sex (M/F)	1/15	1/15	
Weight (kg)	106±5	102±5 [†]	-3±1 [*]
BMI (kg.m ⁻²)	37±2	36±1 [†]	-2±1 [*]
Waist (cm)	114±4	110±3 [†]	-3±1 [*]
Glucose (mg.dL ⁻¹)	120±9	106±5	-7±5
Insulin (uU.mL ⁻¹)	30±8	24±4	-8±6
HDL cholesterol (mg.dL ⁻¹)	48±3	48±4	+1±5
Triglycerides (mg.dL ⁻¹)	134±13	129±12	+7±13
Systolic BP (mmHg)	133±3	128±2 [†]	-4±2 [*]
Diastolic BP (mmHg)	80±2	74±1	-7±2
Mean BP (mmHg)	96±2	92±2 [†]	-4±2 [*]
Heart Rate (beat.min ⁻¹)	70±2	64±2 [†]	-8±2 [*]
Physical Activity (kcal.wk ⁻¹)	942±192	1897±591	+238±123 [*]
VCAM-1 (ng.mL ⁻¹)	557±49	494±33 [†]	-8±4 [*]
ICAM-1 (ng.mL ⁻¹)	135±10	127±10	-8±7
E-selectin (ng.mL ⁻¹)	47±6	42±6	-5±4
P-selectin (ng.mL ⁻¹)	31±2	32±3	-1±4

Mean±SE, n=16 unless otherwise specified (VCAM-1, ICAM-1, E-selectin, P-selectin n=12).

BMI = Body Mass Index, BP = Blood Pressure.

[†] p<0.05 vs Visit 1

^{*} p<0.05 vs No change from Visit 1.

Table 3**Limb Hemodynamics**

Leg Hemodynamics			
	Visit 1	Visit 2	Relative Change (%)
Femoral Artery Diameter (cm)	0.91±0.03	0.90±0.03	-1±1
Antegrade SR (s ⁻¹)	82±5	89±5	+12±6*
Retrograde SR (s ⁻¹)	44±3	44±3	+3±5
Mean SR (s ⁻¹)	39±5	45±4	+39±20*
Oscillatory SI (s ⁻¹)	0.35±0.02	0.33±0.01	-6±4
Blood Flow (mL.min ⁻¹)	367±61	416±51	+38±22*
Conductance (mL.min ⁻¹ .100 mmHg ⁻¹)	355±56	483±71	+39±19*
Arm Hemodynamics			
	Visit 1	Visit 2	Relative Change (%)
Brachial Artery Diameter (cm)	0.38±0.01	0.39±0.01	+2±2
Antegrade SR (s ⁻¹)	210±24	184±11	+1±9
Retrograde SR (s ⁻¹)	45±3	55±4 [†]	+32±15*
Mean SR (s ⁻¹)	165±26	129±12	-4±11
Oscillatory SI (s ⁻¹)	0.20±0.02	0.23±0.02	+52±30*
Blood Flow (mL.min ⁻¹)	104±16	88±8	+8±14
Conductance (mL.min ⁻¹ .100 mmHg ⁻¹)	109±18	94±8	+10±14

Mean±SE, n=15. SR = Shear Rate, SI = Shear Index.

[†] p<0.05 vs Visit 1,

* p<0.05 vs No change from Visit 1.