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VASCULAR OCCLUSION AFFECTS GAIT VARIABILITY PATTERNS OF HEALTHY YOUNGER AND OLDER INDIVIDUALS.

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ABSTRACT

Insufficient blood flow is one possible mechanism contributing to altered gait patterns in lower extremity peripheral arterial disease (PAD). Previously, our laboratory found that induced occlusion alters gait variability patterns in healthy young individuals. However the effect of age was not explored. The purpose of this study was to account for age by investigating gait variability following induced vascular occlusion in healthy older individuals and to identify amount of change from baseline to post vascular occlusion between younger and older individuals. Thirty healthy younger individuals and 30 healthy older individuals walked on a treadmill during baseline and post vascular occlusion conditions while lower extremity joint kinematics were captured. Vascular occlusion was induced by thigh cuffs inflated bilaterally on the upper thighs. Amount and temporal structure of gait variability was assessed. Older individuals exhibited significantly increased values of temporal structure of variability post vascular occlusion. Post vascular occlusion values were similar between younger and older individuals after adjusting for baseline measurements. Results show blood flow contributes to altered gait variability. However alterations were less severe than previously documented in symptomatic PAD patients, suggesting that neuromuscular problems in the lower extremities of PAD patients also contribute to gait alterations in these patients.

Key Terms: Gait patterns, nonlinear analysis, nonlinear dynamics, vascular disease, atherosclerosis

INTRODUCTION

Lower extremity peripheral arterial disease (PAD) is a localized manifestation of systemic atherosclerosis affecting the leg arteries resulting in significantly reduced blood to the lower extremities. The gait abnormality caused by PAD, or intermittent claudication, is the end result of arterial insufficiency. PAD limits available blood flow and renders nerves and muscles ischemic, eventually leading to metabolic changes such as mitochondrial dysfunction, nerve damage and muscle structure damage [1, 2], and resulting in a baseline handicap that appears unrelated to claudication pain [3, 4]. PAD affects eight to twelve million people in the US with the majority of these being elderly [5]. PAD has been shown to lead to poor health outcomes, immobility and physical dependence [6, 7]. Gardner and Montgomery [8] have shown that PAD patients have a higher prevalence for falls and an increased risk of falling [9]. Importantly, because of the high occurrence of PAD among the elderly, PAD may be associated with the higher rates of falls in elderly individuals.

Gait variability is defined as the inherent natural fluctuations that occur during continuous gait cycles. Increased gait variability has been associated with unsteadiness during walking and increased risk of falling in elderly individuals [10]. An explanation for these changes in gait variability is the hypothesis of an optimal state of movement variability. This idea proposes that there is a specific "state" of variability which allows healthy individuals to make necessary adjustments to environmental changes during movement. Environmental changes could include differences in walking surfaces, such as an icy sidewalk or uneven terrain. However, changes that are below or above this "optimal" state of variability are generally associated with disease and could be related with several physiological factors [11]. Previous work in our laboratory determined that symptomatic PAD patients have increased amount of gait variability as well as

altered temporal structure of gait variability when compared to healthy matched controls [4, 12]. That study utilized standard deviation and coefficient of variation to assess amount of variability and the largest Lyapunov exponent to measure structure of variability. Considering that the optimal state of variability is represented by the performance of healthy young individuals, and healthy older individuals demonstrate increased levels of variability compared with the young [13], PAD patients have variability levels even further above the optimal state. Therefore, gait variability alterations in PAD patients are consistent with significant deterioration in the locomotor system of PAD patients, as noted by movement away from an optimal state of variability. However, the specific mechanisms leading to this deterioration and its subsequent mobility problems remain unclear.

Insufficient blood flow to the lower extremities is one potential mechanism resulting in functional impairment of PAD patients. A previous study in our laboratory isolated the impact of reduced blood flow in healthy young individuals by examining gait variability alterations post vascular occlusion [14]. Vascular occlusion was induced by thigh cuffs and tourniquet bands placed bilaterally on the upper thighs and inflated to 200 mmHg for three minutes while subjects were standing. After three minutes, the thigh cuffs were removed, the subjects began walking, and the post vascular occlusion condition was recorded. Findings indicated that vascular occlusion alters gait variability patterns in healthy young individuals, but these alterations did not account for the whole of the alterations demonstrated by PAD patients. In particular, healthy young increased from baseline to post occlusion by 21% for the largest Lyapunov exponent, 26% for the standard deviation, and 22% for the coefficient of variation compared to increases of 48% for the largest Lyapunov exponent, 62% for the standard deviation, and 99% for the coefficient of variation in patients with PAD [14]. However, since gait variability changes in elderly

individuals have been well documented [13, 15-17] and PAD occurs more commonly in elderly individuals [5], age is potentially a confounder that was not accounted for in our previous study.

Therefore, the purpose of this study was to investigate gait variability changes post vascular occlusion in healthy older individuals. Additionally, the study sought to determine the effect of age by comparing gait variability during the post vascular occlusion condition between healthy younger individuals and healthy older individuals. We hypothesized that an induced vascular occlusion in the absence of underlying neuromuscular or systemic dysfunction would result in significant gait variability alterations in comparison to baseline gait. Due to the fact that age and vascular occlusion have independently demonstrated increased values of gait variability, we also hypothesized that healthy older individuals would have significantly increased values of gait variability post vascular occlusion compared with the healthy younger individuals.

MATERIALS AND METHODS

Participants

Thirty healthy younger participants (Age: 22.8 ± 4.16 years, Mass: 75.8 ± 13.4 kg, Height: 175.3 ± 8.7 cm, Gender: 26 males, 4 females) and 30 healthy older participants (Age: 60.1 ± 8.03 years, Mass: 86.6 ± 16.1 kg, Height: 176.7 ± 8.3 cm, Gender: 25 males, 5 females) were recruited to participate in the study. The gender composition of both groups and the age composition of the healthy older group recruited was carefully considered in order to reflect the composition of the PAD patients in our previous studies [3, 4, 18]. Informed consent was obtained from all participants before data collection. The study was approved by the Institutional Review Board for human subjects. Subjects were free from any significant health co-morbidities, including arthritis, history of lower extremity joint surgery, history of back or lower extremity injury or surgery that affects the subject's mobility or any other process limiting the ability to

walk, including neurological disease or impairment (stroke, Parkinson's disease, multiple sclerosis). Additionally, all subjects had normal ankle brachial index values (>1.0), and no subjective or objective ambulatory dysfunction. Ankle-brachial index is a ratio of the systolic pressures taken at the brachial artery in the arm and at the dorsal pedis and posterial tibialis arteries at the ankle. Having an ankle brachial index value less than 0.9 is part of the diagnosis for PAD. All subjects reported to our biomechanics laboratory for gait testing.

Experimental procedure and data collection

Upon arrival to the laboratory, lower extremity blood flow was measured by taking the systolic pressures at the brachial artery in the arm and the dorsal pedis and posterior tibial arteries at the ankle to confirm acceptable ankle brachial index values. Next, subjects' height, weight and anthropometric measures were taken. Before data collection reflective markers were placed on the lower extremities based of the system used by Vaughan et al [4, 19] .. Participants wore a tightly fitting running suit to allow markers to be placed as close to the anatomic position as possible. Specifically, markers were placed bilaterally at the following anatomical locations: anterior superior iliac spine, posterior superior iliac spine, greater trochanter, mid thigh, lower front thigh, lateral knee joint center, medial knee joint center, mid shank, lower shank, lateral and medial malleolus, lateral side of head of fifth metatarsal, medial side of head of first metatarsal, toe, and lateral calcaneus. In addition, one marker was placed on the interface of the fifth lumbar vertebrae and sacrum, but this marker was not collinear with the posterior superior iliac spine markers. After the markers were placed, participants were given ample time to get accustomed to walking on the treadmill (BodyGuard Fitness, St-Georges, QC (Canada)), during which time they were asked to select a comfortable walking speed. This speed was identified as the self-selected walking speed and was used for all subsequent testing. Self-selected speed is the most

desirable speed to evaluate gait variability because asking subjects to walk a predetermined speed that is not comfortable may lead to alterations in variability, rather than the optimal level of variability that occurs with the self-selected pace [20-25].

Three-dimensional kinematics were acquired at 60 Hz using EVART software (Motion Analysis Corp, Santa Rosa, Calif) while participants walked on the treadmill. First the participants walked at their self-selected speed for three minutes. This was the baseline condition. Next, vascular occlusion was induced by placing thigh cuffs (Omron® Exactus Aneroid Sphygmomanometer Model 108MLNL, Kyoto, Japan) bilaterally on the upper thighs and occlusion tourniquets (CyberTech™ Mechanical Advantage Tourniquet MAT01, Maharashtra, India) just above the knee while subjects stood on the treadmill. The cuffs were inflated to 200 mmHg and maintained for three minutes. The chosen level of pressure and time of occlusion are standard ranges used in the literature to induce ischemia in the legs [26-28]. After three minutes of occlusion, the thigh cuffs were removed and the subjects immediately began walking on the treadmill. Three minutes of treadmill walking was recorded post vascular occlusion.

Data analysis

Coordinate trajectories of each marker were exported and processed in custom software using MATLAB software (MathWorks Inc, Natick, Mass). This software was used to calculate relative joint angle time series from the kinematic data for the ankle, knee and hip for all trials. Joint kinematic variability was examined, because it has been shown that variability of stride characteristics (i.e. stride time, step time) offers a less sensitive measure of differences between groups than does variability of the joint kinematics [29]. All trials were cropped to 3300 data points, which is long enough to allow 30 continuous footfalls which is considered adequate for

nonlinear analysis [30]. It was determined that 3300 data points was long enough to ensure 30 continuous footfalls for 30 strides by graphing the knee joint angle for all collected trials and counting the number of cycles from the maximum knee flexion. The trial the required the most data points to reach 30 cycles was 3300 points long and all trials were cropped to this point. The data were analyzed unfiltered to achieve the most accurate representation of the variability within the locomotor system [31, 32]. The same data collection system was used for all participants, and therefore we assumed the level of measurement noise was consistent between subjects. However, the skin motion artifact is also a known contributor to measurement error when utilizing motion capture and is a limitation of this technique. Regardless, skin motion artifact should be similar within subject, and any differences between conditions can be recognized as differences in the locomotor system following the vascular occlusion.

Gait Variability: Linear analysis

Gait variability was evaluated with a linear and a nonlinear analysis. The linear analysis provides information about the amount of variability present in the gait patterns and is used to complement the nonlinear analysis. Range of motion of the ankle, knee, and hip angles were calculated for each gait cycle and for every time series. Means, standard deviations, and coefficients of variation were then calculated for each variable and for each participant. Custom made laboratory MATLAB software was used for these calculations.

Gait Variability: Nonlinear Analysis

Nonlinear analysis methods included in the study were the largest Lyapunov exponent and approximate entropy. Unlike the linear measures, both the largest Lyapunov exponent and the approximate entropy take into account the entire time series of the joint angle, exploring its temporal structure, rather than examining a few specific points in the series [30]. The largest

Lyapunov exponent is a measure of the rate of divergence of neighbored state-space trajectories and it estimates the sensitivity of the locomotor system to perturbations. The largest Lyapunov exponent quantifies the exponential separation of nearby trajectories in the reconstructed state space of the joint angle time series. As nearby points of the state space separate, they diverge rapidly and can produce instability. The largest Lyapunov exponent from a stable system with little to no divergence will be zero (eg, sine wave). Alternatively, the largest Lyapunov exponent for an unstable system that has a high amount of divergence will be positive with a larger value (>0.5; Figure 1) [13, 30, 33]. A deterministic signal exhibiting mathematical chaos will have a largest Lyapunov exponent value between 0 and 0.5. The *Chaos Data Analyzer* professional version (American Institute of Physics [34]) was used to numerically calculate the largest Lyapunov exponent for each joint angle time series for each participant. A detailed description of the actual calculation of the largest Lyapunov exponent is as follows:

To reconstruct the state space, a state vector was created from the joint angle time series. This vector is composed of mutually exclusive information about the dynamics of the system [33, 35] (Eq. (1)).

$$\mathbf{y}(t) = [x(t), x(t-T_1), x(t-T_2),\ldots] \qquad \text{Equation (1).}$$

where $\mathbf{y}(t)$ is the reconstructed state vector, $x(t)$ is the original joint angle data and $x(t-T_i)$ is the time delay copies of $x(t)$. The time delay (T_i) for creating the state vector was determined by estimating when information about the state of the system at $x(t)$ is different from the information contained in its time-delayed copy. If the time delay is too small then no additional information about the dynamics of the system would be contained in the state vector. Conversely, if the time delay is too large then information about the dynamics of the system may be lost and can result in random information [33]. Selection of the appropriate time delay was

performed by using an average mutual information algorithm [33] (Eq. (2)).

$$I_{x(t),x(t+T)} = \sum P(x(t), x(t+T)) \log_2 \left[\frac{P(x(t), x(t+T))}{P(x(t))P(x(t+T))} \right] \quad \text{Equation (2).}$$

where T is the time delay, x(t) is the original joint angle data, x(t+T) is the time delay data, P(x(t), x(t+T)) is the joint probability for measurement of x(t) and x(t+T), P(x(t)) is the probability for measurement of x(t), P(x(t+T)) is the probability for measurement of x(t+T). The probabilities were constructed from the frequency of x(t) occurring in the joint angle time series. Average mutual information was iteratively calculated for various time delays and the selected time delay occurred at the first local minimum of the iterative process [30, 33]. This selection is based on previous investigations that have determined that the time delay at the first local minimum contains sufficient information about the dynamics of the system to reconstruct the state vector [33].

It was also important to determine the number of embedding dimensions to unfold the dynamics of the system in an appropriate state space. An inappropriate number of embedding dimensions may result in a projection of the dynamics of the system that has orbital crossings in the state space that are due to false neighbors and not the actual dynamics of the system [33]. To unfold the state space we systematically inspected x(t) and its neighbors in various dimensions (e.g. dimension = 1, 2, 3,…etc.). The appropriate embedding dimension occurs when neighbors of the x(t) stop being un-projected by the addition of further dimensions of the state vector (Eq. (3)).

$$\mathbf{y}(t) = [x(t), x(t + T), x(t + 2T), \ldots x(t + (d_E\text{-}1)\, T)] \quad \text{Equation (3).}$$

where d_E is number of embedding dimensions, $\mathbf{y}(t)$ is the d_E-dimensional state vector, x(t) is the original joint angle data, and T is the time delay. A global false nearest neighbors algorithm with

the time delay determined from the local minimum of the average mutual information was used to determine the number of necessary embedding dimensions to reconstruct the joint angle time series [33]. The calculated embedding dimension indicates the number of governing equations that are necessary to appropriately reconstruct the dynamics of the system [33]. Custom MATLAB (Mathworks Inc, MA) software was used to calculate the embedding dimension. The embedding dimension used for calculating the largest Lyapunov Exponent was 10 and the average time delay for all time series was 15.

After calculating the appropriate time delay and embedding dimension and reconstructing the joint angle time series, the largest Lyapunov Exponent was calculated using the *Chaos Data Analyzer* (professional version, American Institute of Physics [34]). The *Chaos Data Analyzer* calculates the rate of divergence between two vectors [33] (Eq. (4)).

$$\lambda = \frac{1}{t_M - t_0} \sum_{k=1}^{M} \log_2 \frac{L'(t_k)}{L(t_{k-1})} \qquad \text{Equation (4).}$$

where y(t) is the d_E- dimensional state vector and serves as the reference trajectory. $L(t_0)$ is the distance between y(t) and its nearest neightbor. $L'(t_1)$ is the distance between the y(t) and its nearest neighbor after moving forward n steps (we used n = 3). Then a new state vector replaces the evolved neighboring state vector if it meets the following two conditions:

1) The distance of a replacing vector from the evolved state vector on the reference trajectory denoted as $L(t_1)$ is small.
2) The angular separation between the evolved reference state vector and replacing vector is small.

New vectors are repeatedly generated $M = N - (d_E - 1)$ times where N is the length of the original time series. Then, the largest Lyapunov Exponent is defined by Equation (4), where k = 1, 2, …., M and $n = t_{k+1} - t_k$.

A method to determine the repeatability present in time series is to compute the approximate entropy [30, 36]. Approximate entropy is a measure that can quantify the regularity or predictability of a time series [37]. The approximate entropy measures the logarithmic probability that a series of data points a certain distance apart will exhibit similar relative characteristics on the next incremental comparison with the state space [38, 39]. Time series with a greater likelihood of remaining the same distance apart upon comparison will result in lower approximate entropy values, while data points that exhibit large differences in distances between data points will result in higher values. Values closer to zero are consistent with great periodicity (more predictable and repeatable). Conversely, values nearing two represent maximum irregularity (less predictable and repeatable). The approximate entropy value for a periodic time series such as the sine wave will be close to zero, for a random signal such as white noise will be close to two, while a deterministic signal exhibiting mathematical chaos will be somewhere in between (Figure 1).

The approximate entropy calculation presented has been adapted from the extensive mathematical definition described in great detail by Pincus [37]. Approximate entropy has parameters N, m, and r, where N is the number of input data points $u(1)$, $u(2)$, …, $u(N)$, m is the length of compared runs, and r is a tolerance. The following steps summarize the approximate entropy calculation using these three parameters.

- Vector sequences $x(1)$ through $x(N - m - 1)$ from the joint angle time series $\{u(i)\}$ are formed, defined by $x(i) = [u(i), \ldots, u(i + m - 1)]$. These vectors are basically m consecutive u values, beginning with the i-th point.
- Distance $d[x(i), x(j)]$ between vectors $x(i)$ and $x(j)$ is defined as the largest difference in their respective scalar components.

- Vector sequences $x(1)$ through $x(N - m - 1)$ to create (for each $i \leq N - m + 1$) are used to create

  $C_i^m(r)$ = (number of $x(j)$ such that $d[x(i), x(j)] \leq r$) / $(N - m + 1)$

  The $C_i^m(r)$ values measure (within the tolerance r) the regularity of patterns similar to a given pattern of window length m.

- $\phi^m(r)$ is defined as the average value of $\ln C_i^m(r)$, where ln is the natural logarithm. Lastly, approximate entropy is defined as

  $$\mathrm{ApEn}(m, r, N) = \phi^m(r) - \phi^{m+1}(r)$$

Using approximate entropy we calculate the logarithmic probability that runs patterns that are close (within the tolerance r) for m observations remain close (with the same tolerance r) on the next incremental comparison. In all human movement studies using approximate entropy, the m value used was equal to two and r tolerance levels ranged between 0.20 and 0.25. Therefore, for the current study an m value of two and r value of 0.2 were used. Approximate entropy was calculated by implementing the algorithms above in Matlab (The MathWorks, Inc., MA).

When using nonlinear analysis techniques, it is important to validate results against surrogate data to distinguish a deterministic origin from randomness. Surrogation is also an important measure used to determine if the source of the variation is deterministic in nature [13, 30, 40, 41]. This method compares the original time series data set and an equivalent random data set with similar structure. Surrogation removes the deterministic characteristics from the actual data set, leaving a random series with the same mean, variance and power spectra as the original data. Significant differences between largest Lyapunov exponent values for the original and surrogate time series indicate that the variations observed in the actual data series are not random in nature and have deterministic properties [30]. Surrogation analysis was performed on every continuous

joint angle using the method described by Small et al. [40, 41] for periodic time series.

Statistical analysis

Means for the standard deviation and the coefficient of variation ((standard deviation / mean) * 100) of the range of motion, largest Lyapunov exponent values, and approximate entropy values were calculated for the ankle, knee, and hip joints for the baseline and post vascular occlusion conditions for both healthy younger and healthy older groups. Dependent t-tests were used to test for significant differences between the baseline and post vascular occlusion conditions in healthy older adults. To meet the assumption for the dependent t-test that data be normally distributed, the Shapiro-Wilk test for normality was calculated for each dependent variable. The *W* values for most variables suggest that our data is normally distributed (Table 1). Due to the large number of comparisons for the dependent t-tests, a Bonferroni correction was employed and the level of significance was set at 0.006 (0.05/9). To compare the changes of healthy younger individuals and healthy older individuals following induced vascular occlusion, regression models were used with post vascular occlusion values as the dependent variable and adjusting for baseline values by including them as a covariate in the model. Statistical comparisons were performed using SPSS 12.0 software (SPSS Inc, Chicago, Ill).

RESULTS

Baseline versus post vascular occlusion in healthy older

There were no significant differences exhibited by healthy older individuals exhibited for standard deviation and coefficient of variation values during the post vascular occlusion condition for any of the joints (Table 1). These results indicate amount of variability remained relatively consistent between baseline and post vascular occlusion conditions. Regarding temporal structure of variability, there were significant differences between baseline and post

vascular occlusion conditions for the ankle, knee, and hip joint angle time series (Table 1). Specifically, the approximate entropy values increased at all three joint angle time series during the post vascular occlusion condition, and the largest Lyapunov exponent values also increased at the knee and hip joint angle time series post vascular occlusion. Increases in the approximate entropy values represent greater irregularity of the joint angle time series post vascular occlusion. For the largest Lyapunov exponent, larger values show that joint movement patterns exhibited more divergence in consecutive strides. Notably, in all parameters, even those parameters not significantly different between conditions, the larger values were always seen during the post vascular occlusion condition.

For the surrogation analysis, the surrogate data series had significantly increased largest Lyapunov exponent values than the original data for the ankle and knee joint angle time series during walking in the baseline condition. Regarding the post vascular occlusion condition, only the surrogate data series of the ankle joint time series had significantly higher largest Lyapunov exponent values than the original time series (Table 1). Lack of significant differences between original and surrogate data could affect the values for the largest Lyapunov exponent and approximate entropy and this should be considered when interpreting results of the study.

Comparison of baseline adjusted post vascular occlusion between healthy younger and healthy older groups

There were no differences in post vascular occlusion measurements between healthy younger and healthy older groups for standard deviation or coefficient of variation after adjusting for baseline values (Table 2). These results indicate that changes in amount of variability between conditions are similar for both healthy younger and healthy older individuals. Regarding the measures of temporal structure of variability, there were no differences in post vascular

occlusion measurements between younger and older groups for the largest Lyapunov exponent after adjusting for baseline values (Table 2). For approximate entropy, only the ankle joint angle time series was significantly different between healthy younger and healthy older groups after adjusting for baseline values. The approximate entropy value was significantly greater in the healthy older group post occlusion, even after adjusting for baseline differences. These results demonstrate that overall, the structure of variability post occlusion is similar in healthy younger and healthy older individuals, after adjusting for differences in baselines.

DISCUSSION

The purpose of this study was to determine how gait variability changes due to an induced vascular occlusion in healthy older individuals. This was assessed by examining lower extremity joint kinematic variability before and after inducing lower extremity vascular occlusion. We hypothesized that an induced vascular occlusion would produce significant gait variability alterations compared to baseline gait. The study also sought to determine the effect of age by comparing the baseline adjusted post vascular occlusion variability measures between healthy younger and healthy older individuals. We hypothesized that healthy older individuals would have increased change between baseline and post vascular occlusion conditions compared with the healthy younger individuals. Our variability results showed that all lower extremity joint angles have significantly altered gait variability patterns post vascular occlusion. Specifically, the approximate entropy values significantly increased at all three joint angle time series and the largest Lyapunov exponent values significantly increased at the knee and the hip joint time series post vascular occlusion. For the linear measures, the standard deviation and coefficient of variation values were not significant different between baseline and post vascular occlusion conditions, indicating that the amount of gait variability in the ankle, knee and hip ranges of

motion remains relatively consistent, even if blood flow is insufficient.

For the comparison of post vascular occlusion conditions between healthy younger and healthy older groups, one of twelve possible comparisons were significantly different. Therefore, the vascular occlusion procedure produced similar changes to both amount and structure of gait variability in healthy younger and healthy older individuals. It is important to note that the comparisons between groups were adjusted for differences in baseline variability values. It is well established in the literature that older individuals have increased amount and altered structure of variability measures as compared to healthy younger individuals [13, 17]. This was also true of the current study, and therefore to correctly compare the changes following the vascular occlusion procedure between healthy young and older individuals, post occlusion values were adjusted for differences in baseline values.

Our results demonstrate significant gait variability alterations for all lower extremity joint angle time series post vascular occlusion in healthy older individuals. The motivation for this study was derived from a previous study that identified gait variability differences in patients with PAD. Interestingly, the direction of differences found in this study is similar to those studies comparing gait variability of healthy matched controls and patients with PAD [4, 12]. However, direct comparison of the magnitude of change in largest Lyapunov exponent values show that interruption of blood flow does not account for the total amount of changes in gait variability exhibited by patients with PAD. To compare values directly, the mean differences from the healthy older baseline condition was expressed as percent change averaged across the ankle, knee, and the hip joint angle time series for the post vascular occlusion condition. A previous study that included PAD patients was used to make comparisons between PAD patients during pain free walking compared with the older baseline condition. The post vascular occlusion

condition had an average increase of 11.9% for the largest Lyapunov exponent as compared with the baseline condition. Values for PAD patients during pain free walking had average increases of 34.2% for the largest Lyapunov exponent.. Thus, our findings support the idea that interruption of blood flow results in significant gait alterations in otherwise healthy individuals, but patients with PAD experience additional alterations in variability. Consequently, gait variability impairments must reflect a combination of inadequate blood flow and other pathological mechanisms of PAD, which could include identified alterations in function of the nervous and muscular systems [42, 43] . The contributions of such mechanisms to gait variability are currently unknown, but should be the topic of future investigations.

The presence of the optimal "state" of variability is thought to reflect a healthy neuro-musculoskeletal system that can adjust to stresses encountered during daily movement activities. Induced perturbations, like the interruption of blood flow used in the current study, challenge the neuro-musculoskeletal system to respond. Our study results showed the changes in gait variability measures post vascular occlusion, when adjusted for baseline differences, were not significantly difference between the healthy older and healthy younger groups. Both groups demonstrated increases in the values of nonlinear and linear measures of variability. Previous studies of gait variability in the elderly have consistently shown that older individuals have an altered "state" of variability during normal walking [10, 13, 16, 17, 44] . Thus, we hypothesized older individuals would have more dramatic changes in variability when presented with a perturbation like the vascular occlusion used in our study. However, healthy older individuals appeared to temper their response to vascular occlusion, so that the amount and temporal structure of gait variability post occlusion is similar to that of healthy younger individuals when differences in baseline values were considered. A potential explanation is that healthy individuals strive to stay

within a certain level or "state" of variability. Because healthy older individuals start at the higher end of the variability spectrum at baseline, there is less room for these individuals to change while actually staying within the desired "state" of variability. A similar phenomena is also seen in PAD patients, who exhibit altered gait variability during pain free walking and whose locomotor system is so affected that patients actually make no additional adaptations using gait variability, even though they were experiencing claudication pain [12].

Another potential reason for these findings is that there is a limit to the effect of reduced blood flow on neuromuscular control. More specifically, blood flow could alter gait variability to a specific state, but once that level of variability is reached, the neuromuscular system produces no additional response to the vascular occlusion. It is quite possible that the significant baseline gait variability alterations that are present in older individuals restrict the gait variability adaptations of the locomotor system. Therefore, the effect of vascular occlusion on gait is comparable in the younger and older individuals.

The results of the surrogation analysis found the largest Lyapunov exponent values of the original data to be significantly different than the values of their surrogate counterparts for the ankle and the knee joint angle time series during the baseline condition, but only for the ankle joint time series during the post vascular occlusion condition. Previous studies of healthy young and healthy elderly found significant differences between original and surrogate time series during normal walking conditions [13, 40] and in healthy young in response to induced vascular occlusion [14]. Gait variability patterns that exhibit deterministic characteristics are reflective of individuals who can make necessary modifications in reaction to perturbations (i.e. tripping on a curb, slipping on a wet surface) [45, 46]. The lack of significant differences between the original and surrogate data series in the post vascular occlusion condition demonstrates a degradation of

the variability structure of the healthy older individuals following induced vascular occlusion. These results further support the idea that a certain range of variability is good, but when pushed to maximum levels, individuals are pushed outside of the healthy range, resulting in gait variability patterns that lose deterministic characteristics. This is the case in our healthy older group, which demonstrated deterministic patterns during baseline walking, but lost that pattern at some joints following induced vascular occlusion.

An interesting finding is that induced vascular occlusion resulted in gait variability changes in hip joint angle time series, even though the vascular occlusion was induced distal to the hip joint. Potential reasons for these differences include compensation by the hip for changes seen at the ankle and knee or ischemia of the quadriceps and hamstrings contributing to altered hip joint variability. These results suggest that in addition to the distal musculature, muscles and joints proximal to the area experiencing limited blood flow could make adaptations to adjust for changes in the distal joints.

The main limitation of the study is related with the ability to specifically isolate the effect of reduced blood flow. Because it is not feasible to temporarily occlude the arterial circulation in an invasive manner, the approach utilized in the study sought to create a similar situation to the resting state of demand-supply imbalance that is commonly experienced by claudicating PAD patients. The current study was designed based on techniques used in the vascular laboratory to induce vascular ischemia and produce a low grade ischemia in the distal muscle beds. Even though reperfusion in healthy older individuals likely occurs quickly, our study yielded enough power to demonstrate significant alterations in gait during the post vascular occlusion condition. Lack of significant differences between original and surrogate data series at the hip is likely due to the limitations in calculating the hip joint angle. This includes potential marker movement due

to the large amount of adipose tissue around the hip joint and the necessity to mathematically calculate hip marker location because the actual coordinate data can be lost when covered by the arms swinging.

Conclusions

Collectively, our study shows that reduced blood flow, in the absence of pathology significantly alters gait variability patterns of healthy older individuals. Post occlusion gait variability patterns, when adjusted for baseline differences, were not different between young and older individuals, likely reflecting an attempt to maintain a preferred level of variability by the healthy older group, or a limit to the contribution of blood flow to neuromuscular control. Of interest, the change in the gait variability patterns in healthy older individuals was in the same direction previously documented in symptomatic PAD patients. Future studies will determine the contribution of other pathological mechanisms to gait variability alterations of PAD patients.. Regardless, blood flow is one mechanism contributing to altered gait variability patterns,

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Conflict of Interest

No benefits in any form have been or will be received from a commercial party related directly or indirectly to the subject of this manuscript.

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