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Plasma nitrite response in older women to a physical function test

Devon A. Dobrosielski^{1,*}, Tennille Presley², Andreas Perlegas², Anthony P. Marsh¹, Daniel Kim-Shapiro², and W. Jack Rejeski¹

¹Department of Health and Exercise Science, Wake Forest University, Winston-Salem, NC, USA

²Department of Physics, Wake Forest University, Winston-Salem, NC, USA

Abstract

Background and aims—Nitric oxide (NO) may play a critical role in facilitating the delivery of blood to active skeletal muscle, ultimately impacting functional health in older adults. Plasma nitrite is a useful marker of vascular NO bioavailability. The aim of the current investigation was to examine the effect of a widely used physical function test on plasma nitrite concentrations in older adults.

Methods—Venous blood was drawn before, immediately following, and 10 minutes following the completion of a 400-m walk test. Blood samples were added to heparin and frozen for subsequent analysis of nitrite levels using chemiluminescence.

Results—Twenty six (79±4 yrs) women participated in this study. Plasma nitrite levels decreased approximately 22% from baseline following a 400-m walk. Percent change in plasma nitrite was related to walking speed ($r=-0.550$, $p=0.004$).

Conclusions—These data suggest an alteration in plasma nitrite concentration following a functional test which may impact functional health.

Keywords

Acute exercise; nitric oxide; plasma nitrite; 400 meter walk

INTRODUCTION

Aging is believed to confer the highest risk for cardiovascular disease, in part, because there is an age-related decline in endothelial function (1). Cardiovascular disease is known to influence physical function among older adults, possibly through the effects of aerobic capacity on functional reserve (2). Furthermore, others have reported a direct link between endothelial dysfunction and physical function (3).

Nitric oxide may play a critical role in facilitating the delivery of oxygenated blood to active skeletal muscle (4), ultimately impacting the functional health of older adults. Nitrite has been determined to be an effective biomarker of NO production *via* endothelial NO synthase (5). Recently, it has been reported that post-exercise nitrite concentrations predict exercise capacity (6, 7), yet aging is related to impaired capacity of the vascular system to

sufficiently increase plasma nitrite to an exercise stress, which may, in turn, limit exercise performance (6, 8). However, the effect of an acute exercise bout on plasma nitrite concentration in the “oldest” old is unknown. Accordingly, the aim of this study was to determine whether performing a physical function test decreased nitrite concentration in older women. This group is at a greater risk for mobility limitations than men (9).

METHODS

Study participants

Women over the age of 70 years who completed the lifestyle interventions and independence for elders (LIFE) pilot study (10) were recruited to participate in this investigation. Participants were not screened for peripheral arterial disease. A complete list of the exclusion criteria has been published previously (10). The study protocol was approved by the Wake Forest University Institutional Review Board, and all participants gave written informed consent.

Experimental protocol

Since plasma nitrite levels are affected by the amount of digested nitrate (11) participants were instructed to refrain from eating nitrate/nitrite rich foods for at least 24 hours prior to testing. Participants completed a 400-m walk in a gymnasium as fast as possible without running. The 400-m walk was chosen because it is a validated measure of mobility limitation in older adults and is an important prognostic indicator of cardiovascular disease (12, 13). Furthermore, we were interested in examining plasma nitrite response to a task that is more relevant to daily activities than a maximal stress test.

Prior to, immediately following and 10 minutes following the completion of the walk, 5 ml of blood was drawn from the cephalic vein, and processed for subsequent measurement of basal plasma nitrite levels. The nitrogen oxide content of blood plasma was measured by chemical reduction-linked chemiluminescence using a Sievers 280i NO analyzer (NOA) as per instructions by the manufacturer (Sievers Instruments, Boulder, CO). Consistent with recent reports (7, 14) chemical reduction was achieved using 50 mg of KI in 6 ml of concentrated acetic acid.

Statistical analysis

All analyses were performed using SPSS 16.0 (SPSS Inc, Chicago, Illinois, USA). Paired sample *t*-tests were used to evaluate differences in plasma nitrite concentrations as a result of the acute exercise challenges. Relationships between variables were determined using the Pearson product moment correlation. Nitrite concentrations were expressed as means $\pm$ SEM. Statistical significance was set at the $p=0.05$ level.

RESULTS

Twenty six (79 ± 4 yrs) women at risk for mobility disability (15) completed the protocol. No symptoms of undue fatigue or claudication were reported during walking. In our lab, the between days reproducibility in basal plasma nitrite in this elderly group is 0.08 ± 15 nM with a correlation coefficient of 0.476 ($p=0.014$). No significant difference between immediate post walk and 10 minute post walk plasma nitrite concentrations were observed as illustrated in Figure 1. Subsequently, the effect of the 400-m walk on nitrite concentrations was determined by comparing baseline levels to the levels 10 minute post walk. This approach is consistent with previous reports in the literature (6, 8). The 400-m walk resulted in an approximate 22% decrease in plasma nitrite concentrations (Pre: 129 ± 16 nM vs Post: 100 ± 12 nM, $p=0.005$). The individual changes from baseline to 10 minutes post walk are

displayed in Figure 2. Percent change in plasma nitrite following the 400-m walk was inversely related to walking speed ($r=-0.550$, $p=0.004$), (Fig. 3).

DISCUSSION

The recognition that plasma nitrite reflects acute changes in vascular NO bioavailability (16, 17) has resulted in several studies which have examined the role of nitrite and other NO metabolites in the adaptation to acute exercise (6–8, 16, 18). To our knowledge, our study is the first to report on both basal plasma nitrite levels and responses to a widely used measure of lower extremity physical function in a relatively healthy cohort of advanced age women (range: 73–85 yrs).

The steady-state level of plasma nitrite is affected by both nitrite production (related to eNOS production of NO, some of which then becomes nitrite (19)) and nitrite consumption through its reaction with deoxygenated hemoglobin (4). In addition, the steady-state level of nitrite can be lowered through the reaction of reactive oxygen species and NO (decreasing the amount of NO that could become nitrite (19)). In young, healthy adults, physiological stress leads to an upregulation of eNOS that increases nitrite production, which outweighs nitrite consumption and other factors, so that nitrite levels increase (7). However, it was previously reported that nitrite levels fail to increase among older adults with endothelial dysfunction after undergoing a maximal graded exercise test (8). This decrease in nitrite levels could thus be attributed to one or a combination of the following: 1) inability to up-regulate eNOS production during physiological stress, 2) increased local hemoglobin desaturation, or 3) NO interactions with reactive oxygen species.

The present study revealed a reduction in mean plasma nitrite concentrations in our sample of older women after the completion of a 400-m walk, implying a diminished ability to produce NO or increased consumption during exercise. However, this group averaged data obscured an interesting observation. In approximately 20% of the older women, plasma nitrite concentration actually increased following the exercise challenge. Moreover, the percentage change in plasma nitrite from baseline levels was inversely related to walking speed. This paradoxical phenomenon may be related to the absolute exercise intensity achieved by our women during the walking challenge. We speculate that in women who walked slowly, possibly as a result of increased body mass, an increase in nitrite may be observed simply as a result of increased blood flow, or shear stress, due to ambulation. However, for women who walked faster during the walking task, there may be greater NO scavenging through free radicals produced at high intensities (20), resulting in decreased nitrite, or increased nitrite consumption *via* the reaction with deoxyhemoglobin, secondary to increased local hemoglobin desaturation.

There are limitations to the present investigation. Several of the medications taken by the study participants may have influenced plasma nitrite concentrations independent of performance on the physical function test. Second, baseline plasma nitrite concentrations were highly variable between participants. Plasma nitrite is affected by diet (21) and the study may have benefitted from having the subjects fast before being tested. Third, we did not directly assess flow-mediated dilation of the brachial artery, which precludes us from determining with certainty whether these individuals exhibited endothelial dysfunction. Finally, we did not have a younger control group, which makes it difficult to compare our results with previous works. However, we believe these limitations are balanced by the unique population under investigation.

CONCLUSION

The role of vascular function as a determinant of the accelerated decline in functional capacity in older adults in the 7th and 8th decades of life (22) has received little attention in the literature. This study has provided two unique findings. First, we observed that mean plasma nitrite levels in a sample of older women decreased significantly from basal levels following a 400-m walk and second, plasma nitrite levels were inversely related to walking speed. These observations lead to a number of hypotheses for future work focused on the role of NO in vascular and physical function in older adults.

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Fig. 1. The time course for change in plasma nitrite; prior to (129 ± 16 nM), immediately following (102 ± 13 nM) and 10 minutes following (100 ± 12 nM) the 400-m walk. Data presented as mean $\pm$ SEM.

Fig. 2.
Individual changes in plasma nitrite following a 400-m walk.

Fig. 3.

The relationship between change in plasma nitrite concentration following the 400-m walk and walking speed ($r=-0.550$, $p=0.004$).