The Journal of Physiology

JOURNAL CLUB

# A 'passive' movement into the future of assessing endothelial dysfunction?

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Nitric oxide (NO) is a ubiquitous gaseous molecule that plays an important role in many biological processes in the human body, but perhaps none more important than its vasoprotective role within blood vessels. For many years researchers and clinicians have assessed the role of NO on blood vessel health using a variety of invasive and non-invasive techniques. The most popular non-invasive 'assay' to assess NO-mediated endothelial vascular function is flow-mediated dilatation (FMD) via reactive hyperaemia. This particular modality has grown in popularity as changes in conduit artery diameter due to reactive hyperaemia have been associated with future cardiovascular risk. While the FMD technique may be considered partially NO mediated, and therefore likely to reflect endothelium-dependent vascular function, this particular model may not be an appropriate method to measure systemic vascular health and, therefore, its efficacy remains limited. Thus, there is a growing interest in examining alternative methods with which to assess cardiovascular health across the lifespan. Recently, studies from the lab of R. S. Richardson have employed the technique of passive leg movement (PLM) as a novel and potentially practical assessment of systemic vascular function via NO-dependent mechanisms. In this context, PLM presents a unique modality for investigating vasodilatory mechanisms (e.g. mechanical or endothelial) while confounding factors such as skeletal muscle contraction-induced metabolites are minimized.

Ageing is associated with a progressive decline in peripheral blood flow and vasodilatation, largely attributed to reductions in NO bioavailability and/or signalling. Older adults consistently show decrements in FMD compared to young adults. A caveat to previous investigations utilizing FMD or PLM is that these assessments were typically performed while participants were in a supine position. Movement from a supine to an upright position increases femoral perfusion pressure (FPP) (= arterial pressure – venous pressure), and thereby increases the driving force for blood flow and shear rate within the femoral artery, attributed largely to the effect of gravity. This change in blood flow and shear rate is attenuated with $N^G$-monomethyl L-arginine (L-NMMA), an inhibitor of nitric oxide synthase (NOS), resulting in reductions in anterograde shear rate. Previously, it has been shown that in response to PLM, older adults exhibit a reduced NO bioavailability or signalling (Trinity *et al.* 2015). Increasing perfusion pressure (supine-to-upright) elicits a vasodilator reserve in young adults, whereas older adults show no improvement in blood flow or vasodilatation when measured in the upright position (increased FPP). In a recent article published in *The Journal of Physiology*, Groot *et al.* (2015) explored whether the contribution of NO to the vasodilator capacity was altered as a result of changes in FPP during PLM in healthy young *versus* older individuals.

To test the hypothesis that inhibition of NOS would ablate the vasodilator reserve observed with increases in FPP, Groot *et al.* (2015) assessed leg vascular conductance (LVC) during PLM with FPP manipulation. Results revealed that in both the upright and supine position, older adults exhibited attenuated peak LVC and vasodilator reserve when FPP was increased compared to young adults. When L-NMMA was infused, peak LVC was reduced in the young, while no change was observed in older adults. Furthermore, the magnitude of peak change LVC reduction was greater when young subjects were upright compared to supine; however, during L-NMMA infusion in the supine position, there were no age differences in peak LVC. These results suggest that the increase in peak LVC during PLM with concomitant increases in FPP are largely NO dependent, and that the attenuated response in older adults is presumably due to reductions in NO bioavailability or signalling.

Groot *et al.* (2015) further explored the onset of the vasodilator responses to PLM, namely, the rapid vasodilatation seen within the first 7 s of PLM. Older adults exhibited an attenuated rapid vasodilator response compared to young adults across L-NMMA and position trials. Interestingly, and in contrast to previous research utilizing contraction-induced rapid vasodilatation in the forearm (Casey *et al.* 2013), NOS inhibition had no effect on the rapid vasodilator response in the supine position of either young or older adults. However, NOS inhibition attenuated the rapid dilator response in young adults in the upright position, with no change in older adults. These results suggest that within the PLM model with greater FPP, NO plays a distinct role in the rapid dilator response in young adults only.

Contraction-induced rapid onset vasodilatation (ROV) is blunted in both the arm and leg with ageing (Hughes *et al.* 2015). Mechanistically, reduced NO bioavailability and/or signalling has been implicated in the blunting of ROV within the forearm with age; however, these findings have yet to be extended to the leg (Casey *et al.* 2013). The ROV response to single contractions *versus* PLM appears to be similar, yet each modality utilizes different techniques of analysing the rapid hyperaemia and vasodilatation elicited. Given the heterogeneity of vasodilator responses between the arm and leg, evidence from the PLM model suggests that reduced NO bioavailability contributes to impairments in ROV, independent of metabolic influences. Therefore, based on the aforementioned evidence and current findings by Groot *et al.* (2015), the attenuated ROV in the leg of older adults is most likely due to a reduced NO bioavailability or signalling.

Based on the above discussion, the findings from Groot *et al.* (2015) appreciably improve our understanding of the mechanisms underpinning PLM, NO-mediated vascular function and FPP with age. However, it also raises the question with more insistence of potential experimental considerations that may accompany PLM-induced hyperaemia, which may warrant further research. Indeed, the primary goal of the study by Groot *et al.* (2015) was to further advance the clinical relevance of

 DOI: 10.1113/JP271888

PLM by providing mechanistic insight into PLM with age. However, because PLM is still in its preliminary stages of mechanistic evidence and experimental interpretation, we would like to respectfully present other noteworthy considerations, which may serve to further enhance the current field of knowledge and progress its efficacy into future clinical practice.

Firstly, based on the experimental design by Groot *et al.* (2015) it is not entirely certain that PLM is truly passive, because no steps were taken to characterize the true passive nature of the protocol. This is an important consideration as any extraneous muscle movement or myoelectrical activity during PLM can significantly contribute to the hyperaemic responses and consequently confound the overall interpretation of the results. In support of this contention, there is some evidence demonstrating that passive leg movement has the capacity to induce muscular activity (as assessed using electromyography) in the lower limbs (Kawashima *et al.* 2005). Although this aspect of the protocol does not diminish the significance of the findings of Groot *et al.* (2015), it does emphasize the need for future studies to employ electromyography techniques during PLM, which may provide researchers and clinicians with a confident interpretation that blood flow is only influenced by passive movement.

Additionally, Groot and colleagues (2015) report that PLM is a novel methodology to assess vascular function across a lifespan, yet only two distinct age groups have been utilized. That is, we know that older individuals (∼75 years of age) have an attenuated blood flow and LVC response to PLM relative to younger males and females (∼25 years), an age discrepancy of ∼50 years. Therefore, in order to gain a better understanding of the temporal relationship of PLM-induced alterations in blood flow across the lifespan, it might be useful for future research to utilize the PLM protocol in different age groups (e.g. 30–40, 40–50, 50–60 years). Such information may then provide a more comprehensive, representation on when to expect significant NO-mediated reductions in blood flow. This may then provide clinicians with more accurate information toward the timing of appropriate training modalities to counteract the age-related deficits in blood flow.

In conclusion, commendations are given to Groot and colleagues as the first to demonstrate the contribution of NO to PLM-induced rapid vasodilatation during increased FPP with age, thus adding further important mechanistic evidence in an attempt to progress this modality into clinical practice. While passive movement-induced hyperaemia is clearly attenuated with age, contributions, or lack thereof, to the vasodilatory response remain in the early stages of mechanistic evidence. Further research addressing broader experimental considerations and interpretations will be needed to provide such information.

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## Additional information

### Competing interests

None to declare.

### Author contributions

Both authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

### Acknowledgements

The authors apologize for not citing all relevant articles due to reference limitations. The authors thank Dr Darren P. Casey for his critical evaluation and insightful comments for this manuscript.