The Age-Old Tale of Skeletal Muscle Vasodilation: New Ideas Regarding Erythrocyte Dysfunction and Intravascular ATP in Human Physiology

To the Editor:

We are honored that *Circulation Research* highlighted our recent investigation with a companion Editorial article by Dr Chilian and colleagues. This undoubtedly will bring further attention to the significant and assorted cardiovascular dysfunctions that accrue with advancing age in humans, and for this we are appreciative. As within all good scientific practice, the authors raise a few considerations that they feel are important to bear in mind when assessing our experimental design and the associated conclusions. In this letter, we aim to simply address some of the issues raised in the Editorial with the goal of providing the readers of the journal with relevant scientific references and ultimately allow independent assessment of our work, as well as the work of others in this field.

A principal observation from our investigation was that healthy older adults demonstrated remarkably impaired skeletal muscle vasodilation during systemic hypoxia as well as small-muscle mass exercise, which coincided with a lack of increased plasma [ATP] during these conditions compared with young adults. This was not explained by augmented ATP hydrolysis in whole blood but was clearly associated with an impaired ability of isolated erythrocytes to release ATP in response to deoxygenation. Together, these data are of the first to identify that aging explains all age-related impairments demonstrated in vivo. This is an interesting alternative explanation; however, no scientific references are provided to support this. In contrast, ample evidence indicates that ATP released extraluminally (eg, muscle or nerve) binds to purinergic receptors on vascular smooth muscle and evokes vasoconstriction. Moreover, ATP is rapidly degraded by endothelial cell ectonucleotidases and further, that the authors question the theory that whereas plasma [ATP] increases significantly during exercise, intravascular ATP is no longer elevated when eliminating perfusion of the contracting tissue (via proximal blood pressure cuff inflation greater than systolic pressure). Finally, in our study, plasma [ATP] increased in young adults during isolated systemic hypoxia (in the absence of muscle contractions) where only blood oxygenation is altered and muscle metabolism is unchanged. Thus, ATP release from muscle is most likely not the source of intravascular ATP, and cannot explain all age-associated impairments.

With regard to active muscle (skeletal or cardiac) blood flow control, we all agree that this is a very integrative response involving many systems and would be remiss to believe that only one mechanism explains exercise hyperemia. We acknowledged this but it should not detract from the collective findings in our study on human aging.

The specific limitations and questions raised by Dr Chilian and colleagues are highlighted below, followed by brief responses.

1. **Lack of use of a P2 receptor antagonist to determine cause and effect.** We openly acknowledge this limitation in our manuscript and are working to develop a selective antagonist. However, with respect to hypoxic vasodilation in resistance vessels, a number of important observations support our data: (1) perfusion of isolated resistance vessels with a buffer lacking erythrocytes fails to elicit vasodilation when extraluminal oxygen tension is reduced; (2) perfusion of isolated skeletal muscle resistance vessels with erythrocytes not capable of releasing ATP does not elicit vasodilation in response to this stimulus; (3) rescuing low O2-induced ATP release from diseased erythrocytes restored vasodilation when extraluminal oxygen tension is reduced. These studies to date have not been performed within contracting muscle, and although this would be technically difficult, is certainly an area in need of future investigation. In line with the authors’ suggestion, we, too, would support in vivo animal experimentation aimed at deciphering the critical role of red cells to vasodilation through use of pannexin-1 knockout, but it must be acknowledged that ATP release is not universal to a single channel, and impaired ATP release appears to depend on the specific signaling pathway. (2) Is the magnitude of increase in plasma ATP observed in young adults sufficient to cause vasodilation? Studies have demonstrated that a 2-fold increase in ATP effluent collected after perfusing erythrocytes through isolated vessels exposed to reduced oxygen tension elicits significant vasodilation. Our data during systemic hypoxia and exercise are within this range. It should be noted that we discuss how the in vivo situation may be much more complex as a result of large increases in erythrocyte delivery to the low oxygen environment in addition to augmented ATP hydrolysis, and, as with any circulating substance, it is difficult to know the concentration available to bind to a given receptor within the microcirculation.

2. **Intravascular ATP reflects that released from working muscle (not erythrocytes) and this generalized mechanism of aging explains all age-related impairments demonstrated in vivo.** This is an interesting alternative explanation; however, no scientific references are provided to support this. In contrast, ample evidence indicates that ATP released extraluminally (eg, muscle or nerve) binds to purinergic receptors on vascular smooth muscle and evokes vasoconstriction. Moreover, ATP is rapidly degraded by endothelial cell ectonucleotidases and would further mitigate the exchange from muscle to plasma. Additionally, we have unpublished data indicating that whereas plasma [ATP] increases significantly during exercise, intravascular ATP is no longer elevated when eliminating perfusion of the contracting tissue (via proximal blood pressure cuff inflation greater than systolic pressure). Finally, in our study, plasma [ATP] increased in young adults during isolated systemic hypoxia (in the absence of muscle contractions) where only blood oxygenation is altered and muscle metabolism is unchanged. Thus, ATP release from muscle is most likely not the source of intravascular ATP, and cannot explain all age-associated impairments.

3. **Experimental design.** We do not ignore that ATP is extremely multifarious in its vascular actions and effects; however, it should not be ignored that ATP is extremely multifarious in its vascular effects by containing potent antiadhesive and anticoagulative properties, in addition to vasomotor effects. Thus, the fundamental observation that aged humans demonstrate low [ATP] during hypoxemia should not be discounted. When placed in the...
proper context, our novel findings provide great impetus for future study and lay the physiological framework for links between human aging, circulating (intravascular) ATP, and elevated cardiovascular disease risk.

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Disclosures
None.

References
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