A ngiogenesis and its regulation is a crucial physiological response to chronic tissue ischemia causing increased microvessel growth along with collateral arteriolar remodeling to restore blood flow to deprived tissues. Vascular occlusion, as seen in coronary and peripheral artery disease, often progresses to chronic tissue ischemia without effective vascular remodeling responses. Thus, therapeutic revascularization has an important role to play in the management of patients with coronary or peripheral artery disease to facilitate ischemic tissue reperfusion and limit cellular injury and loss. Unfortunately, successful realization of therapeutic angiogenesis remains elusive despite numerous experimental and clinical studies.

**Article see p 1983**

Nitric oxide (NO) regulates several important vascular responses in chronic tissue ischemia, including angiogenesis, vasodilation, anti-inflammation, and apoptosis, to name a few. Potentiation of NO augments therapeutic angiogenesis; however, the activity of nitric oxide synthase enzymes and its cofactors are compromised during ischemia. Despite that fact, several studies indicate that NO metabolites nitrite (NO$_2^-$) and nitrate (NO$_3^-$), once considered to be inert by-products of NO, can be chemically reduced back to NO in ischemic tissues through multiple reduction mechanisms. In this way, the nitrate/nitrite/NO pathway may serve as an alternative (perhaps archaic) NO-generating pathway under low oxygen conditions like chronic tissue ischemia. Thus, conversion of nitrate/nitrite to NO can influence various vascular responses from systemic blood pressure to protection against ischemia-reperfusion injury.

In this issue of Circulation, Hendgen-Cotta et al present the concept of a nitrate-rich nutrition-based therapeutic approach to restore chronic ischemic injury and revascularization of tissue. The authors demonstrate that dietary nitrate can augment ischemic vascular remodeling responses and restore blood flow in a nitrate/nitrite/NO-dependent manner. This effect occurs through the recently identified nitrate/nitrite enteroSialaviry system whereby commensal nitrate-reducing bacteria present in the oral cavity convert nitrate to nitrite that is swallowed and reduced to NO in the stomach. Evidence supporting this conclusion comes from the fact that use of an antiseptic mouthwash blunted nitrate-mediated restoration of ischemic vascular remodeling, alterations in NO metabolites, and restoration of ischemic limb blood flow. These findings provide further support to the notion that manipulation of nitrate/nitrite/NO metabolism may effectively modulate vascular function as suggested.

Dietary inorganic nitrate is present in numerous green leafy vegetables, such as lettuce, celery, and broccoli, but is especially abundant in beets. Recent studies have revealed that nitrate consumption in the form of beet root juice can significantly elevate plasma nitrite levels that influence blood pressure and exercise tolerance. Importantly, the majority of studies using nitrate-based approaches have revealed that sustained administration at doses of 0.05 to 0.3 mmol/kg/d elevate plasma nitrite and NO metabolite levels consistent with the current study.

However, early studies examining the therapeutic effects of nitrite during tissue ischemia found that equimolar concentration of nitrate administration was unable to confer protection equivalent to nitrite. This is likely due to several reasons, in that (1) the beneficial effects of nitrate involving nitrite or nitrosothiol formation takes longer to accomplish versus direct nitrite therapy, (2) in previous studies, administration of nitrate was performed shortly before or after tissue injury, whereas the current study preadministered nitrate for several days before induction of tissue ischemia, and (3) only ~25% of consumed nitrate enters the nitrate/nitrite enteroSialaviry system, with the remainder predominantly excreted in the urine. Together, these facts and current findings provide evidence that dietary consumption of nitrate/nitrite-containing foods could be helpful for various cardiovascular disease conditions.

Useful NO-based therapeutics have been sought after for decades. However, previous therapeutic approaches aimed at NO supplementation have experienced various difficulties, such as drug tolerance, systemic side effects, lack of tissue specificity, or increased tissue toxicity. Inorganic nitrite/nitrate-based therapies appear to circumvent many of these issues with a lack of tolerance to nitrite therapy, minimal side effects, and demonstrable tissue specificity. Interestingly, Hendgen-Cotta and colleagues show that nitrate pretreatment therapy selectively benefits ischemic tissue reperfusion similar to a report by Kumar et al using nitrite therapy. However, it remains unclear whether nitrate pretreatment therapy selectively augments tissue nitrite levels or NO metabolites as previously reported with nitrite therapy.

Perhaps the most distinct difference between nitrate- versus nitrite-based therapy is the importance of oral bacteria for 1939
the physiological effects of nitrate. Previous work from several groups has demonstrated that oral bacterial nitrate reductase activity is crucial for nitrate elevation of plasma nitrite and associated responses.\(^1\)\(^2\) Thus, it is likely that oral nitrate-mediated changes in plasma nitrite and NO metabolites may be influenced by the presence of different bacterial species that requires future investigation. Nonetheless, it is clear that inorganic nitrite/nitrate-based therapeutics affords several advantages over previous NO donor-based approaches.

Finally, the current work by Hendgen-Cotta et al\(^1\)\(^1\) implicates interesting mechanisms of action involving decreased ischemia mediated apoptosis and increased endothelial cell progenitor mobilization. These results are consistent with NO-dependent mechanisms of tissue protection against ischemic injury. Several reports have documented that nitrate-dependent NO formation confers significant protection against ischemic tissue injury involving reduction of apoptosis and prevention of mitochondrial dysfunction.\(^1\)\(^6\)\(^,\)\(^1\)\(^9\) Moreover, Heiss et al\(^2\)\(^0\) reported that inorganic dietary nitrate stimulates mobilization of circulating endothelial cell progenitors both in human subjects and mice involving nitrite/NO and cytokine functions. Together, the associated protection mechanisms are clear; however, it will be important to further understand precisely how dietary nitrate affects various signaling pathways regulating these responses.

From this and other studies discussed above, it is now even clearer that dietary nitrate is an important contributor to cardiovascular health and protection that again leads to the conclusion: Don’t forget to eat your vegetables!

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**Disclosures**

Dr Kevil is a participant on a US patent (No. 61/003150) for the use of nitrite salts in chronic tissue ischemia and has commercial interest in Theravasc Inc.

**References**


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Beets, Bacteria, and Blood Flow: A Lesson of Three Bs
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