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Reply to Comment by D A Guimaraes and J E Tanus-Santos on “Cardiac effects of 6 months’ dietary nitrate and spironolactone in patients with hypertension and with/at risk of type 2 diabetes, in the factorial design, double-blind, randomised-controlled, VASERA TRIAL”

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Conflict of interest/Disclosures: AJW holds shares in HeartBeet Ltd, which receives a royalty from James White Drinks Ltd who manufacture the beetroot juice used in this study. The other authors have stated explicitly that there are no conflicts of interest in connection with this article

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To the Editor:

We thank Guimaraes and Tanus-Santos for their suggestion that the beneficial effects of nitrate supplementation that we observed on cardiac remodelling in hypertensive patients,¹ may be via nitrate-mediated downregulation of the reactive oxygen species (ROS)-mammalian target of rapamycin (mTOR) signalling pathway.² This is based on their recent preclinical work, showing that treatment with oral nitrite prevents cardiac remodelling in rats, associated with antioxidant effects and downregulation of the mTOR signalling pathway.³ We agree that this mechanism may have contributed to our findings. Indeed, we recently reviewed the mechanisms of nitrate/nitrite including antioxidant/ROS-inhibition.⁴⁵ Furthermore, the emerging application of mTOR modulation to human disease was recently highlighted in a Themed Issue in the Journal.⁶ Thus, the demonstration that nitrite prevents cardiac hypertrophy via mTOR inhibition represents a compelling recent finding.³ However, there are several key differences which are worth considering further. These include: the nature of the cardiac remodelling, the blood pressure-dependency, concentrations of nitrite achieved and response relative to disease characteristics.

With respect to the nature of the cardiac remodelling, Guimaraes et al., found that oral nitrite protected against hypertension-induced cardiomyocyte hypertrophy, assessed by heart weight/body weight ratio and myocyte diameter.³ In our Vasera study,¹ chronic administration of dietary nitrate as beetroot juice significantly decreased cardiac volumes and the mass/volume ratio with no effect on left ventricular mass. Therefore, the nature of the cardiac remodelling was fundamentally different.

Regarding the blood pressure-dependency, Guimaraes et al., reported that the nitrite-induced protection against hypertension-induced cardiomyocyte hypertrophy was by mechanisms that are independent of blood pressure (BP)-lowering effects of nitrite.² However, this effect on hypertrophy was only significant with the higher dose of nitrite (15 mg/kg for four weeks), which also had a
marked effect on lowering tail systolic blood pressure (SBP) by about 20 mmHg (assessed by tail-cuff plethysmography). The lower nitrite dose (1 mg/kg) was without significant effect on BP or LVH, though it produced a trend to a decrease in the latter. By contrast, in our Vasera study, the decreases in LV volumes were seen in the absence of a decrease in SBP, suggesting a BP-independent mechanism.

Whilst dietary nitrate was not additive to the antihypertensive effects of spironolactone or doxazosin, this permitted determination of the independent effects of nitrate versus placebo as part of the factorial design; similarly, no interactions were detected with respect to the echocardiographic parameters.

If one considers the concentrations of nitrite achieved, and the responses relative to the disease characteristics, there were several key differences. In the study of Guimaraes et al., the plasma [nitrite] increased from ~0.5 µmol/L in control, to ~3 µmol/L and ~15 µmol/L with sodium nitrite 1 and 15 mg/kg, respectively, representing ~6-fold and 30-fold elevations; the latter associated with the 20 mmHg decrease in SBP, in the hypertensive (non-diabetic) model of 2 kidney-1 clip. By contrast, in our Vasera study, plasma [nitrite] only approximately doubled with active (nitrate-containing) versus placebo (nitrate-depleted) beetroot juice. Whether this modest increase in plasma [nitrite] would be sufficient to affect the mTOR pathway is not known, and not obvious that it would extrapolating from the work in rats by Guimaraes et al. (since the ~6-fold increase in plasma [nitrite] did not inhibit mTOR). Whilst increases in plasma [nitrite] of this magnitude are typically associated with BP-lowering effects in humans, this appears not to be the case in patients with/at risk of type 2 diabetes mellitus. Thus, the disease characteristics may influence the cardiac response to nitrate/nitrite.
Thus, whilst signalling via the mTOR pathway is clearly a possible mechanism of nitrate/nitrite in humans in need of substantiation, given the differences in the nature of the cardiac remodelling, the blood pressure-dependency, and the concentrations of nitrite achieved and response relative to disease characteristics, we think that the haemodynamic changes we observed currently provide a reasonable explanation for the findings in our study.

As we described in the paper, our main explanation for our results relate to haemodynamic actions of dietary nitrate on cardiac pre-load, rather than actions on myocardial cells.\(^1\) In fact, the observed reduction of cardiac volumes is likely to mediate a significant decrease in myocardial wall stress. Myocardial wall stress has been described as the most important stimulus for cardiac hypertrophy in hypertension.\(^9\) Of note, using an experimental model of non-invasive pre-load reduction in humans, we have demonstrated that ventricular volume, rather than pressure is the main determinant of the wall stress.\(^10\)

We have also recently demonstrated new actions of inorganic nitrite in epicardial coronary arteries providing an insight into the mechanisms of dietary nitrate in humans.\(^11\) Taking these novel findings together it appears that inorganic nitrite elicits complex cardiovascular actions ranging from “afterload”,\(^7\) vasodilatation\(^11,12\) and “preload”\(^1\); the latter also consistent with the peripheral venodilatory effects of inorganic nitrite.\(^12\)

We acknowledge the technical limitations of our study,\(^1\) since the sensitivity of the conventional 2D cardiac ultrasound in detecting changes in left ventricular mass is closely related to the magnitude of intra-operator variability. Clearly further work is needed to determine to what extent antioxidant effects and an action via mTOR on cardiomyocytes may contribute to the remodelling effects of nitrate-derived nitrite in humans.
References


