Hellenic Journal of Cardiology 59 (2018) 207–208

Contents lists available at ScienceDirect

Hellenic Journal of Cardiology

journal homepage: http://www.journals.elsevier.com/hellenic-journal-of-cardiology/

Editorial

Mechanisms of testosterone deficiency-related endothelial dysfunction
Invited commentary for the Hellenic Journal of Cardiology on: Tsikas et al. "Associations between asymmetric dimethylarginine, nitrite-dependent renal carbonic anhydrase activity and plasma testosterone levels in hypogonadal men"

Keywords:
Testosterone
Asymmetric dimethylarginine
Endothelial dysfunction
Nitric oxide
Hypogonadism

A B S T R A C T

Evidence from clinical studies suggests that patients with low testosterone levels are at increased cardiovascular disease risk. Even though the exact mechanisms remain poorly understood, a low plasma testosterone level is associated with a pro-atherogenic lipid profile, insulin resistance and increased levels of pro-inflammatory mediators and vascular dysfunction, which is typically observed in patients with hypogonadism. Furthermore, recent evidence suggests that testosterone deficiency has also direct adverse effects on the endothelium and nitric oxide (NO) bioavailability. Observations from studies in patients with hypogonadal hypogonadism imply that the mechanisms of endothelial dysfunction related to testosterone deficiency may involve changes in asymmetric dimethylarginine (ADMA) levels, a known endogenous inhibitor of NO synthase. Evidence suggests that testosterone replacement therapy is not only a safe but also an effective means to reduce atherosclerotic risk and reverse endothelial dysfunction in patients with hypogonadal hypogonadism. Further research in the field is expected to clarify whether changes in ADMA metabolism constitute the central mechanism through which a low testosterone level leads to endothelial dysfunction.

Endothelial health is largely determined by the vascular bioavailability of nitric oxide (NO), which regulates vascular tone and exerts anti-inflammatory, anti-thrombotic and anti-proliferative effects.[1,2] Endothelial function assessment by either invasive (e.g. intracoronary acetylcholine infusions) or non-invasive means (e.g. the flow-mediated dilation of brachial artery)[3] offers prognostic information about the risk of cardiovascular events. In human vasculature, NO is produced by endothelial NO synthase (eNOS), whose activity and biochemical coupling is controlled by its phosphorylation, S-glutathionylation status, the bioavailability of L-arginine and tetrahydrobiopterin (BH_4), vascular oxidative stress and the presence of endogenous competitive inhibitors of eNOS such as asymmetrical dimethylarginine (ADMA) or N^G-methyl-L-arginine (L-NMMA).[1,4]

In this issue of the *Hellenic Journal of Cardiology*, Tsikas et al.[5] provide some pilot data on the potential role of ADMA in atherosclerosis development in men with hypogonadal hypogonadism. It is known that men with hypogonadal hypogonadism have higher plasma ADMA levels than age-matched men.[6] Tsikas et al.[5] now show that replacement of testosterone in 10 males with hypogonadal hypogonadism lowered plasma ADMA levels. These findings on the effects of testosterone replacement on plasma ADMA levels are in agreement with those reported by a previous study in a similar population with idiopathic hypogonadal hypogonadism.[6] Interestingly, in the study of Tsikas et al.,[5] the effect of testosterone on ADMA levels was observed in men with primary, but not secondary, hypogonadal hypogonadism, although the small number of patients included in the study precludes any safe conclusions to be drawn on potential differences between the two subgroups.

It should be noted that gender differences in the prevalence of coronary artery disease have led to the widespread misconception that high testosterone levels may adversely affect atherosclerosis progression. However, clinical evidence suggests that at population level, a low (rather than high) testosterone level is a risk factor for cardiovascular events.[7] Testosterone deficiency has been associated with arterial stiffening, endothelial dysfunction and accelerated atherosclerosis development.[7] In males with hypogonadism, testosterone replacement therapy appears to be a safe and effective means to reverse metabolic abnormalities and improve vascular function.[8] The exact mechanisms by which testosterone

Peer review under responsibility of Hellenic Society of Cardiology.

https://doi.org/10.1016/j.hjc.2018.06.001

replacement therapy confers beneficial effects remain poorly clarified, but they include changes in plasma lipid profile, visceral adiposity, insulin resistance status and the levels of pro-inflammatory mediators.[8] In this aspect, the findings of Tsikas et al.[5] are important, and they show that the increased ADMA levels in males with hypogonadal hypogonadism can be lowered by testosterone replacement, thus highlighting another mechanism by which testosterone beneficially affects vascular function.

There is ample basic and clinical evidence on the role of ADMA in endothelial dysfunction development.[9] ADMA inhibits NOS either by competitive inhibition of L-arginine oxidation or by inhibition of cellular L-arginine uptake. Alternatively, ADMA may also uncouple eNOS, thereby leading to a reduced NO production and rendering the enzyme a source of superoxide radicals. In cell culture studies, incubation of human umbilical endothelial cells with ADMA increases superoxide radical generation.[10] We have previously shown that plasma ADMA levels are inversely associated with brachial artery flow-mediated dilation in both healthy individuals and patients with established CAD.[9] Additionally, in our previous studies, we have demonstrated that high plasma ADMA levels are associated with reduced ex-vivo vasorelaxation of human arteries and veins to acetylcholine or bradykinin.[10] These effects are related to eNOS uncoupling and increased superoxide generation in the presence of increased plasma ADMA concentrations in patients with advanced atherosclerosis.[9] Moreover, our observations in clinical models of chronic and acute inflammation suggest that ADMA may also play a role in inflammation-induced endothelial dysfunction.[9] In prospective clinical studies, plasma ADMA is a biomarker of increased cardiovascular risk and independently associated with cardiovascular mortality in high-risk subjects such as patients who are on haemodialysis and those who have stable CAD. In a recent systematic review, plasma ADMA levels were also associated with all-cause mortality irrespective of pre-existing renal or cardiovascular disease.[11] Therefore, interventions that reduce ADMA levels in populations at risk for atherosclerosis development, such as males with hypogonadal hypogonadism, could potentially be important therapeutic strategies for cardiovascular risk reduction.

Another interesting issue raised by the findings of the study of Tsikas et al.[5] is the mechanism by which testosterone leads to the normalisation of ADMA levels in men with hypogonadal hypogonadism. ADMA metabolism in humans is complex. ADMA plasma levels are determined by the rate of L-arginine methylation by the redox-sensitive S-adenosylmethionine-dependent protein arginine N-methyltransferases (PRMTs). Increased oxidative stress up-regulates PRMT expression and ADMA synthesis, whereas, on the other hand, dimethylarginine dimethylaminohydrolase (DDAH), the enzyme responsible for ADMA degradation, exhibits reduced activity in the presence of increased systemic inflammation and oxidative stress.[5] Whether testosterone supplementation in men with hypogonadal hypogonadism directly affects any of these pathways remains unclear. Nonetheless, it is possible that testosterone deficiency in males with hypogonadal hypogonadism could lead to endothelial dysfunction by affecting ADMA metabolism through the modulation of inflammation.[5] For example, in experimental rat models of testosterone deficiency, castration leads to oxidative stress and inflammation as well as the elevation of ADMA levels and related endothelial dysfunction.[12] Given the known negative regulation of DDAH by inflammatory stimuli, modulation of inflammation and DDAH expression could be the pathway by which testosterone supplementation lowers ADMA levels.[9] Nevertheless, there is still limited evidence on the effects of testosterone on ADMA metabolism through the regulation of either DDAH or PRMT pathways.

Given the small numbers of patients enrolled in the present study, the findings of Tsikas et al. can be viewed only as preliminary data on the potential role of ADMA in mediating the increased cardiovascular risk of patients with hypogonadal hypogonadism. Further research in the field is expected to confirm this preliminary evidence, shed light on the mechanism by which testosterone affects ADMA, and clarify whether the changes in ADMA levels are predominantly responsible for the improvement in endothelial function following testosterone replacement therapy.

Disclosures

None.

Conflict of interest

There is no conflict of interest.

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22 May 2018
Available online 8 June 2018