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EDITORIAL

Testosterone to estradiol ratio and plaque inflammation: mechanistic insights and biomarker potential?

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This editorial refers to 'Testosterone to oestradiol ratio reflects systemic and plaque inflammation and predicts future cardiovascular events in men with severe atherosclerosis', by I.D. van Koeverden *et al.*, pp. 453–462.

Despite the now clear role of inflammation in atherosclerotic plaque development, progression and instability, our understanding of endogenous factors regulating this is limited. Our strategies for measuring inflammation relevant to the arterial wall remains relatively primitive, e.g. the highly sensitive, but non-specific C-reactive protein (CRP). Improved understanding of the specific factors upstream of inflammation has the potential to identify markers with higher specificity for atherosclerosis risk. New insights may also aid the development of novel therapeutic targets, which may act in a synergistic manner with current standard pharmacotherapy targeting lipids and neurohormonal abnormalities.

The focus of this editorial is the paper in this issue by van Koeverden *et al.*[1] Here, the authors demonstrate a novel association of low testosterone to estrogen ratio (T:E2) ratio in males with both increased systemic and plaque inflammation, as well as risk of related cardiovascular events compared with counterparts with a normal ratio. They unravel an interesting potential mechanism pointing to the T:E2 ratio reflecting aromatase activity. This moves away from more traditional approaches that have investigated the individual functional effects of testosterone, estrogen and related steroid hormones, and raises the possibility that the aromatase activity and resulting T:E2 ratio in males may be an excellent marker of specific subsets of adipose tissue (AT) driving vascular inflammation. This is very timely given the rapid expansion of knowledge regarding the contribution of specific AT subtypes on cardiometabolic risk.[2–5]

Testosterone and estrogen-related steroid hormones (including 17β estradiol) have long been known to have a host of direct and indirect effects on arterial health. These are mediated by both traditional transcriptional effects, as well as more rapid, non-transcriptional effects mediated by membrane receptors and downstream signalling pathways.[6–8] The association of low T:E2 ratio with adverse atherosclerotic phenotype may plausibly be explained by direct effects of a low T, or elevated E2. However, whilst both E2 and T have been reported to have a variety of benefits on endothelial function, lipid profile, and inflammation in preclinical and clinical studies, E2 is generally considered to have a broader range of benefits protective against atherosclerosis.[9] Therefore, the lower T:E2 ratio would not be expected to be causally related to higher inflammation and greater plaque vulnerability through effects of the hormones themselves. Interestingly, in women, the reverse regarding T:E2 ratio has been observed with higher T:E2 ratio in postmenopausal women being associated with worse CVD outcomes/events,[10] likely reflecting more complex estrogen metabolism.

White adipose tissue (WAT) has been shown to secrete various adipokines including inflammatory cytokines, complement-like factors, chemokines, and acute phase proteins as well as anti-angiogenic factors, including VEGF-A165b, which in turn has been linked to 'metabolically unhealthy' phenotype.[3,11] Furthermore, increase in WAT-mediated VEGF-A165b production has been shown to confer higher atherosclerotic risk in both animal and human studies.[12] AT has also been found to undergo a phenotypic shift in the development of cardiovascular disease that results in acquisition of pro-oxidant and pro-inflammatory properties.[3] Thus, it is plausible that the T:E2 ratio is a measure of aromatase activity and abundance as well as phenotype of AT. This is further supported by an observation in this study of association of higher body mass index with higher quartiles of T:E2 ratio and most likely underpins the observed association of T:E2 with higher CRP, higher leucocyte counts in blood, and higher degree of inflammatory features of interleukin-6 (IL-6) and IL-6 receptor expression, as well as neutrophil infiltrate in the plaque. A recent clue from pre-clinical work shows that aromatase-overexpressing WAT, is associated with decreased inflammatory markers and increased adiponectin in mice, leading to protection against insulin resistance.[13] Therefore, the role of WAT in cardiovascular regulation and atherosclerosis is an emerging concept, and the T:E2 ratio, reflective of aromatase activity, may be helpful in risk stratification of individuals, at least in males. The ratio in females is more complex given the variable origins of estradiol and related sex steroids in various life stages and the higher degree of subcutaneous WAT, which has a differing endocrine profile.

Beyond reflecting total WAT volume, it may be that the activity of the aromatase reflects specific subgroups of WAT in which inflammatory/metabolic signalling is particularly adverse (as schematically illustrated

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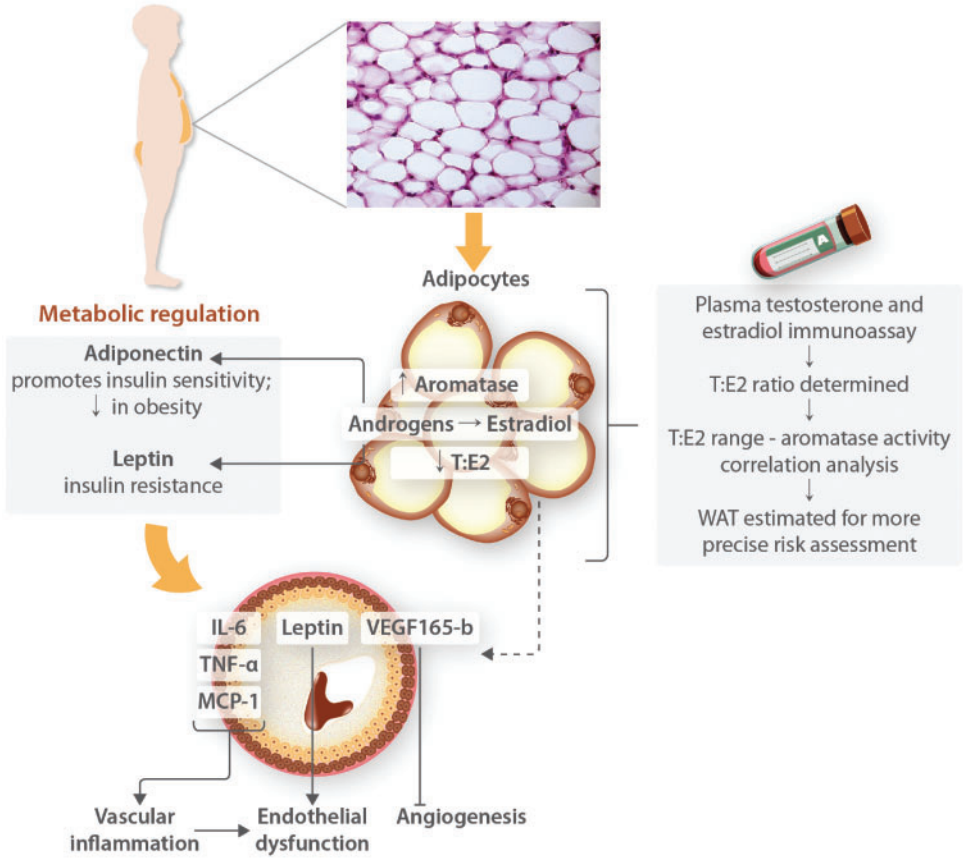

Figure 1 Schematic figure illustrating the potential of T:E2 ratio to reflect aromatase activity, and thus abundance of white adipose tissue in males. Given the role of WAT in production of adipokines related to systemic inflammation, this may have a causal role in atherosclerosis, and may be a better marker of risk than standard markers of obesity such as body mass index. The distribution of WAT is illustrated in the upper panel. Fat, distributed in different locations in the body, is known to have different functions. Excessive visceral or gut fat, composed of retroperitoneal fat ('behind the peritoneum'), omental fat (adipose in a sheet of connective tissue hanging as a flap originating at the stomach and draping the intestines), and mesenteric fat (adipose in the sheets of connective tissue holding the intestines in their looping structure), has been shown to be a risk factor for diabetes and cardiovascular disease. IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; PAI-1, plasminogen activator inhibitor-1; TNF-α, tumour necrosis factor-α.

Figure 1). Previous investigators have identified several local WAT subgroups, including visceral, muscle, epicardial, perivascular, and kidney.[14] which have been postulated to have specialised roles, with differential degrees of inflammatory, metabolic and immune signalling. An example is the inflammatory phenotype of 'central obesity' that is much in excess of that seen with 'gynoid obesity' (involving excess WAT in lower body sites).[14,15] Significant differences in a number of relevant genes have been observed across the different subgroups of WAT in both animal models and humans, and the differential drainage of fat (e.g. into portal circulation vs. systemic circulation) may also influence systemic impact. The dysfunctional WAT promotes not only metabolic dysfunction, but alters sympathetic outflow, glucose handling and insulin sensitivity. Elegant recent work has also demonstrated dynamic interplay between white and beige/brown adipocytes within perivascular visceral AT that results in unique metabolic and pro-inflammatory properties influencing vascular function and plaque stability.[4,5] It is feasible that altered T:E2 ratio reflects abundance of specific subsets of AT, and explain some of the findings observed in the Athero-Express Biobank in the current issue. Further understanding of the relative contribution of specific subsets of AT and their related inflammatory phenotype would be helpful.

The main platform facilitating this research is the Athero-Express Biobank, established in 2002. This demonstrates the long-term benefits of well-governed and designed biobanks for discovery research. Atherosclerotic plaques, blood and patient clinical data were collected over a 14-year period from patients undergoing carotid endarterectomy at St Antonius Hospital Neiuwegein and the University Medical Centre Utrecht. The founding investigators should be congratulated, and research communities and their research governance teams should examine and learn from the benefits provided from such resources, and work to ensure current and future resources have maximal support that allows them to benefit a wide array of researchers and research questions directly relevant to the patient. Discoveries made in well-designed human biobanks have the potential to rapidly translate into new biomarkers, as well as to assist in paradigm shifts of mechanistic understanding and potential novel therapeutic targets. This has tended to be a feature of oncology research, a field where collaborative international biobanks have provided the platform for great leaps in knowledge. The complex nature of the common cardiovascular diseases of atherosclerosis and heart failure require large, well-coordinated studies, with optimal clinical phenotyping and will dramatically benefit from the tide of new

'omics' technologies and computational bioinformatics.[16] Access to the tissue (e.g. arterial wall, atherosclerosis) where available (such as redundant surgical specimens in this study) and rigorous protocols for storage is invaluable.

Given the relative paucity of markers, we have available in the clinic that reflect the underlying biology of atherosclerosis, it will be important to validate this marker, and to test its ability to guide preventative strategies—both in regard to aggressive lifestyle changes, as well as pharmacotherapy. The observations emphasize the importance of ongoing research in the metabolic and immune pathways regulated by AT. Improved knowledge of the relative contributions of different AT subsets to the T:E2 ratio in males would be beneficial to understanding its use as a potential biomarker in the clinic.

Conflict of interest: none declared.

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