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RESEARCH HIGHLIGHT

# Androgens exert sexually dimorphic effects on angiogenesis: novel insight into the relationship between androgens and cardiovascular disease

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The effects of androgen exposure on cardiovascular disease (CVD) risk in men remain poorly understood. Given the earlier incidence of CVD among men relative to women, androgens historically have been assumed to potentiate CVD in men. However, mounting clinical data challenge this assumption and increasingly implicate low levels of circulating testosterone as a risk factor for CVD and mortality.[1,2] In their recently published report 'A sex-specific role for androgens in angiogenesis',[3] Sieveking and colleagues make striking observations regarding the impact of androgens on angiogenesis and recovery from ischemic injury, important components of vascular repair which might provide a mechanism whereby androgens could exert protective cardiovascular effects. Moreover, these findings were sex-specific in both *in vitro* and *in vivo* model systems, suggesting a sexually dimorphic effect of androgens in modulating CVD.

In order to better elucidate the role of androgens in CVD, the authors investigated the effects of differential androgen exposure on angiogenesis, a critical reparative process that occurs subsequent to ischemic injury. Whereas estrogens are known to modulate angiogenesis,[4,5] a comparable role for androgens has yet to be established. Using the non-aromatizable androgen dihydrotestosterone (DHT), the investigators first examined the effects of androgen exposure on endothelial cell (EC) migration, a pivotal step in angiogenesis. In male ECs, DHT conferred a dose-dependent increase in migration, and importantly, this effect was blocked by the addition of an androgen receptor (AR) antagonist, indicating that the increased migration was mediated in an AR-dependent fashion. DHT treatment also potentiated increased male EC proliferation and tubulogenesis, the formation of vascular networks. These effects similarly were abrogated in the presence of the AR antagonist. Consistent with these findings, DHT treatment increased EC production of vascular endothelial growth factor, a pivotal molecule in angiogenesis,[6] and vascular endothelial growth factor receptor 2. In aggregate, these data compelling demonstrate pro-angiogenic effects of androgens in male ECs that might help protect men from CVD.

In striking contrast, DHT treatment of ECs derived from females did not induce comparable changes in migration, proliferation, or tubulogenesis. As this differential effect could be a consequence of reduced AR expression in female ECs, the authors generated AR-overexpressing female ECs. This manipulation increased tubulogenesis in female ECs but not to an extent comparable to that seen in male ECs. Thus, this series of experiments offers strong evidence for sexual dimorphism in EC androgen signaling pathways.

These *in vitro* findings provided the basis for follow-up studies that employed *in vivo* models of angiogenesis. In both male and female mice, gonadectomy significantly reduced Matrigel plug vascularization. Whereas DHT treatment completely rescued this angiogenic impairment in orchidectomized male mice, it conferred no improvement in vascularization in the ovariectomized female mice. Parallel findings were generated after hindlimb ischemia, a model of revascularization that specifically assesses angiogenesis in response to ischemic injury. Through assessment by Doppler perfusion imaging and motor function, the investigators found that orchidectomized mice exhibited significantly impaired recovery from ischemic insult. DHT treatment conferred substantial improvements in both perfusion and motor function, findings that occurred in association with increased capillary density in the adductor muscles subject to ischemia. These improvements also occurred in tandem with corresponding changes in the expression of genes known to play a role in angiogenesis and hypoxic response.

Finally, the authors examined myeloid progenitor cells in the bone marrow and spleen of orchidectomized and control mice, as these cells have been implicated as a critical source of growth factors required for angiogenesis. In orchidectomized mice, immunohistochemical staining revealed markedly diminished numbers of these progenitor cell populations, whereas DHT treatment restored them. Similar results were obtained when progenitor cells were examined in the bone marrow of intact and orchidectomized mice subject to hindlimb ischemia. Again, DHT treatment rescued the diminished numbers of progenitor cells evident in orchidectomized animals 3 days subsequent to ischemic injury. These data therefore suggest that androgens may exert critical effects not only on ECs but also on other cell types involved in angiogenesis. Importantly, this is not the first study to observe changes in a progenitor cell population implicated in vascular repair. Reduced number of circulating endothelial progenitor cells has been associated with increased risk of recurrent CVD events,[7] and hypogonadal men appear to have reduced numbers of these cells which normalize with testosterone replacement.[8]

While the above findings are certainly profound, it is unclear from these studies what

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concentrations of DHT are required to mediate these effects. Normal circulating concentrations of DHT in men are approximately 1–3 nmol $l^{-1}$. In the case of the *in vitro* studies, DHT concentrations of 4–400 nmol $l^{-1}$ were used to demonstrate effects on angiogenesis. For the *in vivo* data, DHT was administered *via* silastic implants, and the corresponding serum DHT levels were not provided. Thus, whether the observed effects are mediated with physiological or pharmacologic doses of DHT cannot be determined from the data presented.

Further, despite the impressive effects of androgen replacement in orchidectomized male mice, DHT treatment did not completely restore a normal response to ischemic injury. Most notably, the DHT-treated orchidectomized animals exhibited reduced vessel diameter compared to intact controls. This finding underscores the complexity of angiogenic regulation and suggests that other gonadal steroids or their metabolites similarly may play a modulatory role in vascular remodeling. As estrogen has pro-angiogenic effects in females, it would have been interesting to test whether estradiol could have rescued any of the orchidectomy effect in the *in vivo* model systems. Nonetheless, these results collectively demonstrate potent, vascular effects of androgens that are consistent with a protective function for CVD and appear specific to males.

The historical notion that androgens potentiate CVD risk derives in part from the earlier incidence of CVD among men and the long-recognized effects of androgens on lipids, in particular their lowering of high density lipoprotein cholesterol. However, the contribution of high density lipoprotein cholesterol to overall CVD risk is relatively small, and ongoing research has generated new appreciation of the heterogeneous biological processes that underlie CVD. As this mechanistic understanding has grown, clinical data in parallel have challenged previous assumptions about the role of androgens in atherogenesis and CVD risk. For example, men undergoing androgen deprivation therapy for management of prostate cancer exhibit a disproportionate prevalence of CVD, suggesting that androgen depletion rather than androgen exposure augments CVD risk.[9] This association between low circulating androgens and increased CVD risk in men is in striking contrast to the augmented CVD risk associated with excess androgen exposure in women with polycystic ovarian syndrome.[10] As the work of Sieveking and colleagues elegantly demonstrates, the biological effects of sex steroids appear contingent on sex, lending insight into this apparent clinical paradox. Sex steroid effects also may depend on additional variables including age or the chronicity and magnitude of steroid exposure. Moreover, sex steroids appear to modulate a variety of biological processes implicated in CVD and therefore simultaneously could exert both protective and detrimental effects. Continued research is necessary to better define the mechanistic roles of sex steroids in CVD and to explore how these effects may vary across clinical populations.

1 Laughlin GA, Barrett-Connor E, Bergstrom J. Low serum testosterone and mortality in older men. *J Clin Endocrinol Metab* 2008; **93**: 68–75.
2 Vikan T, Schirmer H, Njolstad I, Svartberg J. Endogenous sex hormones and the prospective association with cardiovascular disease and mortality in men: the Tromso Study. *Eur J Endocrinol* 2009; **161**: 435–42.
3 Sieveking DP, Lim P, Chow RW, Dunn LL, Bao S *et al*. A sex-specific role for androgens in angiogenesis. *J Exp Med* 2010; **207**: 345–52.
4 Morales DE, McGowan KA, Grant DS, Maheshwari S, Bhartiya D *et al*. Estrogen promotes angiogenic activity in human umbilical vein endothelial cells *in vitro* and in a murine model. *Circulation* 1995; **91**: 755–63.
5 Losordo DW, Isner JM. Estrogen and angiogenesis: a review. *Arterioscler Thromb Vasc Biol* 2001; **21**: 6–12.
6 Ahn A, Frishman WH, Gutwein A, Passeri J, Nelson M. Therapeutic angiogenesis: a new treatment approach for ischemic heart disease—part I. *Cardiol Rev* 2008; **16**: 163–71.
7 Werner N, Kosiol S, Schiegl T, Ahlers P, Walenta K *et al*. Circulating endothelial progenitor cells and cardiovascular outcomes. *N Engl J Med* 2005; **353**: 999–1007.
8 Foresta C, Caretta N, Lana A, de Toni L, Biagioli A *et al*. Reduced number of circulating endothelial progenitor cells in hypogonadal men. *J Clin Endocrinol Metab* 2006; **91**: 4599–602.
9 Keating NL, O'Malley AJ, Smith MR. Diabetes and cardiovascular disease during androgen deprivation therapy for prostate cancer. *J Clin Oncol* 2006; **24**: 4448–56.
10 de Groot PC, Dekkers OM, Romijn JA, Dieben SW, Helmerhorst FM. PCOS, coronary heart disease, stroke and the influence of obesity: a systematic review and meta-analysis. *Hum Reprod Update*; e-pub ahead of print 18 Feb 2011; doi: 10.1093/humupd/dmr001.