The oncogenic murine AKT8 retrovirus was identified almost 25 years ago, and the presence of the causative oncogenic agent v-akt was subsequently demonstrated in transformed cells or tumors from both mice and humans.1,2 The cellular homolog of v-akt was cloned in the early 1990s and was termed c-Akt (or simply Akt). Largely on the basis of its sequence similarity to both protein kinases A and C, it was concluded that Akt (alternatively known as protein kinase B [PKB]) represented a Ser/Thr-protein kinase. Three mammalian genes have been identified, with transcripts of akt1 and akt2 being highly expressed in heart. The explosion of interest in Akt was the direct consequence of the realization that it is an effector of the lipid signaling molecule phosphatidylinositol 3,4,5-trisphosphate [PtdIns(3,4,5)P3] (Figure). 6 Phosphorylation of Thr 32 and Ser 253 in FKHRL 1, and AFX) are recognized substrates of Akt, 2 which phosphorylates Thr24, Ser256, and Ser19 in FKHR (Figure). 6 Phosphorylation of Thr32 and Ser253 in FKHL1 (equivalent to Thr24 and Ser256 in FKHR) retains it in the cytoplasm through sequestration by 14-3-3 proteins and prevents it from activating transcription of proapoptotic genes. 7 In concert with the greater degree of Akt phosphorylation and activation, Camper-Kirby et al 4 detected significantly greater amounts of cytoplasmic FKHR(phospho-Ser256) in myocytes of adult female mouse hearts than in adult males, as established by immunohistochemistry or Western blotting. In support of a role for estrogen in promoting Akt signaling, exposure of rat cardiac myocyte cultures to 17β-estradiol or estrogens in vivo has been reported to increase Akt phosphorylation.8,9,15

Gender Differences in Cardiovascular Disease

In humans, the risk of developing cardiovascular disease is considerably less in premenopausal females than in age-matched males. 3 The cause of this difference is unclear and may depend more on the properties of the vascular tree than on the cardiac myocyte. Cardiovascular factors strongly associated with gender include vascular function (endothelium-dependent flow-mediated dilatation and aortic compliance are greater in females) and left ventricular mass index (LVMI), which is greater in males. After menopause, the rates of cardiovascular disease converge, and once affected by ischemic heart disease, females may fare worse than their male counterparts. The differences in susceptibility are widely held to be related to estrogen status. The biological basis for these effects of estrogen is not fully understood, but one factor may be its ability to induce systemic vasodilation.3

In this issue of Circulation Research, Camper-Kirby et al 4 describe an additional facet of gender differences in the cardiovascular system that could be involved in modulating vulnerability to cardiovascular disease. Using an immunohistochemical approach with an antibody that recognizes only the Ser473/Ser474-phosphorylated species of Akt1/2, they show that adult premenopausal women display a significantly greater frequency of staining of Akt1/2(phospho-Ser473/474) (suggestive of increased Akt activity) in the nuclei of their cardiac myocytes than men or postmenopausal women. These differences are also seen in adult mice and in juvenile transgenic mice that cardiospecifically overexpress IGF-1 [which stimulates the PI3K/PtdIns(3,4,5)P3 signaling pathway]. These transgenic mice are protected against myocardial infarction.5 Administration of the phytostrogen genistein, which has several biological actions, including estrogen receptor agonism, also increased nuclear staining of Akt1/2(phospho-Ser473/474). The immunohistochemical findings were confirmed by Western blotting for Akt1/2(phospho-Ser473/474) and by immunokinase assays for Akt activity.

The Forkhead family of transcription factors (FKHR, FKHL1, and AFX) are recognized substrates of Akt, 2 which phosphorylates Thr24, Ser256, and Ser19 in FKHR (Figure). 6 Phosphorylation of Thr32 and Ser253 in FKHL1 (equivalent to Thr24 and Ser256 in FKHR) retains it in the cytoplasm through sequestration by 14-3-3 proteins and prevents it from activating transcription of proapoptotic genes. 7 In concert with the greater degree of Akt phosphorylation and activation, Camper-Kirby et al 4 detected significantly greater amounts of cytoplasmic FKHR(phospho-Ser256) in myocytes of adult female mouse hearts than in adult males, as established by immunohistochemistry or Western blotting. In support of a role for estrogen in promoting Akt signaling, exposure of rat cardiac myocyte cultures to 17β-estradiol or...
How Might the Estrogen-Mediated Activation of Akt Be a Factor in Gender Differences of Susceptibility to Cardiovascular Disease?

Most previous studies on gender-associated risk have emphasized the importance of the vascular system. Estrogen activates endothelial nitric oxide (NO) synthase through an extranuclear estrogen receptor. Two signaling pathways have been implicated in this response to estrogen: the extracellular signal-regulated kinase cascade and the PI3K/Akt pathway. Clearly, estrogen-dependent stimulation of NO production could decrease vascular resistance, and such a response could be protective. The estrogen-dependent activation of Akt in the cardiac myocyte demonstrated by Campen-Kirby et al. is that exposure to estrogens increases the activity of Akt in cardiac myocytes. Hypothetically, this could protect females against cardiovascular disease by increasing the resistance of their myocytes to cytotoxic stimuli.

Regulation of Apoptosis

As mentioned above, Akt plays a central role in cell survival and resistance to apoptosis. Space does not permit us to discuss mechanisms of apoptosis, but it has been the topic of recent reviews. Although controversial, evidence of apoptosis has been detected in myocytes of heart failure patients, animal models of heart failure, and after acute myocardial infarction. In isolated cardiac myocytes, earlier work has shown that 17β-estradiol reduces staurosporine-induced apoptosis and that adenoviral infection of constitutively activated PI3K increases Akt phosphorylation and decreases doxorubicin-induced apoptosis.

Apoptosis is regulated by two distinct but interrelated pathways: the mitochondrial and receptor-mediated pathways. The former involves release of cytochrome c from the mitochondria into the cytoplasm and activation of procaspase 9 in the apoptosome complex. Release of cytochrome c is regulated by the Bcl-2 family proteins, which can be either antiapoptotic (eg, Bcl-2 itself and Bcl-XL) or proapoptotic (eg, Bad and Bax). After activation, caspase 9 cleaves and activates caspase 3, an end-effector caspase, and degradation of cellular macromolecules results. In the receptor-mediated pathway, proapoptotic factors, such as tumor necrosis factor-α or the cell surface Fas ligand (FasL), interact with their cell-surface receptors (the tumor necrosis factor receptor and Fas/CD95, respectively) to activate procaspase 8, thence caspase 3.

How activation of Akt increases resistance to apoptosis is incompletely understood. A variety of schemes have been proposed. All are controversial, and none have been shown to be operative in the cardiac myocyte. All or any of the mechanisms described below could potentially increase cardioprotection, although some (eg, Bad or caspase 9 phosphorylation) would require participation of extranuclear Akt. This is important, because Campen-Kirby et al. detected significant activation of Akt only in nuclei, and it is not clear whether there was any significant activation of Akt in the cytoplasm at any stage.

Antiapoptotic Effects of Akt

Proapoptotic Bad is phosphorylated by Akt, the major site of phosphorylation being Ser136. This phosphorylation promotes retention of Bad in the cytoplasm through sequestration by 14-3-3 proteins and prevents initiation of the mitochondrial pathway of apoptosis. However, phosphorylation of Ser136 in Bad (which possesses other additional antiapoptotic phosphorylation sites) was not detectable in cardiac myocytes that expressed constitutively-activated PI3K and showed phosphorylation of Akt. A second possibility is that Akt phosphorylates and inhibits caspase 9. This has been demonstrated for the human enzyme, but the phosphorylation site is not conserved in several other species, and the significance of these findings remains to be established. Although other antiapoptotic effects of Akt on the mitochondrial pathway have been reported, these are even less well characterized mechanistically than those mentioned above. Akt may also inhibit the receptor-mediated pathway of apoptosis. As mentioned above, Akt phosphorylates members of the Forkhead family of transcription factors, causing them to be retained in the cytoplasm. Dethosphorylation removes this restraint, and Forkhead translocates to the nucleus, where one of its roles may be to drive expression of FasL. However, it is not clear whether the Fas/FasL pathway operates in the cardiac myocyte. Whereas the recombinant soluble form of FasL may increase apoptosis in isolated cardiac myocytes, cardio-specific overexpression of full-length FasL in mice in vivo does not demonstrably increase cardiac myocyte apoptosis.
Pathways exist whereby Akt could inhibit both the mitochondrial and receptor-mediated pathways of apoptosis. Inhibitor-of-apoptosis proteins (IAPs) inhibit caspases, and the transcription factor nuclear factor-κB (NF-κB) increases expression of IAP genes.\(^{20}\) NF-κB is normally retained in the cytoplasm in unstimulated cells through sequestration by inhibitor κB (IκB). After its phosphorylation by IκB kinases, IκB undergoes proteasomal degradation, and this releases NF-κB from inhibition, allowing it to migrate to the nucleus and regulate transcriptional activity. Akt has been reported to associate with and activate IκB kinases, although the mechanisms are unclear.\(^{20}\)

**Other Actions of Akt**

Although an explanation based on resistance to apoptosis is undoubtedly in tune with the contemporary zeitgeist, more mundane scenarios for gender-related cardioprotection by Akt can be devised. For example, Akt promotes glycogen synthesis (again a cytoplasmic process) by phosphorylating and inhibiting glycogen synthase kinase 3 (GSK3), one kinase responsible for phosphorylating and inhibiting glycogen synthase.\(^{21}\) Inhibition of GSK3 thus promotes glycogen synthesis. An increase in cardiac glycogen might increase resistance to cellular hypoxia during ischemia by providing a greater pool of fuel reserve for anaerobic glycolysis. Indeed, there is evidence that administration of 17β-estradiol to female mice increases cardiac glycogen content.\(^{22}\) Activation of Akt and inhibition of GSK3 have also been reported to influence of the vasculature) and to examine whether any link between estrogen status, Akt activation, and cytoprotection against this hypothesis, it is not clear whether the greater LVMI is a consequence of myocyte size or number, with males simply possessing a greater number of myocytes of smaller average size than females.

Clearly, the next steps in the study of the gender-related differences in Akt activity will be to establish a functional link between estrogen status, Akt activation, and cytoprotection at the level of the isolated myocyte (to remove any influence of the vasculature) and to examine whether any signaling molecules involved show estrogen-dependent differences in their biological activities.

**References**

Akt Like a Woman: Gender Differences in Susceptibility to Cardiovascular Disease

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