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Commentary

Lymphangiogenesis: A new player in the heart's adaptive response to exercise

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The heart's lymphatic network is essential for maintaining fluid and immune cell homeostasis. Lymphatic vessels return interstitial fluid to the bloodstream, transport macromolecules such as proteins and lipids, and clear the heart of inflammatory cells.[1] Several studies have demonstrated the importance of cardiac lymphatics and lymphangiogenesis (the formation of new lymphatic vessels from pre-existing vessels) in settings of cardiac pathology.[2-6] Blocking endogenous lymphangiogenesis during myocardial infarction exacerbates myocardial edema and cardiac inflammation and fibrosis, while enhancing lymphangiogenesis attenuates ischemia-induced heart failure and pressure overload-induced cardiac dysfunction.[3-5] Collectively, these studies identify stimulation of lymphangiogenesis as a potential therapeutic strategy for the treatment of cardiovascular diseases and heart failure.

Physiological cardiac hypertrophy is an adaptation that occurs in response to chronic exercise training or pregnancy. Unlike pathological hypertrophy, which occurs in settings of cardiac injury (e.g., myocardial infarction) or sustained hemodynamic overload (e.g., hypertension), physiological hypertrophy is not associated with interstitial fibrosis or cardiomyocyte apoptosis. Heart function is preserved or enhanced, and increases in heart muscle mass are reversible with detraining or following pregnancy.[7] Physiological cardiac hypertrophy is mediated by the insulin-like growth factor 1 (IGF1)-phosphoinositide 3-kinase-protein kinase B (Akt) pathway, and enhancing signaling via this pathway using exercise training, gene therapy approaches or in genetically modified mice is shown to attenuate pathological cardiac remodeling and dysfunction in preclinical animal models (reviewed in[7]). Thus, understanding the mechanisms by which exercise induces physiological hypertrophy and cardioprotection will inform the development of exercise interventions and exercise-based therapeutics for the treatment of cardiovascular disease.[8,9]

In this issue of the *Journal of Sport and Health Science*, Bei and colleagues[10] provide evidence for cardiac lymphangiogenesis in a well-established mouse model of exercise-induced cardiac hypertrophy (3 weeks of twice-daily swim training). Their results are consistent with a previous report from a rat model of eccentric exercise training (downhill treadmill running).[11] Robust characterization of both male and female mice revealed that hypertrophied hearts from exercised mice had a higher density of lymphatic vessels, as determined by immunofluorescent staining of lymphatic vessel endothelial hyaluronic acid receptor 1 (LYVE-1)-positive lymphatics in cardiac cross-sections. This was accompanied by increases in protein expression of the lymphatic vessel markers LYVE-1 and podoplanin, an effect that appeared to be more pronounced in female hearts. Two homologous ligands, vascular endothelial growth factors (VEGF) C and D, induce lymphangiogenesis via VEGF receptor 3 (VEGFR3).[12] Expression of both growth factors and VEGFR3 was elevated in heart tissue from exercised mice, and administration of a VEGFR3-selective inhibitor, SAR131675, blocked exercise-induced increases in lymphatic vessel density and expression of VEGFR3, LYVE-1, and podoplanin. A key finding of this study was that SAR131675 treatment also blocked exercise-induced increases in heart mass, suggesting that VEGFR3-dependent lymphangiogenesis is required for physiological hypertrophy induced by exercise.

To investigate whether cells of the lymphatic system can signal to cardiomyocytes to regulate cell size, the authors

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applied conditioned media from human dermal lymphatic endothelial cells (LECs) to neonatal rat cardiomyocytes (NRCMs). NRCMs are a well-established model for investigating the impact of hypertrophic stimuli on cardiomyocyte biology. Application of LEC-conditioned media to NRCMs induced significant increases in cardiomyocyte area, an effect that was blocked by pre-treating LECs with SAR131675. Further investigation revealed that LECs secrete IGF1 and reelin (RELN), an effect that was also attenuated by SAR131675 treatment. RELN is an extracellular matrix protein that was recently shown to regulate cardiomyocyte proliferation and survival via activation of integrin β1−Akt signaling in cardiomyocytes.[13] Consistent with previous reports,[14,15] the authors of the current study[10] found that exercise training increased the number of Ki67-positive cells (suggestive of cardiomyocyte proliferation; other markers of cardiomyocyte proliferation such as Aurora B kinase and phospho-histone H3 were not examined), and that this was blocked by SAR131675. *In vitro*, application of LEC-conditioned media to NRCMs also increased the number of Ki67-positive cells. Collectively, these experiments suggest that LECs can influence cardiomyocyte growth and proliferation, likely via VEGFR3-dependent secretion of growth factors such as IGF1 and RELN.

Finally, the authors performed loss- and gain-of-function experiments in NRCMs to manipulate downstream components of the IGF1−phosphoinositide 3-kinase signaling pathway. These experiments confirmed the involvement of Akt and transcription factors C/EBPβ and carboxylterminal domain 4 in mediating the hypertrophic and proliferative response of NRCMs to LEC-conditioned media. In summary, this study: (1) provides evidence that exercise-induced physiological cardiac hypertrophy is associated with lymphangiogenesis, (2) provides important validation of previously reported molecular changes in the exercised heart (i.e., enhanced IGF1 levels, increased Akt phosphorylation, and reciprocal regulation of CCAAT enhancer-binding protein beta and CBP/p300-interacting transactivators with E (glutamic acid)/D (aspartic acid)−rich-carboxylterminal domain 4 expression), (3) identifies VEGFR signaling as an important regulator of the heart's adaptive response to exercise, and (4) provides evidence for cross-talk between LECs and cardiomyocytes, which regulates cardiomyocyte growth and proliferation.

Single cell RNA-sequencing of exercised hearts to identify potential intercellular connection networks (as has been done in a setting of pathological cardiac remodelling[16]), will be valuable for obtaining a more complete understanding of the paracrine and autocrine mechanisms that modulate the heart's adaptive response to exercise. Cardiomyocytes are the predominant source of VEGFA in the heart.[16] VEGFA activates VEGFR2 on endothelial cells (also expressed in LECs[17]), and is the main ligand responsible for inducing angiogenesis (the formation of new blood vessels from existing blood vessels).[18] Angiogenesis is an important feature of both pathological and physiological hypertrophy, and a seminal study from Kenneth Walsh's group showed that inhibition of angiogenesis contributes to the progression from adaptive cardiac hypertrophy to heart failure.[19] A limitation of the current study is that a single inhibitor was used to interrogate the role of VEGFR3, and its selectivity for VEGFR3 was not investigated. In a non-cardiac setting, SAR131675 was highly selective for VEGFR3 at the same dose that was used in the current study (100 mg/kg/d), and only inhibited VEGFR2 and angiogenesis when administered at a higher dose (300 mg/kg/d).[20] The use of 2 structurally distinct inhibitors and/or a genetic loss-of-function mouse model would have strengthened the findings of the current study.[10] Nevertheless, this study provides an important foundation for understanding how cardiomyocytes, LECs, blood endothelial cells, and other cardiac cells communicate with one another to coordinate the hypertrophic, lymphangiogenic, and angiogenic response to hemodynamic challenge.[10]

Further work is also needed to investigate possible sex differences in the context of cardiac lymphangiogenesis and hypertrophy. Although sex was not a major focus of the current study, inspection of the pertinent data reveals greater exercise-induced increases in cardiomyocyte area, lymphatics/cardiomyocyte ratio, LYVE-1 expression, and podoplanin expression in female hearts compared with male hearts. Previous studies have shown that female rodent hearts display a greater hypertrophic response to exercise training and have higher levels of Akt phosphorylation than male hearts.[21,22] Another study reported a higher density of LYVE-1-positive vessels in female hearts compared with male hearts.[23] The biological significance of these differences is unclear, but warrants further investigation.

To conclude, this study provides convincing evidence that exercise-induced physiological cardiac hypertrophy is associated with increased lymphangiogenesis and that factors released by LECs can stimulate cardiomyocyte hypertrophy and proliferation. The experiments using SAR131675 suggest an important role for VEGFR3 in mediating these effects, although further work is needed to examine a possible concomitant role of VEGFR2 and angiogenesis in the development of exercise-induced cardiomyocyte hypertrophy and proliferation. Investigation of sex differences may also provide insight into the mechanisms regulating cardiac lymphangiogenesis in the context of exercise-induced heart growth, an important pursuit to facilitate the development of new strategies to treat and protect against various myocardial pathologies.

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Authors' contributions

SD, JVJ, and KLW drafted, reviewed, and edited the manuscript. All authors have read and approved the final version of this manuscript, and agree with the order of presentation of the authors.

Competing interests

The authors declare that they have no competing interests.

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