Enhancing Treatment for Cardiovascular Disease: Exercise and Circulating Angiogenic Cells

Sarah Witkowski 1, Nathan T. Jenkins 2, and James M. Hagberg 2

1 The University of Massachusetts, Department of Kinesiology, Amherst, MA; and 2 The University of Maryland, Department of Kinesiology, College Park, MD

INTRODUCTION

Physical activity is recognized for its profound benefits on cardiovascular health, although the mechanisms for these effects are less clear. The improvement in endothelial function as a result of exercise is likely a central factor contributing not only to decreased rates of cardiovascular disease (CVD) in active individuals but also to beneficial changes in vascular function in persons with established CVD who undergo exercise therapy. Indeed, overall CVD risk may be influenced to a greater degree by changes in endothelial function than traditional CVD risk factors (19).

Discovery of circulating cells with cardiovascular regenerative effects has transformed dogma related to the maintenance and repair of cardiovascular tissues and ignited exciting research in cardiovascular adult cell therapy. Circulating angiogenic cell populations support vascular repair and reendothelialization. The ability of circulating angiogenic cells (CAC) to contribute to cardiovascular maintenance and repair is dependent on multiple processes including liberation of CAC from their compartments, proliferation, differentiation, homing to areas of injury or angiogenesis, and engraftment or incorporation into the vasculature. Investigations are underway to use CAC in regenerative medicine to treat vascular deficits associated with CVD (20). In some instances, these treatments have not been as efficacious as hoped, which may be related to intrinsic cellular defects in CAC relative to the pathogenesis of disease. Understanding strategies to improve CAC function may aid current efforts to optimize cell therapy with CAC.

We and others have shown beneficial effects of acute and chronic exercise on circulating CAC. Many investigations have revealed increases in circulating number of CAC in response to exercise. Although increased CAC in the blood are related to decreased rates of CVD and improved endothelial function and are therefore clinically relevant, we hypothesize that physical activity influences intrinsic CAC cellular characteristics that may be critical in the promotion of vascular health and repair. In this paper, we will review new data supporting the hypothesis that acute exercise and exercise training influence changes in CAC biology that may have implications for endothelial repair. Specifically, we will show that improvements in intercellular nitric oxide (NO), decreases in oxidative stress-generating factors, and promotion of signaling for cellular proliferation are influenced by physical activity in CAC. We also discuss the necessity of future studies to directly determine whether enhancement of CAC characteristics through acute and/or chronic exercise lead to improved vascular repair and function. Elucidating the nature of improvements in cellular characteristics of CAC with acute and chronic exercise will help us to understand cell behavior such that physical activity may be used in conjunction with cell therapy to optimize the effectiveness of treatment for CVD.

CIRCULATING ANGIO-SUPPORTIVE CELLS

The development of new blood vessels, or angiogenesis, and reendothelialization were traditionally believed to occur...
via existing vascular and endothelial cells. On the other hand, vasculogenesis is the formation of new vessels from stem cells or progenitors. For the past decade, circulating cells have been identified with the capacity to differentiate into endothelial cells and incorporate into the sites of angiogenesis. These cells, termed endothelial progenitor cells (EPC), have their origin in the bone marrow and target the vascular endothelium as indicated by the cell surface markers CD34+ and VEGFR2+, respectively, and/or their characteristics after in vitro culture, such as the formation of tube-like structures, or formation of vessels in vivo. Endothelial progenitor cells have been studied extensively with respect to their role in endothelial regeneration and repair, and researchers are becoming increasingly interested in these cells for their therapeutic potential. In addition to neovascularization and reendothelialization, these cells have been implicated in tumor vascularization, wound repair, and recovery from cardiac and limb ischemia.

Current evidence suggests that the process of vascular maintenance and repair likely is accomplished by a heterogeneous group of cells, with some playing a direct role in vessel formation and others having a more supportive role. In particular, the term EPC is now considered a broad term that may apply to a number of specific cell subpopulations. Two subpopulations of EPC, termed early and late EPC, have been studied extensively and seem to perform their repair functions in distinct ways. Early EPC form colonies in culture after approximately 1 wk and promote vascular repair by secretion of proangiogenic growth factors at sites of endothelial injury but have a low capacity to self-replicate and do not contribute to the formation of new vessels in vitro or in vivo. Conversely, late EPC form colonies after extended time in culture (~2–4 wk), have robust proliferative capacity, and contribute directly to neovascularization (34).

Additional circulating mononuclear subpopulations with angiogenic potential that display substantial overlap with EPC in their origin and phenotype have been discovered. For example, some subpopulations of T-cells and monocytc macrophages coexpress endothelial antigens, home to sites of endothelial injury, and secrete angiogenic growth factors in a manner similar to EPC (12). A commonly used putative EPC colony-formation assay (10) has been shown to consist of early EPC and angiogenic T-cells that are necessary to support EPC colony formation and endothelial cells through the secretion of cytokines (12). Finally, although EPC are commonly thought of as being bone marrow derived, the vessel wall itself has been implicated as a source of circulating late EPC (35). Taken together, we believe that the currently available information related to the origin and phenotype of these different cell types warrants substantial caution when interpreting data of a given study as well as in the choice of terms to describe the cells under investigation. As the previous evidence suggests, there is likely more than one population of cells that contributes to vascular repair, and as such, we hypothesize that physical activity likely influences the function and contribution of each cell type. Therefore, for this review, we use the term circulating angiogenic cells in a broad sense to refer to any circulating mononuclear cells that support vascular repair and reendothelialization. Current data indicate that these cells comprise, at least, the following: 1) bone marrow–derived stem/progenitor cells, 2) proangiogenic macrophages and T-cells, and 3) circulating cells that originate from the vessel wall itself. Excellent reviews that further detail the definition and function of CAC can be found elsewhere (11,34).

**COMPONENTS OF REGENERATIVE POTENTIAL**

The regenerative potential of CAC is likely to be determined by multiple factors. These may include liberation from their niche compartment, differentiation into a mature cell phenotype, proliferation or expansion, cell survival, recruitment (homing) to a site of action, and engraftment (Fig. 1). In addition, as previously mentioned, CAC may assist vascular

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**Figure 1.** The efficacy of CAC to support vascular growth and regeneration is dependent on the successful participation in several functions. Exercise has the potential to affect each separately, in combination, or all of these functions. Characterization of the mechanisms by which exercise may influence CAC function may help optimize cardiovascular regenerative medicine.
repair via an indirect humoral-like response in which cells release angio-supportive factors to encourage regeneration. Therefore, the efficacy of CAC-induced repair relies on the coordinated and successful function through these processes. Although the liberation and number of CAC in circulation is important and has been related to cardiovascular risk (10), strategies to maintain and/or improve proper cell function and survival within the environments they reside (origin and target tissue) and travel should be a primary focus for cell therapy.

Exercise has the potential to influence any one, a combination, or all of these processes. The majority of the literature on CAC and exercise to date details the enumeration of cells in circulation. The appearance of CAC in the bloodstream most likely represents the balance between the liberation of cells from their niche compartment, incorporation into the target tissue, and degradation. With respect to cell number in circulation, exercise could influence any or all of these factors, having potential to influence the niche compartment regulation of CAC emergence, degradation as cells travel through the circulation, or engraftment at the terminal site of action.

Some studies have examined changes in CAC function by analysis of cell migration, or chemotaxis toward a stimulus such as vascular endothelial growth factor (VEGF). Chemotaxis is an important measure of cell function because a multitude of factors (such as chemical sensing via receptor-mediated signaling, actin-based molecular motor function, and cellular energy systems) must be intact for proper migratory activity. Similarly, cell functions, such as differentiation and proliferation, require coordinated and controlled sensing, signaling, and response. Studies only recently have begun to address the mechanisms by which exercise influences the survival and function of CAC.

**CAC AND PHYSICAL ACTIVITY**

Regular physical activity is associated with decreased risk of development of atherosclerosis and CVD, improvement in CVD risk factors, and improved endothelial health and vascular function in patients with CVD. In general, exercise, both acute and exercise training, has been found to stimulate increases in circulating CAC in healthy subjects and patients with ischemic coronary artery disease and CVD risk factors, although there are few studies that lend insight into the mechanisms and signaling that connect exercise with CAC release and action. Three central questions for optimizing cell therapy with exercise are as follows: 1) do various sub-populations of CAC with different angiogenic capacity have distinct responses to exercise? 2) What are the effects of CVD and/or CVD risk factors on the CAC response to exercise? 3) Can exercise reverse the dysfunction observed in CAC in patients with disease.

**Acute Exercise**

The number of CAC in the blood has been demonstrated to increase in response to acute exercise in healthy volunteers (18), patients with CVD risk factors (21), and overt CVD (1). However, particularly in healthy volunteers, not all studies have reported an acute exercise-induced CAC response. Important factors, such as characterization of CAC kinetics after exercise, the influence of exercise on various CAC populations, and effects of variations of intensity and duration of exercise, still are being resolved in both healthy and patient populations.

In younger healthy individuals, it seems that during a prolonged bout of exercise, CAC number increases progressively and rapidly decreases toward baseline values as soon as 30 min after the acute exercise (18). Our group, however, did not observe any significant increase in CAC number in older healthy men 30 min after an acute bout of treadmill exercise (16). Thijssen et al. (27) found that different CAC populations responded differently to acute exercise in healthy men and that the increase in cells expressing the hematopoietic stem cell marker CD34+ was greater in younger men compared with older men 10 min after exercise. Therefore, it seems that the exercise-induced appearance of CAC may be blunted in older healthy populations. However, the kinetics of the response of various CAC populations to acute exercise in older individuals and clinical populations has yet to be resolved.

There is evidence to suggest that various subpopulations of CAC, defined by different cell surface markers, respond differently to acute exercise. This could be due to factors, such as differential release from the niche compartment; cellular differentiation; or selective degradation, survival, and/or engraftment of CAC. In healthy participants, CD34+ CAC increased to a greater degree compared with CAC positive for the marker CD133 during acute exercise. However, CAC positive for CD133 (CD133+ or CD133+/CD34+) remained elevated in the blood longer than CAC without (CD34+ or CD34+/ KDR+) after the bout of cycling exercise (18). In volunteers with various CVD risk factors, CAC (CD133+/VE-Cadherin+) increased nearly four-fold 5–10 min after exhaustive treadmill or bicycle exercise; CAC that were positive for CD133 and negative for VE Cadherin, which are early progenitors, increased only 40%, and cultured CAC that express monocyte/macrophage markers increased 2.5 fold (21). These results may indicate either greater release of a differentiated cell population or cellular differentiation in response to acute exercise. Furthermore, this work demonstrates that monocyte/macrophage CAC subpopulations clearly are increased with an acute bout of exercise in people with CVD risk factors. At this time, the regenerative potential and roles of the various CAC populations are not clear entirely. However, understanding whether exercise preferentially leads to the liberation of cells with higher angiogenic capacity, or cells that have a more supportive or enhanced paracrine role, and the fate of particular cell types involved is important to determine how exercise could be coupled with cell therapy to benefit patient outcomes.

Circulating angiogenic cells from patients with disease are known to be dysfunctional compared with those from healthy patients. Therefore, factors relevant to CAC biology and function may be different in healthy patients compared with patients with disease. More research is necessary to understand factors that may improve CAC function in clinical populations. For example, the mechanisms of CAC liberation in response to acute exercise may be different in patients with disease compared with healthy individuals. Vascular endothelial growth factor is recognized as a stimulus for CAC liberation. Adams et al. (1) quantified the response of CAC...
to an acute bout of maximal exercise in patients with CAD with exercise-induced ischemia, asymptomatic patients with no exercise-induced ischemia, and healthy age-matched controls. They found that CAC number increased in response to maximal exercise in CAD patients with exercise-induced myocardial ischemia, and the increases were correlated with the increases in plasma VEGF levels. However, CAD patients without exercise-induced myocardial ischemia and healthy subjects did not display a significant increase in CAC number, and they also did not increase plasma VEGF levels. These data may indicate that, although exercise and VEGF each have been shown to increase CAC, an exercise-induced increase in VEGF may influence CAC liberation only in certain patient populations.

Although there are several studies on the effects of acute exercise on the number of CAC in the blood, there are relatively few on the effects of acute exercise on CAC functional capacity in patients with CVD. Recently, it was reported that acute exercise improved CAC migration in severe and mild congestive heart failure patients to a level similar to that evident in healthy controls (29). Therefore, even in patients with disease, acute exercise can have profound effects on the function of CAC, which could represent a potentially important mechanism to improve vascular deficits associated with heart disease.

**Exercise Training**

Exercise training is the accumulation of repeated bouts of acute exercise. In this way, exercise training is recognized as a regular intermittent stress stimulus that elicits an adaptive response. Whereas an acute bout of exercise seems to improve CAC in CVD patients and may therefore serve as a useful method to improve cell therapy, understanding whether exercise training will continue to deliver beneficial effects on CAC is relevant for exercise prescription.

When evaluating whether CAC respond to exercise training, the effects of CVD and/or CVD risk factors on the CAC response to exercise, and whether exercise reverses the dysfunction observed in CAC in patients with disease, some evidence indicates that the effect of exercise training on CAC may be enhanced in individuals with disease compared with healthy populations. Exercise is well known for its multitude of beneficial effects on health status, particularly influences on each CVD risk factor independently. However, exercise also is a global stimulus that influences multiple systems simultaneously. Given these facts, in patients with cardiovascular-related diseases, who likely have several compounded CVD risk factors, exercise training may elicit a more robust improvement in CAC compared with healthy individuals. In fact, Thijsen et al. (27) reported no change in resting CAC in healthy young and older participants after 8 wk of moderate cycle exercise training. Similarly, we found no difference in CAC numbers between healthy older inactive and active men (31). However, several studies have shown beneficial effects of exercise training on CAC in clinical populations.

In patients with disease, most studies have shown improvements in circulating cell number at rest after exercise training. Laufs et al. (15) found in patients with stable coronary artery disease (CAD) that CAC number increased 78 ± 34% and CAC apoptosis decreased 41 ± 11% with 4 wk of exercise training. In patients with heart failure, Sarto et al. (23) reported a significant increase in CAC number and CAC colony-forming capacity with 8 wk of supervised aerobic training and a return to baseline of CAC number 8 wk after discontinuation of aerobic training demonstrating a close relationship between exercise training and detraining and the number of CAC in this population.

Improvements in CAC with exercise training in patients have been associated with markers of improved vascular function. For example, Steiner et al. (25) found that 12 wk of exercise training in patients with asymptomatic CAD and/or CVD risk factors resulted in a 2.9 ± 0.4-fold increase in CAC that was correlated with an increase in flow-mediated dilation and NO synthesis. Recent reports in patients with chronic heart failure show that exercise training increased CAC migratory activity, which was related to improvements in endothelial function (30). Therefore, although current evidence supports only an association between number of CAC and endothelial function, it is possible that exercise improves vascular function at least in part through improvements in CAC.

Recent work by Van Craenenbroeck et al. (30) revealed that the increase in CAC function with acute exercise before training was absent after training in these heart failure patients. Our group recently studied the importance of the
repeated acute bout of physical activity. We showed that when highly active older men with a long-term history of endurance exercise stopped training for 10 d, the number of CAC drops off precipitously (Fig. 2) to or below the level evident in chronically sedentary men of the same age (31). Therefore, although CAC demonstrate robust changes in response to acute exercise in patients with disease, adaptations with exercise training lead to an absence of the response to acute exercise, similar to results seen in healthy populations. It is an attractive hypothesis to speculate that the adaptation to repeated bouts of acute exercise leads to sufficient repair in patients with disease such that they demonstrate a CAC phenotype similar to healthy individuals.

One of the most important questions for the potential of exercise to enhance cardiovascular regenerative cell therapy is whether training leads to enhanced CAC efficacy to engraft to the sites of injury or angiogenesis. Because of methodological and ethical issues with tracking CAC in humans, the influence of exercise training on successful CAC engraftment has, for the most part, been shown by indirect measures. For example, a correlation between improvements in CAC number or function and beneficial changes in endothelial function is consistent with the possibility that training partly improves endothelial function through CAC-mediated vascular repair. In a mouse model of vascular injury, compared with sedentary animals, those exposed to exercise training had significantly improved angiogenesis (15), and aged mice exposed to swim training exhibited enhanced homing of implanted CAC compared with sedentary mice (2). Therefore, we have limited evidence from studies in animals and correlational evidence from humans that exercise may improve homing of CAC to cardiovascular tissues.

**PUTATIVE MECHANISMS**

**Nitric Oxide and CAC**

Many studies have addressed the effects of acute and chronic exercise on CAC; however, the mechanisms by which exercise stimulates the mobilization and altered function of CAC remains to be determined, as well as whether these mechanisms differ between healthy and diseased individuals. Nitric oxide (NO) has been well characterized with respect to endothelial function, and deficits in the NO system are related to endothelial dysfunction and CVD.

Flow-mediated dilation, a measure of peripheral vascular NO bioavailability and endothelial function, is improved with acute exercise and exercise training. The effects of exercise training on endothelial function are seen most consistently in patients with CVD risk or overt CVD. In healthy populations, studies showing improvements in endothelial function with exercise training are less consistent and may be dependent on the intensity and duration of exercise training (7). A single bout of high-intensity acute exercise improves endothelial-dependent dilation in patients with metabolic syndrome and in the aortas of rats after an oxidative stress-related transient decline (8).

NO also seems to be an important factor in the liberation and function of CAC in health and disease (9,15). Flow-mediated dilation has been associated with CAC colony formation (10) and also is correlated with CAC mobilization with exercise (3). The relationship between endothelial function, exercise, and CAC was highlighted when we recently reported changes in CAC with short-term detraining that were related to dynamic changes in flow-mediated dilation (31). These results, along with data showing that the beneficial effects of exercise on the vascular endothelium operate largely through improvements in NO bioavailability, provide a strong rationale to further explore the contribution of NO to the regulation of CAC with exercise.

Liberation of CAC from niche compartments with exercise seems to be, at least in part, regulated by NO availability. Granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) are two cytokines known to be powerful stimulants of CAC release and are used to encourage CAC liberation in autologous cell therapy. Circulating angiogenic cell liberation with acute exercise is not accompanied by increased G-CSF or GM-CSF (21), providing evidence that other factors are involved with CAC appearance in the blood with exercise. Endothelial NO synthase (NOS) knockout mice (NOS3-/-) showed a greatly diminished increase of CAC in the blood after an acute bout of exercise compared with wild-type mice (15). In humans, systemic infusion of the NOS inhibitor, L-NMMA eliminated the exercise-induced appearance of CAC in the blood (3). Local NO in the bone marrow compartment may be responsible for CAC liberation with exercise, perhaps in response to changes in blood flow; however, this has yet to be proven.

For CAC to contribute to reendothelialization and angiogenesis, intrinsic CAC function must be maintained. Circulating angiogenic cell functional capacity has been measured via cell migration or chemotaxis, differentiation, and incorporation into tube-like structures, with endogenous NO potentially playing a significant role in the maintenance of all of these functions. It has been established that CAC migration in patients with CVD related to endothelial dysfunction is deficient (29). New data reveal that endogenous NO inhibition in CAC decreased, whereas the addition of an NO donor recovered migratory activity (9). This group also showed that CAC from CVD patients lacked endothelial NOS (eNOS) expression compared with CAC from healthy participants, and deficient migration of CAC to VEGF in patients was recovered as the result of treatment with an NO donor. Endogenous NO production also can be affected by asymmetric dimethylarginine (ADMA), which inhibits NO synthase. In patients with CAD, plasma ADMA was associated with CVD severity, decreased CAC, and impaired CAC colony formation. These authors showed that ADMA diminished endogenous CAC NO, and in vitro ADMA exposure diminished CAC differentiation and incorporation into tube-like structures (28). Therefore, as in mature endothelial cells, there is strong evidence that endogenous NO plays a central role in CAC function, influencing differentiation, migratory capacity, and incorporation into vascular-like structures.

Exercise is related to improvements in CAC function in healthy and diseased persons. Acute exercise elicited an acute improvement in CAC migratory capacity, and exercise training in the same group elicited a 77% increase in migration...
that was related to improved endothelial function in patients with heart failure (30). Work from our laboratory showed that CAC eNOS messenger RNA (eNOS mRNA) and intracellular NO (NOi) are greater at rest in active trained compared with inactive untrained men (Fig. 3), revealing an effect of exercise training (14). The CAC eNOS mRNA was not changed with an acute bout of exercise in either group; however, NOi increased after acute exercise in inactive men, whereas in trained men, in whom endogenous NO levels were higher, there was no further increase in NO with acute exercise. These data show that endogenous CAC NO can be increased via chronic exercise training and, in inactive persons, by an acute bout of exercise. Importantly, it has yet to be determined whether alterations in CAC NO with exercise represent a central mechanism contributing to improving CAC function in patients with disease and the optimal exercise prescription for enriching CAC NO levels.

Oxidative Stress and CAC

Oxidative stress has emerged as an important mechanism in the development of endothelial dysfunction, atherosclerosis, and progression of CVD. Oxidative stress occurs with disruptions of the redox balance, leading to damage to DNA and lipids, cellular senescence, and death. Reactive oxygen species also are recognized as cell signaling factors for growth, survival, and apoptosis. Oxidative stress influences CAC function, and numerous investigations have demonstrated oxidative stress–related deficiencies in CAC in patients with various cardiovascular diseases (33). Notably, highly atherogenic oxidized LDL (oxLDL), a marker for CVD, has been investigated for its deleterious effect on endothelial cells and CAC (17) because of the presence of the oxidized lipoprotein receptor, lectin-type oxidized LDL receptor 1 (LOX1), on the surface of these cell types. Although oxLDL and CAC are related to CVD, and exercise diminishes circulating oxLDL (24), we reported in a healthy population that plasma oxLDL was a significant predictor of CAC colony-forming capacity independent of physical activity (31). This leaves open the possibility that exercise may decrease oxLDL load in patients with CVD, which may have a positive influence on CAC function.

Similar to other cells with regenerative potential, CAC exhibit higher expression of the antioxidant enzymes, such as catalase, glutathione peroxidase, and manganese superoxide compared with differentiated mature cells. This characteristic may make them uniquely suited to survive in areas of vascular damage with high oxidative stress (4). However, there is evidence that CAC subpopulations may be differentially sensitive to oxidative stress, and cellular age may play a role in CAC sensitivity to oxidative stress (13). Regardless, the antioxidative capacity of CAC is clearly related to CAC function. For example, the inhibition of CAC antioxidant enzymes increased CAC reactive oxygen species and decreased CAC survival and migration (4). In addition, mice deficient in GPX-1, the gene that encodes glutathione peroxidase, had decreased CAC function, decreased angiogenesis in response to hindlimb injury, and increased sensitivity to oxidative stress-inducing peroxide to induce EPC apoptosis (6). The antioxidant capacity of CAC may be an important characteristic for the paracrine function of CAC. Yang et al. (32) recently reported that human umbilical vascular endothelial cells incubated with CAC and exposed to oxidative stress displayed less apoptosis and increased expression of several antioxidant enzymes compared with human umbilical vascular endothelial cells incubated without CAC. Therefore, the beneficial effects of improved antioxidant capacity in CAC may encourage cell survival and function in CAC themselves as well as other cells involved in the regenerative process.

Exercise training is hypothesized to enhance antioxidant capacity and confer a positive effect on the vascular endothelium. Chronic physical activity has been repeatedly shown to preserve or improve systemic antioxidant capacity in association with endothelial function. However, there is little direct evidence that physical activity alters CAC antioxidant capacity. Our data have shown that drastically changing physical activity via detraining in highly active men elicited changes in CAC senescence that were significantly related to changes in plasma total antioxidant capacity (31). However, we also found similar levels of expression of various antioxidant genes in CAC from young active and inactive participants at rest and in response to acute exercise (14). We do not know whether CAC from older individuals display lower antioxidant activity or whether chronic exercise training in older men has the potential to influence the antioxidant capability.
expression in CAC in a similar fashion to its effects on preserving vascular endothelial function and aortic antioxidant expression with age (5). Furthermore, it has yet to be revealed whether CAC antioxidant capacity is altered with aging and chronic exercise and whether exercise plays a role in paracrine antioxidant enrichment of other angiosupportive cells.

There is a great deal of crosstalk between oxidative stress and the NO pathway as a number of oxidative stress factors have been shown to influence NO bioavailability. For example, oxidized LDL can be taken into endothelial cells via the LOX1 receptor and activate the enzyme nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase), a major source of oxidative stress in endothelial cells, or uncouple eNOS (22). The NADPH oxidase activation in patients with disease has been shown to influence CAC function, and exercise reduced oxidative stress and NADPH oxidase in aortas from older rats compared with sedentary animals (5). We found significantly greater expression of CAC gp91<sup>phox</sup>, the major catalytic subunit of NADPH oxidase, in inactive men compared with active men, which was reduced with an acute bout of exercise in both groups (Fig. 3B). To test whether changes in CAC NO were dependent on NADPH oxidase, cells from active and inactive men before and after an acute bout of exercise were cultured in the presence or absence of a NADPH oxidase inhibitor. The data revealed that endogenous NO increased with NADPH oxidase inhibition in the inactive group to a similar extent as occurred with acute exercise, but the effects were not additive (Fig. 4). Although eNOS and NOi were higher in the active group at baseline, there was no change in eNOS mRNA in either group with exercise, implicating an NADPH oxidase-dependent mechanism for the inactive men and training-induced enhancement of CAC NO in the active group (Fig. 3A). Therefore, preliminary studies implicate NO, oxidative stress, and their interaction in the CAC responses with acute exercise and exercise training. For patients with disease, who likely have high levels of cardiovascular NADPH oxidase activity and oxidative stress, the acute and chronic effects of exercise on NADPH oxidase, oxidative stress, and CAC NO could be significant for improving CAC regenerative potential (Fig. 5). Furthermore, given our data and the recent results indicating that exercise acutely reverses CAC dysfunction in CHF patients and NO mediates a variety of CAC functions, we propose the hypothesis that restoration of impaired NO-dependent CAC function will be a mechanism by which preconditioning with acute exercise and posttherapy exercise training will increase the efficacy of cell therapy in future clinical trials.

**The Role of Thrombin**

Although thrombin is known for its role in fibrin formation and platelet activation, thrombin also has been shown to be involved in angiogenesis. Both mature endothelial cells and CAC express the receptor for thrombin, protease activated receptor 1 (PAR-1), and the angiogenic actions of endothelial cells are regulated through the PAR-1 receptor. Thrombin influences CAC differentiation, migration,
and proliferation (26); therefore, we have begun to explore the potential role of thrombin as a signaling molecule for CAC function with acute exercise and exercise training.

In mature endothelial cells, thrombin has a mitogenic effect influencing angiogenesis via endothelial cell proliferation. Plasma thrombin levels are known to increase with acute exercise. We exposed CAC cultured from inactive and highly active older men to various concentrations of thrombin to evaluate whether thrombin stimulated changes in the expression of genes related to cellular proliferation and differentiation. We found that gene expression of the cell cycle genes cyclin A2 and cyclin D1 was up regulated, and p27, a negative inhibitor of the cell cycle, was down regulated with thrombin treatments indicating activation of a proliferative gene program with thrombin exposure (16). We also measured gene expression and markers of thrombin production with an acute bout of strenuous exercise and found increased plasma thrombin and a similar proliferation gene expression pattern in CAC. Therefore, thrombin treatment and a thrombin-inducing acute bout of exercise appear to increase CAC proliferation gene expression.

Exercise training has been shown to influence homeostasis by improving sensitivity to environmental perturbations. For example, exercise training increases sensitivity to insulin such that a lower insulin output is necessary for the same glucose clearance from the blood compared with the untrained state. Thrombin production with acute exercise in inactive men was 3.6 times higher compared with highly active men (16) at the same relative intensity of exercise, which may reflect the exercise training-induced adaptation in thrombin production. We also observed increased expression of VE-Cadherin and VEGFR2, two markers of mature endothelial cells, in CAC from high-active men in response to low levels of thrombin treatment in vitro. These results show that markers of differentiation are expressed in CAC from older high-active men exposed to low levels of thrombin but not in CAC from older low-active men. These observations lead us to hypothesize that exercise training may increase sensitivity of CAC to thrombin to signal the expression of genes involved with differentiation. Therefore, our preliminary work reveals that thrombin production with exercise influences CAC proliferation, and exercise training may influence the sensitivity of CAC to thrombin signaling for differentiation. It is possible that exercise training could improve the sensitivity of CAC to other signaling molecules, thereby improving homeostatic balance. Production of cells with enhanced capability to respond to perturbations in the environment should be a goal for the optimization of cell therapy that likely can be achieved through exercise.

CONCLUSIONS AND FUTURE DIRECTIONS

CAC are recognized as contributors to the regeneration of the cardiovascular system. Harnessing and optimizing the potential of CAC to repair cardiovascular tissues has been challenging as researchers work to understand the identity, phenotypes, and actions of these cells. Regardless, regenerative therapy with CAC is likely to become an available option in the future. The benefits of exercise on the cardiovascular system in patients with disease are well recognized. Exercise has the potential to improve several cellular characteristics important for the successful navigation of CAC from their source to their terminus. With data from our laboratory and others on the beneficial influence of acute exercise and exercise training on CAC and the potential mechanisms involved, we can envision the use of exercise as an accompanying modality to cardiovascular regenerative cell therapy to enhance CAC and the efficacy of treatment. However, because there are few data on the effect of exercise on CAC in patients, one of the major future directions in this effort should be to determine whether exercise could reverse CAC dysfunction observed in individuals with cardiovascular-related diseases. In addition, we only have begun to understand the ways in which exercise influences CAC and the potential mechanisms underlying these responses. Gathering information on timing of delivery and optimal prescription of acute exercise and/or exercise training may enhance the efficacy of exercise-assisted cell therapy with CAC in future trials. Finally, new populations of cells that may contribute to vasculogenesis and maintenance of the endothelium continue to emerge (i.e., adipose-derived stem cells). Future studies will continue to evaluate their contribution to overall vascular health and the influence of exercise on these populations.

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