

## REVIEW ARTICLE

# Endothelial Regenerative Capacity and Aging: Influence of Diet, Exercise and Obesity

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**Abstract: Background:** The endothelium plays an important role in cardiovascular regulation, from blood flow to platelet aggregation, immune cell infiltration and demargination. A dysfunctional endothelium leads to the onset and progression of Cardiovascular Disease (CVD). The aging endothelium displays significant alterations in function, such as reduced vasomotor functions and reduced angiogenic capabilities. This could be partly due to elevated levels of oxidative stress and reduced endothelial cell turnover. Circulating angiogenic cells, such as Endothelial Progenitor Cells (EPCs) play a significant role in maintaining endothelial health and function, by supporting endothelial cell proliferation, or *via* incorporation into the vasculature and differentiation into mature endothelial cells. However, these cells are reduced in number and function with age, which may contribute to the elevated CVD risk in this population. However, lifestyle factors, such as exercise, physical activity obesity, and dietary intake of omega-3 polyunsaturated fatty acids, nitrates, and antioxidants, significantly affect the number and function of these circulating angiogenic cells.

**Conclusion:** This review will discuss the effects of advancing age on endothelial health and vascular regenerative capacity, as well as the influence of diet, exercise, and obesity on these cells, the mechanistic links and the subsequent impact on cardiovascular health.

**Keywords:** Diet, obesity, endothelial regeneration, progenitor cells, angiogenesis, exercise.

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## ARTICLE HISTORY

Received: February 26, 2018  
Revised: June 20, 2018  
Accepted: June 22, 2018

DOI:  
10.2174/1573403X14666180726112303

## 1. INTRODUCTION: THE AGING ENDOTHELIUM

The inner lining of all blood vessels consists of a single monolayer of endothelial cells. These cells play a key role in diffusion and transport of nutrients, gases from the blood to surrounding tissues, as well as being central to the control of blood flow *via* the endothelium's ability to secrete vasoactive substances, such as Nitric Oxide (NO) and prostacyclin (PGI<sub>2</sub>). The endothelium also plays a role in our immune system, whereby it controls the adhesion, rolling and trans-endothelial migration of leukocytes to sites of tissue damage and/or infection. The maintenance of the endothelium is key for optimal health, and specifically cardiovascular health, as endothelial dysfunction often precedes Cardiovascular Disease (CVD).

Advancing age is associated with endothelial dysfunction [1-4], which is highly predictive of cardiovascular event risk and mortality [5, 6]. Aging is also associated with increased endothelial susceptibility to apoptosis [7]. These aging effects are potentially due to elevated levels of vascular tissue oxidative stress [8] which may contribute to uncoupling of endothelial NO Synthase (eNOS) [9], key for NO bioavailability *via* the conversion of L-arginine to NO. Elevated

levels of pro-oxidant free radicals, such as superoxide, have been found in vascular tissue from aged compared to younger rats [8]. This elevated production of superoxide leads to the formation of peroxynitrite [10], which has been observed to stimulate the uncoupling of eNOS [9].

A recent meta-analysis also demonstrated that peripheral vascular resistance is also elevated in aging populations, with negative alterations in smooth muscle function in older compared with younger men and women [11]. Upon specific dilator administration, such as nitroglycerine or sodium nitroprusside, older adults display reduced dilator capacity, indicative of reduced smooth muscle function. This has been attributed to decreased expression of soluble guanylyl cyclase in smooth muscle cells [12], attenuating the cell's ability to relax, subsequently leading to impaired vasodilation and peripheral blood flow. It is clear that the deleterious impact of aging on vascular resistance is due to, in part, alterations in endothelial NO release, as well as smooth muscle function, but there are also data to suggest that changes in vascular resistance due to age may be related to abnormal responses to the metaboreflex [13].

In addition, angiogenic capabilities are reduced with advancing age in both mice [14-17] and human studies [18], which may contribute to the increased CVD risk amongst the elderly [19, 20] due to insufficient repair or replacement of damaged endothelial cells. This is

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highlighted in animal models, with the ability to re-vascularisation in response to vascular trauma or occlusion is reduced with age [21, 22], suggestive of an impaired endothelial regenerative capacity.

## 2. ENDOTHELIAL REGENERATION AND ADVANCING AGE

It was previously thought that endothelial cell turnover was wholly maintained by the proliferation of vascular resident endothelial cells. However, in 1997, researchers discovered a circulating cell subset which had the ability to differentiate into mature endothelial cells *in vitro* [23], and these researchers termed these cells 'endothelial progenitor cells' (EPCs). These cells were human CD34<sup>+</sup> cells, and after a period of 7 days in culture expressed mature endothelial cell markers (VEGFR2, CD31, E-selectin, eNOS). These cells could also form tubes on fibronectin-coated plates *in vitro* [23, 24]. A number of studies have shown that such EPCs have the ability to stimulate neovascularization in rodent [25, 26] and human models [21, 27]. However, the origin of these cells has been widely debated. Some studies show that these CD34<sup>+</sup> vasculogenic progenitors are derived from the bone marrow using tracking models [25, 26], however, there is some evidence to suggest that progenitor cells within tumour vasculature did not derive from bone marrow [28].

These cells may maintain endothelial integrity and health *via* differentiating into mature endothelial cells, therefore replacing damaged or apoptotic endothelial cells, or *via* paracrine means by secreting vasculogenic growth factors such as VEGF and IL-8 [29]. However, *via* cellular tracking, EPCs from humans transplanted into a mouse hindlimb ischemic model were found to stimulate neovascularisation and were later found incorporated in the injured vasculature [21], suggesting that the integrity of the endothelium may be partly dependent upon the reparative capacity of such EPCs [30]. It is now generally accepted that circulating EPCs that act in a paracrine manner, or as genuine endothelial precursors, are phenotypically distinct, with the former expressing CD34, CD133, being CD45<sup>bright</sup> as well as expressing an endothelial cell surface antigen, such as VEGFR2 or CD31 [29, 31]. Circulating CD34<sup>+</sup> progenitors that have been shown to have potential to differentiate into mature endothelial cells express CD34, dimly express CD45 (CD45<sup>dim</sup>), lack CD133 expression whilst also expressing endothelial cell surface antigens [29, 31]. These two distinct phenotypes of EPCs have been termed 'early' and 'late' outgrowth endothelial cells because of the time of their appearance in culture. Early-outgrowth endothelial cells (EOC) appear early in culture, and function primarily *via* paracrine means, whereas late outgrowth endothelial cells (LOC) appear late in culture and have the ability to differentiate into endothelial cells *in vitro* [29]. Together, both EOC and LOC can be considered as contributing to the maintenance of endothelial cell integrity, just *via* differing means. For this review, EOC and LOC will be grouped together as 'EPCs'. For a more in-depth review on EPC subsets and physiological functions, see review by Medina *et al.* [32].

Circulating EPCs are rare in peripheral blood, often making up to 0.05% of all mononuclear cells in humans

[33], however, despite their small number, they remain, independent predictors of endothelial function [34, 35], and mortality in patient populations [36, 37], with lower numbers, often reflecting endothelial dysfunction and heightened cardiovascular mortality risk. Many studies have demonstrated lower circulating number and function of EPCs in vascular-related disease states (such as stroke, cerebrovascular disease, atherosclerosis) compared to age-matched healthy controls [30, 34, 35, 38-47]. The reduction in these cells in the circulation may be due to an exhaustion of the bone marrow progenitor cell pool due to an increased need for vascular repair [46], and increased apoptosis of these cells [43, 48].

Older adults display reduced number and function of circulating EPCs [21, 27, 49-54] which may play a role in the increased CV risk with advancing age [20]. Advancing age is linked with reduced vascular repair mechanisms, as observed by Torella *et al.* [22] who found that endothelial repair after balloon injury in a rat model was significantly reduced in older *vs.* younger rats. Our laboratory has shown that older adults display significantly reduced circulating angiogenic cells compared to younger counterparts, independent of several cardiometabolic risk factors (*e.g.* fasting glucose, triglycerides, LDL, HDL) [54]. Thijssen *et al.* [49] also observed reduced circulating CD34<sup>+</sup>VEGFR2<sup>+</sup> EPCs in old (67-76 years) *vs.* younger men (19-28 years), but the reasons for these differences remain unclear.

EPC function appears to be affected also by advancing age. EPC migration, proliferation and tube forming capacity is reduced in older individuals [21, 27, 50-53, 55-57]. In an elegant study, Xia, Yang [21] took human EPCs from young and older adults, and investigated their re-endothelialization ability in a hindlimb ischemia model in mice, and found that transplanted EPCs from older adults did not stimulate endothelialization or recovery of perfusion to the same extent as transplanted EPCs from younger individuals. The underlying mechanisms explaining the age-related reduction in both EPC number and function are still unclear. It is highly likely that a combination of age-related increases in oxidative stress [58], bone marrow niche alterations [59], telomere shortening [56] and other circulating factors [60] may explain these observations.

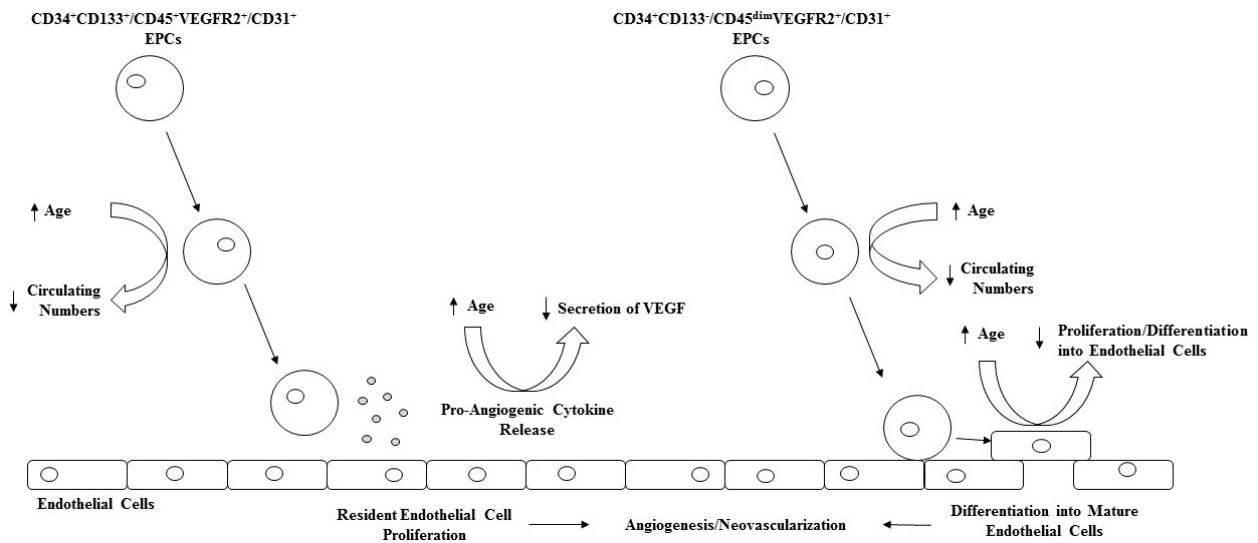
Together, this data strongly suggests a deleterious effect of aging on EPC number and function (Table 1 and Fig. (1) for a summary of the effect of age on EPC number and function), and studies have investigated the effect of pharmacological interventions to improve EPC number and function in at-risk individuals [61-64]. However, as a preventative measure, lifestyle modifications may hold significant promise as these cells are significantly affected by lifestyle factors such as smoking [65, 66], physical activity/inactivity, and exercise [67-69].

In this review we will cover the influence of various dietary factors on EPC number and function, and the potential negative impact obesity has on EPCs, finally reviewing the literature on dietary strategies to induce weight loss, and the subsequent impact this may have on circulating EPCs to promote cardiovascular health in an at-risk, aging population.

**Table 1. Influence of age on circulating endothelial progenitor cell number and function.**

References	Subjects	EPC Assay	Findings
Xia <i>et al.</i> , 2012a [21]	10 young, 10 older males.	Flow cytometry CD34 <sup>+</sup> VEGFR2 <sup>+</sup> EPC migration and adhesion Human EPC re-endothelialization in mice	Lower CD34 <sup>+</sup> VEGFR2 <sup>+</sup> cells in elderly. Reduced migration, adhesion and re-endothelialization capacity in elderly vs. young males.
Xia <i>et al.</i> , 2012b [27]	25 young, 22 elderly males. Resting	Flow cytometry CD34 <sup>+</sup> VEGFR2 <sup>+</sup> /CD133 <sup>+</sup> VEGFR2 <sup>+</sup> EPC migration and adhesion Human EPC re-endothelialization in mice	Lower CD34 <sup>+</sup> VEGFR2 <sup>+</sup> /CD133 <sup>+</sup> VEGFR2 <sup>+</sup> cells in elderly. Reduced migration, adhesion and re-endothelialization capacity in elderly vs. young males.
Thijssen <i>et al.</i> , 2006 [49]	8 young, 8 older sedentary males.	Flow cytometry CD34 <sup>+</sup> VEGFR2 <sup>+</sup>	Lower CD34 <sup>+</sup> VEGFR2 <sup>+</sup> EPCs in older vs. younger males
Thum <i>et al.</i> , 2007 [50]	10 young, 16 middle-aged, 12 older males.	Flow cytometry CD133 <sup>+</sup> VEGFR2 <sup>+</sup> EPC migration and eNOS gene expression.	Lower EPC number and migration in older vs. middle-aged and younger males. Lower EPC eNOS gene expression in older vs. younger adults.
Heiss <i>et al.</i> , 2005 [51]	20 young and 20 older male and female subjects.	Flow cytometry CD34 <sup>+</sup> VEGFR2 <sup>+</sup> /CD133 <sup>+</sup> VEGFR2 <sup>+</sup> EPC survival, migration and proliferation assays.	No difference in EPC number between young and older subjects. Lower survival, migration and proliferation of EPCs in older subjects.
Hoetzer <i>et al.</i> , 2007 [52]	10 young, 15 middle-aged, 21 older men.	EPC EC-CFU assay. EPC migration	Lower EC-CFU in older and middle-aged adults compared to young subjects. Lower migration of EPCs from older subjects vs. middle-aged and younger adults.
Williamson <i>et al.</i> , 2013 [53]	EPCs from 5 young, and 4 older subjects.	EPC apoptosis, migration, and tube formation assays	No difference in proliferation, apoptosis and tube formation of EPCs from young and older subjects. EPC migration lower in older subjects vs. younger subjects.
Ross <i>et al.</i> , 2018 [54]	107 males, aged 18-75yrs.	Flow cytometry CD34 <sup>+</sup> CD45 <sup>dim</sup> VEGFR2 <sup>+</sup> Cell surface expression of CXCR4	Age inversely associated with EPC number and cell surface CXCR4 expression.
Yang <i>et al.</i> , 2013 [55]	10 young, 10 older male subjects.	Flow cytometry: CD34 <sup>+</sup> VEGFR2 <sup>+</sup> EPC migration and proliferative assays.	Lower EPC number, migration and proliferation in older vs. younger subjects.
Kushner <i>et al.</i> , 2009 [56]	12 young, 12 middle-aged, and 16 older sedentary males.	EPC telomere length	Lower EPC telomere length in older vs. middle-aged and younger males.
Kushner <i>et al.</i> , 2010 [57]	17 young and 20 older males.	Stimulated release of EPC-derived pro-angiogenic cytokines and growth factors	Lower release of G-CSF from EPCs from older vs. younger subjects.

EPC- Endothelial Progenitor Cells, eNOS- endothelial nitric oxide synthase, EC-CFU- Endothelial Cell Colony-Forming Units. CXCR4- C-X-C Chemokine Receptor 4.



**Fig. (1).** Effect of aging on circulating endothelial progenitor cell number and vasculogenic function.

### 3. NITRIC OXIDE-MEDIATED MOBILIZATION OF EPCS: POTENTIAL FOR DIETARY NITRATE

Recently, the therapeutic role of dietary nitrate (in the form of beetroot [as a root vegetable or in concentrated form], watercress, and spinach) in vascular health has been explored due to the potential to modulate NO bioavailability. Acute and chronic supplementation of inorganic dietary nitrate has been shown to improve arterial vasomotor function [70-72], reduce blood pressure in healthy young [73] and older subjects [72] and reduce arterial stiffness as measured *via* pulse wave velocity [72]. The potential mechanisms by which dietary nitrate may improve these vascular health markers include an increase in NO bioavailability. Once ingested, nitrate is reduced to nitrite in the mouth and gut [74], where it can be absorbed into the circulation. Elevations of plasma nitrate and nitrite are observed as quickly as within 2 hours of ingestion of a high concentrated nitrate dose (in the form of beetroot juice) which subsequently results in significant alterations in both systolic and diastolic blood pressure within this timeframe [75, 76].

Recent epidemiological evidence suggests that nitrate has a strong positive effect on human health. In 2017, researchers found that plasma nitrate was inversely associated with all-cause mortality in the Offspring cohort of the Framingham Heart Study [77]. Interestingly, there was no such association with incidence CVD mortality, with their data also suggesting that the effect of plasma nitrate on mortality was attenuated after controlling for glomerular filtration rate, suggestive of a protective effect on renal function. In mice, 3 months of nitrate deficient diet resulted in greater visceral adiposity, reduced glycaemic control and vascular function [78]. Levels of eNOS were downregulated in the mice fed with the nitrate-depleted diet which may contribute to the reduced vascular function in these mice.

In addition to having an impact on endothelial function *via* modulating NO bioavailability, dietary nitrate may also impact on circulating angiogenic cells. The mobilization of EPCs has been shown to be eNOS dependent [79], and

additionally, NO itself can mobilise these cells *via* activation of bone marrow matrix metalloproteinase-9 [80] which itself cleaves membrane-bound Kit ligand from bone marrow stromal cells, leading to the extravasation of progenitor cells into the circulation [81]. This led researchers to investigate if dietary nitrate can influence progenitor cell number and function. Indeed, the ingestion of a single dose of nitrate-rich solution led to the mobilisation of  $CD34^+VEGFR2^+$  and  $CD133^+VEGFR2^+$  cells into the circulation within 1 hour in healthy humans, which was accompanied by increases in Stem Cell Factor (SCF) and stromal-derived factor-1 $\alpha$  (SDF-1 $\alpha$ ) [82]. Within the same study, this effect was abolished with the co-infusion of a NO scavenger (cPTIO) in a mouse model. A chronic supplementation study in hypercholesterolemic rabbits found that supplementing with L-arginine, the precursor to NO synthesis led to a significantly greater number of circulating  $CD34^+VEGFR2^+$  EPCs than control hypercholesterolemic rabbits [83]. This data has been supported elsewhere, with a diet supplemented with L-arginine, in combination with exercise training, resulted in elevations in EPCs in mice, compared to exercise alone which also resulted in increases in circulating EPCs [84].

Together, these data suggest a potential role for nitrate diets to potentially mobilize EPCs from the bone marrow to maintain or improve vascular health. However, there is a paucity of data in humans, and in clinical conditions whereby such an intervention may have greater health implications. Future research must also include data on the functionality of such EPC populations to determine potential cellular effects outside of the bone marrow mobilisation itself.

### 4. OMEGA-3

Omega-3 polyunsaturated fatty acids (PUFA) have recently emerged as potential vascular protective foods. Omega-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are primarily found in oily fish, but also found in plant sources, such as nuts and seeds. Epidemiological data suggested that the ingestion of

omega-3 fatty acids may reduce CVD rates [85]. This study observed a 10x reduced risk of myocardial infarction amongst Greenland Inuits compared to a Danish population, which may be due to their vastly different intake of omega-3 fatty acids per day (14g vs. 3g) [86]. However, clinical trial data of the impact of omega-3 fatty acids on cardiovascular and all-cause mortality are mixed with regards to the efficacy of these fatty acids on health [87-90].

Omega-3 fatty acids may influence health through affecting plasma membrane phospholipid composition, which may impact cell signalling *via* altering membrane fluidity, lipid raft structure and substrate availability [91, 92]. DHA upregulates eNOS phosphorylation in human endothelial cells *in vitro* [93] and suppresses cytokine-induced endothelial adhesion molecule expression [94], suggestive of a potent vascular benefit. There is also evidence that both EPA and DHA can attenuate H<sub>2</sub>O<sub>2</sub>-induced DNA damage in human aortic endothelial cells *via* reductions in intracellular oxidative stress as a result of upregulated levels of heme oxygenase-1, thioredoxin reductase 1, and manganese superoxide dismutase [95]. However, the evidence for a strong effect on vascular endothelial function is absent in human studies [96].

Interestingly, omega-3 fatty acids may play a role in angiogenesis. Recent studies showed that in aged mice, a diet rich in omega-3 PUFA was associated with improved post-ischemic stroke angiogenesis and neurogenesis [97], and transgenic mice that overproduce n-3 PUFAs were protected against ischemic stroke, displayed enhanced post-ischemic angiogenesis and greater survival than control mice [98]. Potential contributions to the augmented revascularization may be due to enhanced VEGF signalling in resident endothelial cells [98] or *via* mobilization of angiogenic cells to the infarct zone and manipulation of their angiogenic functions. In an *in vitro* study, incubation with EPA or DHA significantly improved EPC colony forming units and tube formation of these regenerative cells *in vitro* [99]. However, migratory capacity of these cells, reflective of the ability to migrate to ischemic tissue *in vivo*, only improved upon co-incubation with both EPA+DHA [99]. These results were somewhat supported by Tikhonenko *et al.* [100] who found that supplementing with DHA in a type 2 diabetes mouse model rescues EPCs in blood and bone marrow, as well as displaying protective effect of DHA on EPC migration *in vitro* further suggestive of protective effect of omega-3 fatty acids on EPC number and angiogenic function [100, 101]. In only two human supplementation studies, an eight- and six-week fish oil supplementation period significantly increased the number of circulating CD34<sup>+</sup>VEGFR2<sup>+</sup> cells [102, 103], and also significantly reduced markers of vascular damage and platelet aggregation [103]. These changes in EPCs were not accompanied by changes in circulating biochemical markers of vascular health, such as total cholesterol, LDL, HDL, triglycerides or fasting glucose [103], suggestive of a direct effect on cellular survival [104] and/or mobilization. These effects of omega-3 fatty acids on EPCs are not long-lasting, as six weeks after cessation of the omega-3 fatty acid-rich diet, circulating EPCs returned to pre-diet levels [102].

These data strongly suggest despite not having clear benefits on vascular function in humans, omega-3-rich diets may augment the number and function of circulating EPCs which may have clinical significance for endothelial repair and may be of interest to older adults who display such EPC dysfunction.

## 5. MEDITERRANEAN DIETS

Mediterranean diets typically contain high levels of olive oil, fruits, nuts, vegetables and cereals, and often include moderate intake of fish and poultry, with low intake of red and processed meats. There is strong evidence that supports the use of a diet rich in olive oil, fruit, nuts, vegetables and low in red meats for the prevention of CV events and CVD [105, 106], with a meta-analysis indicating a reduced risk ratio for CV incidence or mortality, cancer incidence or mortality, and neurodegenerative disease incidence with for those adhering to such a diet [106]. The proposed mechanism for such effect on cardiovascular health may be due to specific effects on reducing atherosclerosis-associated inflammation [107], such as circulating high sensitivity C-reactive protein (CRP) and interleukin-6 (IL-6) [107, 108]. After 2 years of a Mediterranean diet, an improvement in endothelial function was observed, as well as a reduction in carotid intima-media thickness (cIMT) [107] and insulin sensitivity also improved significantly [107, 108].

One year of a 'Mediterranean' diet, rich in olive oil, fruit, vegetables, fish, legumes, and wholegrain foods improved vascular conductance in a group of older adults (mean age: 56 years) more so than a year-long exercise training intervention [109], indicating that the diet could be a beneficial strategy for preventing CV issues with aging, once again, potentially due to reductions in inflammatory biomarkers or improvement in antioxidant status [110, 111]. Considering also, the high omega-3 content of such a diet, potential vascular health benefits of a Mediterranean diet may be also due to the reductions in oxidative stress *via* the biological effects of EPA and DHA.

Several studies have investigated the impact of these types of diet on circulating EPCs in a variety of human populations (metabolic syndrome, type 2 diabetics, and the elderly), showing significant promise in modulating endothelial repair capacity. In those with type 2 diabetes mellitus, 4 years of the diet resulted in a significant increase in CD34<sup>+</sup>VEGFR2<sup>+</sup> and CD34<sup>+</sup>CD133<sup>+</sup>VEGFR2<sup>+</sup> EPCs at both year 2 and year 4 time-points [108]. There was an absence of any change in these markers of endothelial repair capacity in a parallel low-fat diet. The elevations in EPCs were concomitantly observed alongside reductions in inflammatory biomarkers CRP, and reductions in cIMT. Interestingly, the increases in EPCs were inversely associated with cIMT in the Mediterranean diet group [108]. After only 8 weeks, such a diet resulted in significant increases in CD34<sup>+</sup>VEGFR2<sup>+</sup> EPCs in individuals with the metabolic syndrome, however, this increase was superseded by a combination of diet plus exercise intervention over the same duration [112].

In an aging population of both men and women (>65yrs), a 4 week dietary intervention resulted in >100% increases in circulating CD34<sup>+</sup>CD133<sup>+</sup>VEGFR2<sup>+</sup> EPCs in participants undertaking a diet rich in olive oil, vegetables, and fish, as

opposed to a low carbohydrate diet enriched with PUFA, and a significant reduction in endothelial microvesicles (indicative of endothelial damage and/or activation) [113]. Once again, these changes were irrespective of cardiometabolic risk factor changes. Cesari *et al.* [114] found that circulating number of EPCs ( $CD34^+VEGFR2^+/CD34^+CD133^+VEGFR2^+$ ) were related to olive oil consumption, dietary vegetable servings and ‘Mediterranean diet score’ (a score of adherence to a Mediterranean diet devised by Panagiotakos *et al.* [115]) in a large population of nonagenarians. However, longer duration interventions in this aging population, as well as a functional assessment of endothelialization are lacking and thus are required to fully elucidate the impact of such diet on endothelial regeneration and repair. It must also be acknowledged that it is difficult to attribute the improvements in these vascular reparative cells to a certain aspect of the diet due to the wide variety of components of the diet.

## 6. PHYSICAL ACTIVITY AND EXERCISE EFFECTS ON ENDOTHELIAL PROGENITOR CELLS

Exercise and physical activity have potent cardiovascular effects. These include the prevention or reversal of plaque formation in the vasculature [116, 117], improved endothelial function [118-121], and angiogenesis [122-124] in a variety of human populations. Single bouts of exercise have the remarkable ability to stimulate the mobilization of EPCs from peripheral tissues such as the bone marrow, into the circulation for up to 72 hours post-exercise [54, 68, 125-129]. However, some studies have failed to show any changes in circulating EPCs in the post-exercise recovery period [49, 130]. The response to exercise is not only duration and intensity-dependent [127], but also dependent on human population investigated, with the evidence showing those with CVD [131-133], and older adults [54] display an attenuated response. EPC mobilization in response to exercise is said to be due to changes in circulating chemoattractants, such as VEGF, G-CSF and SDF-1 $\alpha$  [68, 129, 134], however, mechanistic studies in exercise and EPC mobilization are lacking.

It is not just single bouts of exercise which may have this profound effect on EPCs, but studies investigating the effect of regular exercise and physical activity on these cells generally report increase in EPC number and/or function [27, 52, 69, 122, 135-139], even in older adults [27, 52]. After a 3-month home-based aerobic exercise intervention, older men, who had displayed significantly reduced basal EPC number and migratory function, improved their EPC number and function nearly 2-fold [52]. Xia *et al.* [27] reported improvements in both *in vitro* and *in vivo* function of EPCs from older adults who had undergone a 12-week aerobic exercise program using a carotid artery injury mouse model. The researchers took EPCs from older adults before and after the exercise program and injected these into the left carotid of athymic nude mice after inducing carotid injury. Endothelial regeneration was evaluated by measuring the area of re-endothelialization in the denuded artery 3 days post-injection. The improvement observed in re-endothelialization due to EPCs from older individuals post-training was accompanied by improvements in intracellular CXCR4 signalling, which is key for EPC homing to sites of injury [41].

It is clear that single bouts and regular prolonged exercise can improve circulating number and function of these vasculogenic cells in humans. This improvement has been aligned with improvements in vascular function, and reduced arterial stiffness, offering a key mechanism by which exercise may benefit cardiovascular health in older populations. The potential effects of exercise and physical activity on EPCs in aging have been reviewed in depth elsewhere [67].

## 7. OBESITY

Obesity is heavily linked with the development of key variables of the metabolic syndrome and type 2 diabetes mellitus (T2DM). The worldwide incidence of CVD and metabolic abnormalities, such as T2DM is increasing, and obesity is a significant risk factor. Data suggests that those who are overweight or obese are 50-75% more likely to develop CVD than those who are ‘normal weight’ [140]. This is likely to be driven by inflammatory pathways, including adipose tissue-derived tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) [141], which may affect endothelial function specifically *via* activation of NADPH oxidase and subsequent production of superoxide [142]. Endothelial dysfunction with obesity precedes the development of atherosclerosis, with impaired vasodilator functions apparent [143], potentially as a direct result of impairments in the L-arginine-NO pathway. Therefore, obesity-induced endothelial dysfunction may be a primary cause of the increased CVD risk in this population.

Obesity may promote endothelial dysfunction *via* effects on endothelial regeneration and repair mechanisms, such as bone marrow-derived EPCs. Fadini *et al.* [144] observed a negative association between components of the metabolic syndrome, and  $CD34^+$  progenitor cell count, with accumulative scores of the metabolic syndrome strengthening this inverse relationship. Several studies report an inverse relationship between BMI and circulating total progenitor cells and EPC count [144, 145]. Furthermore, other studies have reported that obese men with metabolic syndrome had 40% fewer circulating EPCs than healthy age-matched controls [146]. Interestingly, EPC proliferative capacity reflected reductions in circulating EPCs in obese compared to lean individuals [147]. The same group showed that the *in vitro* pro-angiogenic function of EPCs was also impaired with obesity in 50+year-old individuals, with impaired stimulated the release of both VEGF and G-CSF, which may be linked to the finding that these EPCs displayed higher expression of caspase-3, a pro-apoptotic intracellular signal [148]. In a murine model of obesity, obese animals displayed impaired *in vitro* angiogenesis, suppressed EPC mobilization in response to limb ischemia, and reduced incorporation into aortic vessels after LPS-induced vascular damage [149], confirmed by other animal models also showing impaired recovery of blood flow after limb ischemia accompanying the reductions in ischemia-induced PC mobilization [150]. In humans, EPC adhesion, migration and angiogenesis *in vitro* were significantly lower than in lean individuals [151]. The ability of EPCs to home to sites of ischemia, adhere and migrate are key roles of EPCs in order for these cells to exert their vasculogenic function. These findings suggest that obesity suppresses the angiogenic potential of human EPCs to home to sites of vascular damage or tissue ischemia, and to promote blood vessel growth and repair.

There is clear evidence for obesity-mediated EPC dysfunction, which may be as a result of associated inflammation, impaired glucose tolerance and elevated oxidative stress. The resultant endothelial dysfunction and suppressed endothelial repair capacity increase the risk of atherosclerosis in this population. Interventions designed to stimulate weight loss may have significant health benefits by improving vascular endothelial health *via* modulating EPC number and functional capacity.

## 8. CALORIE RESTRICTION/WEIGHT LOSS DIETARY INTERVENTIONS TO COMBAT OBESITY-MEDIATED EPC DYSFUNCTION

Recently, calorie restriction diets have been touted as a potential intervention to improve health and enhance longevity [152]. Recent reports suggest that calorie restriction may reduce CVD risk by modulating oxidative stress levels [153], and DNA damage [154]. Such diets have been proven to be beneficial for weight loss in overweight and obese individuals [155, 156] due to the stark effects on reducing oxidative stress [157] and improving the metabolic profile of obese and older humans [156, 158-160].

A 24-week low carbohydrate diet resulted in significant reductions in endothelial damage biomarkers in overweight post-menopausal women despite no changes in metabolic profiles [161], suggesting vascular benefit effect of such a diet is independent of metabolic changes. Added to this, there is a wealth of evidence showing vascular function benefits of calorie restriction/weight loss diets in obese and older individuals [162-168]. Mechanisms include reductions in NADPH oxidase activity, increased activation of sirtuin-1, a powerful intracellular antioxidant complex [169], increased antioxidant capacity (increased levels of manganese superoxide dismutase) and increasing tissue eNOS content and NO bioavailability [170]. Furthermore, improvements in vascular function with weight loss strategies may be preceded by improvements in endothelial regenerative capacity.

Indeed, preliminary data showed that weight loss strategies may be beneficial for improving EPC number [171]. The extent of reductions in body fat composition in response to a weight loss diet relates to the extent of EPC improvement in humans [172]. Xin *et al.* [173] exposed mice to prolonged fasting after cerebral ischemia. They observed significant upregulation of the antioxidant enzyme MnSOD, as well as eNOS in bone marrow-derived EPCs, increased capillary number in the infarct zone, and improved EPC migratory and tube formation capacity in the fasted mice compared to control mice. These observations were accompanied by reductions in the volume of infarct zone, which was also further improved by intravenous administration of EPCs from fasted mice compared to control mice [173], strongly suggesting a protective role of periodic fasting to improve EPC vascular regenerative capacity. Interestingly, exercise and diet may act synergistically to promote EPC number and function in obese populations [174]. An 8-week combined exercise and calorie restricted diet resulted in significant improvements in circulating EPCs, and EPC migratory capacity in obese populations [174]. However, the effect of combined strategies in older adults is yet to be investigated

but may hold promise due to the already significant impact of exercise on EPC number and function [49, 54, 128].

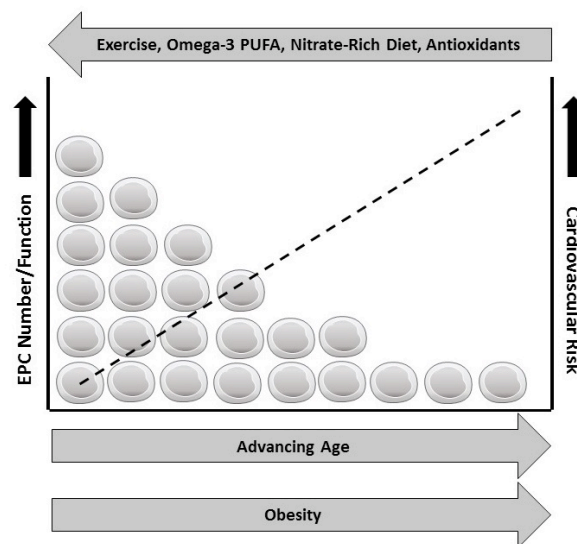
## 9. FUTURE DIRECTIONS

Currently, large-scale cohort interventional studies in dietary influence on vascular regenerative capacity are lacking, especially in aging adults with or without CVD, and are thus warranted. In addition, other angiogenic cell populations, such as angiogenic T-cells [175, 176] and mesenchymal stem/progenitor cells [177] are being investigated for their influence on endothelial function and repair through their potent pro-angiogenic capacity and may be targeted for such therapeutic interventions, such as diet and/or exercise.

Additionally, the role of physical activity and exercise for cardiovascular benefit is clear, however, more studies are required to elucidate the benefit for older, and frail populations who are specifically at-risk of CVD and vascular-related disorders.

## SUMMARY & CONCLUSION

Age-related increased CVD risk is due to a plethora of factors. Reductions in endothelial repair capacity *via* alterations in both EPC number and functions may explain the aging impairments in endothelial function, thus promoting atherosclerotic disease risk. However, lifestyle factors such as diet, exercise and obesity (Fig. 2) can have a significant impact on these vascular regenerative cells, and thus older populations may be able to attenuate CVD risk through lifestyle modifications.



**Fig. (2).** Possible effects of lifestyle factors on aging circulating endothelial progenitors and cardiovascular risk.

## CONSENT FOR PUBLICATION

Not applicable.

## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

## ACKNOWLEDGEMENTS

Declared none.

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