

## REVIEW

# Nutraceuticals and cardiovascular risk: potential role of EPCs modulation

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According to WHO cardiovascular diseases (CVDs) are the first cause of death in the world: more people die annually from CVDs than from any other cause. Vascular endothelium plays a pivotal role in the onset and progression of these pathologies and cardiovascular risk factors are frequently associated to the levels of endothelial progenitor cells (EPCs), bone marrow-derived circulating progenitors for the endothelial lineage. Since EPCs not only preserve vascular endothelium homeostasis, but might directly participate to re-endothelization and neovascularization, these cells represent an emerging protagonist in vascular competency and as such a cell model of great interest. An unhealthy diet is one of the main cardiovascular risk factor, while there is a great interest in the potential protective effects of "nutraceuticals", food-derived compounds that exert beneficial effects on human and animal health. The characterization of the endothelial effects of different nutraceuticals may provide fresh insights into their potential role in CVDs prevention. Several studies have already showed the protective effects of natural antioxidants on EPCs levels and functionality; some examples are resveratrol, catechin and folic acid. Fermentation has recently shown interesting roles in cardiovascular prevention since this process gave origin to a new class of foods, rich in bioactive compounds, the fermented foods. Consumption of fermented legumes and cereals, but also fermented beverages (such as beer and wine) was found to protect endothelial function through both lipid-lowering, as well anti-inflammatory and antioxidative mechanisms. Little is known about the effects of fermentation-derived nutraceuticals on EPCs. Given the important role of this cardiovascular biomarker, further analysis in this field can improve opportunities for CVDs prevention and treatment.

**Keywords:** endothelial function; endothelial progenitor cells; diet; antioxidants; cardiovascular diseases

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## Vascular endothelium and EPCs: new insights

For many years vascular endothelium has been considered a mere physical barrier between blood and underlying tissues; recent studies have demonstrated new and interesting roles of endothelium in vascular health <sup>[1]</sup>.

Endothelial cells composing this monolayer have therefore paracrine, endocrine and autocrine functions, by secreting

several factors with vasodilatory and antiproliferative effects, such as endothelium-derived hyperpolarization factor (EDHF), nitric oxide (NO) and prostacyclin (PGI<sub>2</sub>), and others inducing vasoconstrictor response, for instance endothelin-1 (ET-1), angiotensin II and reactive oxygen species (ROS) <sup>[2]</sup>.

These evidences make the vascular endothelium a multifunctional unit that regulates vascular homeostasis by

maintaining the equilibrium between vasodilation and vasoconstriction; when this finely regulated balance is compromised, endothelium dysfunction occurs.

Beyond setting of vascular tone and integrity, the maintenance of vascular health includes also regulation of angiogenesis, hemostasis, immune and inflammatory responses, vascular growth and remodeling <sup>[2]</sup>.

One key actor in vascular repair and homeostasis, is the endothelial progenitor cell (EPC) system, bone marrow-derived circulating progenitors for the endothelial lineage, first described in 1997 by Asahara and colleagues <sup>[3]</sup>. EPCs circulate from bone marrow to incorporate into vascular endothelium and thus contribute to postnatal physiological and pathological neovascularization <sup>[4]</sup>. EPCs not only ensure the correct turnover of endothelial cells, but are also involved in the development, repair and regeneration of the systemic vasculature, as demonstrated by Prater and colleagues <sup>[5]</sup>. Indeed, in this study, authors suggest that postnatal neovascularization does not rely exclusively on sprouting from preexisting blood vessels (angiogenesis).

During the last decade, significant research has been performed to elucidate the physiological role of EPCs in tissue repair and to correlate these cells to several disease states.

EPCs are mainly located within the stem cell niche in bone marrow, along with some circulating populations in the peripheral blood. The process by which EPCs contribute to new vessel formation in adults consists of four interrelated steps. When injury or tissue damage occurs, EPCs are mobilized from the bone marrow into the circulation and home to tissue repair sites under the induction of signals such as hypoxia, growth factors, chemoattractant, and chemokines. EPCs then invade and migrate at the same sites, and differentiate into mature endothelial cells (ECs) and/or regulate pre-existing ECs via paracrine or juxtacrine signals <sup>[6]</sup>. These progenitors also improved the activity of endothelial NO synthase (eNOS), thereby restoring the correct functionality of endothelium <sup>[7, 8]</sup>. The success of each step depends on the ability of EPCs to interact, adapt, and respond to multiple molecular stimuli <sup>[9]</sup>.

Thus EPCs, playing an important role in endothelium maintenance, re-endothelialization and neovascularization, represent an emerging actor involved in vascular fitness and as such a cell model of great interest <sup>[4]</sup>.

Despite the great enthusiasm aroused by these cells, several areas of controversy still persist in the study of EPCs, related to their identification, characterization and effective

role in vascular biology. In particular, the lack of an unique and specific EPCs marker and the functional and phenotypical overlap between these cells and hematopoietic and mature endothelial cells, make it very difficult and controversial to define EPCs real identity <sup>[10]</sup>.

### **Endothelium dysfunction and cardiovascular diseases: role of EPCs**

Functional impairment of vascular endothelium is found in cardiovascular diseases <sup>[11]</sup> and also in patients with type 2 diabetes, insulin resistance and obesity <sup>[12]</sup>; therefore the integrity and functional properties of the endothelial monolayer play a critical role in atherogenesis, while cardiovascular risk factors induce endothelial injury and a cascade of pro-inflammatory events <sup>[13]</sup>.

As mentioned before endothelium dysfunction is caused by an imbalance between vasoconstrictor and vasodilator molecules, and between pro-atherogenic and anti-coagulant states <sup>[14]</sup>. This pathological condition is characterized by one or more of the following features: impaired NO bioavailability, increased oxidative stress, enhanced cell turnover, increased production of growth factor, over-expression of adhesion molecules and inflammatory genes, hemodynamic deregulation and increased permeability of the cell layer <sup>[15]</sup>.

Several factors contribute to the outcome of endothelial dysfunction, for example high levels of LDL cholesterol, glucose, insulin, triglycerides and omega-6 to omega-3 ratio, low concentration of HDL and Vitamin D and all wrong life habits <sup>[16]</sup>. Most of these factors are related to diet. This is why a dietary approach may reduce the risk of cardiovascular disease, also by affecting and ameliorating the endothelial function.

The endothelium is an important target for CVDs therapy, since it is rapidly exposed to systemically administered agents establishing a link with the underlying tissues <sup>[1]</sup>. Recent studies have shown a strong correlation between endothelial progenitor cells levels and cardiovascular risk; these cells have been indeed proposed as novel biomarker of endothelial function <sup>[17]</sup>. Accumulated evidences suggest that a balance between the damaged effects of cardiovascular risk factors and the ability of circulating progenitors to improve endothelial function, affects the cardiovascular risk <sup>[17-19]</sup>.

Decreased amounts of EPCs are correlated with atherosclerotic disease progression and increased cardiovascular risk, thus supporting an important role for endogenous vascular repair in modulating the clinical outcome of CAD. Age, sex, exercise, smoking, lipid profile,

insulin resistance, inflammation, hypertension and genetic background have emerged as important factors correlated with EPCs number and function<sup>[20]</sup>. So, monitoring the levels of circulating EPCs as a surrogate biological marker might be specifically useful to identify novel approaches to enhance vascular repair capacity and thereby affect the progression of cardiovascular diseases<sup>[21]</sup>.

Several studies have shown the beneficial effect of drugs on EPCs, often isolated from patients with cardiovascular disease (CVD).

Treatment with statins, for example, leads to an increase in both number and functional activity of EPCs, thus contributing to the re-endothelization of damaged vessels<sup>[22]</sup>; moreover, a recent study of Fadini and colleagues showed that a brief statin withdrawal increases circulating EPCs and functional proangiogenic cells in T2D patients<sup>[23]</sup>. Treatment with sitagliptin, a dipeptidyl peptidase-4 inhibitor, increases the number of circulating CD34<sup>+</sup>CXCR4<sup>+</sup> cells, candidate markers for endothelial progenitor cells (EPCs) definition, by approximately 2-fold in patients with T2D<sup>[24]</sup>. Exendin-4, a human glucagon-like peptide 1 analogue, was shown to protect against ischemia in obese mice under hypoxic stress by enhancing angiogenesis capacity and circulating EPCs levels<sup>[25]</sup>.

Experimental evidence demonstrated an induction, by angiotensin-II, of senescence in endothelial progenitor, through an increase of oxidative stress; starting from these results, recent studies have shown the beneficial effect of the ACE inhibitors and also of angiotensin receptor blockers, on the mobilization and functional status of EPCs in conditions associated with vascular endothelial damage<sup>[26, 27]</sup>. Spigoni and colleagues demonstrated that also pioglitazone, an insulin-sensitizing agent currently used in type 2 diabetic patients, is able to increase both viability and tubule formation capacity of endothelial progenitor cells isolated from pre-diabetic patients (patients with IGT impaired glucose tolerance)<sup>[28]</sup>. Continuous infusion of insulin in T1D patients enhances EPCs number, suggesting a novel mechanism of vascular damage by glucose fluctuations<sup>[29]</sup>.

Even treatment with erythropoietin seems to have beneficial effects on the functional properties of EPCs in both animals and humans<sup>[30]</sup>, while a study of Iwakura and colleagues showed an increase in the mobilization of EPCs after treatment with estradiol<sup>[31]</sup>.

### **Endothelial health maintenance: the nutraceutical approach**

An "unhealthy" diet is one of the main causes of increased atherosclerotic cardiovascular disease in the industrialized countries. There is indeed a great interest in the potential cardiovascular protective effects of "nutraceuticals", food-derived substances that seems able to exert beneficial effects on health. Whether the endothelial dysfunction plays an important role in development and progression of atherosclerosis, the characterization of the endothelial effects of several nutraceuticals may provide important insights into their potential role in cardiovascular prevention<sup>[32]</sup>.

Several nutrients and phytochemicals in fruits and vegetables, including fiber, vitamins and antioxidant, could be independently or jointly responsible for the reduction in cardiovascular risk<sup>[33]</sup>. Since endothelial dysfunction is considered a risk factor for CVDs and an independent prognostic marker, maintaining correct endothelial function with dietary approaches has become a key therapeutic goal<sup>[34]</sup>.

Dietary nitrite supplementation, for example, has been suggested to treat vascular aging, by reducing oxidative stress and inflammation thus preventing CVDs<sup>[35]</sup>, omega-3 fatty acid supplementation improves endothelial health reducing CVD risk<sup>[36]</sup>, while L-arginine, a precursor of NO synthesis, is able to normalize endothelial function when long-term administered<sup>[37]</sup>. Since oxidative stress is one of the major risk factor of endothelial dysfunction, several studies were performed using antioxidant molecules in healthy or ill subjects.

Antioxidant vitamin E and C supplementation showed an improvement of endothelial function in non-obese T2D subjects<sup>[38]</sup>, while vitamin D intake improved vasculature and kidney function in postmenopausal women<sup>[39]</sup>. Lipoic acid, a naturally occurring antioxidant, reduced serum glucose in diabetic patients<sup>[40]</sup> and enhanced endothelial function in subjects with metabolic syndrome<sup>[41]</sup>.

Since EPCs play an essential role during endothelial homeostasis and repair, a healthy diet is probably able to affect endothelium health also by modulating the number and the functional activity of endothelial progenitors; active and functional EPCs are indeed able to better and faster counteract to all external stimuli and risk factors.

Ginkgo Biloba, for instance, showed in vitro beneficial effects on endothelial progenitors: treatment with its extract inhibits EPCs senescence, through augmentation of telomerase activity<sup>[42]</sup>. Moreover, folic acid supplementation modulates EPCs transcriptome in diabetic subjects<sup>[43]</sup>. Fish oil intake, in a different study, revealed no significant effect on vascular health<sup>[44]</sup>, while a recent research showed

beneficial actions a of fish-oil supplementation on cellular markers of endothelial function: increased numbers of EPCs and reduced numbers of EMPs (endothelial microparticles), thus favoring the maintenance of endothelial integrity<sup>[45]</sup>.

Recent studies focused on polyphenols, a large class of natural compounds abundant in fruits, vegetables and spices. Several researches<sup>[46, 47]</sup> have found an inverse correlation between polyphenols assumption and CVD risk and mortality. In fact such healthy effects may explain the protective action on CVD exerted by food and beverages rich in polyphenols such as wine and tea or fruit juice<sup>[48, 49]</sup>. The beneficial effects of polyphenols are mediated by many biochemical pathways; these compounds indeed improve endothelial function and increase NO release, modulate inflammation and lipid metabolism, protect against atherothrombotic events and platelet aggregation, and increase antioxidant status<sup>[33]</sup>. All these functions lead to normalization of vascular tone and also an overall antihypertensive effect<sup>[50]</sup>.

Epicatechin and catechin, two of the major and most investigated polyphenols, recently showed beneficial effects on patients with peripheral artery disease, by modulating endothelial activation induced by platelets, thus preventing atherosclerosis development<sup>[51]</sup>. A member of catechin family, epigallocatechin gallate, the most abundant polyphenol in green tea, seems to exert an insulin-mimetic action that counteract endothelial dysfunction and ameliorate insulin resistance in liver and skeletal muscle<sup>[52]</sup>. Since the main actions of polyphenols are based on their scavenging ability, recent both *in vivo* and *in vitro* studies focused on their protection from oxidative stress. Campos and colleagues, for example, showed that lemon grass polyphenols protect HUVECs (human umbilical vein endothelial cells) from oxidative stress induced by several stimuli<sup>[53]</sup>; another study analyzed the effects of olive oil polyphenols using an *in vitro* model that simulates T2D (ECV304 cells) and demonstrated that such compounds protect against endothelial dysfunction induced by high glucose and free fatty acids through modulation of nitric oxide and endothelin-1<sup>[54]</sup>. Although several polyphenols are well known nowadays, the most famous is still resveratrol, an important antioxidant in red wine, which probably contributes to the potential of this beverage to prevent cardiovascular diseases. Wang and collaborators recently showed that this compound not only increases number and activity of endothelial progenitor cells, but also delays their senescence by enhancing telomerase activity<sup>[55, 56]</sup>.

In recent years, many *in vivo* studies have been carried out, based on polyphenols-enriched diet; an improvement of

endothelial function was shown in diabetic mice<sup>[57]</sup>, in hyperlipidemic rats<sup>[58]</sup> and also in dyslipidemic pigs<sup>[59]</sup>.

Although the great interest on the single bioactive food component, such as resveratrol in wine, flavonoids in tea or allicin in garlic, one of the main challenge of agro-alimentary research is to understand the effective action of whole food on human and animal health, to further advice the composition of an healthy diet<sup>[60]</sup>. In order to fulfill this need, several clinical studies have been carried out to identify the different effects of single component and whole food, for example lycopene vs tomato<sup>[61]</sup> or resveratrol vs red wine<sup>[62]</sup>, with controversial results. Therefore further studies need to be carried out to fully understand all individual and synergistic effects of food components.

### Fermented food: a new class of nutraceuticals?

Shortly, the fermenting process can be defined as a set of chemical reactions that transform substances into simpler and more easily digestible compounds that activates digestive enzymes and friendly bacteria. This process has been always employed to get a new class of food, rich in bioactive compounds, the fermented food, which recently showed interesting roles in cardiovascular prevention.

Consumption of traditional fermented legumes, for example, has shown protective effects against CVD<sup>[63]</sup>, indeed, fermentation of legumes brings several advantages since it reduces the non-nutritional factors, improves nutrient digestibility and decreases their allergenicity<sup>[64]</sup>. This technological process also affects the biological functionality of legumes, as microbial enzymes perform the bioconversion of polyphenols into more active compounds<sup>[65, 66]</sup>. A recent study showed that fermentation enhances the content of bioactive compounds in kidney bean extracts, thus encouraging the consumption of fermented legumes as a source of healthy substances<sup>[66]</sup>.

*Phaseolus Vulgaris* species, known to decrease both LDL-cholesterol and oxidative stress, well-known risk factors for CVD, and able to exert beneficial effects on short-term satiety and weight loss<sup>[67]</sup>, demonstrated antistress and antioxidant effect *in vivo* when fermented<sup>[68]</sup>.

La Marca and colleagues showed that an extract obtained from a fermented powder of bean (*Phaseolus vulgaris L.*) tested on primary cultures of rat hepatocytes was able to regulate antioxidant and detoxifying enzymes through the Nrf2 pathway, inhibiting NF-kB activation and reducing H<sub>2</sub>O<sub>2</sub>-induced endoplasmic reticulum (ER) stress<sup>[69]</sup>.



Recently, a *Monascus*-fermented rice extract was found to protect endothelial function through lipid-lowering, anti-inflammatory and antioxidative mechanisms; this extract also affects C-reactive protein concentration<sup>[70]</sup>.

Black tea, the fermented type of tea, was recently shown to protect against endothelial dysfunction through oxidative and endoplasmic reticulum stress modulation<sup>[71]</sup>.

Moreover, HPLC analysis of samples obtained from fermentation procedures showed that the level of major tea catechins epigallocatechin gallate (EGCG) and epicatechin gallate (ECG) dropped increasingly to about 1/3 in the final product<sup>[72]</sup>. Also in kidney bean extracts HPLC analysis showed a huge increase of total bioactive compounds, in particular non-anthocyanin phenolic compounds, after fermentation<sup>[66]</sup>.

Epidemiological evidences demonstrate that moderate alcohol consumption, in particular of fermented beverages such as wine and beer, reduces the risk of morbidity and mortality from CVDs<sup>[73]</sup>; two recent studies suggest an involvement of endothelial progenitor cells in these healthy effects. Indeed, Hang and colleagues, showed an improvement of number and functional activity of EPCs after red wine intake through the enhancement of nitric oxide bioavailability<sup>[74]</sup>. In a further study, Chiva-Blanch and colleagues, compared the effects of moderate consumption of beer, non-alcoholic beer and gin on the number of circulating EPCs and EPC-mobilizing factors in subjects at high cardiovascular risk<sup>[75]</sup>; since non-alcoholic fraction of beer positively affects EPCs number, the authors suggested that the greater cardiovascular protection conferred by fermented beverage (such as beer or wine) is due to their high polyphenolic content.

A recent *in vivo* study, showed an augmentation of EPCs in healthy subjects after supplementation with a food supplement, Stem-Kine<sup>[76]</sup>, produced by fermentation of a combination of green tea, astragalus and goji berry extracts.

Another fermentation product, an extract of Zijuan Pu-erh tea, revealed an elevated antioxidant activity in vascular endothelial cells exposed to oxidative stress probably related to a different concentration of bioactive compounds such as polyphenols<sup>[77]</sup>. The same extract showed antiangiogenic effects on HUVECs and anticancer activity on HT-29 cells<sup>[78]</sup>.

Such evidences suggest a positive effect of food fermentation on endothelial progenitors number and functions.

In our study EPCs treatment with a fermented grain, Lisosan G, increased not only viability but also cell function through the enhancement of antioxidative defenses, involving Nrf-2 pathway<sup>[79]</sup>. Whole grain cereals contain a lot of nutrients and bioactive substances that have health-promoting effects and epidemiological studies extensively demonstrated that whole grains consumption correlates with a reduced risk of type 2 diabetes and cardiovascular diseases<sup>[80]</sup>.

In our work, we explored the effects of Lisosan G. This product not only presents the above mentioned features of cereals, but is also enriched in bioactive compounds such as flavonoids, phenolic components and alpha-lipoic acid, since it derives from a fermentation process<sup>[81]</sup>. There, we evaluated the viability and cell function of endothelial progenitors and showed beneficial effects on both properties.

Lipoic acid (LA) is a natural antioxidant synthesized in the mitochondria which is able to directly scavenge ROS, chelate metals and react with, and regenerate, other antioxidants such as Vitamins E and C<sup>[82]</sup>; it also showed anti-inflammatory properties and is soluble both in fat and water which allows it to travel to and to enter all parts of the body<sup>[83]</sup>. Recent studies proposed LA as protective agent against CVDs outcome and progression, since it reinforces natural defenses and resistance to several types of stressors<sup>[84, 85]</sup>. Although the mechanisms underlying these protective effects remain still unclear, a recent study of Ying and colleagues<sup>[86]</sup> demonstrated that LA activates eNOS in endothelial cells in a PI3-kinase/Akt-dependent manner, thus counteracting endothelial dysfunction.

We further suggest that Lisosan G ability to increase functional properties of this cardiovascular disease marker, is related to its components, such as linoleic and linolenic acids, alpha-lipoic acid, flavonoids and phenols.

## Conclusions

The area of nutritional modulation of the stem cell compartment seems to offer significant benefit in treatment of a wide variety of cardiovascular, metabolic and degenerative diseases.

Fruits, cereals, legumes and vegetables are currently recognized as a source of antioxidant compounds that ameliorate EPCs viability and function, thus preventing endothelial dysfunction.

However, given the commercial pressures associated with this largely unregulated field, we suggest that detailed

scientific investigations had to be performed before disease associated claims are released by the scientific community.

One area of recent interest in the biomedical field has been that of functional foods and nutraceuticals. While it is known that alteration of diet may modulate vascular responses, to our knowledge, little work has been reported to date on fermented foods and their capacity in improving levels and functions of circulating EPCs.

Fermentation is known to increase the bioavailability of minerals, proteins, peptides, antioxidants, flavanols and other organic molecules and several studies reported an association between fermented foods and human endothelial functionality and in particular EPC levels and function.

The mechanisms involved in these processes may be associated with a reduced production of free radicals and less oxidative stress, due to the protective effect of the antioxidant components of a fermented food rich diet.

In conclusion, consumption of fermented foods, enriching the diet of antioxidants thus reducing oxidative stress, improve the regenerative capacity of the endothelium as a result of a better balance between damage and repair.

### Conflict of interest

The authors have no conflict of interest to declare.

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### Abbreviations

CVDs: cardiovascular diseases; EPCs: endothelial progenitor cells; NO: nitric oxide; ROS: reactive oxygen species; ALA: alpha-lipoic acid; T2D: type 2 diabetes; T1D: type 1 diabetes; HPLC: High Performance Liquid Chromatography; HUVECs: Human Umbilical Vein Endothelial Cells; EMPs: endothelial microparticles; LDL: Low Density Lipoprotein; eNOS: endothelial nitric oxide synthase; ECs: endothelial cells.

### Authors contributions

Contributed to the writing of the manuscript: RR. Contributed to the revision and submission of the manuscript: LP. Read and approved the final draft: DL, VL,

MG and GP.

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