Nonpharmacological Treatment of Hypercholesterolemia Increases Circulating Endothelial Progenitor Cell Population in Adults

To the Editor:

Hypercholesterolemia represents a major cardiovascular risk factor because of its ability to promote and sustain proatherogenic inflammation of vascular wall.1 A reduction of number and activity of bone marrow–derived endothelial progenitor cells (EPCs) could participate in the development of vascular damage in hypercholesterolemic patients.2 Indeed, EPCs serve as a cellular reservoir to replace dysfunctional endothelium and to form a cellular patch at the site of denuding injury.1 According to this, the level of circulating EPCs predicts the occurrence of cardiovascular events and death from cardiovascular causes.4 Nonpharmacological treatment represents the first-line approach to primary prevention in hypercholesterolemia because of its effects on lipid profile and cardiovascular outcomes.5 Despite the wealth of evidence derived from epidemiological and interventional trials, there is limited understanding of the underlying molecular mechanisms. To clarify this topic, we evaluated whether or not changes in dietary habits, alone or in association with regular physical activity, were able to affect the number of circulating EPCs in patients with isolated hypercholesterolemia.

We studied 38 never-treated hypercholesterolemic patients (LDL cholesterol between 4.1 and 4.9 mmol/L) without additional cardiovascular risk factors and/or concomitant diseases, including clinical conditions in which neovascularization might be present, such as cardiovascular disease, retinopathy, wound healing, or cancer. Patients were consecutively recruited among those who met the above conditions in which neovascularization might be present, such as cardiovascular disease, retinopathy, wound healing, or cancer. Patients were consecutively recruited among those who met the above criteria and referred to our Outpatient Unit for Cardiovascular Prevention between October 2004 and June 2005. After enrollment, all patients were randomly assigned to a 4-week treatment period based either on diet alone (10F/10M, 46.8±8.3 years) or on diet+physical training (8F/10M, 47.8±6.2 years). For this purpose, patients were asked to exercise daily for 30 minutes (including 5-minute warm-up and cool-down periods during each session, respectively) on a bicycle ergometer at 50% to 70% of their maximum heart rate.6

At baseline and after the intervention period blood samples were taken for routine hematochemical check and assessment of circulating number of EPCs. For this purpose, mononuclear cells were isolated using Ficoll density-gradient centrifugation from 20 mL of peripheral blood, washed three times in PBS, resuspended in EGM-2 Bullet kit (Cambrex, Milan, Italy). Then, 10⁶ mononuclear cells per cm² were seeded on fibronectin-coated culture dishes (Becton & Dickinson). After 4 days of culture, nonadherent cells were discarded by washing with PBS while adherent cells were maintained in culture for further 3 days and then underwent cytochemical analysis. To confirm the EPC phenotype, adherent cells were incubated with Dil-labeled acLDL (Molecular Probes), at a concentration of 2.4 µg/mL for 1 hour at 37°C. Cells were then fixed with 1% paraformaldehyde for 10 minutes and incubated with fluorescein isothiocyanate (FITC)-labeled Ulex europaeus agglutinin I (Ulex-lectin; Sigma) at a concentration of 10 µg/mL for 1 hour. Dual-staining cells positive for both Dil-acLDL and FITC-labeled Ulex-lectin were judged as EPCs.7 EPCs were counted manually in 10 randomly selected microscopic fields by two independent investigators with an inverted fluorescent microscope (×20).

Compared with baseline, two factor analysis of variance on one factor demonstrated a significant increase of EPC number in both treatment groups (Figure, panel A). Increments of EPCs were more evident in patients on diet+physical activity than in those on diet alone (Figure, panel A). LDL cholesterol levels significantly decreased after 4 weeks under both nonpharmacological treatment strategies being the decrement more evident in patients on diet+physical activity than in those on diet alone (Figure, panel B).

Our study provides the first evidence that a nonpharmacological approach to hypercholesterolemia is associated with a significant increase of circulating EPC number in adults. This effect is more evident if dietary intervention is combined with physical training and mainly depends on LDL cholesterol reduction. In this regard, it is worth mentioning the recent demonstration that oxidized LDLs interfere with differentiation8 while increasing senescence9 of EPCs, thus potentially reducing their circulating pool. Because hypercholesterolemia is associated with increased lipid peroxidation,10,11 it is intriguing to speculate that a reduction of oxidative stress after nonpharmacological correction of hypercholesterolemia could have played a role in the observed increase in EPC population.

Considering the suggested critical role of EPCs in restoring and maintaining multiple endothelial functions and counteracting atherogenesis and acute vascular complications of atherosclerosis,2 our findings shed new light on the mechanisms underlying the observed benefits deriving from a healthy lifestyle in hypercholesterolemic patients.3

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