Nutrition, Metabolism & Cardiovascular Diseases (2019) **29**, 604–610

Available online at www.sciencedirect.com

Nutrition, Metabolism & Cardiovascular Diseases

journal homepage: www.elsevier.com/locate/nmcd

Mediterranean, but not lacto-ovo-vegetarian, diet positively influence circulating progenitor cells for cardiovascular prevention: The CARDIVEG study

F. Cesari [a], M. Dinu [b], G. Pagliai [b], A. Rogolino [a], B. Giusti [b,c], A.M. Gori [b,c], A. Casini [b,d], R. Marcucci [b,c], F. Sofi [b,d,e,*]

[a] Central Laboratory, Careggi University Hospital, Florence, Italy
[b] Department of Experimental and Clinical Medicine, University of Florence, Italy
[c] Unit of Atherothrombotic Diseases, Careggi University Hospital, Florence, Italy
[d] Unit of Clinical Nutrition, Careggi University Hospital, Florence, Italy
[e] Don Carlo Gnocchi Foundation, Onlus IRCCS, Florence, Italy

Received 6 December 2018; received in revised form 29 January 2019; accepted 14 February 2019
Handling Editor: A. Siani
Available online 26 February 2019

KEYWORDS
Mediterranean diet;
Vegetarian diet;
Progenitor cells

Abstract *Aim:* To evaluate the possible association between dietary habits and progenitor cells using data obtained from a randomized crossover trial using two different diets, lacto-ovo-vegetarian (VD) and Mediterranean (MD), the CARDIVEG study.
Methods and results: Eighty clinically healthy subjects with a low-to-moderate cardiovascular risk profile (61 F; 19 M; mean age: 50.7 ± 11.6 years) were randomly assigned to isocaloric VD and MD diets lasting three months each, and then crossed. The two diets showed no effects on endothelial progenitor cells and circulating endothelial cells but opposite effects on circulating progenitor cells. In fact, VD determined significant ($p < 0.05$) and negative changes on circulating progenitor cells, with an average geometric variation of −130 cells/10^6 events for $CD34^+/CD45^{-/dim}$, −80 cells/10^6 events for $CD133^+/CD45^{-/dim}$, and −84 cells/10^6 events for $CD34^+/CD133^+/CD45^{-/dim}$ while MD determined significant ($p < 0.05$) and positive changes for $CD34^+/CD45^{-/dim}$ levels, with a geometric mean increase of +54 cells/10^6 events. No significant correlations were observed between changes in progenitor cells and changes in inflammatory parameters during the VD phase. On the other hand, during the MD phase negative correlations between changes of $CD34^+/CD45^{-/dim}$ and interleukin-6 ($R = -0.324$; $p = 0.004$) as well as interleukin-8 ($R = -0.228$; $p = 0.04$) and monocyte chemotactic protein-1 ($R = -0.277$; $p = 0.01$), were observed. These correlations remained significant also after adjustment for confounding factors only for $CD34^+/CD45^{-/dim}$ and interleukin-6 ($\beta = -0.282$; $p = 0.018$) and monocyte chemotactic protein-1 ($\beta = -0.254$; $p = 0.031$).
Conclusions: MD, but not VD, reported a significant and positive effect on circulating progenitor cells in a group of subjects at low-to-moderate cardiovascular risk, probably acting through the modulation of inflammatory parameters.

* Corresponding author. Department of Experimental and Clinical Medicine, University of Florence, Largo Brambilla 3, 50134 Florence, Italy.
E-mail address: francesco.sofi@unifi.it (F. Sofi).

https://doi.org/10.1016/j.numecd.2019.02.005
0939-4753/

Introduction

Vascular endothelial function is essential for the proper maintenance of cardiovascular health in humans [1]. An increasing number of evidences indicate that circulating bone marrow-derived progenitor cells play an important role in regulating endothelial function since they have been shown to increase neovascularization, repair endothelial lesions, and improve endothelial function [2]. Indeed, these cells can be considered as active players in preserving integrity of the cardiovascular system and it has been suggested that their circulating levels represent a novel biomarker of future cardiovascular events [3].

Some studies have shown in recent years a beneficial effect of the diet on the number of endothelial progenitor and circulating progenitor cells [4–7]. In particular, it has been shown that the Mediterranean diet (MD) is able to determine a significant increase in the number of these circulating cells. More recently, a study conducted by our group in a large cohort of nonagenarians showed that adherence to the MD can significantly increase the number of endothelial progenitor and circulating progenitor cells, with a significant influence especially for some components of the diet such as olive oil and fruit and vegetables [4]. These food components are some of the categories of food in common with two of the most beneficial dietary models available, MD and lacto-ovo-vegetarian diet (VD).

In the last decades VD has attracted a growing interest in both scientific and lay communities [8]. The typical VD pattern includes all categories of food with the exception of direct animal products, i.e. fresh and processed meat, and fish. Some results have shown an important role of VD in reducing the risk of cardiovascular disease, probably mediated by abstinence of meat and meat products, that on the other hand are somehow linked to the occurrence of cardiovascular disease [9], but no data are available on the possible effect of the VD in modulating progenitor cells. Therefore, we aimed at this study to evaluate the possible effect of both VD and MD in the same group of clinically healthy subjects, using data obtained from a randomized controlled dietary intervention study, the CARDIVEG study [10].

Methods

Study population

The analysis presented in this paper is based on data obtained from 80 clinically healthy subjects (61 women, 19 men; mean age: 50.7 $\pm$ 11.6 years) enrolled on the frame of the CARDIVEG study, a dietary intervention study conducted with the aim of comparing VD and MD in the same group of subjects for cardiovascular prevention [10]. The study design and general characteristics of the participants are described in a previous article [11]. Briefly, clinically healthy subjects with a low-to-moderate cardiovascular risk profile according to the European Society of Cardiology were recruited to enter into a randomized, open, crossover dietary trial with two intervention periods each lasting 3 months. The intervention comprised two isocaloric diets, a lacto-ovo-VD and a MD. Both diets were hypocaloric with respect to the energy requirements of subjects, but isocaloric between them, and consisted of approximately 50–55% of energy from carbohydrate, 25–30% from total fat and 15–20% from proteins. The VD was characterized by abstinence to consume meat and meat products, poultry, fish and seafood, and flesh from any other animal, but including eggs and dairy products. The MD was characterized by the consumption of all the food groups including meat and meat products, poultry, and fish.

The primary outcomes were changes in total body weight, body mass index, and fat mass from baseline, while the secondary outcomes were changes on all the circulating cardiovascular risk parameters from baseline (lipid profile, glycaemic profile, oxidative stress profile, inflammatory profile, and progenitor cells). The study was approved by the Ethic Committee (SPE 15.054) of the Tuscany Region, Careggi University Hospital, was registered at clinicaltrials.gov (identifier: NCT02641834), and adhered to the principles of the Declaration of Helsinki and the Data Protection Act.

Compliance

Compliance to the VD was evaluated through unannounced telephone calls, during which a 24-h diet recall interview was conducted, and through a modified version of the National Health and Nutrition Examination Survey food questionnaire, with the aim of confirming the total absence of any animal flesh in the diet [10]. Adherence to the VD was defined as the absence of the consumption of any animal flesh, reported both through a 24-h diet recall and food frequency questionnaire. Compliance to the MD was evaluated at the baseline and during the follow-up visits, using the MD adherence score that was recently released and validated by our group [10]. Participants in the MD group were considered adherent if they reported $\geq$10 points in a scale ranging from 0 to 18.

Data collection

Data-collection and follow-up measurements were performed at the Clinical Nutrition Unit of Careggi University Hospital. All the participants were examined between 6:30 am and 9:30 am after an overnight fast. Participants were asked not to undertake strenuous physical activity on the day before the examination. The baseline assessment for both groups included a questionnaire on demographic information, risk factors, and comorbidities. All participants were asked to report the frequency (times per week), duration (months), and intensity of recreational and physical activities performed during the preceding year. A physical activity grade was derived for each participant based on frequency, type, and duration of the physical activity and described in terms such as absent or light (ie, inactive or either occasional walking or recreational activity only) and

moderate (ie, frequent recreational activity, regular walking for 30 min 3–5 times per week, or sporting exercise at least once a week). The grade was not a measure of the total time spent in physical activity; it was a relative qualitative measure of how much physical activity was undertaken.

Laboratory analyses

Venous blood samples were collected at baseline and at the end of each intervention phase in evacuated EDTA plastic tubes (Vacutainer, Beckton Dickinson, Plymouth, UK). Endothelial progenitor, circulating progenitor, and circulating endothelial cells were assessed using flow cytometry, as previously described [4].

Briefly, 200 μl of peripheral venous blood was incubated for 20 min in the dark with:

- Fluoresceine isothiocyanate (FITC)-labelled monoclonal antibodies against human CD34 (BD Pharmingen, San Diego, California, US)
- Allophycocyanin (APC)-labelled monoclonal antibodies against human AC133 (Miltenyi Biotec, Bergisch Gladbach, Germany)
- Phycoerythrin (PE)-labelled monoclonal antibodies against human VEGFR2-KDR (R&D Systems Inc, Minneapolis, US)
- Allophycocyanin-Cyanin7(APC-Cy7)-labelled monoclonal antibodies against human CD45 (Becton Dickinson, San Jose, US)
- PE-labelled monoclonal antibodies against human CD146 (BD Pharmingen, San Diego, California, US)
- FITC-labelled monoclonal antibodies against human CD31 (BD Pharmingen, San Diego, California, US)
- PerCP-labelled monoclonal antibodies against human CD61 (Becton Dickinson, San Jose, US)
- LDS751, a nucleic acid dye, (Molecular Probes, Invitrogen, Eugene, Oregon, US)

Mouse isotype-identical antibodies served as controls (Becton Dickinson, San Jose, CA, US). Red blood cells and platelets were subsequently lysed by NH_4Cl lysing solution (Autolyse solution; BioSource International, Camarillo, US). For analysis, 500,000 cells within the leukocyte gate were acquired using a FACSCanto analyser (Becton Dickinson, San Jose, US) and data were processed using BD FacsDiva software. Endothelial progenitor cells were identified through their expression of CD34, KDR, and CD133 and were considered as EPCs cells CD34+/KDR+/$CD45^{dim}$, CD133+/KDR+/$CD45^{dim}$ and CD34+/CD133+/KDR+/$CD45^{dim}$. Circulating progenitor cells were defined as cells forming a cluster with low side scatter and low-to-intermediated CD45 staining and positive for CD34+, CD133 + and CD34+/CD133+. Circulating endothelial cells were identified through their expression of CD146, and CD31 and were considered as cells CD146+/CD31+/CD45−/CD61−.

Pro- and anti-inflammatory cytokines were determined by Bio-Plex cytokine assay (Bio-Rad Laboratories Inc., Hercules, CA, US), according to the manufacturer's instructions.

Statistical analyses

The statistical package PASW 20.0 for Macintosh (SPSS Inc., Chicago, IL, US) was used. The results were expressed as mean ± standard deviation (SD), median and range, or geometric mean with 95% confidence intervals (CIs) as appropriate. Means were compared by the two-sample t-test or, when appropriate, by paired t-tests. Dichotomous variables were analysed by the chi-square test. Mann–Whitney test was used to test for comparisons among groups. Categorical variables were presented in terms of frequencies and percentages. All data were treated as paired samples from a crossover study. The 2 interventions were analyzed combining the results obtained in the 2 phases of both groups. The results were analyzed within each group using a 2-tailed Student's t test. Absolute change (mean baseline value subtracted from mean value after intervention) was estimated by an independent sample t test. The Spearman (r) test was used to estimate the correlation between the changes in progenitor cells and inflammatory parameters. To compare the effect of the 2 different diets, a general linear model, adjusted for age, gender, smoking habit, order of treatment and weight change was conducted. Because these tests assume normal data distribution, nondistributed data were transformed into logs, and further analyses were performed with the processed data. However, to facilitate interpretation, the log data were again converted to the original scale (antilog) and presented as geometric means with 95% CIs. Furthermore, a linear regression analysis was conducted to evaluate the relationship between changes in circulating progenitor cells and inflammatory parameters, after adjustment for age, gender, smoking habit, order of treatment and weight change. A P-value <0.05 was considered to indicate statistical significance.

Results

The study population comprised only subjects who reported an optimal adherence to the prescribed diets. Forty-one subjects were randomized to start with VD and 39 with MD. Baseline demographic and clinical characteristics of the study population according to the first dietary intervention are reported in Table 1. No significant differences were observed.

Regarding progenitor cells subjects did not report significantly different levels compared to the order of the first dietary intervention (Table 2). Analysing the possible variations obtained after 3 months of dietary intervention with VD and MD, a general linear model was conducted for repeated measurements after adjustment for possible confounders (Table 3). A significant difference was observed between the changes of all the three types of circulating progenitor cells obtained during VD and MD. In particular, VD determined significant and negative changes for circulating progenitor cells' levels, with an

Table 1 Baseline characteristics of the study population according to first dietary intervention.

	VD (n = 41)	MD (n = 39)	p
Age, years[a]	50 (24–70)	52 (26–74)	0.6
Females, n (%)	32 (78)	29 (74.4)	0.7
BMI, kg/m^2[b]	29.6 ± 4.8	30.9 ± 4.7	0.2
Obese (BMI >30), n (%)	16 (39)	21 (53.8)	0.2
Fat mass, kg[b]	31.1 ± 12.2	31.9 ± 10.9	0.3
Current smokers, n (%)	7 (17.1)	10 (25.6)	0.3
Absent or light physical activity, n (%)	16 (39)	18 (46.2)	0.5

MD = Mediterranean Diet; VD = Lacto-ovo-vegetarian diet.
[a] Median and (range).
[b] Mean ± SD.

average geometric variation of −130 cells/10^6 events (95% CI −196; −66) for $CD34^+/CD45^{-/dim}$, −80 cells/10^6 events (95% CI −140; −20) for $CD133^+/CD45^{-/dim}$, and −84 cells/10^6 events (95% CI -152; −18) for $CD34^+/CD133^+/CD45^{-/dim}$. MD, on the other hand, determined significant and positive changes for $CD34^+/CD45^{-/dim}$ levels, with a geometric mean increase of 54 cells/10^6 events (95% CI 2; 104). With regard to endothelial progenitor cells, MD phase reported a significant increase of $CD34^+/KDR^+$.

To study the possible mechanisms underlying the effects of diets on circulating progenitor cells' levels, correlation analyses were studied between all the changes of the different parameters investigated in the study. During the VD phase, no significant correlations were reported between the changes in progenitor cells and changes in both anthropometric parameters and the inflammatory parameters. During the MD phase, however, we were able to demonstrate a negative and significant correlation between changes in circulating progenitor cells $CD34^+/CD45^{-/dim}$ and interleukin-6 ($R = -0.324$; $p = 0.004$) (Fig. 1), interleukin-8 ($R = -0.228$; $p = 0.04$) and monocyte chemotactic protein-1 ($R = -0.277$; $p = 0.01$). Furthermore, a negative and significant correlation was observed between changes in circulating endothelial cells' levels and interleukin-8 only during MD ($R = -0.296$; $p = 0.008$).

After adjustment for possible confounding factors such as age, gender, smoking habit, treatment order and weight change, linear regression analysis confirmed the relationship between changes of $CD34^+/CD45^{-/dim}$ and interleukin-6 levels ($\beta = -0.282$; $p = 0.018$), and monocyte chemotactic protein-1 ($\beta = -0.254$; $p = 0.031$), as well as the relationship between changes in circulating endothelial cells and interleukin-8 levels ($\beta = -0.311$; $p = 0.009$). In contrast, the relationship between changes of $CD34^+/CD45^{-/dim}$ and interleukin-8 levels lost its significance ($\beta = -0.214$; $p = 0.179$).

Discussion

The present is the first study that investigated the possible effect of VD on the number of progenitor cells in a clinically healthy population. Based on a randomized controlled dietary intervention study aimed at comparing two diets in terms of cardiovascular prevention [10], we were able to demonstrate that the adoption of VD for 3 months did not result in significant improvement of progenitor cells, instead reporting a significant and negative influence on all three types of circulating progenitor cells. On the other hand, we were able to confirm that the adoption of a MD leads to a significant increase in circulating progenitor cells, further supporting the beneficial role of MD in the maintenance of vascular function.

The CARDIVEG study was a randomized controlled open crossover study aimed at comparing for the first time two of the most beneficial diets, namely VD and MD, for the cardiovascular prevention of clinically healthy subjects at low-to-moderate cardiovascular risk profile [10]. The main results of this study were that the two diets were substantially similar in reducing the cardiovascular risk profile, with different effects on the lipid profile, oxidative stress parameters and inflammatory cytokines. In particular, VD determined a greater reduction of total cholesterol, LDL-cholesterol, and oxidative stress, while MD showed a more significant effect on triglycerides and inflammatory cytokines.

Regarding the secondary endpoints of the study, we measured the number of progenitor cells in a subgroup of subjects. This because both endothelial progenitor and circulating progenitor cells have been labelled as good surrogate indicators of cardiovascular health since they appear to constitute a natural system for the maintenance of vascular function, improving endothelial repair and

Table 2 Baseline endothelial progenitor and circulating progenitor cells according to first dietary intervention.

	VD (n = 41)	MD (n = 39)	p
CPCs			
$CD34^+/CD45^{-/dim}$, cells/10^6 events	650 (250–1132)	680 (168–1462)	0.603
$CD34^+/CD133^+/CD45^{-/dim}$, cells/$10^6$ events	566 (156–1018)	618 (166–1396)	0.421
$CD133^+/CD45^{-/dim}$, cells/10^6 events	566 (156–1200)	646 (166–1396)	0.326
EPCs			
$CD34^+/KDR^+$, cells/10^6 events	12 (0–58)	10 (0–50)	0.350
$CD133^+/KDR^+$, cells/10^6 events	12 (0–56)	10 (2–50)	0.463
$CD34^+/CD133^+/KDR^+$, cells/10^6 events	10 (0–50)	10 (0–50)	0.421
CECs, cells/10^6 events	4 (0–24)	6 (0–20)	0.950

CPCs = Circulating Progenitor Cells; EPCs = Endothelial Progenitor Cells; CECs = Circulating Endothelial Cells; MD = Mediterranean Diet; VD = Lacto-ovo-vegetarian diet.

Table 3 Baseline endothelial progenitor and circulating progenitor cells according to dietary intervention.

	VD pre (n = 41)	VD post (n = 41)	p	MD pre (n = 39)	MD post (n = 39)	p	p (Δ_{MD} vs. Δ_{VD})[a]
CPCs							
$CD34^+/CD45^{-/dim}$, cells/10^6 events	700 (640–760)	570 (506–632)*	<0.0001	608 (548–668)	662 (595.2–730)	0.041	<0.0001
$CD133^+/CD45^{-/dim}$, cells/10^6 events	598 (544–650)	516 (462–572)*	0.008	552 (496–608)	562 (510.6–614)	0.668	0.013
$CD34^+/CD133^+/CD45^{-/dim}$, cells/$10^6$ events	640 (578–704)	556 (490–622)*	0.014	590 (532–648)	624 (558–692)	0.191	0.004
EPCs							
$CD34^+/KDR^+$, cells/10^6 events	14 (12–16)	16 (12–20)	0.451	11(8–14)	14 (12–18)*	0.005	0.351
$CD133^+/KDR^+$, cells/10^6 events	12 (10–16)	14 (12–18)	0.09	12 (10–14)	14 (10–16)	0.345	0.553
$CD34^+/CD133^+/KDR^+$, cells/10^6 events	12 (10–14)	14 (10–18)	0.131	11 (9–14)	12 (10–16)	0.102	0.775
CECs, n (%)	6 (4–8)	4 (2–6)	0.831	6 (4–8)	6 (4–8)	0.277	0.942

CPCs = Circulating Progenitor Cells; EPCs = Endothelial Progenitor Cells; CECs = Circulating Endothelial Cells; MD = Mediterranean Diet; VD = Lacto-ovo-vegetarian diet.
Data are reported as geometric mean and 95% confidence interval (CI).
General linear model adjusted for age, sex, smoking habit, randomization order and weight change.
*$p < 0.05$ for paired t-test.
[a] Independent t-test.

neovascularization [2–4]. After incorporation into the vascular system, they are able to differentiate into mature endothelial cells and release angiogenic growth factors that act in a paracrine fashion to support local angiogenesis and mobilize residual tissue progenitor cells.

In recent years some studies have suggested that diet can lead to an improvement in cardiovascular health, not only in the mediation and reduction of cardiovascular risk factors, but also in the increase in surrogate indices such as inflammatory parameters and progenitor cells [4–7]. The first finding to support the possible influence of diet on circulating progenitors that could explain indirect beneficial mechanisms on cardiovascular disease comes from a study by Foo et al. who suggested that a high-protein diet promotes atherosclerosis through other pathophysiological mechanisms in addition to the modification of classic cardiovascular risk factors [12]. They studied mice fed a Mediterranean-like diet or a high-protein diet and found that mice that fed a high-protein diet had almost twice the level of the arterial plaque as mice fed a Mediterranean-like diet. The classic risk factors did not differentiate these two groups of mice, but the mice that fed a high-protein diet had markedly less circulating endothelial progenitor cells than mice fed a Mediterranean-like diet.

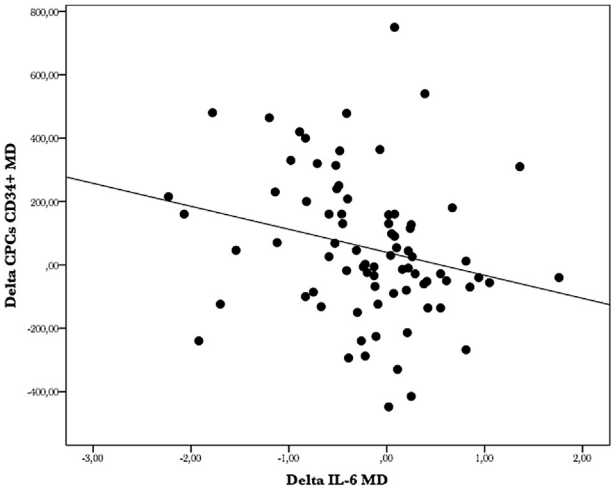

Figure 1 Correlation between variations of CPCs $CD34^+/CD45^{-/dim}$ and interleukin-6 during the MD intervention.

Subsequently, some other studies have investigated this possible effect of diet, highlighting the beneficial effect in particular of the MD model on the number of endothelial progenitor and circulating progenitor cells. Marin et al. studied the effect of 3 diets, each for 4 weeks with a crossover design, a saturated fatty acids diet, a low-fat diet, MD, and a high-carbohydrate diet by reporting that only MD had a beneficial effect on endothelial progenitor cells [5]. Similarly, another study by Fernandez and colleagues demonstrated a beneficial effect of a hypocaloric MD along with exercise in a group of patients with metabolic syndrome [6]. Recently, we and other groups were able to demonstrate that dietary and lifestyle habits can determine an increase of progenitor cells in different study groups, such as diabetic patients and elderly [4,7]. Interestingly, in our cohort of nonagenarians we also found a significant effect on endothelial progenitor and circulating progenitor cells from increased consumption of olive oil and fruit and vegetables [4], suggesting that also certain categories of foods may be responsible, at least in part, for the beneficial effect observed by MD on progenitor cells.

Olive oil and fruit and vegetables are two of the food categories shared by MD and VD. VD in recent decades has been attributed to many beneficial effects on many diseases, showing a greater effect in particular on cardiovascular diseases, as reported by a recent meta-analysis of our group that showed a 25%-reduction in the occurrence of cardiovascular diseases for the subjects following a vegetarian model [8]. Among the beneficial properties of VD there are also the reduction of classic cardiovascular risk factors such as high blood cholesterol, high body mass index and high blood glucose. To date, no data on the effect of VD on progenitor cells are available.

In the present study we were able to show a negative and significant effect of the adoption of VD for 3 months in

previously omnivorous subjects at low-to-moderate cardiovascular risk profile. In particular, we found a significant reduction of all the three types of circulating progenitor cells during the VD phase, in contrast to the beneficial effect observed in some of the other cardiovascular risk factors. These results can be interpreted in light of the differences between the two intervention diets. In the CARDIVEG study the two diets were completely similar in terms of calories and consumption of all food categories, apart from fresh and processed meat and fish [10]. On the one hand, the absence of meat and meat-products is known to determine many healthy effects in terms of cardiovascular prevention, but on the other hand it can lead to a relative pro-inflammatory state through the reduction of vitamin B12 levels [13]. In fact, after only 3 months of dietary intervention with VD we observed a small but significant reduction of vitamin B12 levels, which was significantly and inversely correlated with the levels of interleukin-6 [10]. It could therefore be possible that the reduction of vitamin B12 levels influenced inflammatory parameters, thus reducing the circulating levels of progenitor cells. To date, the significant and inverse relationship between changes in circulating progenitor cells and inflammatory parameters observed only during MD and not during VD allow us to hypothesize that moderate consumption of animal products is able to maintain normal circulating levels of vitamin B12, not increasing so the inflammatory parameters. Another possible mechanism that can explain this apparent paradoxical result of VD on progenitor cells is based on the absence of fish in the diet. Some studies have recently observed a beneficial role played by the n-3 polyunsaturated fatty acids typical of the fish food group in the function and bioavailability of endothelial progenitor cells [14]. Therefore, it is also possible that the absence of such food group was detrimental to the number of endothelial progenitor cells and circulating progenitor cells in these subjects.

This study has limits and strengths. The number of subjects was not so large and the duration of dietary intervention was limited to 3 months. We are aware that 3 months of intervention are a limited period and only allows us to suggest a possible interpretation of the results. Therefore, more extensive studies are needed to support these preliminary findings and clarify this problem. However, the study has several strengths since the data come from the first randomized controlled trial with a crossover design that was done comparing VD and MD in the same group of subjects. Another strength is the fact that the group of subjects was composed of omnivores that modified their dietary habits for the intervention study and were not previously vegetarian.

In conclusion, the results of the present study confirm the positive effect of MD on progenitor cells extending the results also to a clinically healthy population at low-to-moderate risk of cardiovascular diseases, while reporting for the first time the negative influence of a short period of intervention with VD on progenitor cells. The relationships observed during the MD phase, and not during the VD phase, between circulating progenitor cells and some inflammatory parameters allow us to hypothesize some pathophysiological mechanisms that need to be confirmed by further studies.

Authors' contributions

FC was responsible for the evaluation of all the laboratory parameters, wrote the paper and participated in the design of the study. MD participated in the design of the study, participated in the clinical evaluations, conducted the statistical analyses and revised the manuscript. GP participated in the clinical evaluations, conducted the statistical analyses and revised the manuscript. AR conducted the laboratory analyses and participated in the writing of the study. AMG and RM participated in the writing of the study protocol and in the critical revision of the manuscript. AC participated in the design of the study and critical revision of the manuscript for important intellectual content. FS conceived the study, participated in the design of the study, wrote the study protocol, and revised the final version of the manuscript. He has been responsible for recruitment, clinical evaluations and statistical analyses. All authors read and approved the final manuscript.

Conflict of interest disclosure

All the authors have reported that they have no relationships relevant to the contents of this paper to disclose.

Funding

This research received no specific grant from any funding agency, commercial or not-for-profit sectors.

References

[1] Paneni F, Diaz Cañestro C, Libby P, Lüscher TF, Camici GG. The aging cardiovascular system: understanding it at the cellular and clinical levels. J Am Coll Cardiol 2017;69:1952–67.
[2] Bianconi V, Sahebkar A, Kovanen P, Bagaglia F, Ricciuti B, Calabrò P, et al. Endothelial and cardiac progenitor cells for cardiovascular repair: a controversial paradigm in cell therapy. Pharmacol Ther 2018;181:156–68.
[3] Rigato M, Fadini GP. Circulating stem/progenitor cells as prognostic biomarkers in macro- and microvascular disease: a narrative review of prospective obesravational studies. Curr Med Chem 2018; 25:4507–17.
[4] Cesari F, Sofi F, Molino Lova R, Vannetti F, Pasquini G, Cecchi F, et al. Aging process, adherence to Mediterranean diet and nutritional status in a large cohort of nonagenarians: effects on endothelial progenitor cells. Nutr Metab Cardiovasc Dis 2018;28:84–90.
[5] Marin C, Ramirez R, Delgado-Lista J, Yubero-Serrano EM, Perez-Martinez P, Carracedo J, et al. Mediterranean diet reduces endothelial damage and improves the regenerative capacity of endothelium. Am J Clin Nutr 2011;93:267–74.
[6] Fernández JM, Rosado-Álvarez D, Da Silva Grigoletto ME, Rangel-Zúñiga OA, Landaeta-Díaz LL, Caballero-Villarraso J, et al. Moderate-to-high-intensity training and a hypocaloric Mediterranean diet enhance endothelial progenitor cells and fitness in subjects with the metabolic syndrome. Clin Sci (Lond) 2012;123:361–73.
[7] Maiorino MI, Bellastella G, Petrizzo M, Gicchino M, Caputo M, Giugliano D, et al. Effect of a Mediterranean diet on endothelial

progenitor cells and carotid intima-media thickness in type 2 diabetes: follow-up of a randomized trial. Eur J Prev Cardiol 2017;24:399–408.
[8] Dinu M, Abbate R, Gensini GF, Casini A, Sofi F. Vegetarian, vegan diets and multiple health outcomes: a systematic review with meta-analysis of observational studies. Crit Rev Food Sci Nutr 2017;17:3640–9.
[9] Wang X, Lin X, Ouyang YY, Liu J, Zhao G, Pan A, et al. Red and processed meat consumption and mortality: dose-response meta-analysis of prospective cohort studies. Public Health Nutr 2016;19:893–905.
[10] Sofi F, Dinu M, Pagliai G, Cesari F, Gori AM, Sereni A, et al. Low-calorie vegetarian versus mediterranean diets for reducing body weight and improving cardiovascular risk profile. CARDIVEG study. Circulation 2018;137:1103–13.
[11] Sofi F, Dinu M, Pagliai G, Cesari F, Marcucci R, Casini A. Mediterranean vs. Vegetarian diet for cardiovascular prevention (the CARDIVEG study): study protocol for a randomized controlled trial. Trials 2016;17:233.
[12] Foo SY, Heller ER, Wykrzykowska J, Sullivan CJ, Manning-Tobin JJ, Moore KJ, et al. Vascular effects of a low-carbohydrate high-protein. Proc Natl Acad Sci USA 2009;106:15418–23.
[13] Lee YJ, Wang MY2, Lin MC3, Lin PT. Associations between vitamin B-12 status and oxidative stress and inflammation in diabetic vegetarians and omnivores. Nutrients 2016;8:118.
[14] Chowdhury R, Stevens S, Gorman D, Pan A, Warnakula S, Chowdhury S, et al. Association between fish consumption, long chain omega 3 fatty acids, and risk of cerebrovascular disease: systematic review and meta-analysis. BMJ 2012;345:e6698.