

SUPPLEMENTAL MATERIAL

Impact of following a healthy dietary pattern with co-consuming wolfberry on number and function of blood outgrowth endothelial cells from middle-aged and older adults

Xia et al.

It includes:

1. Supplementary Figures 1–4
2. Supplementary Tables 1–2

Supplementary Figures

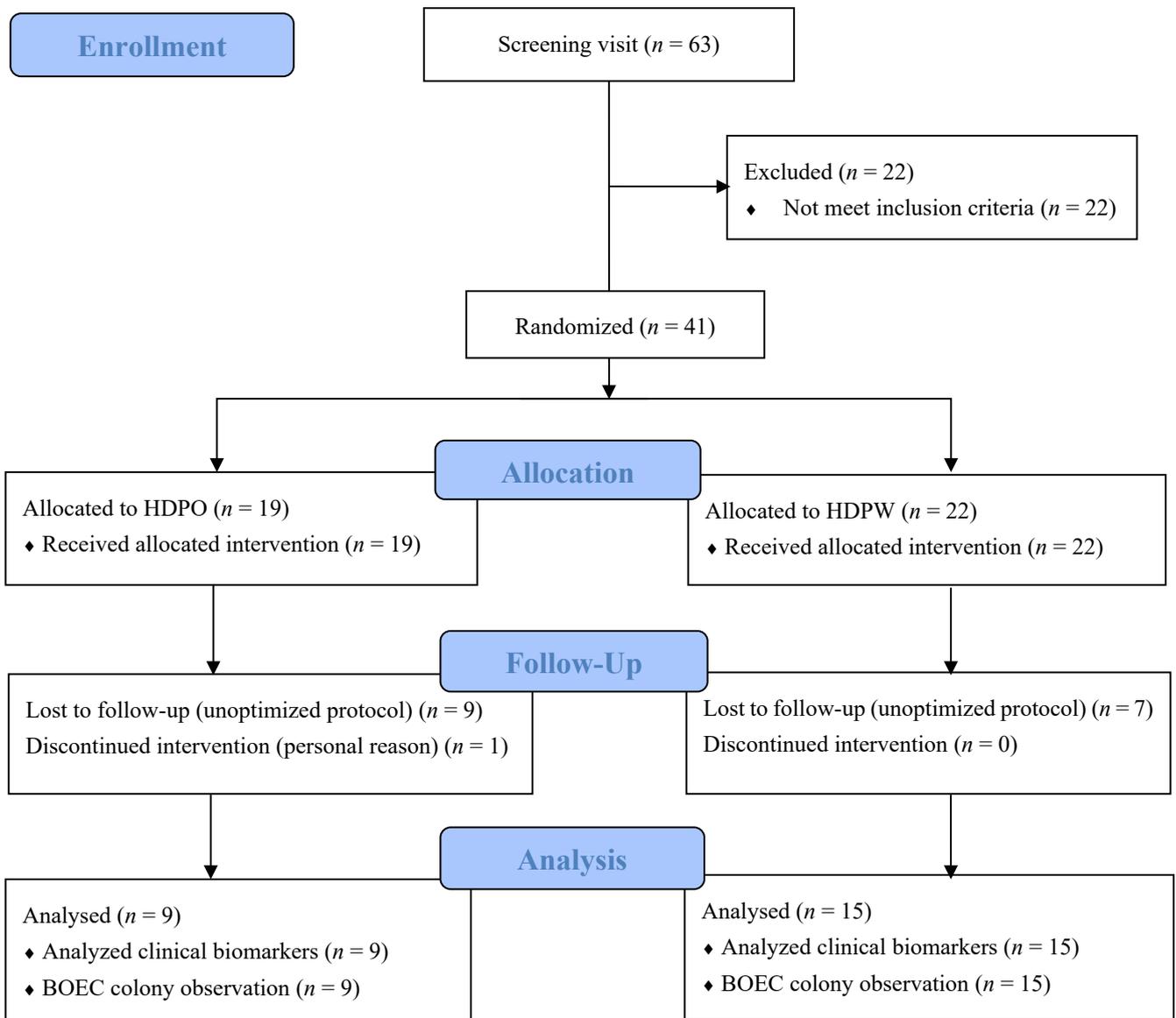


Figure S1 Flow of participants through the study. HDPO, healthy dietary pattern only;

HDPW, HDP supplemented with wolfberry; BOEC: blood outgrowth endothelial cell.

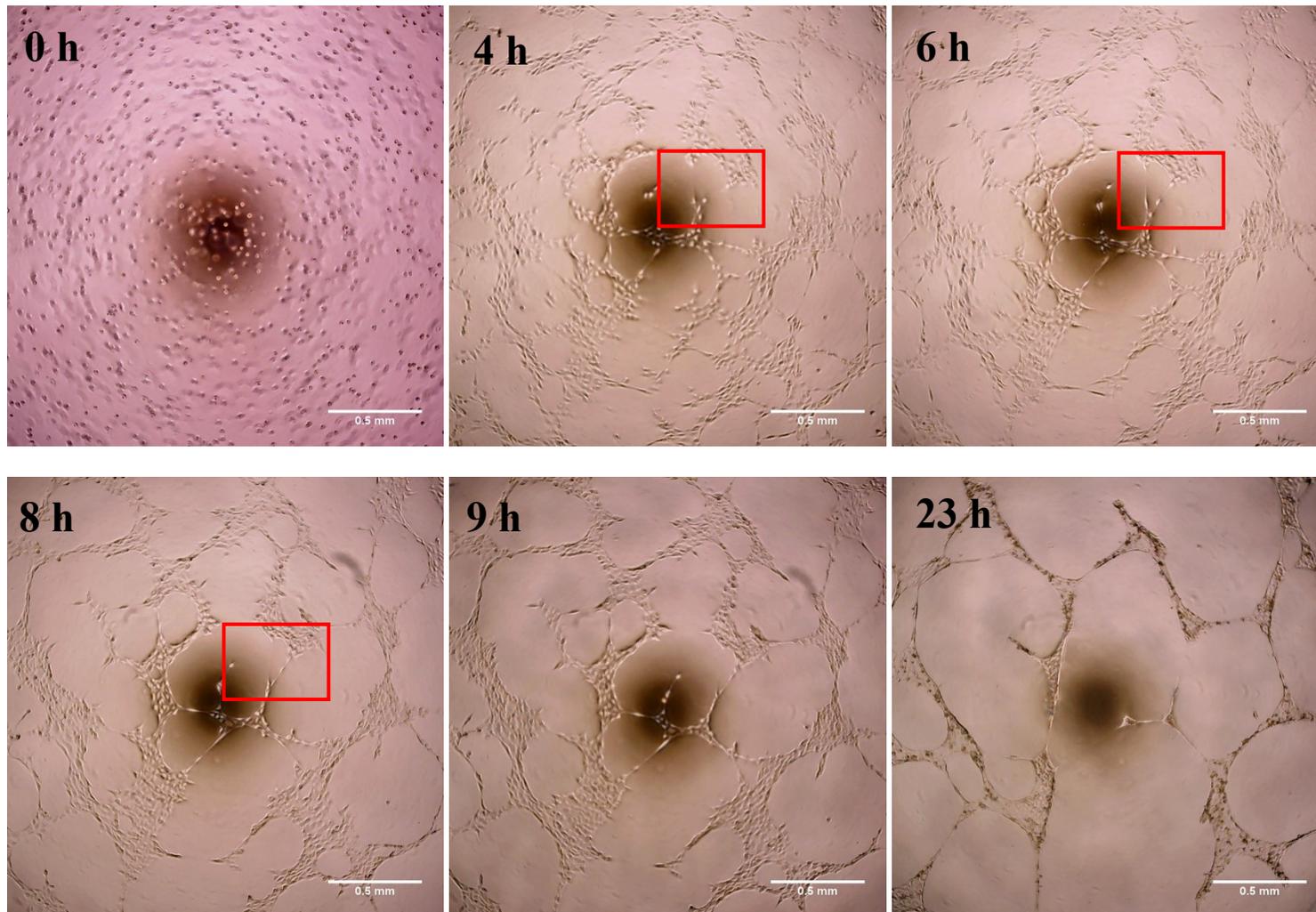


Figure S2 Tube formation of BOECs on gelled basement membrane extract at different time points. Red squares highlight the tiny tube structures,

which began to decline at the 8 h time-point. Therefore, 6 h duration was selected for tube formation assay. Abbreviations: BOEC, blood outgrowth endothelial cell. Images were taken by EVOS XL Core Cell Imaging System (Life Technologies, USA) with 4× objective.

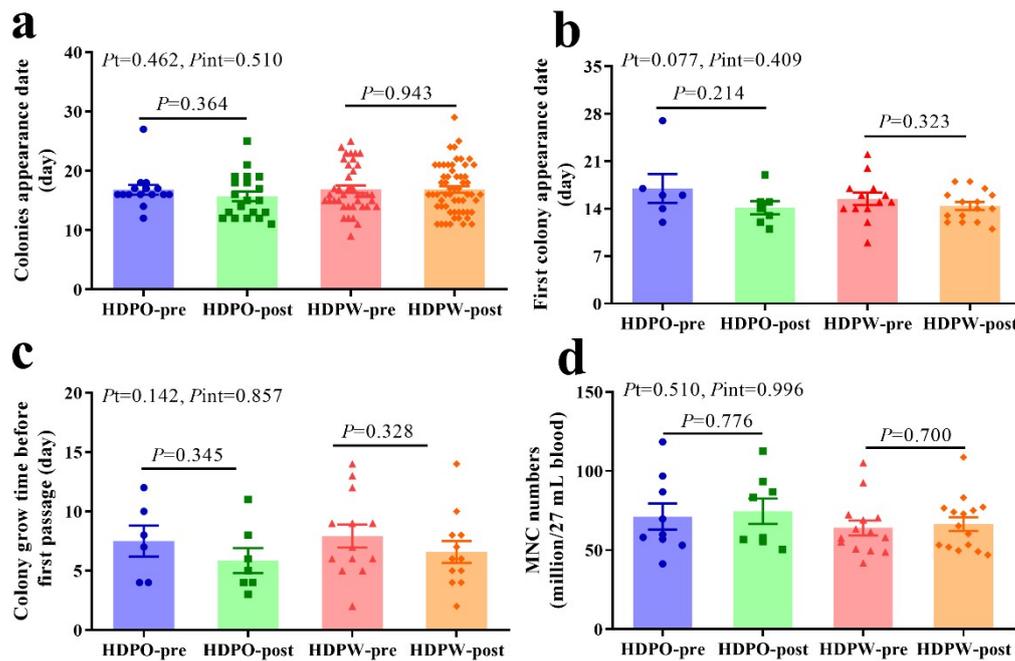


Figure S3 Parameters show growth rate of BOECs (**a–c**) and peripheral blood MNC number (**d**). Graphs are plotted as means with SEM. Statistical comparisons of pre- and post-intervention within groups were performed by unpaired *t*-test or Mann–Whitney test. Two-way ANOVA was used to determine the *P* values of time (*P_t*) and interaction (*P_{int}*) effects. Abbreviations: BOEC, blood outgrowth endothelial cell; HDPO, healthy dietary pattern only; HDPW, HDP supplemented with wolfberry; MNC, mononuclear cell.

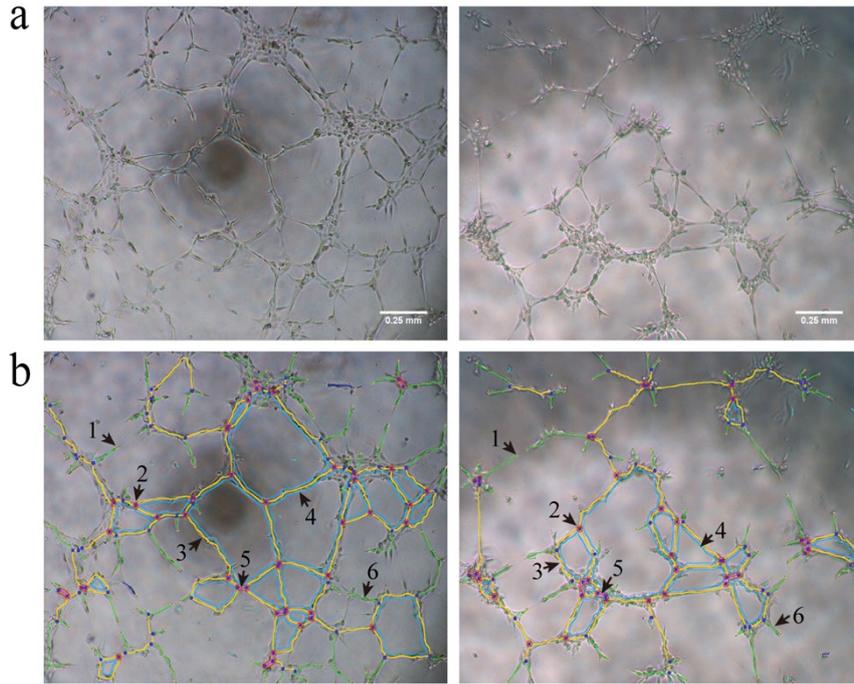


Figure S4 Representative images of capillary-like structure of BOECs. **(a)** Capillary-like structures of BOECs with different tube formation capacities. **(b)** Characterization of the structures using Angiogenesis Analyzer. Arrows 1–6 point representative extremities, master junctions, master segments, meshes, segments and branches, respectively. Abbreviations: BOEC, blood outgrowth endothelial cell.

Supplementary Tables

Table S1 CD34 and KDR expression in BOECs and MNCs

Parameter	HDPO			HDPW		
	Pre-	Post-	# <i>P</i>	Pre-	Post-	# <i>P</i>
BOECs						
CD34⁺ (%)						
Range	9.0–95.9	35.7–99.9		37.6–97.0	15.5–88.9	
Average (SEM)	45.0(20.2)	60.1(14.1)	0.556	56.4(10.9)	56.5(9.1)	0.993
KDR⁺ (%)						
Range	99.6–100.0	98.4–100.0		99.8–100.0	98.0–100.0	
Average (SEM)	99.8(0.1)	99.6(0.3)	0.786	99.9(0.0)	99.7(0.2)	0.936
CD34⁺KDR⁺ (%)						
Range	9.0–95.5	35.7–99.8		37.6–97.0	15.5–97.0	
Average (SEM)	44.9(20.1)	59.8(13.9)	0.551	56.4(10.9)	56.3(9.0)	0.996
MNCs						
CD34⁺ (%)						
Range	0.9–48.5	1.3–67.8		1.3–51.3	1.2–67.6	
Average (SEM)	26.7(5.1)	32.9(10.1)	0.566	22.7(5.0)	27.0(6.0)	0.636
KDR⁺ (%)						
Range	1.1–2.0	0.8–3.2		0.7–3.7	0.8–3.5	
Average (SEM)	1.6(0.1)	1.5(0.3)	0.250	1.9(0.2)	1.4(0.2)	0.191
CD34⁺KDR⁺ (%)						
Range	0.1–1.4	0.0–1.6		0.1–1.1	0.1–1.1	
Average (SEM)	0.6(0.1)	0.6(0.2)	0.973	0.6(0.1)	0.5(0.1)	0.672

#Statistical comparisons of pre- and post-intervention within groups were performed by unpaired *t*-test or Mann–Whitney test. No significant differences were observed from baseline data comparisons within groups and two-way ANOVA analyses (data not shown). Sample number for BOEC analysis in HDPO-pre, HDPO-post, HDPW-pre, and HDPW-post groups were 4, 5, 5 and 10, respectively. Sample number for MNC analysis in HDPO and HDPW groups were 8 and 13, respectively. Abbreviations: BOEC, blood outgrowth endothelial cell; HDPO, healthy dietary pattern only; HDPW, HDP supplemented with wolfberry; MNC, mononuclear cells.

Table S2 Other parameters that characterized BOECs' tube formation capacity.

Parameter	HDPO				HDPW			
	Pre- (<i>n</i> =5)	Post- (<i>n</i> =5)	Change (%)	# <i>P</i>	Pre- (<i>n</i> =8)	Post- (<i>n</i> =11)	Change (%)	# <i>P</i>
Number nodes	196.3 ± 43.3	192.9 ± 34.5	-1.8	0.952	244.7 ± 23.6	238.0 ± 24.0	-2.8	0.848
Number junctions	56.8 ± 12.4	55.6 ± 9.8	-2.1	0.942	70.2 ± 6.7	68.8 ± 6.9	-1.9	0.893
Number pieces	123.5 ± 17.2	115.5 ± 14.2	-6.5	0.730	146.8 ± 12.0	139.6 ± 11.0	-4.9	0.668
Number segments	59.8 ± 17.3	60.5 ± 13.2	1.1	0.976	74.0 ± 8.0	76.1 ± 9.2	2.8	0.873
Number branches	50.6 ± 3.6	46.0 ± 3.6	-9.2	0.392	62.1 ± 5.1	54.4 ± 2.8	-12.5	0.167
Number isolated elements	13.1 ± 3.1	9.1 ± 2.3	-30.8	0.324	10.7 ± 2.0	9.2 ± 0.9	-14.1	0.462
Total length (mm)	15.8 ± 2.2	15.7 ± 1.9	-1.0	0.958	17.3 ± 1.0	17.6 ± 1.4	1.5	0.888
Total segment length (mm)	7.9 ± 2.3	8.4 ± 1.9	6.6	0.867	8.7 ± 0.8	9.8 ± 1.1	13.2	0.461
Total branch length (mm)	6.3 ± 0.5	6.0 ± 0.4	-3.7	0.708	7.3 ± 0.5	6.6 ± 0.4	-9.5	0.492
Total isolated branch length (mm)	1.6 ± 0.4	1.2 ± 0.4	-27.5	0.445	1.3 ± 0.3	1.1 ± 0.1	-14.6	0.444
Branching interval (μm)	152.0 ± 43.3	179.7 ± 39.3	18.2	0.648	144.8 ± 15.8	179.7 ± 18.5	24.1	0.192
Mesh index (mm)	0.435 ± 0.028	0.443 ± 0.022	1.8	0.828	0.426 ± 0.011	0.426 ± 0.028	0.0	0.999

Instruction of parameters can be found from the online description of Angiogenesis Analyzer for ImageJ

(<http://image.bio.methods.free.fr/ImageJ/?Angiogenesis-Analyzer-for-ImageJ&lang=en>). Picture analyze area is 3.50 mm². Values are presented

as means ± SEM. #Statistical comparison of pre- and post-intervention within groups was performed by unpaired *t*-test or Mann–Whitney test. No

significant differences were observed from baseline data comparisons and two-way ANOVA analyses (data not shown). Abbreviations: BOECs,

blood outgrowth endothelial cells; HDPO, healthy dietary pattern only; HDPW, HDP supplemented with wolfberry.