EXTRA VIRGIN OLIVE OIL AND APPLES ENRICHED-DARK CHOCOLATE CONSUMPTION AND ENDOTHELIAL FUNCTION: A RANDOMIZED CROSSOVER TRIAL IN PATIENTS WITH CARDIOVASCULAR RISK FACTORS

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none

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Declaration of interest

- I have nothing to declare

Background

The polyphenols equivalence

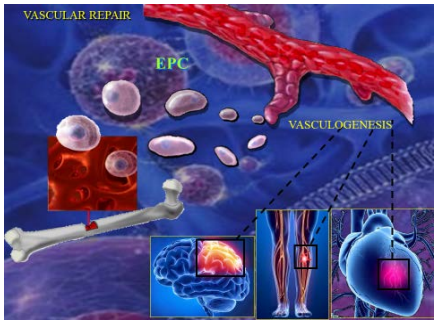
- **Chocolate** represents functional properties due to its high level of flavanols, namely cathechins and procyanidins

- **Consumption of cocoa** products has been correlated to a range of cardiovascular-protective properties

The Kuna population

- Several clinical studies have assigned to **Extra Virgin Olive Oil (EVOO)** as well as to **Apples** polyphenols , beneficial effect on human health.

- **Bone marrow- derived endothelial progenitor cells (EPCs)** are critical for vascular repair and maintenance of endothelial function and result **reduced in subjects with cardiovascular risk factors**



The endothelial progenitor cells

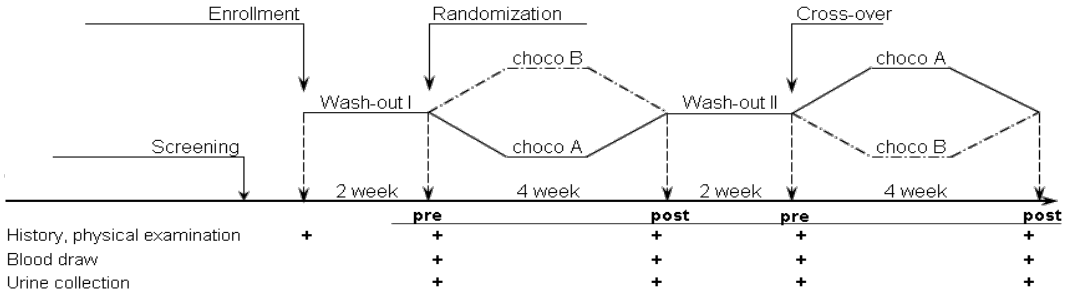
KI WON LEE et al. Cocoa Has More Phenolic Phytochemicals and a Higher Antioxidant Capacity than Teas and Red Wine. *J. Agric.Food Chem.* 2003, *51,* 7292-7295

McCullough Ml et al .Hypertension, the Kuna , and epidemiology of flavonols *J Cardiovasc Pharmacol 2006 :S103-s109*

Hill JM, Zalos G, Halcox JP, et al. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N Engl J Med 2003;348:593-600*.

Purpose and methods

- **To examine the effects of EVOO and Panaia red apple-enriched dark chocolate (70% cocoa) intake on EPCs blood levels and endogenous metabolites in free living subjects carrying cardiovascular risk factors.**
- In a randomized cross-over trial, 30 adults (51 ± 9 years of age) with at least **three cardiovascular risk factors** among smoke, dyslipidemia, hypertension, overweight or family history for cardiovascular disease, received a solid dark chocolate bar (**40 g/day**) containing **10% EVOO** or a solid dark chocolate bar containing **2.5% dry apple** in random order **over 28 days**
- Urine and blood samples were collected at baseline and after the intervention and analyzed by proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy for **endogenous metabolites** and by flow cytometry for blood **circulating EPCs (CD133+/KDR+/CD34+) levels**
- Age, sex, smoking status, body mass index (BMI), blood pressure (BP), glycaemia and lipid profile were monitored in all subjects.



Results : clinic and biochemical parameters

26 volunteers completed the study. Tables show pre-post intervention biochemical parameters :
EVOO-chocolate : an increase in HDL levels and a moderate decrease of diastolic blood pressure
Apple-chocolate : an increase in LDL levels and a moderate decreased in triglyceride levels

Characteristic	Value
N (male/female)	26 (14/12)
Age (years, mean ± SD)	51 ± 9
Body mass index (kg/m^2)	29 ± 6
Cardiovascular Risk Factors	
CAD family history (n)	19
Overweight (n)	20
Hypertension (n)	14
Dyslipidemia (n)	15
Active smokers (n)	9

CAD, coronary artery disease; Data are expressed as number (n) and as mean and SD.

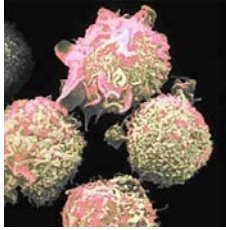
Biochemical parameter	EVOO-dark chocolate bar		
	Pre-treatment	Post-treatment	P-value
BMI (Kg/m^2)	29 ± 6	29 ± 6	0.80 §
Glucose (mg/dl)	90 ± 9	91 ± 13	0.78 §
Total cholesterol (mg/dl)	216 ± 32	213 ± 33	0.49 ǂ
HDL cholesterol (mg/dl)	50 ± 13	51 ± 16	0.28 ǂ
LDL cholesterol (mg/dl)	139 ± 31	133 ± 31	0.09 ǂ
Triglycerides (mg/dl)	134 ± 61	142 ± 94	0.58 §
Systolic BP (mmHg)	128 ± 13	128 ± 17	0.90 ǂ
Diastolic BP (mmHg)	84 ± 10	80 ± 9	0.07 ǂ

BMI: Body mass index; HDL: High-density lipoprotein, BP: blood pressure; LDL: Low-density lipoprotein. ǂ t-test. § Wilcoxon test.

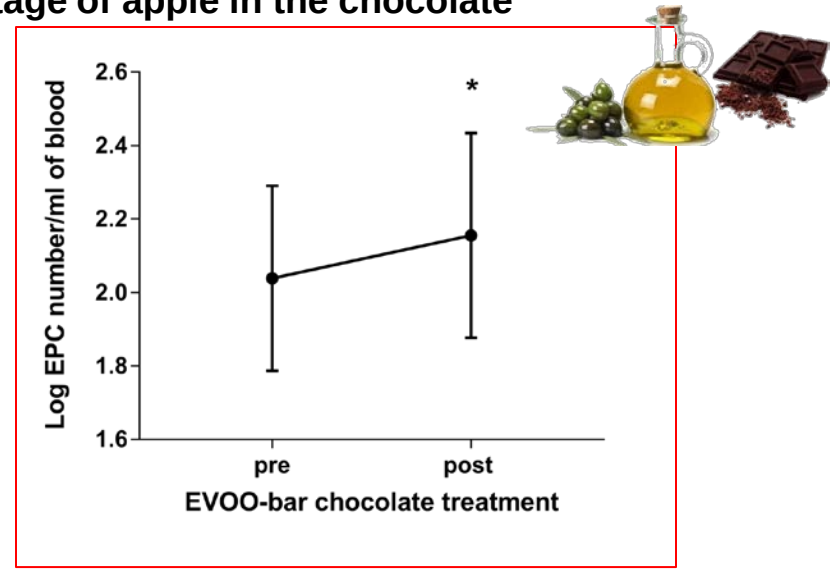
Biochemical parameter	Panaia red apple-dark chocolate bar		
	Pre-treatment	Post-treatment	P-value
BMI (Kg/m^2)	29 ± 6	28 ± 6	0.08 §
Glucose (mg/dl)	91 ± 12	93 ± 8	0.44 §
Total cholesterol (mg/dl)	213 ± 32	220 ± 34	0.16 ǂ
HDL cholesterol (mg/dl)	50 ± 15	50 ± 14	0.43 ǂ
LDL cholesterol (mg/dl)	132 ± 32	142 ± 33 *	0.03 ǂ
Triglycerides (mg/dl)	152 ± 80	135 ± 48	0.37 §
Systolic BP (mmHg)	128 ± 21	123 ± 15	0.18 ǂ
Diastolic BP (mmHg)	81 ± 10	79 ± 8	0.34 ǂ

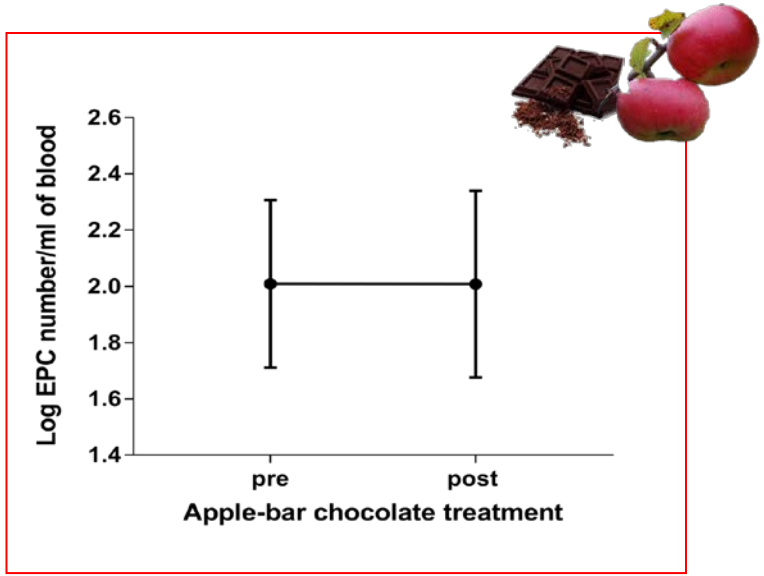
*BMI: Body mass index; HDL: High-density lipoprotein, BP: blood pressure, LDL: Low-density lipoprotein. ǂ t-test. § Wilcoxon test. * P < 0.05*

Results : Endothelial Progenitor Cells (EPCs)



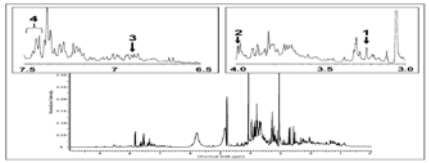
EVOO-dark chocolate consumption induces **a significant increase of blood EPC** levels
Apple- dark chocolate was not effective
These results may be due to peculiar **EXTRA VIRGIN OLIVE OIL polyphenols** or to a too **low percentage of apple in the chocolate**



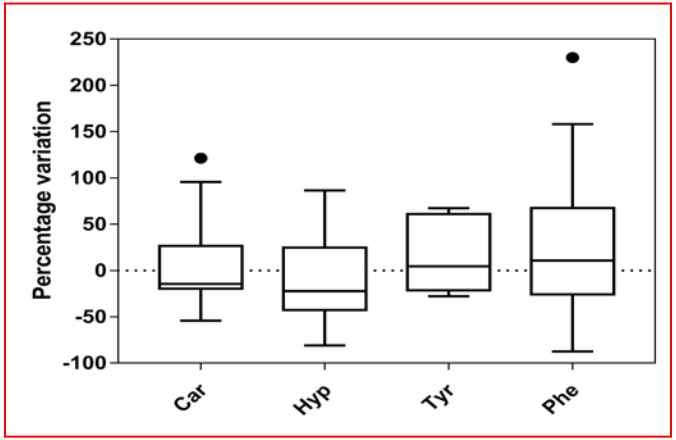


EVOO-dark chocolate consumption induced a significant increase of circulating EPC levels compared to apples-dark chocolate consumption ($P < 0.05$).

Results : endogenous metabolites



^{1}H-NMR spectroscopy of urine samples revealed a decrease of at least 2 metabolites involved in cardiovascular risk, linked with carnitine (-14.5%) and hippurate (-22%) metabolism



Percentage variation of four biomarkers (Car, carnitine; Hyp, 2-hydroxyhyppurate; Tyr, L-tyrosine; and Phe, phenylalanine from patients after EVOO-enriched dark chocolate consumption

Conclusions

Daily intake of extra virgin olive oil enriched -dark chocolate for 2 weeks resulted in an increase of circulating EPCs , known to be associated with improvement of endothelial function and a modification of metabolism, with potential long-term healthy consequences on cardiovascular system.

These results may motivate clinical trials to evaluate if to indulge in **a small piece of dark** (70% cocoa or more **) chocolate** each day may help **longevity**

UNIVERSITÀ DI PISA

Laboratorio di Ricerca Cardiovascolare

Laboratorio di NMR

GRAZIE !

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