
Cardiovascular System
ISSN 2052-4358

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Research Open Access

Dark chocolate intake improves endothelial function in young healthy people: a randomized and controlled trial

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Abstract

Introduction: The aim of this study was to assess the effect of the administration of dark chocolatein the endothelial and vascular function in a healthy and young population.

Methods: A randomized and controlled trial was carried out involving 30 healthy young individuals of Portuguese nationality, mean age 19.99±1.02 years, randomized into two groups: control group (CG) and intervention group (IG). The IG underwent a month ingestion of 8gr/daily of 70% cocoa chocolate. All the individuals were submitted to two clinical assessments, basal and after one month of intervention, in which their weight, height, body mass index (BMI), Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), heart rate (HR) and flow-mediated dilation (FMD) were assessed. In the second moment glycaemia was also assessed.

Results: CG and IG groups had similar baseline clinical and demographic characteristics. The basal FMD was similar in the IG and the CG, but was significantly different in the second evaluation, with the IG showing higher FMD values (23±7% versus 13±6%, respectively for the IG and the CG; $p<0.001$). Endothelial function improved in the IG after the 1 month flavonoid intake, with the FMD increasing from 14±5% (baseline) to 23±7% (post-intervention; $p<0.001$), with no significant differences in the CG. No significant variation was observed vis-à-vis BMI, blood pressure, HR and glycaemia.

Conclusion: The daily ingestion of 8g/day of 70% cocoa chocolateduring a month improves the endothelial function of young people, improving the endothelium-dependent vasodilatation.

Keywords: Endothelial function, flow mediated dilatation, flavonoids, dark chocolate

Introduction

The study of the endothelial function is quite important as the major cardiovascular events that explain the largest proportion of death and morbidity worldwide express pathophysiological contexts that have endothelial dysfunction as the common denominator. The endothelial function is indeed the first to be modified as response to risk factors and can lead to vascular damage and the first atherosclerosis lesions [1,2]. Hence, the endothelial damage plays a critical role in all the stages of atherosclerosis. The traditional markers of cardiovascular risk, such as age, arterial hypertension, hyperlipidemia, diabetes mellitus and smoking are associated with the damaged endothelium in asymptomatic individuals. However, the changes in these risk factors induce an improvement in the endothelial function [3].

Therefore, the endothelial function can be looked at as the common way between clinical risk factors and the development of atherosclerosis. Moreover, the endothelial function plays a fundamental part on the clinical manifestations of coronary disease through the development of vasoconstriction and thrombosis [3].

Being so, the implementation of strategies for endothelial protection should be a clinical imperative considering the potential benefits that can come from there. To this end, several substances have been identified by their positive impact at the endothelium level, namely the flavonoids [4].

The flavonoids are compounds of natural origin of the group of the secondary metabolite, quite abundant in the Plant Kingdom. They are present in human diet, however they cannot be synthesized in our organism, and so they are obtained through food as fruit, vegetables, greens and also herb tea, wine and honey. We can also find this compound in the form of glycosides, which promote a better intestinal absorption [4].

The flavonoids have a vast biological action that goes beyond the simple food fact, showing also an important medicinal action. In fact, part of the benefits of ingesting fruit and vegetables is due to this compound as it helps the absorption of vitamin C and can also have anti-inflammatory, antiallergic, anti-hemorrhagic and antioxidant action [4-7]. This antioxidant action definitely explains the vast interest that these compounds have capitalized given the potential protector role they can play in the cardiovascular system [4-7].

In the last few years, several randomized short studies have been published assessing the effects of food containing cocoa in intermediary endpoints of cardiovascular disease. The consumption of chocolate or cocoa drinks rich in flavonoids

doi: 10.7243/2052-4358-2-3

lowered blood pressure and improved the endothelial function and insulin sensitivity [5,6]. Observations made by Hollenberg et al., [7] indicated that the Kuna Indians in Panamá had a very lowincidence of hypertension and cardiovascular diseases, but when the members of that tribe moved to urban places in Panamá, their blood pressure increased. This migration led to cultural changes, including a relevant decrease in cocoa consumption, suggesting the influence of cocoa in the changes observed in blood pressure and in the occurrence of significant cardiovascular events.

Even though the benefit mechanism is not yet clear, flavonoids have in fact a number of properties that can really contribute for cardiovascular protection. The dietetic intervention with flavonoids to revert endothelial dysfunction in any early stage or when atherosclerosis is already present thus becomes a priority in scientific research, in that this strategy could reduce the amount of complications resulting of the endothelium dysfunction [8].

Considering the current state of the art and the need to consubstantiate the preliminary data that have indicated the potential benefits of flavonoids, we draw a randomized and controlled clinical trial with the aim of understanding if the daily ingestion of a determined amount of cocoa rich chocolate (>70%) improves the endothelial function in young and healthy individuals. There are nowadays several devices for the assessment of the endothelial functionso the possibility to carry through measurements of the endothelial function in a rapid, safe and reproducible manner allows the application of this method full scale, as this can be considered as an element of stratification of cardiovascular risk in population [9,10]. In this study, we use the flow mediated dilatation (FMD) technique to evaluate the endothelial impact of the diet in study.

Methods

Population

Between October and December 2011, 30 clinically healthy individuals (65% women), under 25 years of age, were recruited to take part in this study. All individuals were free of any pharmacological therapy. They were then randomized into two groups: intervention group (IG) and control group (CG). All individuals were instructed to follow their normal daily routines. The aims of the trial were explained to all participants and their informed consent was obtained. All procedures were done in respect with the Helsinki Declaration.

Study design

In order to study the vascular effects of dark chocolate in young people,a randomized and controlled trial was carried out including 30 young and healthy individuals randomized into two groups (CG and IG), having similar clinical features and followed up throughout a month with FMD assessments at two moments: basal and after a month, during which the IG daily ingested 8 grams of dark chocolate with over 70% cocoa. This chocolate was ingested daily before the subjects went to bed. All participants were instructed not to eat other forms of chocolate or, for the IG, not to ingest more than the determined dosage. The compliance to the prescribed ingestion regime for the IG was assessed weekly through individualized counting.

In the first evaluation (basal) all the individuals were clinically assessed and answered a short clinical questionnaire that focused on personal and family background, smoking habits, alcohol and medication consumption. In the two different moments of clinical evaluation (basal and after a month), we also assessed height, weight and body mass index (BMI). The brachial Blood Pressure (BP) and Heart Rate (HR) were measured in supine position, after a 5 minute rest, always using the same operatorand using a clinically validated automatic blood pressure monitor (Colson MAM BP 3AA1-2®; Colson, Paris) [11]. We used the mean of three BP and HR for analysis. In both groups were assessed the diameters of the brachial artery for the calculation of the FMD (described below), BP, HR, in the two defined assessment periods (basal and after 30 days), and glycaemia only after 30 days.

Flow mediated dilatation

FMD was determined by using an echograph Vivid 3® (General Electric Company, USA) with a linear vascular transducer of 7 MHz, with a previously described technique [5]. The FMD was established with the young person in a supine position, after resting in a tranquil environment. Some restrictions were made, such as the ingestion of caffeine and / or smoking in the 2 hours prior to the assessment. The study was upon the right brachial artery, which was identified by using a color flow Doppler imaging, 2 to 5 cm above the cubital crease in a longitudinal plan.

The diameter of the brachial artery was measured by calculating the distance between the inner proximal and distal (D1) at diastole. Ischemia was provoked through pneumatic compression of the radial and cubital arteries during 5 minutes (cuff positioned over the braquial artery, and cuff was insufflated pressure 30 mmHg above the previously measured systolic blood pressure) and the measurement of the artery was repeated 60 to 90 seconds after the interruption of the compression (D2), at diastole, with the help of a pulsed Doppler. The endothelium-depended function was obtained according to the formula:$(D2 - D1)/D1 \times 100$. The higher the numeric value obtained, the better the endothelial function. Endothelium-independent vasodilation to Nitroglycerin was not performed. All evaluations were performed by the same operator, highly trained and experienced with this technique, and blinded regarding the cocoa intake.

Statistical analysis

The data concerning the subjects of the cohort were computerized and treated with SPSS for Windows, version 17.0. The distribution of the variables was tested for normality

 doi: 10.7243/2052-4358-2-3

Table 1. Baseline study cohort characteristics according to the group.

	Total	Control Group (n=15)	Intervention Group (n=15)	p
Gender (male/female, n)	14/16	6/9	8/7	0.464
Age (years, mean±SD)	19.99±1.02	19.90±1.01	20.07±0.80	0.524
BMI (Kg/m^2, mean±SD)	22.01±0.32	21.45±0.27	22.57±0.36	0.342
Hip measure (cm, mean±SD)	98.40±7.85	96.93±6.60	99.87±8.92	0.315
Waist measure (cm, mean±SD)	74.57±8.30	75.67±8.23	73.47±8.51	0.478
HR (bpm, mean±SD)	74.67±10.85	74.80±11.07	74.53±10.00	0.948
SBP (mmHg, mean±SD)	106.73±16.46	113.07±17.16	110.40±13.45	0.532
DBP (mmHg, mean±SD)	66.67±8.12	65.60±8.62	67.73±7.74	0.482
Personal Background (N/S; %)	93.3/6.7	93.3/6.7	93.3/6.7	1.000
Family History (N/Y; %)	40.0/60.0	53.3/46.7	26.7/73.3	0.136
Smoking (N/Y; %)	96.7/3.3	93.4/6.6	100.0/0.0	0.309
Occasional Drinking (N/Y; %)	36.7/63.3	26.7/73.3	66.6/33.4	0.256
Oral Contraception (n)	16	9	7	0.617

BMI: body mass index; HR: heart rate; SBP: systolic arterial pressure; DBP: diastolic arterial pressure; N: no; Y: yes

using the Shapiro-Wilks test and for homogeneity of variance by Levene's test. Simple descriptive statistics were used to characterize the sample and the distribution of variables. Quantitative variables are reported as mean ± standard deviation (SD).

The comparisons between groups were made through the $\chi 2$ test, for categorical variables, or the Student's *t* test for independent samples, for quantitative variables.

For *within-subject* comparisons we used Student's *t* test for pairwise samples, or repeated measures ANOVA, carried through the several considered measures, as best suited. For the repeated measures ANOVA, the Greenhouse-Geisser correction for the degrees of freedom was adopted whenever *sphericity* violation was verified. All multiple comparisons meant to localize the significant effects of a factor were based on the Bonferroni correction.

The criterion of statistical significance used was $p \leq 0.05$ for a confidence interval of 95%.

Results

The cohort consisted of 30 individuals, with a similar proportion of males and females, with a mean age of 19.90±1.01 years (ranging from 18 to 21 years) in the CG, and 20.07±0.80 years (ranging from 19 to 21 years) in the IG(p=0.524). The groups did not differ significantly in the several anthropometric and clinical characteristics taken into account. Basal study cohort characteristics are shown in **Table 1**. The adherence rate of dark chocolate was above 99%.

Table 2 shows the data obtained for each group in the two different moments of assessment. Comparing the values of the baseline study and the follow up after 1 month, there are no significant variations on the weight, BMI, HR, SBP and DBP in both groups. Glycaemia values collected in the second assessment were not different in the individuals from CG (87.13±4.83 mg/dl) and IG (85.40±4.51 mg/dl) (p=0.812).

Considering the variation of the brachial artery diameter during the determination of the FMD (**Figure 1**), it was observed, in the IG, that after the 5 minutes of ischemia, there was a considerable difference between the basal diameter (3.61±0.54mm) and the post-intervention (after 1 month cocoa ingestion) diameter (3.78±0.48mm; p<0.001). The baseline diameters didn´t differed significantly in the two moments of follow up. In the CG, there were no statistical relevant differences in any of the measurements of the considered brachial diameter (**Figure 1**).

Considering now the estimated indicator of endothelial function (FMD) there were no meaningful differences between the baseline values in both groups (p=0.535). However, significant differences for the FMD after 30 days intervention were depicted between IG and CG (p<0.001), as depicted in **Figure 2**. CG showed a mean FDM of 13.23±5.76% and IG 23.22±7.64% (**Figure 2**). As seen in **Table 3**, the FMD in IG improved considerably after a month of daily ingestion of cocoa, going from 13.91±4.71% to 23.22±7.64% (p<0.001). Mean difference at 1 month was 8.93 (IC: 13.01-4.84), in favor of the IG.

Discussion

The dysfunction of endothelial cells is probably the most precocious event in the atherosclerotic process, leading to the concept that the evaluation of the endothelial function can be an useful means to stratify the risk of coronary disease, mainly in patients with chronic inflammation, as the persistence of the dysfunction makes the prognosis gloomy [**12**,**13**]. On the other hand, the integration of endothelial function analysis in clinical practice could also contribute to the optimization

doi: 10.7243/2052-4358-2-3

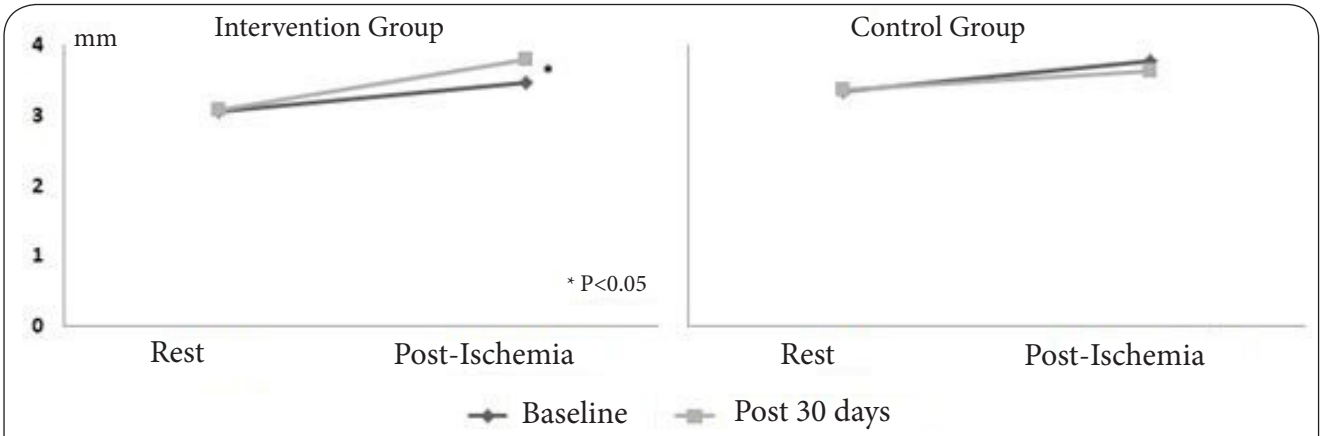

Figure 1. Variation of the Diameters of the Brachial Artery in the intervention group and control group in the two assessment moments. *-p<0.05 for mean post ischemia diameter comparing the baseline and post 30 days in the intervention group.

Table 2. Variation of the clinical parameters per group and per assessment moment.

	Control Group (n=15)			Intervention Group (n=15)		
	Baseline	**30 days**	**p**	**Baseline**	**30 days**	**p**
Weight (kg, mean±SD)	66.00±10.56	66.20±10.75	0.458	60.27±10.98	59.67±10.00	0.144
BMI (Kg/m², mean±SD	21.45±0.27	21.50±0.27	0.529	22.57±0.36	22.34±0.36	0.126
HR (bpm, mean±SD)	74.80±11.07	71.13±9.98	0.204	74.53±10.00	76.87±9.86	0.402
SBP (mmHg, mean±SD)	113.07±17.16	107.00±17.39	0.754	110.40±13.45	101.47±13.36	0.134
DBP (mmHg, mean±SD)	65.60±8.62	62.00±7.52	0.173	67.73±7.74	66.93±8.31	0.732
Glycaemia (mg/dL, mean±SD)	--	87.13±4.83	--	--	85.40±4.51	--

BMI: body mass index; HR: heart rate; SBP: systolic arterial pressure; DBP: diastolic arterial pressure

of preventive strategies in a primary prevention concept that should be taken as a priority regarding public health in modern societies.

There is no doubt that one of the obstacles for the dissemination of the assessment of the endothelial function lies on the inexistence of a reference method. Nonetheless, there is evidence that sustains the FMD as an adequate, simple and relatively well tolerated method for the assessment endothelial function [**12**]. This technique is independent of physical characteristics such as gender, age and BMI [**14**], and there are several studies that highlight an association of the FMD to several markers of active atherosclerotic disease, and even cardiovascular events. For instance, Yeboah et al., [**15**] have shown that FMD is a predictor of the incidence of cardiovascular events in adults with no prior cardiovascular diseases (CVD), and Gokce et al., [**16**] pointed it out as a predictor for the occurrence of cardiovascular events in post-surgery.FMD is a useful measure of the endothelial function and, if applied in a meticulous manner, it can be used as a marker for the exposition of cardiac risk factors and their functional biological effects on healthy people [**17**].

These features made natural choosing this method to fulfill the main goal of this study, focused on the assessment of eventual endothelial benefits of dark chocolate. The results clearly indicate a positive modulation of the endothelial function associated to the daily ingestion of cocoa in chocolate. In fact, the young individuals that ate a square (8 grams) of dark chocolate with over 70% cocoa during 30 days, revealed an obvious improvementin FMD values. This improvement reflects an optimization of the endothelium function, fact already demonstrated in similar studies. For example, Engler et al., [**18**,**19**] indicate that dark chocolate improves endothelium-dependent vasodilatation, what upholds the observation, during the study, of the improvement of the endothelial function after two weeks of intervention with chocolate rich in cocoa, even though no difference in arterial pressure was found. In a recent meta-analysis, Ried et al., [**20**], documented theinexistence of alterations on the tensional profile in normotensive individuals, after the ingestion of products rich in cocoa, fact that is along the line with the results obtained in the present study, during which no differences between SBP and DBP were observed in the CG versus IG at both moments of assessment. The oral ingestion of a cocoa drink resulted in an increase in the

 doi: 10.7243/2052-4358-2-3

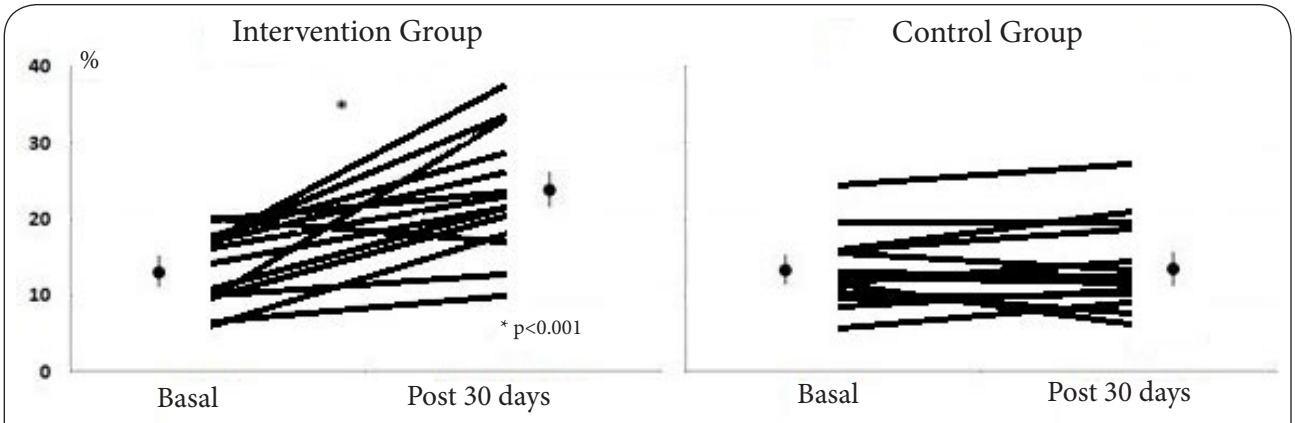

Figure 2. FMD results of basal assessment and after 1 month follow up in control group and intervention group. Legend: Data presented as mean±SD; *-p<0.001 for mean FMD comparison between the basal and post 30 days in the intervention group.

Table 3. Variation of the FMD per group and per assessment moment.

	Control Group(n=15)		Intervention Group (n=15)		
	Baseline	**1 Month**	**Baseline**	**1 Month**	**Mean Difference (95% IC) at 1 month**
FMD (mean±SD %)	12.85±4.51	13.23±5.76	13.91±4.71	23.22±7.64	8.93 (4.84-13.01)

FMD: Flow Mediated Dilation

concentration of nitric oxide (NO) derived species in plasma and urine of healthy individuals that were associated with the improvement of FMD [19,21]. Grassi et al., [22,23] verified that after consuming 100g of chocolate during 2 weeks, FMD improved in hypertensive individuals (with or without impaired glucose tolerance), thus concluding that by balancing the total ingestion of calories, the natural active compounds from cocoa products can provide cardiovascular benefits if included as part of a healthy diet for hypertensive patients. Mechanistic studies also suggest that dark chocolate can be responsible for the improvement of the endothelial function. The consumption of chocolate or cocoa rich in flavonoids is indicated to increase arterial [18,24], and peripheral [25] dilatation, nonetheless, this effect is less or absent after the consumption of chocolate or cocoa poor in flavonoids [7,18,25]. In the Zutphen Elderly Study [5], it was verified that the usual ingestion of cocoa was associated to a 45% to 50% lower risk of cardiovascular mortality and all other death causes, not observing a positive association of the ingestion of cocoa with the BMI. This study consists on the continuation of the Zutphen Study, the Netherlands contribution for the Seven Countries Study. This is an epidemiological longitudinal study and was the first study to analyze systemically the relations between lifestyle, diet, coronary diseases and stroke in different populations of different regions of the world.

Strikingly, the endothelial benefits were documented for relatively low doses of dark chocolate, as compared with other studies [21-23]. This interesting finding may translate the particular characteristics of the cohort in terms of good vascular health, as documented by remarkably high basal FMD values (as compared to previously data [21]). Therefore, the naturally optimized vasomotricity in these individuals should make the endothelium more reactive to the vasomotor compounds in dark chocolate. On the other hand, and in opposition to other studies [19,21], dark chocolate was used because it's formulated with a higher percentage of cocoa bean liquor, therefore contains greater amounts of flavonoids as compared with other alternatives [25].

Another aspect that must be stressed has to do with the fact that the glycaemia values obtained in the second assessment moment do not show differences on both studied groups, what indicates that the daily consumption of a square of dark chocolate does not increase glycemic values.

To conclude, the daily ingestion of 8 grams of dark chocolate (>70% cocoa) for a month improves endothelium-dependent vasodilatation, which indicates a clear improvement of the endothelial function in young and healthy individuals. One must recognize that the long-term clinical benefits of the alterations in the endothelial function detected in the present study are not known, consequently, it would be quite interesting to verify if these results are maintained in a longer follow-up period. On the other hand, the benefits of dark chocolate in other clinical sets where cardiovascular risks is manifest are also to be demonstrated; however, a the observation of a positive modulator effect can be anticipated. Furthermore, there is the need of clinical trials with other sources of flavonoids besides chocolate, in a larger scale, in more diversified clinical contexts and with longer follow up periods.

doi: 10.7243/2052-4358-2-3

Authors' contributions

Authors' contributions	TP	MVB	JC
Research concept and design	✓	✓	✓
Collection and/or assembly of data	✓	--	--
Data analysis and interpretation	✓	✓	✓
Writing the article	✓	✓	✓
Critical revision of the article	✓	✓	✓
Final approval of article	✓	✓	✓
Statistical analysis	✓	✓	✓

Competing interests

The authors declare that they have no competing interests.

Acknowledgement

The authors sincerely thank Dr. Cristina Serra for her linguistic assistance.

Publication history

Received: 04-Jun-2013 Revised: 06-Jul-2013
Re-Revised: 24-Nov-2013 Accepted: 19-Feb-2014
Published: 01-Mar-2014

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Citation:
Pereira T, Vilas Boas M and Conde J. **Dark chocolate intake improves endothelial function in young healthy people: a randomized and controlled trial**. *Cardio Vasc Syst*. 2014; **2**:3.
http://dx.doi.org/10.7243/2052-4358-2-3