Nutrition 63–64 (2019) 175–183

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Applied nutritional investigation

Randomized study of the effects of cocoa-rich chocolate on the ventricle–arterial coupling and vascular function of young, healthy adults

Telmo Pereira Ph.D. [a,*], Jacqueline Bergqvist B.Sc. [b], Carla Vieira B.Sc. [a], Bente Grüner Sveälv Ph.D. [b,**], Joaquim Castanheira Ph.D. [a], Jorge Conde Ph.D. [a,b]

[a] Polytechnic Institute of Coimbra, Coimbra Health School, LabinSaude, Portugal
[b] University of Gothenburgh, Salgrenska Academy, Sweden

ARTICLE INFO

Article History:
Received 1 October 2018
Received in revised form 12 February 2019
Accepted 24 February 2019

Keywords:
Cocoa
Flavanols
Arterial stiffness
Ventricle–arterial coupling

ABSTRACT

Objectives: The aim of this study was to evaluate and explore the benefits of long-term dark chocolate intake in young, healthy adults by measuring cardiovascular function.
Methods: A randomized study was conducted with 30 healthy participants ages 18 to 27 y. Half of the participants ingested a 20-g dose of lower cocoa chocolate (LCC; ~55%; 12.6 ± 1.4 mg equivalent of epicatechin/g) and the others ingested a daily dose of 20 g of higher cocoa chocolate (HCC; ~90%; 18.2 ± 2.6 mg equivalent of epicatechin/g). A baseline evaluation was performed before the participants started ingesting the assigned chocolate for a 30-d period, after which a final evaluation was performed. Each evaluation included heart ultrasonography, carotid-femoral pulse wave velocity (PWV) and carotid pulse wave analysis, flow-mediated slowing (FMS), and an analysis of the ventricular–arterial coupling (VAC), which reflects the matching between the aorta and the left ventricle (ratio of arterial elastance to left ventricle elastance).
Results: The baseline evaluation presented similar values within normal range in both groups. The positive vascular effects were overall more distinct in the group eating the HCC. No structural modifications on the heart were found after the intervention, notwithstanding cardiac function was improved on certain functional parameters in the HCC group only. A statistically significant improvement was depicted over the brachial and central systolic and pulse pressures in the HCC group, and a trend for improvement in the reflected waves component (Aix) and the FMS was also observed in the HCC, but not in the LCC group. VAC parameters were similar at baseline between groups, but showed a significant improvement in the HCC group after intervention, increasing from 0.674 to 0.719 ($P = 0.004$), so that the post-intervention VAC was significantly higher in the HCC group than in the LCC group ($P < 0.05$). In addition, significant variation was observed in both groups regarding arterial and left ventricle elastances, stroke work, and potential energy, with greater mean differences identified in the HCC group.
Conclusion: This study demonstrated that regular consumption of HCC has beneficial effects on the cardiovascular system in young, healthy adults, improving vascular function by reducing central brachial artery pressures and promoting vascular relaxation, and thus enhancing the matching of the arterial system with the left ventricle.

Introduction

The healthy properties of cocoa, and its derivatives, have been the subject of extensive research, and evidence has accumulated that identifies benefits in the cardiovascular system [1–4] These benefits are in large measure due to the fact that cocoa, compared with other foods, contains a large amount of polyphenolic antioxidants, flavanols in particular. The most represented flavanols in cocoa are epicatechin and catechin, which are also present in wine, tea, and berries, and recently have been considered a biologically active food constituent by the European Food Safety Authority [1,2].

Several intervention studies have demonstrated that flavanol-rich cocoa exerts beneficial effects on health in general [3–17], such as protecting endothelial function, reducing insulin resistance and blood pressure, improving lipid profile, and assisting in the regulation of body weight [3,4]. Cutaneous benefits and benefits at

* Corresponding author: Tel.: +351 239 802 430; Fax: +351 239 802 430.
** Co-author.
E-mail address: telmo@estescoimbra.pt (T. Pereira).

https://doi.org/10.1016/j.nut.2019.02.017
0899-9007/

the cognitive level have been identified, particularly in protecting cognitive deterioration inherent to aging [5,6].

Regarding the benefits of cocoa flavanols at the cardiovascular level, it is known that they are largely due to a beneficial effect on endothelial and vascular function [1–3,7,8]. In fact, the endothelium contributes to cardiovascular homeostasis, influencing factors such as tonus and vascular permeability, platelet aggregation, and thrombosis [9,13]. Because abnormal endothelial function is a key event in the atherosclerosis process, it is not surprising that endothelial dysfunction is a hallmark of many cardiovascular disease states [7]. On the other hand, the increase in vascular stiffness closely follows endothelial dysfunction, being a characteristic process of aging [18–21]. All segments of the arterial vascular system are involved in this process of vascular senescence, although the underlying mechanisms remain largely to be defined and have particular significance at the level of large elastic arteries (such as the aorta and carotid arteries). Thus, there is a high interest in dietary interventions, among others, that are capable of delaying or even reversing the pattern of vascular changes that follow biological aging, a more significant aspect when we consider the demographic trends of modern times [8,22].

In line with these arguments, there is evidence to show that both acute and chronic consumption of flavanol-rich cocoa compared with a flavanol-free dietary control increases endothelium-dependent vasodilation in humans [1–15], and the effect seems to be dependent on the dose ingested as a function of the percentage of flavanols that are present in cocoa [10,11,13,14,16]. Most of the data suggest that the mechanisms underlying this response are related to the increased bioavailability of nitric oxide by a direct activation of the nitric oxide-endothelial synthetase, largely provided by the epicatechins [1,2,9,13]. On the other hand, these have the capacity to reduce the production of reactive oxygen species, which are responsible for reducing the bioavailability of nitric oxide and impairing normal endothelial function, thus disturbing the balance between vasodilators and vasoconstrictors [9–12]. In addition to these aspects, cardioprotection also may result from factors such as reduction of oxidative stress, inhibition of low-density lipoproteins, oxidation and platelet aggregation, vasodilation of blood vessels, inhibition of monocyte adhesion in vascular endothelium, the promotion of fibrinolysis, anti-inflammatory activity, and improved mitochondrial structure or function [13,23,24].

Although the benefits of cocoa on endothelial function are well documented in the general population, and in several clinical contexts, it is notoriously less representative in young, healthy populations. On the other hand, less is known about its effect on the matching of heart and vascular function, as expressed with ventricle–arterial coupling (VAC) measurements [25–27], which provides an integrated insight into the mechanistic implications of changes in the arterial dynamics, as measured with aortic pulse wave velocity (PWV) and carotid pulse wave analysis (PWA), in the overall heart function. Thus, the aim of this study was to evaluate and explore the benefits of long-term dark chocolate intake in young, healthy adults by simultaneously measuring its effect on the heart and arterial function.

Methods

Study design

A randomized, double-blind design was used, comparing two objects of study: a control group assigned to chocolate with lower cocoa content (LCC; comparatively lower flavanol content) and an intervention group assigned to chocolate with higher cocoa content (HCC; higher in flavanol content). Participants were randomized into each group and a baseline evaluation was performed before they began the intervention period, eating 20 g/d of the assigned chocolate type for 30 d in a row. To study the effects of long-term intake and not the acute effects, the final evaluation was accomplished 2 d after participants stopped eating the chocolate.

Study population

Participants between 18 and 35 y of age were recruited from the academic population at the Polytechnic Institute of Coimbra. Exclusion criteria included presence of any chronic diseases, including hypertension, diabetes, and dyslipidemia; taking any sort of medication or dietary supplement; high to moderate consumption of alcoholic beverages; pregnancy; and allergy or intolerance to dark chocolate or its constituents. A standard questionnaire and a clinical evaluation were assembled to categorize the conditions for each participant. Thirty-eight volunteers were evaluated for eligibility. Four individuals did not meet the inclusion criteria and four declined to complete the trial. Finally, 30 healthy individuals (mean age 19.9 ± 1.7 y) were included in the study (Fig. 1). To assess the food pattern of the participants, the Portuguese version of the PREDIMED questionnaire was used [28,29], and a mean overall score of 6.9 ± 2.2 was obtained for the included participants.

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Health Sciences Research Unit. Anonymity and confidentiality of the collected data were assured because the study was developed for scientific purposes only, free of any financial or economic interests. All participants signed an informed consent before the study.

Intervention: Chocolate

The two types of chocolate used in the study had similar nutritional ingredients, only differing in the percentage of cocoa and the amount of epicatechins

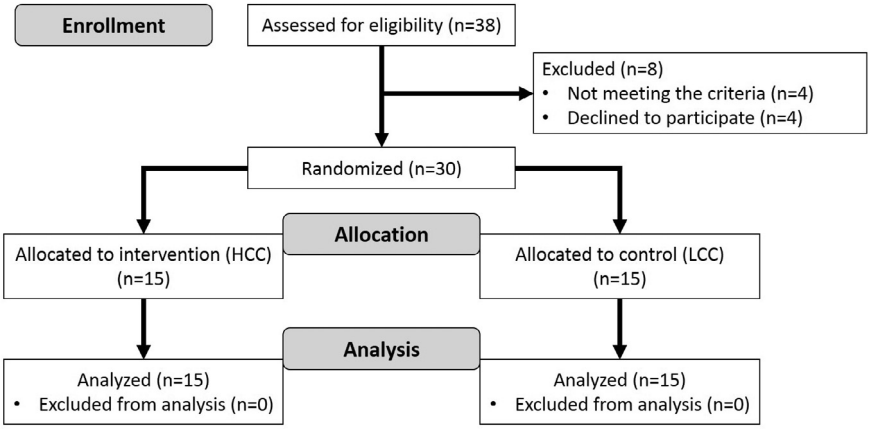

Fig. 1. CONSORT flow diagram of selection of participants HCC, higher cocoa content; LCC, lower cocoa content [30].

Table 1
Nutritional information of the chocolate used in the study

Nutritional information per 20 g	Chocolate 90% cocoa	Chocolate 55% cocoa
Energy value (kcal)	119	108
Lipids (g)	10	7
(of which saturated fatty acids) (g)	6	5
Carbohydrates (g)	4	8
(of which sugar) (g)	2	4
Fibers (g)	3	3
Proteins (g)	3	2
Salt (mg)	4	7
Epicatechin (mg epicatechin/g of sample)	18.19 ± 2.64	12.61 ± 1.35

(Table 1). A sample of both chocolates were analyzed according to a previously described method [31,32]. A conventional sonication extraction technique was employed to extract the polyphenols from cocoa and cocoa products, and afterward the Folin-Ciocalteu reducing capacity was estimated as epicatechin equivalents (EE), expressed as mg/g of sample [31,32]. LCC presented an amount of 12.6 ± 1.4 mg/g of sample, and HCC estimated epicatechin content was 18.2 ± 2.6 mg/g of sample. The chocolate for the intervention group had 90% cocoa and for the control group a chocolate with 55% cocoa was adopted, both types in tablets of 100 g. Participants received the chocolate in dose packages of 20 g covered in aluminum foil, not revealing information of the content. Participants were therefore blinded to the type of chocolate they were eating. Also, the researchers responsible for the clinical measurements were blinded to the type of chocolate for each participant.

Outcomes

Echocardiography

The transthoracic echocardiogram (TTE) was performed using a Vivid I (GE Healthcare, Chicago, IL, USA) and a 1.7/3.4 MHz probe by the same highly experienced examiner. A full TTE examination with standard projections was performed on each participant complying with the international recommendations [33]. The measurements were attained after three repeated cycles. With the probe in a parasternal two-dimensional long-axis view, values on interventricular septum, posterior left ventricle (LV) wall thickness, and internal dimensions of LV were attained according to the guidelines [33]. The apical four- and two-chamber views provided the basis for the volumetric analysis of the LV and of the left atrium (LA). Left atrium volume index was determined with modified Simpson's rule and body surface area correction. Simpson method was applied to evaluate LV ejection fraction (LVEF), and Devereux's formula for LV mass [34]. A combination of LV outflow Doppler method, area of LV outflow tract, and LV time-velocity integral allowed for the estimation of the stroke volume (SV) of the participants [35]. In the four-chamber apical view, pulse-wave Doppler was used to determine diastolic filling velocities for early (E) and late (A) peak, the E-to-A ratio, and E-wave deceleration time. Tissue Doppler imaging of the mitral valve was performed from a septal and lateral view, from which mean values were obtained for early diastolic annular velocities (e'). From the ratio E-to-e' on the left side, the LV filling pressure was assembled [36]. Thoracic ascending (tubular) aorta diameters were measured 2 to 3 cm above the level of the aortic valve, with the leading edge-to-leading edge technique [34]. Dimensions of the aortic arch and descending aorta were measured from the substernal view.

Aortic pulse wave velocity and carotid pulse wave analysis

Carotid-femoral PWV, a measure of aortic stiffness, and carotid PWA were assessed simultaneously with the Complior Analyse device (Alam Medical, Saint-Quentin-Fallavier, France) according to a previously described technique [20]. The measurements were made with the participants in a supine position with the neck in a slight hyperextension, and slightly rotated to the left, after a resting period of 10 min. Brachial blood pressure (bBP) was measured and entered on the Complior Analyse (Alam Medical, Smyrna, GA, USA) software, and then signal acquisition was launched. When the operator observed pulse waveforms of adequate quality, simultaneous carotid and femoral pressure curves were recorded for 15s. The distance travelled by the pulse waveforms was measured between the two recording sites directly on the body surface and was automatically corrected according to the equation "0.8 × direct distance," subtracting the manubrium-to-carotid distance as previously recommended [20,21]. The aortic transit time was calculated according to the intersecting tangent algorithm, as previously recommended [20,21]. PWV was then calculated using measurements of transit time and corrected distance travelled by the pulse wave, between the two recording sites:

$$PWV\ (m/s) = \frac{corrected\ distance\ (meters)}{time\ (seconds)}.$$

The averaged carotid waveform for pulse wave analysis was extracted from the 15s recording window of carotid pulse waves acquired during the PWV procedure and were calibrated with brachial diastolic blood pressure (bDBP) and mean arterial pressure (bMAP). The pressure curve was analyzed, and its morphologic and temporal components were extracted for analysis. Central systolic blood pressure (cSBP), central pulse pressure (cPP), augmentation pressure (AP), augmentation index (Aix), augmentation index corrected for heart rate (Aix@75), amplification of systolic (SAmp) and pulse pressure (PPratio), ventricular ejection time (LVET), diastolic filling time (DT), the dP-to-dt relation, and the Buckberg subendocardial viability index (SEVI) were assessed by analyzing the morphology and timeline of the curves.

All measurements were performed by a highly experienced operator, with high reproducibility scores, as previously published [37], and a noteworthy concordance between invasive arterial parameters and the Complior-based pulse wave analysis method, which also has been previously documented [38].

Ventricle–arterial coupling

VAC reflects the matching between the arterial system and the LV, and is expressed as the ratio of arterial elastance (Ea) to LV elastance (Elv). [26] The Ea is an integrated index of net arterial load that is imposed to LV work, and Elv is an index of contractility and systolic stiffness of myocardium. The matching of both elastances provides greater cardiovascular efficiency and optimal transfer of blood from the LV to the aorta, thus maintaining BP, LV pressure, and cardiac output (CO) in a physiologic range at the highest myocardial energetic efficiency [27]. A VAC of 1 reflects the perfect balancing between the Ea and the Elv [26]. The non-invasive assessment of Ea and Elv was determined using end systolic pressure derived from the arterial waveform using the Complior Analyse (as previously described) and the end systolic volume of the LV and the SV, obtained from the echocardiogram (as described earlier). Table 2 indicates the main equations applied to derive the VAC parameters and other hemodynamic data of interest.

Flow-mediated slowing

The FMS has been suggested as an alternative method to evaluate endothelial function [39–41]. As such, a flow-mediated dilation procedure was implemented complying with the methodological recommendations [42,43], and FMS was calculated according to a previously described technique [39–41]. Resting brachial PWV was acquired in the right arm using the Complior Analyse device, simultaneously with the measure of aortic PWV and PWA. For this, a probe with piezoelectric crystals was placed on the right radial artery using a specific holder, while the two other probes were placed in the ipsilateral carotid and femoral arteries. The probes were adjusted to ensure the acquisition of pulse waves with suitable reproducibility, stability, and amplitude. The distance between the radial and carotid points was measured directly. The brachial PWV corresponded to the distance (d) divided by the carotid-radial pulse wave transit time. Subsequently, a cuff was placed on the forearm and was inflated into a suprasystolic pressure (50 mm Hg above the previously measured systolic blood pressure), keeping the ischemia for a 5-min period, after which the cuff was deflated. Approximately 1 min after complete deflation of the cuff, brachial PWV was repeated. The FMS was calculated as the percentage of variation in brachial PWV, from resting to postreactive hyperemia. According to the Moens-Korteweg equation, PWV is dependent on the arterial diameter, hence, an increase of the arterial radius that follows the FMD protocol in participants with good endothelial function will result in a deceleration (reduction) of brachial PWV after reactive hyperemia [39–41].

Procedure

To avoid an effect on the results from other food products, the participants were told not to consume berries, fruits, jellies and preserves, tea or other infusions, coffee, cocoa, soy products, caffeinated energy drinks, vegetables (excluding potatoes), and alcoholic beverages, on the day before the evaluations. Participants were instructed not to perform any intense physical training or smoking during the 2 h before data collection.

Table 2
Formulas for calculating ventricle–arterial coupling

Ventricle–arterial coupling	Ea/Elv
Arterial elastance	ESP/SV
Ventricular elastance	ESP/ESV
Stroke work	ESP × SV
Potential energy	ESP × ESV/2

Ea, arterial elastance; Elv, left ventricular elastance; ESP, end-systolic pressure; SV, stroke volume.

The baseline and the post-intervention evaluations were completed in the same means in both groups, and each procedure was performed by the same experienced examiner. Sampling of data was carried out in the morning, starting with questionnaires on the Portuguese version of the Profile of Mood States (POMS) [44], perceived happiness through the first item of the Fordyce Happiness Measures [45], and self-satisfaction according to the Organization for Economic Cooperation and Development estimation [46]. Height and weight of the participants was measured with a standard scale. Body mass index (BMI) was estimated as weight/(height^2). Afterward, the participants rested for 10 min and bBP was measured with a validated oscillometric device. Echocardiographic examination and PWV/PWA were then conducted according to the methods described previously. All the operators were blinded for the intervention and group allocation.

At the end of the baseline evaluation, participants were given the individual doses of chocolate, in accordance with the randomized table, and were instructed to comply with the dosage and maintain their habitual lifestyle. The participants were asked to bring any remaining chocolate to the post-intervention evaluation to allow a compliance analysis. The rate of compliance was similar in both groups (>95%), and no side effects were reported by the participants.

Statistical analysis

Data was gathered in Excel 2016 (Microsoft Office, Redmond, WA, USA) and then imported to SPSS version 24 (IBM, Armonk, NY, USA) for statistical analysis.

Categorical variables were reported as frequencies and percentages, and χ^2 or Fisher exact tests were used when appropriate. The Shapiro–Wilks test was used to confirm normal distribution of all continuous variables, expressed as mean + SD. Variables with a non-normal distribution were log-transformed. Student's t test was applied for baseline group comparisons. Individual variables were checked for homogeneity of variance via Levene's test. A two-factor mixed-design analysis of variance was used to evaluate modifications of variables between the baseline to the post-intervention evaluation in each and between groups. The Greenhouse-Geisser correction was used when sphericity was violated, and the Bonferroni adjustment was adopted for multiple comparisons designed to locate the significant effects of a factor. For between-groups comparison, an additional analysis of covariance was performed over the post-intervention data, adjusting for the baseline data (entering as a covariate into the model). A two-tailed $P < 0.05$ was considered significant. The magnitude of the effects was also checked with the η2 value.

Results

Baseline characteristics of the participants

In the sample of 30 participants (26 women and 4 men), the mean age was 19.9 ± 1.7 y, ranging from 18 to 27 y, with a mean BMI of 22.9 ± 3.4 kg/m^2. Risk factors were reported in 5 individuals, family history of cardiovascular disease in 10, smoking habits in 1, and a mild consumption of alcohol and coffee was identified in 20 and 19 participants, respectively. Of the participants, 10% followed a Mediterranean diet, based on the PREDIMED individual score, and the average PREDIMED was 6.9 ± 2.2 for the total population. For the self-assessed questionnaire on psychological variables, the total score on POMS was 78.1 ± 27.2, the degree of happiness was 7.2 ± 1.2, and self-satisfaction was 6.5 ± 1.5 on average. According to the baseline measurements, no statistical significance in between-group differences were found (Table 3).

Vascular effect of the intervention

The effects of the dark chocolate intake on bBP is depicted for both groups in Table 4. As shown, no significant differences were observed regarding the baseline for all considered variables. After intervention, bSBP and bDBP significantly decreased in both groups, although the magnitude of the effect was larger in the HCC group: bSBP mean reduction of 2.4 mm Hg versus 3.5 mm Hg, respectively, in the LCC and HCC groups; bDBP mean reduction of 1.7 mm Hg versus 2.3 mm Hg, respectively, in the LCC and HCC groups. The greater reduction observed in the HCC group led to significant differences in these parameters after intervention, with the HCC group showing lower bSBP and bDBP mean values ($P = 0.036$ and 0.024, respectively). Similar direction of effect was observed for bMAP, but no significant differences were identified regarding bPP and heart rate (HR).

In terms of arterial hemodynamics, no differences were identified in the baseline parameters between groups. After intervention, a significant reduction in aortic PWV was observed in both groups, although reaching statistical significance only in the HCC group (Fig. 2). Post-intervention cfPWV was measured to 6 ± 0.9 m/s in LCC and 5.5 ± 0.8 m/s in HCC ($P < 0.05$). Similar results were observed concerning cSBP, cPP, and the AIx, all showing a trend for a reduction after the intervention, reaching the statistical significance criterion only for the HCC group (Fig. 2). Post-intervention cSBP was 106.9 ± 15.1 mm Hg in LCC and 98.3 ± 5.5 mm Hg in HCC

Table 3
Characteristics of the selected participants

Variables	Total (N = 30)	LCC (n = 15)	HCC (n = 15)	*P*-value*
Age, y	19.9 ± 1.7	20.4 ± 2.1	19.5 ± 1.1	0.140
Male/Female, n (%)	4 (13.3)/26 (86.7)	1 (6.7)/14 (93.3)	3 (20)/12 (80)	0.283
Weight (kg)	62.7 ± 12.6	61.3 ± 13.6	64.2 ± 12.4	0.540
Height (m)	1.7 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	0.497
BMI (kg/m^2)	22.9 ± 3.6	22.6 ± 4.3	23.2 ± 2.9	0.655
Waist (cm)	72.4 ± 9.8	70.8 ± 9.3	74.1 ± 10.1	0.360
Hip (cm)	96.8 ± 8.2	96 ± 10.3	97.6 ± 5.7	0.595
PREDIMED score	6.9 ± 2.2	6.8 ± 2.2	7.1 ± 2.2	0.744
POMS total score	78.1 ± 27.2	77.3 ± 27.4	78.9 ± 28	0.881
Happiness degree	7.2 ±1.2	7.2 ± 1.3	7.2 ± 1.2	0.941
Satisfaction degree	6.5 ±1.5	6.4 ± 1.1	6.5 ± 1.8	0.810
Risk factors				
Yes/No, n (%)	5 (16.7)/25 (83.3)	3 (20)/12 (80)	2 (13.3)/13 (86.7)	0.624
Family history of CVD				
Yes/No, n (%)	10 (33.3)/20 (66.7)	7 (46.7)/8 (53.5)	3 (20)/12 (80)	0.121
Smoking habits				
Yes/No, n (%)	2 (6.7)/28 (93.3)	1 (6.7)/14 (93.3)	1 (6.7 /14 (93.3)	1.000
Alcohol consumption				
Yes/No, n (%)	20 (66.7)/10 (33.3)	10 (66.7)/5 (33.3)	10 (66.7)/5 (33.3)	1.000
Coffee consumption				
Yes/No, n (%)	19 (63.3)/11 (36.7)	9 (60)/6 (40)	10 (66.7)/5 (33.3)	0.705
Diet Mediterranean				
Yes/No, n (%)	3 (10)/27 (90)	1 (6.7)/14 (93.3)	2 (13.3)/13 (86.7)	0.543

BMI, body mass index; CVD, cardiovascular disease; HCC, higher cocoa content; LCC, lower cocoa content; POMS, profile of mood states.
*P-value, LCC vs HCC.

Table 4
Mean values of bBP at baseline and after intervention for the two groups showing the difference between the groups and *P*-value

Variables		LCC (n = 15)	HCC (n = 15)	*P*-value (LCC vs HCC)
bSBP (mm Hg)	Baseline	119.7 ± 12.7	114.7 ± 9.4	0.224
	Post-intervention	117.3 ± 12	111.1 ± 7.6	0.036
	Difference	−2.4	−3.5	
	P-value (ANOVA)	0.047	0.022	
bDBP (mm Hg)	Baseline	72.3 ± 10.6	70 ± 9.7	0.546
	Postintervention	70.6 ± 7.9	67.7 ± 6.6	0.024
	Difference	−1.7	−2.3	
	P-value (ANOVA)	0.049	0.031	
bPP (mm Hg)	Baseline	47.5 ± 8	44.7 ± 9.5	0.391
	Post-intervention	46.7 ± 9.7	43.5 ± 6.1	0.490
	Difference	−0.7	−1.2	
	P-value (ANOVA)	0.687	0.567	
bMAP (mm Hg)	Baseline	88 ± 10.8	84.9 ± 8.6	0.398
	Post-intervention	86.2 ± 8.3	82.2 ± 6.3	0.011
	Difference	−1.8	−2.7	
	P-value (ANOVA)	0.045	0.018	
Heart rate (beat/min)	Baseline	65.1 ± 6.8	69.3 ± 8.8	0.161
	Post-intervention	64.4 ± 7.2	67.1 ± 10.8	0.716
	Difference	−0.7	−2.1	
	P-value (ANOVA)	0.523	0.299	

ANOVA, analysis of variance; bSBP, brachial systolic blood pressure; bDBP, brachial diastolic blood pressure, bPP; brachial pulse pressure; bMAP, brachial mean arterial pressure; HCC, higher cocoa content; LCC, lower cocoa content.

($P < 0.05$), cPP was 40.5 ± 12.8 mm Hg in LCC, and 34.8 ± 9 mm Hg in HCC ($P = 0.17$), and the AIx was −13.2 ± 14.9% in LCC and −21.1 ± 21.1% in HCC ($P < 0.05$).

Other aspects of central arterial hemodynamics were compared and are shown in Table 5. No baseline differences were observed between groups, and no significant variation was depicted for the diastolic time or for the LVET. Importantly, the SEVI increased after the intervention in both groups, with a greater magnitude of the effect in the HCC group: mean increase of 13.7% in the LCC group versus mean increase of 49.6% in the HCC group. The post-intervention mean SEVI was thus significantly greater in the HCC group than in the LCC group (180.3 ± 18.7 and 156.7 ± 17.1, respectively; $P = 0.001$). In addition, FMS as an indirect measure of endothelial function was also positively modulated with the intervention, increasing in both groups, with a significantly larger increase in the HCC. In fact, the FMS increased from 6.8% ± 3.6% to 14.6% ± 4.4% in the HCC (mean increase of 7.8%) and increased from 6.3% ± 2.9% to 8.9% ± 4.7% in the LCC (mean increase of 2.6%), with significant differences in the mean FMS after the intervention between groups ($P < 0.001$).

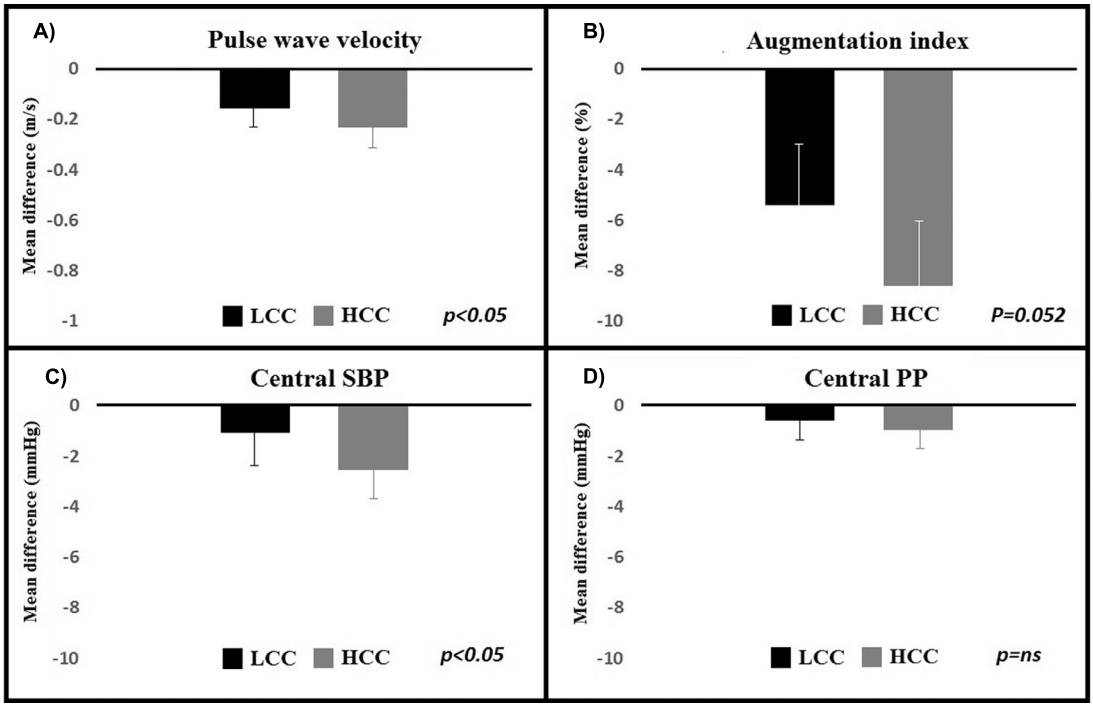

Fig. 2. Central arterial hemodynamics showing the difference from evaluation at baseline and post-intervention for group with LCC and for HCC group in (**A**) pulse wave velocity, (**B**) augmentation index, (**C**) central SBP and (**D**) central PP. HCC, higher cocoa content; LCC, lower cocoa content; ns, not significant; PP, pulse pressure; SBP, systolic blood pressure.

Table 5
Arterial hemodynamics and FMS at baseline and after intervention for the total sample and the two groups; showing the difference between the moments of evaluation and *P*-value

Variables		Total (N = 30)	LCC (n = 15)	HCC (n = 15)	*P*-value
LVET (ms)	Baseline	346.2 ± 99.3	334 ± 52.7	358 ± 131.6	0.521
	Postintervention	348.7 ± 76.1	360.6 ± 104.2	336.1 ± 28.6	0.309
	Difference	2.2	26.3	-21.9	
	P-value (ANOVA)	0.915	0.346	0.47	
DT (ms)	Baseline	565 ± 160.7	608.9 ± 108.3	521 ± 194	0.137
	Post-intervention	583.5 ± 126.7	585.4 ± 90.5	581.5 ± 158.2	0.567
	Difference	18.5	−23.5	60.5	
	P-value (ANOVA)	0.529	0.474	0.22	
SEVI (%)	Baseline	136.9 ± 41.8	143 ± 28.2	130.7 ± 52.3	0.431
	Post-intervention	168.5 ± 21.6	156.7 ± 17.1	180.3 ± 18.7	0.001
	Difference	31.6	13.7	49.6	
	P-value (ANOVA)	0.001	0.031	0.004	
FMS (%)	Baseline	6.6 ± 3.2	6.3 ± 2.9	6.8 ± 3.6	0.698
	Post-intervention	11.8 ± 5.3	8.9 ± 4.7	14.6 ± 4.4	<0.001
	Difference	5.2	2.6	7.8	
	P-value (ANOVA)	<0.001	0.007	<0.001	

ANOVA, analysis of variance; DT, diastolic filling time; FMS, flow-mediated slowing; HCC, higher cocoa content; LCC, lower cocoa content; LVET, left ventricular ejection time; SEVI, subendocardial viability index.

Cardiac structural and functional effect of the intervention

The structural dimensions from TTE at baseline evaluation were within normal range and the mean values did not differ between the groups (Table 6). These data did not change significantly from baseline to post-intervention, indicating no structural effect of the intervention over the heart.

Regarding functional data, the results also showed values within normal range and no statistically difference between the groups at baseline (Table 7). After the intervention, significant changes were observed only in the HCC group, with a significant increase in the LV shortening fraction (from 37.3 ± 3.4% at baseline to 45.3 ± 11.6% after the intervention; $P = 0.034$), a trend for an increase in LVEF (from 67 ± 4.5% at baseline to 69.3 ± 4.7% after intervention; $P = 0.082$), and a significant increase in the mean mitral E wave velocity (from 78.1 ± 12.00 cm/s at baseline to 84.3 ± 11.9 cm/s post-intervention; $P = 0.019$). Notwithstanding the improvement in these variables in the HCC group, no significant differences were seen in the between-group comparison of the post-intervention values. No significant variations were identified in the other analyzed functional parameters. Also, although mean CO increased in the HCC group compared with the LCC group, the difference was not significant.

Additional functional data were extracted from the tissue Doppler imaging, showing no baseline significant differences between groups and values within the normal range. After intervention, no significant differences for the two groups were identified, with the exception of the lateral A wave in the HCC arm ($P = 0.038$). The diameter and flow velocities of the ostial portion of both right and left coronary arteries were also inspected through echocardiography in a parasternal short-axis projection at the level of the aortic valve. No significant variations were depicted in either group after the intervention program.

Effect of intervention on the VAC

VAC parameters were similar at baseline between groups, but showed a significant improvement in the HCC group after intervention, increasing from 0.67 to 0.72 ($P = 0.004$), so that the post-intervention VAC was significantly higher in the HCC group than in the LCC group ($P < 0.05$), as shown in Figure 3 and Table 8.

In addition, significant variation was observed in both groups regarding arterial stroke work and potential energy, with greater mean differences identified in the HCC arm, as depicted in Table 9.

Discussion

The search for health benefits associated with cocoa ingestion has motivated extensive research [1–17], and although many studies provided support of the beneficial properties of cocoa, the full

Table 6
Structural cardiac values at baseline of the two compared groups

Variables	LCC (n = 15)	HCC (n = 15)	*P*-value
Intraventricular septum, D (mm)	6.9 ± 0.3	7 ± 0.5	0.243
Intraventricular septum, S (mm)	10.5 ± 1.1	10.6 ± 1.4	0.831
LV diameter, D (mm)	50.6 ± 5.1	50.8 ± 4.4	0.893
LV diameter, S (mm)	30.7 ± 3	31.9 ± 4	0.377
LV posterior wall, D (mm)	6.9 ± 0.3	6.9 ± 0.4	0.451
LV posterior wall, S (mm)	15.9 ± 1.4	15.3 ± 1	0.162
LV relative wall thickness (mm)	2.7 ± 2	2.7 ± 1.3	0.805
LV volume, D (mL)	124.7 ± 29.8	124.6 ± 26.3	0.998
LV volume, S (mL)	49.5 ± 14.3	54.1 ± 17	0.435
LV mass, D (g)	116.9 ± 27.3	120.3 ± 27.8	0.739
LV index mass, D (b/m^2)	69.7 ± 12	70 ± 10.8	0.951
Aortic root diameter (mm)	25 ± 2.6	25.8 ± 3.2	0.473
Left atrium area (cm^2)	12.5 ± 2.9	12.3 ± 2.1	0.827
Right atrium area (cm^2)	11.7 ± 2.4	12.3 ± 1.8	0.490

D, diastole; HCC, higher cocoa content; LCC, lower cocoa content; S, systole; LV, left ventricle.

Table 7
Functional data from echocardiogram showing the values at baseline and post-intervention and *P*-values

Variables		LCC (n = 15)	HCC (n = 15)	*P*-value
LV ejection fraction (%)	Baseline	69.3 ± 3.1	67 ± 4.5	0.117
	Post-intervention	68.1 ± 5.3	69.3 ± 4.7	0.268
	P-value (ANOVA)	0.456	0.082	
LV shortening fraction (%)	Baseline	39.2 ± 2.7	37.3 ± 3.4	0.094
	Post-intervention	44.3 ± 13.3	45.3 ± 11.6	0.759
	P-value (ANOVA)	0.127	0.034	
Mitral E wave velocity (cm/s)	Baseline	78 ± 11.3	78.1 ± 12	0.975
	Post-intervention	83.6 ± 10.4	84.3 ± 11.9	0.866
	P-value (ANOVA)	0.108	0.019	
Mitral A velocity (cm/s)	Baseline	45.3 ± 7.5	45.9 ± 11.6	0.853
	Post-intervention	44 ± 8.2	45.7 ± 11.1	0.629
	P-value (ANOVA)	0.555	0.913	
E/A	Baseline	1.8 ± 0.3	1.8 ± 0.5	0.891
	Post-intervention	2.1 ± 0.5	1.9 ± 0.5	0.223
	P-value (ANOVA)	0.034	0.514	
E/e'	Baseline	6.5 ± 1.3	6.5 ± 0.9	1
	Post-intervention	6.9 ± 1	9.9 ± 1.1	0.853
	P-value (ANOVA)	0.334	0.014	
Tricuspid E wave velocity (cm/s)	Baseline	58.9 ± 14.1	58.7 ± 9.5	0.964
	Post-intervention	62.4 ± 8.9	60.7 ± 8.9	0.607
	P-value (ANOVA)	0.393	0.568	
Tricuspid A velocity (cm/s)	Baseline	33 ± 8	33.8 ± 12.2	0.833
	Post-intervention	29 ± 7.7	32 ± 7	0.291
	P-value (ANOVA)	0.133	0.585	
Cardiac output(L/min)	Baseline	5.9 ± 1.3	5.9 ± 1.1	0.896
	Post-intervention	5.8 ± 1.4	6.2 ± 1.4	0.417
	P-value (ANOVA)	0.932	0.263	

ANOVA, analysis of variance; HCC, higher cocoa content; LCC, lower cocoa content; LV, left ventricle.

understanding of the underlying mechanisms are not yet completely identified, particularly in young adults. In the present study, the vascular effects observed after the intervention were in line with previous research [1,3,4,6]. The reduction of both diastolic and systolic BPs occurred in both groups, but was larger in the HCC group, thus indicating that a higher cocoa content, and thus higher amount of epicatechin, has a stronger ability to reduce BP. This result correlates with a study conducted on Kuna Indians, where the findings indicated a strong relation with normotensive individuals and a high cocoa intake [11]. This positive association was also observed for aortic PWV, in which a significant decrease was depicted for the HCC group, translating into a less stiff aorta than at basal evaluation, thus resulting in a better overall cardiovascular profile [10,12,20,21]. In line with this argument, the increase of the SEVI, which was significantly higher in the HCC group, reflects an improvement in myocardium perfusion [19] and is intertwined with the results for the FMS, an indirect indicator of endothelial

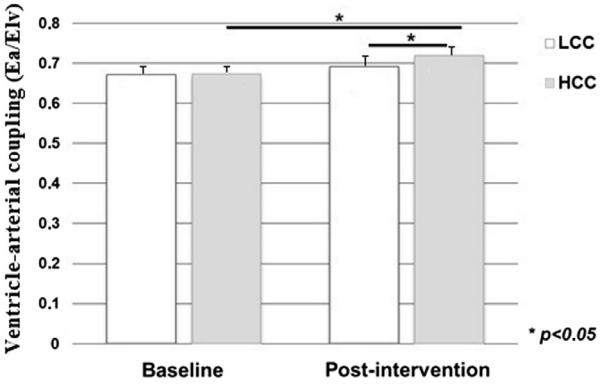

Fig. 3. Comparative variation of the ventricular–arterial coupling estimation in the LCC and HCC groups. Ea, arterial elastance; Elv, left ventricle elastance; HCC, higher cocoa content; LCC, lower cocoa content. *$P < 0.05$.

function [39–41]. This reveals an improvement of the endothelial function, to a greater extent in the HCC group, which is aligned with a large body of previous research [1], and clearly demonstrates the benefits of cocoa on the endothelium. This pinpoints this association as the reason for the majority of the vascular benefits attributable to cocoa, and particularly to the isomer epicatechin, which was shown to endorse the release of nitric oxide, to increase the sensitivity of the nitric oxide receptors to its relaxing effect, and vis-a-vis, leading to vasodilation, less peripheral resistance, more relaxation in the arterial wall, and consequently, reducing BP, PWV, and the reflected wave's magnitude [1,2,7,8,16,17].

The positive vascular modulation associated with the consumption of HCC also produced relevant changes in the interaction of the LV and the aorta, as measured through the VAC parameter. The significant enhancement in the VAC in the HCC group indicates a better matching between the heart and the aorta, contributing to an optimized LV performance, better stroke work, and less energetic cost, indicating more efficient LV mechanical work [25–27]. To the best of our knowledge, this is the first study to identify benefits of cocoa in VAC, integrating benefits in vascular dynamics with heart function. Although an improvement in the mechanical matching of the aorta and the LV was found after the intervention, no significant changes were identified in the heart, as evaluated by several anatomic and functional measures obtained through cardiac ultrasonography. Of course, this finding was expected considering the particular profile of the population, with all participants being quite young and healthy, and also considering the short intervention period (30 d). Even so, significant variations were observed in some functional parameters in the HCC group, although with a small magnitude. Mainly, the shortening fraction and the EF of the LV both increased slightly, but significantly, in the HCC group, accompanied by similar favorable changes in the mitral E wave velocity, which may be a consequence of the improvement in the overall mechanical coupling of the LV and the arterial

Table 8
Variation in hemodynamic endpoints from baseline to 1 mo of daily consumption of 20 g dark chocolate in the LCC and HCC groups

	LCC (n = 15)		HCC (n = 15)		Differences at 1 mo	
	Baseline	1 mo	Baseline	1 mo	HCC vs LCC	*P*-value*
VAC (Ea/Elv)	0.67 ± 0.06	0.69 ± 0.05	0.67 ± 0.06	0.72 ± 0.06	0.04 (0.14 to 0.06)	0.01
PWV (m/s)	6.1 ± 0.9	6 ± 0.9	5.8 ± 0.7	5.5 ± 0.8	−0.58 (−1.20 to −0.06)	0.04
cSBP (mm Hg)	108.4 ± 13.8	106.9 ± 15.1	102 ± 5.3	98.3 ± 5.5	−8.60 (−17.10 to −0.10)	0.04
cPP (mm Hg)	41.1 ± 11.5	40.5 ± 12.8	35.9 ± 9.6	34.8 ± 90	−0.60 (−4.20 to 3.00)	0.72
AIx (%)	−8.7 ± 18.8	−13.2 ± 14.9	−12.1 ± 22.4	−21.1 ± 21.1	−4.90 (−8.60 to −1.10)	0.01

AIx, augmentation index; cPP, central pulse pressure; cSBP, central systolic blood pressure; Ea, arterial elastance; Elv, left ventricle elastance; HCC, higher cocoa content; LCC, lower cocoa content; PWV, carotid-femoral pulse wave velocity; VAC, ventricle–arterial coupling.
Mean ± SD or mean (Bonferroni-corrected 95% CIs).
*Derived by using 1-factor analysis of covariance with baseline value as covariate.

Table 9
Variation in stroke work and potential energy from baseline to post-intervention in the total participants and, comparatively, in the LCC and HCC groups

Variables		Total (N = 30)	LCC (n = 15)	HCC (n = 15)	*P*-value
Stroke work (mm Hg × mL)	Baseline	85.8 ± 13.7	89.8 ± 15	81.8 ± 11.3	0.113
	Post-intervention	78.6 ± 13.9	84.9 ± 13.7	72.3 ±11.2	0.024
	Difference	−7.2	−4.9	−9.5	
	P-value (ANOVA)	<0.001	0.005	0.001	
Potential energy (mm Hg × mL)	Baseline	27.6 ± 10.1	27.2 ± 11.3	28 ± 9.2	0.844
	Post-intervention	26.1 ± 9.3	25.7 ± 10.1	26.4 ± 8.7	0.990
	Difference	−1.6	−1.5	−1.6	
	P-value (ANOVA)	0.001	0.035	0.011	

ANOVA, analysis of variance; HCC, higher cocoa content; LCC, lower cocoa content;

system, resulting in an optimization of both the filling and the contraction of the LV. Some studies have suggested an important role of the ascending aorta in the diastolic component of the cardiac cycle, acting as a spring that contributes, along with the twisting and torsion movements of the LV, to a suction effect that facilitates LV filing and translates into a better diastolic function [27].

The present study had several limitations that should be considered. The small number of participants is a significant aspect, despite the results being consistent in the most relevant outcomes considered in the study. Information on flavanol absorption and plasmatic concentration would also provide further relevant information, but the high cost of such methodology made it unviable for the present study. Furthermore, a more thorough and refined component analysis of the chocolates, based on high-performance liquid chromatography, would provide more detailed information about relevant nutritional differences, but the high cost and lack of funding made this option unviable. The study enrolled only healthy, young participants with good overall cardiovascular health, so the expected degree of improvement with cocoa ingestion was necessarily limited. Even so, most studies addressing the health benefits of cocoa have been conducted mainly in middle-aged adults and patients with cardiovascular diseases; therefore, approaching the biological effects of cocoa in a young population is an innovative feature of potential interest for early primary cardiovascular prevention, taking into account the benefits that were observed in association with the consumption of HCC chocolate.

Conclusion

The present study demonstrated that the regular consumption of a small amount (20 g/d) of HCC dark chocolate provides cardiovascular benefits in young, healthy adults, providing an enhancement in the heart and vascular matching as measured with the VAC parameter. The extent to which cocoa may interact with other healthy lifestyles strategies remains to be explored, and further research is needed to clarify the underlying mechanisms and to define optimal amounts of regular cocoa rich dark chocolate intake.

References

[1] EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). Scientific Opinion on the substantiation of a health claim related to cocoa flavanols and maintenance of normal endothelium-dependent vasodilation pursuant to Article 13 (5) of Regulation (EC) No 1924/2006. EFSA J 2012;10:2809.
[2] Fraga CG, Oteiza P. Flavanols and vascular health: molecular mechanisms to build evidence-based recommendations. Free Radic Biol Med 2014;75(suppl 1):S12.
[3] Perez A, Gonzalez-Manzano S, Jimenez R, Perez-Abud R, Haro JM, Osuna A, et al. The flavonoid quercetin induces acute vasodilator effects in healthy volunteers: correlation with beta-glucuronidase activity. Pharmacol Res 2014;89:11–8.
[4] Rassaf T, Rammos C, Hendgen-Cotta UB, Heiss C, Kleophas W, Dellanna F, et al. Vasculoprotective effects of dietary cocoa flavanols in patients on hemodialysis: a double-blind, randomized, placebo-controlled trial. Clin J Am Soc Nephrol 2016;11:108–18.
[5] Yoon HS, Kim JR, Park GY, Kim JE, Lee DH, Lee KW, Chung JH. Cocoa flavanol supplementation influences skin conditions of photo-aged women: a 24-week double-blind, randomized, controlled trial. J Nutr 2016;146:46–50.
[6] Mastroiacovo D, Kwik-Uribe C, Grassi D, Necozione S, Raffaele A, Pistacchio L, et al. Cocoa flavanol consumption improves cognitive function, blood pressure control, and metabolic profile in elderly subjects: the Cocoa, Cognition, and Aging (CoCoA) study—a randomized controlled trial. Am J Clin Nutr 2015;101:538–48.
[7] Monahan KD, Feehan RP, Kunselman AR, Preston AG, Miller DL, Lott ME. Dose dependent increases in flow-mediated dilation following acute cocoa ingestion in healthy older adults. J Appl Physiol 2011;111:1568–74.
[8] Sansone R, Rodriguez-Mateos A, Heuel J, Falk D, Schuler D, Wagstaff R. Flaviola Consortium, European Union 7th Framework Program. Cocoa flavanol intake improves endothelial function and Framingham Risk Score in healthy men and women: a randomised, controlled, double-masked trial: the Flaviola Health Study. Br J Nutr 2015;114:1246–55.
[9] Steinberg FM, Bearden MM, Keen CL. Cocoa and chocolate flavonoids: implications for cardiovascular health. J Am Diet Assoc 2003;103:215–23.
[10] Vlachopoulos CV, Alexopoulos NA, Aznaouridis KA, Ioakeimidis NC, Dima IA, Dagre A, et al. Relation of habitual cocoa consumption to aortic stiffness and wave reflections, and to central hemodynamics in healthy individuals. Am J Cardiol 2007;99:1473–5.
[11] McCullough ML, Chevaux K, Jackson L, Preston M, Martinez G, Schmitz HH, et al. Hypertension, the Kuna, and the epidemiology of flavanols. J Cardiovasc Pharmacol 2006;47(suppl 2):S103–9. discussion 19–21.

[12] Okamoto T, Kobayashi R, Natsume M, Nakazato K. Habitual cocoa intake reduces arterial stiffness in postmenopausal women regardless of intake frequency: a randomized parallel-group study. Clin Interv Aging 2016;11:1645–52.
[13] Higginbotham E, Taub PR. Cardiovascular benefits of dark chocolate? Curr Treat Options Cardiovasc Med 2015;17:54.
[14] Steinhaus DA, Mostofsky E, Levitan EB, Dorans KS, Hakansson N, Wolk A, et al. Chocolate intake and incidence of heart failure: findings from the cohort of Swedish men. Am Heart J 2017;183:18–23.
[15] Buijsse B, Feskens EJ, Kok FJ, Kromhout D. Cocoa intake, blood pressure, and cardiovascular mortality: the Zutphen Elderly Study. Arch Intern Med 2006;166:411–7.
[16] Taubert D, Roesen R, Lehmann C, Jung N, Schomig E. Effects of low habitual cocoa intake on blood pressure and bioactive nitric oxide: a randomized controlled trial. JAMA 2007;298:49–60.
[17] Fisher ND, Hughes M, Gerhard-Herman M, Hollenberg NK. Flavanol-rich cocoa induces nitric-oxide-dependent vasodilation in healthy humans. J Hyperten 2003;21:2281–6.
[18] Shirwany NA, Zou M. Arterial stiffness: a brief review. Acta Pharmacol Sin 2010;31:1267–76.
[19] Prince CT, Secrest AM, Mackey RH, Arena VC, Kingsley LA, Orchard TJ. Augmentation pressure and subendocardial viability ratio are associated with microalbuminuria and with poor renal function in type 1 diabetes. Diabetes Vasc Dis Res 2010;7:216–24.
[20] Van Bortel ML, Laurent USS, Boutouyrie DP, Chowienczyk DP, Cruickshank DJK, De Backer DT, et al. Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. J Hyperten 2012;30:445–8.
[21] Reference Values for Arterial Stiffness Collaboration. Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: establishing normal and reference values. Eur Heart J 2010;31:2338–50.
[22] Task Force for the Management of Arterial Hypertension of the European Society of Hypertension. Task Force for the Management of Arterial Hypertension of the European Society of Cardiology. 2013 ESH/ESC guidelines for the management of arterial hypertension, 22. Blood Press; 2013. p. 193–278.
[23] 4th RowleyTJ, BF Bitner, Ray JD, Lathen DR, Smithson AT, Dallon BW, et al. Monomeric cocoa catechins enhance β-cell function by increasing mitochondrial respiration. J Nutr Biochem 2017;49:30–41.
[24] Watanabe N, Inagawa K, Shibata M, Osakabe N. Flavan-3-ol fraction from cocoa powder promotes mitochondrial biogenesis in skeletal muscle in mice. Lipids Health Dis 2014;13:64.
[25] Sunagawa K, Maughan WL, Burkhoff D, Sagawa K. Left ventricular interaction with arterial load studied in isolated canine ventricle. Am J Physiol 1983;245(5 Pt 1):H773–80.
[26] Chantler PD, Lakatta EG, Najjar SS. Arterial-ventricular coupling: mechanistic insights into cardiovascular performance at rest and during exercise. J Appl Physiol 2008;105:1342–51.
[27] Bell V, McCabe EL, Larson MG, Rong J, Merz AA, Osypiuk E, et al. Relations between aortic stiffness and left ventricular mechanical function in the community. J Am Heart Assoc 2017;6. pii: E004903.
[28] Martinez-Gonzalez MA, Garcia-Arellano A, Toledo E, Salas-Salvado J, Buil-Cosiales P, Corella D, et al. A 14-item Mediterranean diet assessment tool and obesity indexes among high-risk subjects: the PREDIMED trial. PLoS One 2012;7:e43134.
[29] Afonso L, Moreira T, Oliveira A. Índices de adesão ao padrão alimentar mediterrânico – a base metodológica para estudar a sua relação com a saúde. Revista Factores de Risco 2014;31:48–55.
[30] Schulz KF, Altman DG, Moher D. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. Trials 2010;11:32.
[31] Lee KW, Kim YJ, Lee HJ, Lee CY. Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine. J Agric Food Chem 2003;51:7292–5.
[32] Pimentel FA, Nitzke JA, Klipel AB, de Jong EV. Chocolate and red wine – a comparison between flavonoids content. Food Chem 2010;120:109–12.
[33] Evangelista A, Flachskampf F, Lancellotti P, Badano L, Aguilar R, Monaghan M, et al. European Association of Echocardiography recommendations for standardization of performance, digital storage and reporting of echocardiographic studies. Eur J echocardiogr 2008;9:438–48.
[34] Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr 2015;28. 1–39. e14.
[35] Dubin J, Wallerson DC, Cody RJ, Devereux RB. Comparative accuracy of Doppler echocardiographic methods for clinical stroke volume determination. Am Heart J 1990;120:116–23.
[36] Ommen SR, Nishimura RA, Appleton CP, Miller FA, Oh JK, Redfield MM, et al. Clinical utility of Doppler echocardiography and tissue Doppler imaging in the estimation of left ventricular filling pressures: a comparative simultaneous Doppler-catheterization study. Circulation 2000;102:1788–94.
[37] Pereira T, Maldonado J, Andrade I, Cardoso E, Laranjeiro M, Coutinho R, et al. Reproducibility of aortic pulse wave velocity as assessed with the new Complior Analyse. Blood Press Monit 2014;19:170–5.
[38] Pereira T, Maldonado J, Coutinho R, Cardoso E, Laranjeiro M, Andrade I, et al. Invasive validation of the Complior Analyse in the assessment of central artery pressure curves: a methodological study. Blood Press Monit 2014;19:280–7.
[39] Cauwenberghs N, Heyrman Y, Thijs L, Yang W, Wei F, Zhang Z, et al. Flow-mediated slowing of brachial-radial pulse wave velocity: methodological aspects and clinical determinants. Artery Res 2018;21:29–37.
[40] Torrado J, Farro I, Bia D, Zocalo Y, Valls G, Valero M, et al. Levels and rates of change in carotid-radial pulse wave velocity associated with reactive hyperaemia: analysis of the dependence on transient ischemia length. Conf Proc Annu Int Conf IEEE Eng Med Biol 2010;2010:2865.. e8.
[41] Pereira T, Almeida A, Conde J. Flow-mediated slowing as a methodological alternative to the conventional echo-tracking flow-mediated dilation technique for the evaluation of endothelial function: a preliminary report. Mayo Clin Proc Innov Qual Outcomes 2018;2:199–203.
[42] Harris RA, Nishiyama SK, Wray DW, Richardson RS. Ultrasound assessment of flow-mediated dilation. Hypertension 2010;55:1075–85.
[43] Thijssen DH, Black MA, Pyke KE, et al. Assessment of flow mediated dilation in humans: a methodological and physiological guideline. Am J Physiol Heart Circ Physiol 2011;300:H2–12.
[44] Viana MF, Almeida PL, Santos RC. Adaptação portuguesa da versão reduzida do Perfil de Estados de Humor – POMS. Análise Psicológica 2001;1:77–92.
[45] Fordyce MW. Happiness, its daily variation and its relation to values. Diss Abstr Int 1972;33:1266 B.
[46] Organization for Economic Cooperation and Development. Society at a glance: OECD social indicators. Paris: Author; 2005.