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Cocoa flavanols improve peakVO₂ and exercise capacity in a randomized double blinded clinical trial in healthy elderly people†

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Background: Loss of functional capacity is one of the hallmarks in cardiovascular aging. Cocoa flavanols (CF) exert favorable effects on endothelial function, blood pressure, and inflammation. These cardiovascular health markers worsen with increasing age and limit functional exercise capacity. **Aim:** To investigate the effect of CF on cardiorespiratory-fitness in healthy elderly people. **Methods:** In a randomized, double-masked, placebo-controlled, parallel-group dietary intervention trial, 68 healthy elderly people (55–79 years, 28 female) received either 500 mg of CF or a nutrient-matched control capsule twice a day for 30 days. Primary endpoint was defined as peak oxygen consumption (VO₂) in a cardiopulmonary exercise test (CPET). Secondary endpoints were oxygen pulse (VO₂ per heart rate (HR)), resting blood pressure (BP), and resting vascular function. **Results:** After 30 days of CF intake peakVO₂ increased by 190 ml min⁻¹ (95% CI 1–371 ml min⁻¹) and peakVO₂ per kg by 2.5 ml (min kg)⁻¹ (95% CI 0.30–4.2 ml (min kg)⁻¹). O₂-pulse increased by 1.7 ml (95% CI 0.29–3.2 ml) and max exercise capacity by 9.6 W (95% CI 2.1–17.7 W). CF decreased resting systolic and diastolic BP by 5.4 mmHg (95% CI –10.7 to –0.1 mmHg) and 2.9 mmHg (95% CI –5.5 to –0.4 mmHg), respectively. Flow-mediated vasodilation (FMD) increased by an absolute 1.3% (95% CI 0.76–1.79%) in the CF group. Indexes of pulmonary function were not affected. No changes for primary and secondary endpoints were detected in control. **Conclusion:** CF substantially improve markers of cardiorespiratory fitness in healthy elderly humans highlighting their potential to preserve cardiovascular health with increasing age.

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Introduction

Loss of functional capacity is a hallmark of cardiovascular aging. Age is one of the major risk factors for development of cardiovascular disease (CVD).^{1,2} The aging heart is characterized by increasing mass-to-volume ratio, reduced stroke volume and reduced maximum heart rate resulting in reduced cardiac output, which diminishes cardiac reserve capacity and functional status.³ Reduced cardiac regeneration capacity, oxidative stress and chronic low-grade inflammation are key factors for the development of age associated cardiac disease.⁴ Additionally, aging is

associated with endothelial dysfunction and arterial remodeling culminating in luminal dilation and intimal thickening,^{5,6} which overall predispose for the development of vascular stiffness.

Cardiovascular fitness as a strong predictor of mortality⁷ is of socioeconomic importance in an aging population worldwide, where costs for care and nursing are growing.⁸ Cardiopulmonary exercise testing (CPET) is the standard non-invasive method for the quantification of exercise capacity and discrimination of underlying reasons for functional limitation.⁹ The ability of CPET to assess both peak and submaximal exercise response in a ramp protocol makes it a useful tool in the quantification of capability in daily activities. CPET is a valuable method to estimate prognosis in patients with heart failure and CAD.¹⁰ Peak oxygen consumption (peakVO₂) has been independently associated with all-cause mortality, death from heart failure, sudden cardiac death, and functional deterioration in heart failure and hypertrophic cardiomyopathy.^{3,10} High levels of cardiorespiratory fitness are associated with the lowest risk-adjusted all-cause mortality.⁷

Cocoa flavanols (CF) are a subfamily of flavonoids found in various food groups like berries, chocolate, and wine.

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Flavanols are thought to increase endothelial NO synthase (eNOS) activity and nitric oxide (NO) bioavailability.^{11,12} Its major bioactive components in terms of vascular function improvement are (–)-epicatechin and its metabolites and pro-cyanidins may decrease cholesterol and thereby contribute to long-term vascular effects.^{13,14} The methylxanthines in cocoa and cocoa extracts may contribute to the biological activities of cocoa *via* enhancing the bioavailability.¹⁵ They exert favorable effects on several cardiovascular health markers including low-grade inflammation, oxidative stress,¹⁶ endothelial function,¹⁷ cholesterol,¹³ and blood pressure.¹⁸

Improved endothelial function and reduced vascular stiffness and normotensive blood pressure positively affect ventricular-arterial-coupling¹⁹ leading to improved cardiac output and supposedly better exercise performance. Inorganic nitrate, which also increases NO in the circulation, was shown to improve exercise capacity in heart failure with preserved ejection fraction.²⁰ Several studies have examined the effects of flavanol-rich compounds on exercise performance in healthy volunteers. Decaffeinated green tea extracts have been shown to increase power output and distance covered in a 40 minutes performance trial in recreationally active males.²¹ Also, higher ventilation threshold during exercise was recorded after graded cycle exercise in non-athlete males under 50 years of age.²² There is evidence for reduced oxygen cost and time trial performance in moderately trained subjects.²³ In one study, dark chocolate ingestion containing only 18–36 mg (–)-epicatechin was not associated with overall better performance in high altitude trained cyclists despite significantly lower lactate levels in the chocolate group.²⁴ Our previous work shows that the amount of (–)-epicatechin required to achieve half maximal effects on endothelial function is 31 mg and that the maximal is almost reached after the highest dose at 112 mg (–)-epicatechin (820 mg cocoa flavanols).¹⁵ Additionally, our previous studies showed a sustained improvement of FMD after one-month of ingestion of 900 mg cocoa flavanol (128 mg (–)-epicatechin) daily.²⁵ Recently, we showed that the intake of 1000 mg of cocoa flavanols (160 mg (–)-epicatechin) protects radial arteries against intima hyperplasia after catheterization-induced injury.²⁶ We hypothesized, that CF at a dose sufficient to maximally increase endothelial function have a positive impact on peakVO₂ and exercise capacity in older adults without manifest cardiovascular disease. Therefore, the aim of the current study was to investigate the effect of dietary CF intake on cardiorespiratory fitness and functional status in healthy elderly individuals. Capsules containing a high-flavanol cocoa extract were given in a randomized, placebo-controlled, double-blinded trial to healthy elderly humans following structured exercise testing.

Methods

Study design

In a randomized, double-masked, placebo-controlled, parallel-group dietary intervention trial, 68 healthy elderly humans (55–79 years, 28 female) were recruited by advertisement from

the general population. Posters advertising possible study participation were displayed in hallways of the University Hospital of Duesseldorf and the Heinrich-Heine University Duesseldorf. An initial screening visit was scheduled *via* E-Mail. Additionally, home-care physicians and cardiologists were asked to screen patients for potential participation. Participants underwent vascular function assessment, biomarker testing and CPET in this standardized order starting in the early afternoon. At baseline and after 30 days, several standard laboratory variables including total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, creatinine, HbA1C, CRP, hemoglobin, and cell counts were assessed in whole blood, separated and analyzed by the central laboratory of the University Hospital of Duesseldorf. Blood was withdrawn by standard venous puncture. Inclusion criteria were healthy elderly participants over 55 years of age and without diagnoses of cardiovascular disease. In addition to past medical history, heart rate, electrocardiogram, physical examination and laboratory parameters validated a healthy status in all participants (Table 1). Exclusion criteria included age under 55 or over 80 years, non-cardiac limitations of exercise capacity such as orthopedic or neurological disorders, BMI >35 kg m⁻², active smoking, uncontrolled blood pressure, diabetes, chronic obstructive pulmonary disease, atrial fibrillation, cardiomyopathy, coronary heart disease, valvular heart disease, cardiac pacemaker, acute respiratory tract infections, regular intake of any kind of medication and missing ability or missing cooperation

Table 1 Baseline characteristics of the study cohort. There were no significant differences between groups at baseline, values are reported as means ± standard deviation (SD). BSA = body surface area; HbA1C = glycated hemoglobin; CRP = C-reactive protein; Hb = hemoglobin

Baseline characteristics	Control (n = 33)	Cocoa flavanols (n = 35)	p-Value
Age (y)	64.5 ± 6.6	64.3 ± 5.4	0.8884
Sex			0.3246
Male	17	23	
Female	16	12	
Height (cm)	174 ± 8.4	177 ± 8.9	0.0996
Weight (kg)	80.2 ± 13.0	82.5 ± 14.1	0.4839
BSA (m ²)	1.99 ± 0.20	1.98 ± 0.19	0.8296
Blood Pressure (mmHg) – baseline			
Systolic	139.5 ± 16.8	137.3 ± 14.6	0.3421
Diastolic	81.0 ± 9.5	80.3 ± 8.5	0.7555
Heart rate (b per min) – baseline	69.6 ± 12.7	67.6 ± 10.4	0.4654
Cholesterol (mg dl ⁻¹)			
Total	208 ± 41.6	212 ± 37.7	0.6815
Low-density	126 ± 36.0	133 ± 33.9	0.4125
High-density	65 ± 17.8	64 ± 22.0	0.8500
Triglycerides (mg dl ⁻¹)	170 ± 154.5	163 ± 93.6	0.8034
Creatinine (mg dl ⁻¹)	0.84 ± 0.15	0.89 ± 0.19	0.2194
HbA1C (%)	5.48 ± 0.47	5.43 ± 0.32	0.6214
CRP (mg dl ⁻¹)	0.25 ± 0.25	0.16 ± 0.13	0.0594
Hb (g dl ⁻¹)	14.01 ± 0.76	13.87 ± 2.37	0.7367
White blood cells (×1000 μL ⁻¹)	6.78 ± 1.25	6.64 ± 1.31	0.6442



to participate in the study. Additionally, participants had to be community-dwelling and mobile in day-to-day activities.

After baseline assessment, participants were randomized to either the high-flavanol cocoa or the placebo group. The CF group received 1000 mg of high-flavanol cocoa extract, including 160 mg of (–)-epicatechin. Participants in the placebo group received nutrient-matched control capsules. Mars Edge provided the cocoa flavanol extract and the placebo capsules. Participants were instructed to continue their routines without changing activity or eating behavior. Both groups continued the supplementation of 500 mg twice a day for 30 days. The total amount of flavanols in mg represents the sum of all monomeric flavanols and oligomers, as published before.²⁵ The predominant monomeric flavanol in our capsule was (–)-epicatechin (Table S1†). Capsule format was utilized to simulate the effects of a flavanol-rich diet. Placebo capsules were filled with microcrystalline cellulose and matched for theobromine and caffeine content. Thus, flavanols and (–)-epicatechin were absent in placebo capsules. Overall appearance and weight of placebo capsules were not distinguishable from those of active capsules. After 30 days, the same testing protocol was applied (see Fig. 1A, study protocol). After completing the baseline assessment following a prespecified protocol, participants were randomly assigned to the intervention or placebo group by an independent study nurse in the clinical trial unit. The group allocation was then disclosed *via* telephone. Capsule containers were labeled with double-blinded alphabetical character codes. The unblinding process was performed by a pre-determined protocol after study completion.

Written informed consent was obtained from each participant. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in *a priori* approval the ethics committee of the Heinrich Heine University Düsseldorf (Approval Number R5761R). Initial registration on clinicaltrials.gov was mistakenly done with 410 mg of CF twice daily. After the unblinding process, the registration form was corrected to 500 mg CF twice daily. All participants randomized to intervention (CF) arm received the same dose of CF. Registered on clinicaltrials.gov (NCT 05782309).

CPET

CPET was conducted using a standardized bicycle ergometer test protocol at baseline and after 30 days. The test was performed with an upright cycle ergometer (Ergoselect 100, Ergoline, Bitz, Germany) with corresponding CPET equipment (CORTEX METALYZER 3B, CORTEX Biophysik GmbH, Leipzig, Germany) at the same time of day for all participants. Before examination, there was a 2-minute load free warmup period to get accustomed to the mask and breathing pattern. The testing protocol was an incremental ramp protocol with increases in work rate of 25 watts every 2 minutes. Participants were instructed to try maintaining a 55–70 rounds per minute cadence. Site technicians were trained and certified for the protocol and followed a detailed CPET manual. Gas exchange analysis was done with an online display over 3 breaths and a standard 30 seconds moving average for export of the final

data. The examination was terminated if one of the following criteria appeared: muscular exhaustion, dyspnea, fatigue, dizziness, systolic blood pressure >220 mmHg, ECG changes or arrhythmia, RER >1.15 or a leveling off. Heart rate reserve <5 bpm (predicted) was defined as a criterion for effort, not necessarily for termination. PeakVO₂ was assessed as the primary endpoint. Also, the following prespecified exploratory cardiovascular parameters were recorded: VCO₂, peak minute ventilation (VE), peak RER, peak heart rate, peak exercise capacity, peak oxygen pulse (VO₂ per HR) and VO₂ at ventilatory threshold. A trained Sports Cardiologist in a double-blinded approach analyzed the CPET data.

FMD

FMD was measured as previously described^{27,28} on the participant's right arm. Vessel diameter and flow velocity of the radial artery (RA) were measured using a 12 MHz transducer (Vivid I, GE) and automatic edge-detection software (Brachial Analyzer, Medical Imaging Applications, Iowa City, Iowa) yielding standard deviations of mean differences between repeated measurements of less than an absolute 1.0%. Reactive hyperemia was induced by 5 min of lower arm occlusion with a sphygmomanometer cuff inflated to 200 mmHg. Immediately after cuff deflation, and 20, 40, 60, and 80 s later, RA diameter was assessed and FMD was calculated as maximal relative diameter gain relative to baseline.

Blood pressure and central aortic pressure

Peripheral blood pressure was measured with an automated medical device (boso medicus, BOSCH + SOHN GmbH u. Co. KG) at both participant's left and right brachial artery to calculate the arithmetical mean, which was the used for analyses.

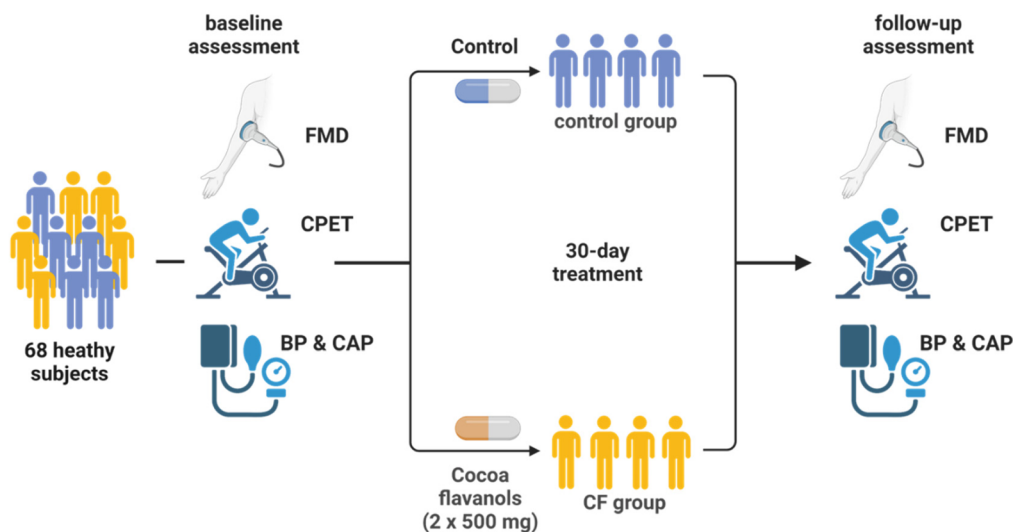
Central aortic pressure was assessed by noninvasive measurement of the central aortic pressure waveform using Sphygmocor® platform (Atcor medical, Sydney, Australia). Calculation of central aortic pressures is calculated *via* an integrated algorithm after entering individual patient characteristics and peripheral blood pressure.²⁵

Statistical analysis

Data are presented as means ± standard deviation (SD). Statistical analyses of baseline demographics were performed using Fisher's exact test (case controls), and unpaired *t*-tests. Statistical analysis of the intervention effects were performed by repeated measurements two- or three-way ANOVA with the Bonferroni *post hoc* test. Significance was assumed if *p* was <0.05. Analyses were performed by GraphPad Prism version 9 and SPSS statistics, version 24 (IBM, Armonk, USA). During planning of the study, the sample size was estimated using G-Power V3.1. (Heinrich Heine University of Duesseldorf). Previous publications showed a mean peakVO₂ per BW of 14.3 ± 4.8 (SD) ml kg⁻¹ min⁻¹ in elderly individuals.²⁹ Since 30-day intake of CF led to a 30% increase in FMD,³⁰ which has a positive relationship to peakVO₂,³¹ we assumed an increase in peakVO₂ per BW of 25% in the intervention group. Based on this data we calculated a sample size of *n* = 28 in each group as



A



B

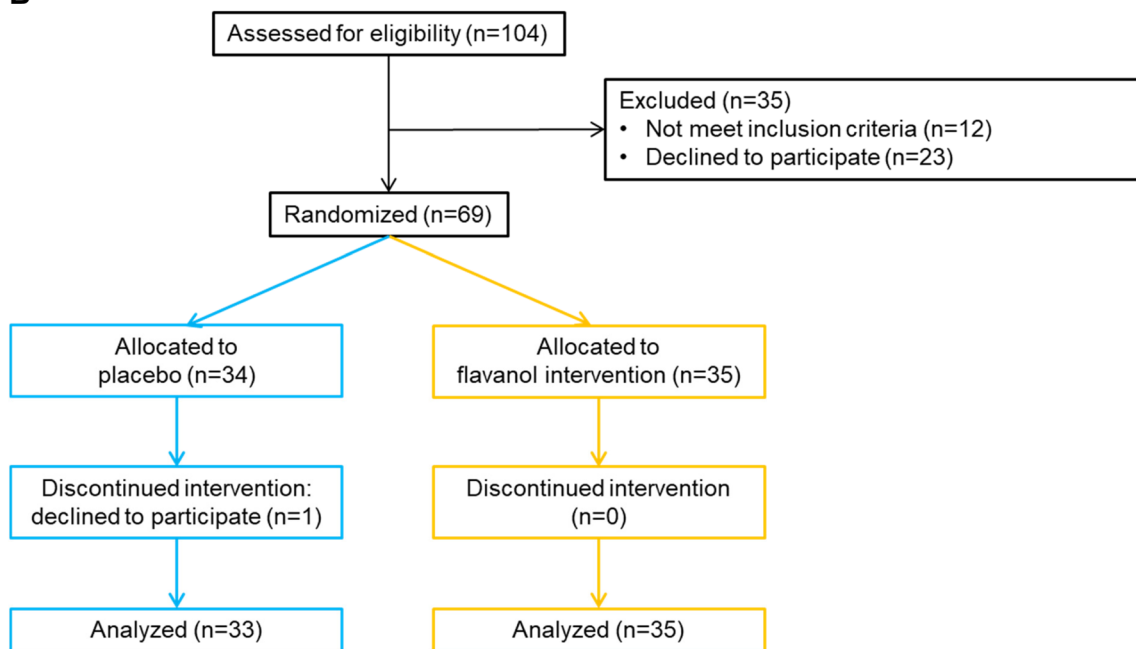


Fig. 1 Study protocol. (A): 68 healthy elderly adults (aged 55 to 79 years) received either 500 mg of Cocoa flavanols (CF) capsule twice daily or a nutrient-matched control capsule for 30 days in a randomized, double-masked, placebo-controlled design. Cardiopulmonary exercise testing (CPET), flow-mediated dilatation (FMD), central aortic pressure (CAP) and blood pressure (BP) measurements were performed at baseline and after 30 days. Primary endpoint was exercise capacity and peak VO_2 . Secondary endpoints included CAP, BP, and resting vascular function, measured as FMD. (B): CONSORT flow diagram.

required with an alpha level of 5% and a power of 80%. To allow dropouts the final total sample size was set at 62 subjects.

Results

Study population

From April 2018 to November 2021, after screening of 104 possible participants, 68 elderly adults (>55 years) fulfilled

inclusion criteria and were defined as healthy. One participant initially randomized to control declined participation after baseline examination and was excluded from the study. Baseline characteristics are presented in Table 1. Baseline exercise parameters were similar for both groups (Table 2). Baseline measures of FMD, peripheral blood pressure and central aortic pressure are presented in Table 3 and were not different between groups.



CF increase cardiorespiratory fitness

CF improved peak oxygen uptake (peakVO₂) by 190 ml min⁻¹ (1.83 ± 0.6 (SD) at baseline *versus* 2.02 ± 0.6 l min⁻¹ at follow-up, Fig. 2A, *p* = 0.0135) and peakVO₂ per kg of bodyweight (VO₂peak per kg) by 2.51 ml (min kg)⁻¹ (22.74 ± 6.88 at baseline *versus* 25.25 ± 6.78 ml (min kg)⁻¹ at follow-up, Fig. 2B, *p* = 0.0124). In a *post hoc* analysis investigating sex as a third variable using a three-way ANOVA, we found a significant rise in peakVO₂ (*p* = 0.0241) and VO₂peak per kg (*p* = 0.0410) of male participants receiving CF. O₂-Pulse increased by 1.74 ml (13.37 ± 4.78 at baseline *versus* 15.11 ± 3.95 ml at follow-up, Fig. 2C, *p* = 0.0175) and maximum exercise capacity by 9.6 W (151.5 ± 45.6 at baseline *versus* 161.1 ± 53.8 W at follow-up, Fig. 2D, *p* = 0.0472). No significant changes were seen for these parameters in the control group (peakVO₂: 1.88 ± 0.6 at baseline *versus* 1.93 ± 0.62 l min⁻¹ at follow-up, Fig. 2A, *p* = 0.7742; VO₂peak per kg: 22.4 ± 6.74 at baseline *versus* 23.4 ± 6.94 ml (min kg)⁻¹ at follow-up, Fig. 2B, *p* = 0.4790; O₂-pulse: 13.29 ± 4.55 at baseline *versus* 14.22 ± 4.55 ml at follow-up, Fig. 2C, *p* = 0.3316; exercise capacity: 150.5 ± 49.1 at baseline *versus* 151.3 ± 52 W at follow-up, Fig. 2D, *p* = 0.0667). Maximum minute ventilation (VE) increased in CF and control group. No changes were observed in maximum heart rate (HR max) and RER max. VO₂ at estimated ventilatory threshold (VO₂ at VT1) remained unchanged in both groups after 30 days (Table 2).

CF decrease blood pressure and improve vascular function parameters

Resting systolic and diastolic BP decreased by 5.4 mmHg (137.3 ± 14.6 (SD) at baseline *versus* 131.9 ± 13.9 mmHg at follow-up, Fig. 3A, *p* = 0.0456) and 2.9 mmHg (80.3 ± 8.5 at baseline *versus* 77.4 ± 8.1 mmHg at follow-up, Fig. 3C, *p* = 0.0362), respectively in the CF group. Also, central systolic and diastolic aortic pressure decreased in the CF group by 6.3 mmHg (126.9 ± 14.2 at baseline *versus* 120.6 ± 13.8 mmHg at follow-up, Fig. 3B, *p* = 0.0118) and 2.6 mmHg (81.1 ± 7.8 at

baseline *versus* 78.5 ± 8.3 mmHg at follow-up, Fig. 3D, *p* = 0.0292), respectively (Fig. 3). FMD increased by an absolute 1.28% (4.59 ± 1.85 at baseline *versus* 5.87 ± 1.93% at follow-up, Fig. 4B–D, *p* < 0.0001) in the CF group. No changes were documented in the control group (Fig. 4). In laboratory analyses levels of NT-proBNP were within the normal range for the assay utilized (ULN 125 ng L⁻¹) and remained unchanged in both groups.

Discussion

The present results demonstrate that dietary CF intake improves exercise capacity in healthy elderly humans. Improvement of cardiorespiratory fitness through dietary intervention may contribute to the maintenance of health in older individuals. The increase in exercise capacity was mainly driven by improvement of cardiac performance while pulmonary indices were not altered. The chosen amount of CFs enhanced endothelial function and lowered arterial blood pressure, both of which may have contributed to improvement in cardiac exercise capacity with significantly increased oxygen carrying capacity per heartbeat.

Exercise capacity in healthy elderly humans

According to the World Health Organization health is considered a “state of complete physical, mental and social well-being and not merely the absence of disease”.³² Participants in this study were screened prior to randomization to ensure the absence of manifest cardiovascular diseases. With increasing age large artery stiffness develops inducing mild increases in central arterial blood pressure.³³ In our study population, elderly participants showed slightly elevated systolic blood pressure with an average of 139 and 137 mmHg. Also, LDL-cholesterol levels were mildly elevated. According to current guidelines lifestyle interventions are recommended as first line option to modify these parameters.^{34–36} Healthy aging

Table 2 Effects of dietary CFs on measures of exercise capacity during CPET. peakVO₂, peakVO₂ per kg, O₂-pulse, and exercise capacity significantly improved with CFs; VE increased in both groups. BL = baseline; FU = follow up; HR = heart rate; RER = respiratory exchange ratio; VE = minute ventilation; peakVO₂ = maximum oxygen uptake, peakVO₂ per kg = maximum oxygen uptake per kg of bodyweight, O₂-pulse = oxygen uptake per heartbeat; VT = tidal volume W = power in Watts; **p* < 0.05; repeated measurements 2-way-(time × intervention) ANOVA with Bonferroni's *post hoc* test

Measures of exercise capacity during CPET

Intervention group	Control (<i>n</i> = 33)			Cocoa flavanols (<i>n</i> = 35)		
	BL	FU	<i>p</i>	BL	FU	<i>p</i>
VO ₂ peak (l min ⁻¹)	1.88 ± 0.60	1.93 ± 0.62	0.7742	1.83 ± 0.60	2.02 ± 0.60	0.0135*
VO ₂ peak per kg (ml min ⁻¹ kg ⁻¹)	22.40 ± 6.74	23.40 ± 6.94	0.4790	22.74 ± 6.88	25.25 ± 6.78	0.0124*
VO ₂ at VT1 (l min ⁻¹)	1.33 ± 0.48	1.41 ± 0.48	0.7150	1.37 ± 0.45	1.36 ± 0.42	0.9396
VO ₂ at VT1/VO ₂ peak [%]	70.1%	70.4%	—	74.9%	67.7%	—
O ₂ -pulse (VO ₂ per HR) (ml)	13.29 ± 4.55	14.22 ± 4.55	0.3316	13.37 ± 4.78	15.11 ± 3.95	0.0175*
Exercise capacity (W)	150.5 ± 49.04	151.3 ± 51.94	0.9667	151.5 ± 45.6	161.1 ± 53.8	0.0472*
HR max (beats per min)	140.6 ± 20.4	140.7 ± 22.1	0.9929	136.0 ± 19.7	131.6 ± 17.7	0.3611
RER max	1.110 ± 0.09	1.091 ± 0.08	0.3310	1.083 ± 0.08	1.075 ± 0.06	0.9992
VE max (l min ⁻¹)	57.55 ± 21.0	64.03 ± 26.4	0.0229*	55.26 ± 17.1	63.69 ± 22.3	0.0025*
VT max (l)	2.058 ± 0.68	2.153 ± 0.65	0.3722	2.025 ± 0.59	2.144 ± 0.63	0.2184



Table 3 Effects of dietary CFs on vascular function. FMD increased with CFs. Peripheral blood pressure and central aortic pressure decreased with CFs at follow up. No changes were seen in the control group. BL = baseline; FU = follow up FMD = flow-mediated-dilation; * $p < 0.05$; repeated measurements 2-way-(time \times intervention) ANOVA with Bonferroni's *post hoc* test

Results in FMD and blood pressure

Intervention group	Control ($n = 33$)			Cocoa flavanols ($n = 35$)		
	BL	FU	p	BL	FU	p
FMD peak change [%]	4.33 \pm 1.16	4.23 \pm 1.18	0.8631	4.59 \pm 1.85	5.87 \pm 1.93	<0.0001*
Peripheral blood pressure						
Systolic (mmHg)	139.5 \pm 16.8	135.3 \pm 17.7	0.1828	137.3 \pm 14.6	131.9 \pm 13.9	0.0456*
Diastolic (mmHg)	81.0 \pm 9.5	79.3 \pm 6.5	0.3949	80.3 \pm 8.5	77.4 \pm 8.1	0.0362*
Central aortic pressure						
Systolic (mmHg)	129.2 \pm 15.6	125.1 \pm 16.1	0.1450	126.9 \pm 14.2	120.6 \pm 13.8	0.0118*
Diastolic (mmHg)	82.6 \pm 9.7	80.7 \pm 6.5	0.3612	81.1 \pm 7.8	78.5 \pm 8.3	0.0292*

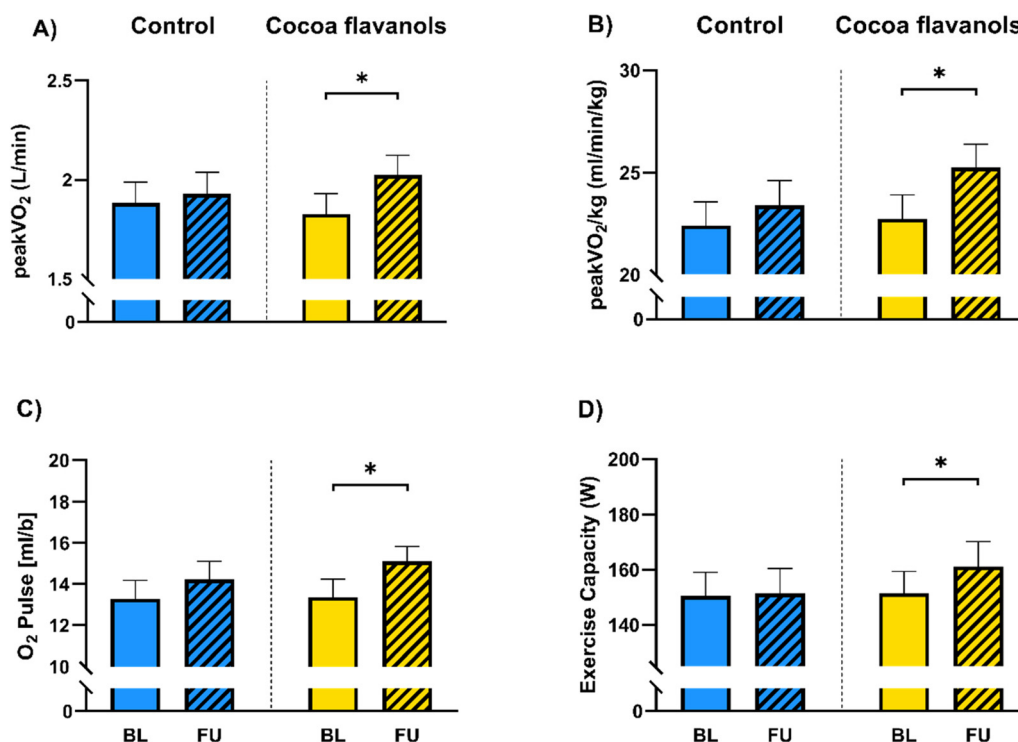


Fig. 2 Dietary CFs increase maximum oxygen uptake and exercise capacity. Significant increase in (A) peakVO₂, (B) peakVO₂ per kg, (C) O₂ pulse, and (D) exercise capacity at maximum workload after 30 days of CF intake compared to baseline. No changes were observed with control. * $p < 0.05$; repeated measurements 2-way-(time \times intervention) ANOVA with Bonferroni's *post hoc* test. BL = baseline; FU = follow-up; peakVO₂ = maximum oxygen uptake, peakVO₂ per kg = maximum oxygen uptake per kg of bodyweight, O₂-pulse = oxygen uptake per heartbeat.

comprises the primary prevention of functional limitations, occult and manifest disease with increasing age. Blood pressure, pulse wave velocity and FMD have been proposed to monitor the transition of healthy to dysfunctional arteries with increasing age.³⁷ Young adults under 25 years of age are generally suspected healthy. After the age of 45 years subclinical disease starts building up depending on previous lifestyle behavior and risk factors. Beyond 65 years of age CVD starts manifesting and eliciting events.³⁸ Age *per se* is considered an independent risk factor for CVD and mortality.^{39,40} CPET is a valuable tool to assess cardiac, circulatory, and pulmonary func-

tion and health. It is a highly reliable and reproducible method for measuring peakVO₂ and to evaluate the functional capacity in healthy and diseased humans. PeakVO₂ is also a strong predictor of long-term all-cause, CV- and cancer mortality.^{7,41} Limitations to cardiopulmonary fitness can often be assigned to one of the primary components: skeletal muscle oxygen extraction, pulmonary oxygen uptake, blood oxygen transport capacity and cardiac output for oxygen distribution. Cardiac performance in this regard is also modulated by preload and afterload. Cardiac afterload is amongst others determined by blood pressure and endothelial function, altera-



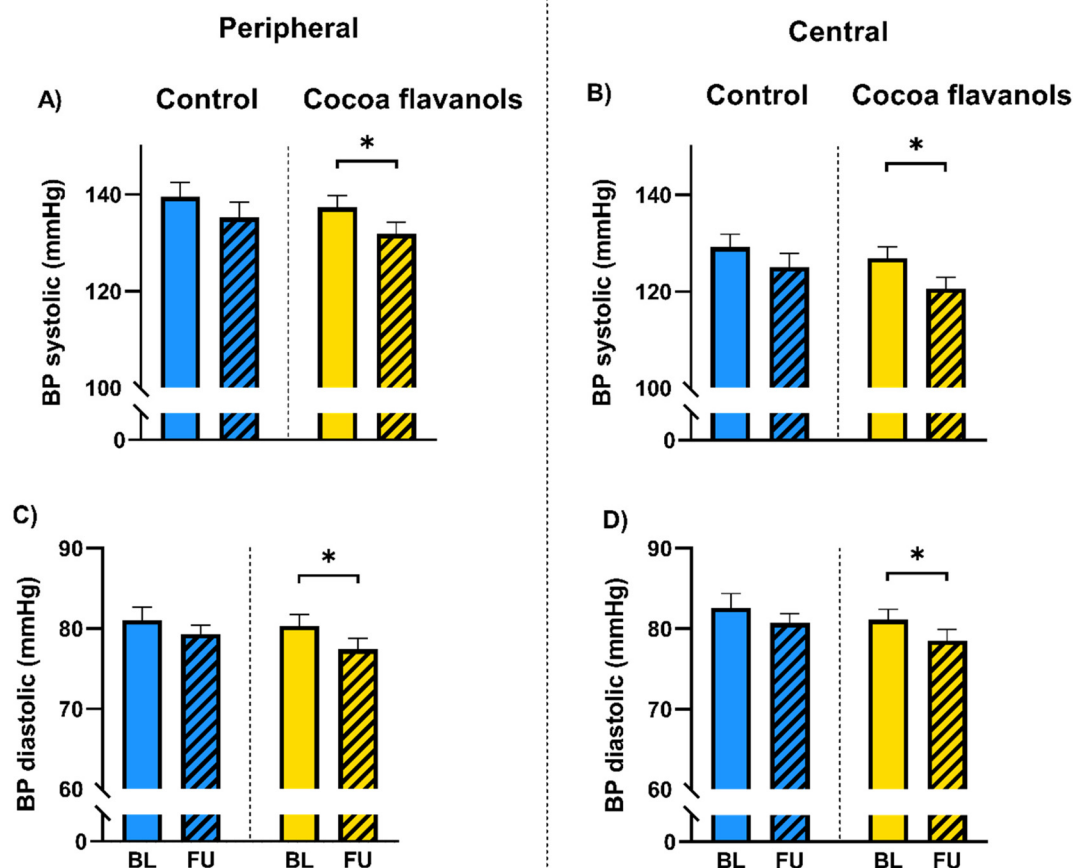


Fig. 3 Dietary CFs decrease blood pressure. Significant decrease in peripheral blood pressure (A & C) and central aortic pressure (B & D) after 30 days of CF intake. No changes occurred with control. * $p < 0.05$; repeated measurements 2-way-(time \times intervention) ANOVA with Bonferroni's *post hoc* test. AP = aortic pressure; BP = blood pressure; BL = baseline; FU = follow up.

tions of both have been associated with the cardiovascular aging process.^{25,37} The aging heart itself is less well understood but comprises a gradual deterioration of functional and structural characteristics including increased mass-to-volume-ratio and a decline in diastolic function.^{3,42} Reduced β -adrenergic response and consecutively reduced maximum heart rate are prevalent in aging hearts.⁴³ Especially, age-related decline in cardiac functional parameters can be positively modified^{44,45} with aerobic interval training, while the effect of dietary interventions with CFs on exercise capacity in the elderly healthy population has not been well characterized.

Cocoa flavanols improve exercise capacity in healthy elderly humans

After a period of 30-days dietary intake CF, CF increased peak $\dot{V}O_2$, peak $\dot{V}O_2$ per kg, O_2 -pulse, and exercise capacity suggesting beneficial effects on cardiovascular fitness. Indeed, the increase in peak $\dot{V}O_2$ of 190 ml or 2.5 ml per kg of body weight that was achieved with dietary intake is comparable to the effects in structured exercise programs.^{46,47} In older adults over 70 years of age, a supervised exercise training with high-intensity intervals improved peak $\dot{V}O_2$.⁴⁸ With increasing age applicability of physical exercise programs may be limited due

to orthopedic diseases and thus dietary interventions may offer complementary tools to maintain cardiovascular health and fitness. Comparable to our results, dark chocolate supplementation previously has shown improvements in peak $\dot{V}O_2$ by 6% *versus* baseline and reduced oxygen cost in moderately trained participants.²³ No overall performance benefit despite lower lactate levels changes appeared in trained cyclists when only 18–36 mg of (–)-epicatechin was administered.²⁴ This suggests that CF induced improvements might depend on baseline fitness, vascular function status and sufficient CF to exert an effect. Similarly, in patients with heart failure with preserved ejection fraction other sources of NO like beet root juice induced about 8% improvement in peak $\dot{V}O_2$.²⁰ In a *post-hoc* analysis, we asked whether sex might affect the benefits of CF intake on exercise capacity. Interestingly, the multiple comparisons of the three-way ANOVA showed a significant result for men receiving CF. Since our study was not powered to consider sex-specific changes, these data cannot answer possible sex-specific effects of CF on exercise capacity. Maximum heart rate was unaffected in our study groups. An increased O_2 -pulse suggests an improvement in stroke volume as a potential primary mechanism for improved oxygen transport. Stroke volume is amongst others dependent on the afterload.



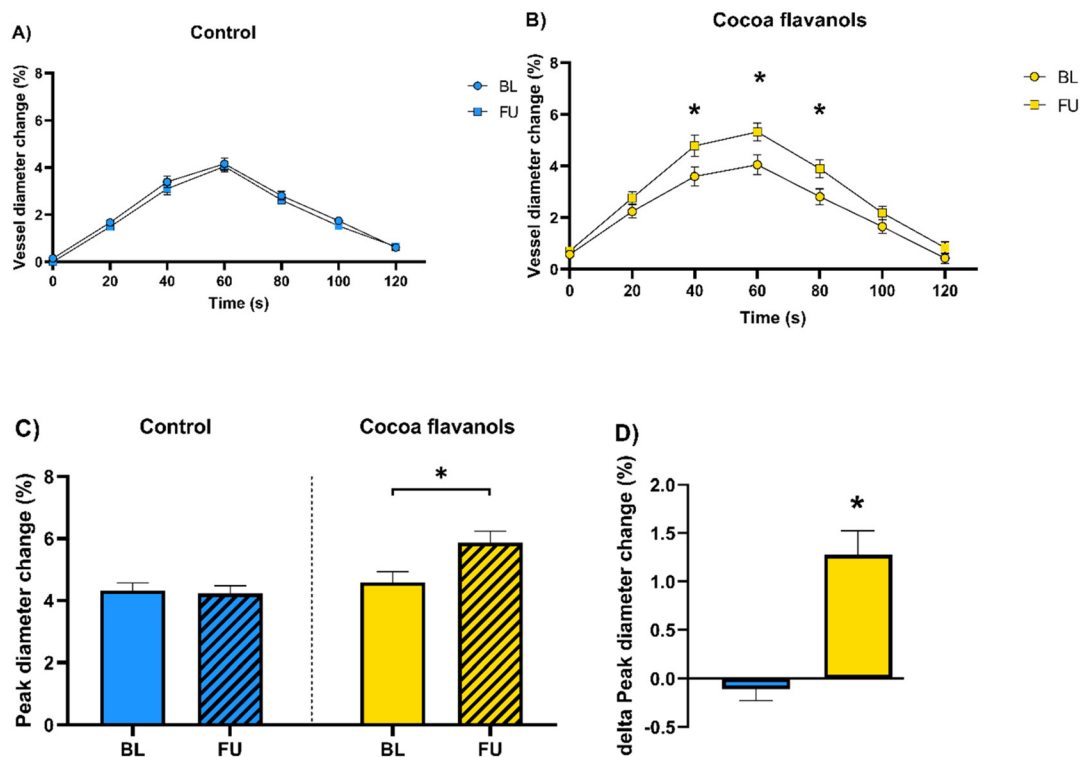


Fig. 4 Dietary CFs improve endothelial function. (A) Unchanged FMD with control (B) significant increase at FU in FMD at 40 s, 60 s and at 80 s after CF intake compared to BL; (C) significantly higher Peak inner diameter change at FU vs. BL with CFs, (D) increased delta in peak diameter change from BL to FU with CFs but not with control; * $p < 0.05$; repeated measurements 2-way-(time \times intervention) ANOVA with Bonferroni's *post hoc* test. BL = baseline; FU = follow up. FMD = flow-mediated-dilation.

Determinants of left ventricular afterload are BP, CAP and aortic stiffness,^{37,49} all of them have been positively modulated through CFs. In our study population a reduction in systolic BP by 5.4 mmHg and CAP by 6.3 mmHg was achieved. Thus, an improved afterload and arterio-ventricular coupling can contribute to the increase in stroke volume. Decreased afterload, in the long term, prevents ventricular remodeling and hypertrophy.⁵⁰ To support this process through a different mechanism, CFs have been linked to decreased inflammatory response,⁵¹ which further reduces progression of left ventricular remodeling, hypertrophy and fibrosis.^{40,52,53}

Effectiveness of CFs to improve vascular function in healthy humans

In our study peripheral blood pressure and CAP decreased, whereas FMD, as a marker for endothelial function, increased in the CF group. These findings corroborate our previous findings that not only in diseased but also in healthy individuals, CFs improve vascular function and cardiovascular risk markers.^{12,25,54} The proposed increase in eNOS activity in healthy adults¹⁴ is one likely mechanism by which CFs and especially (–)-epicatechin improve vascular function, blood pressure^{25,37} and endothelial integrity.⁵⁵ Overall, dietary interventions have been shown to reestablish disturbed redox signaling in the vasculature.⁵⁶ As already demonstrated in the

COSMOS trial⁵⁷ a 27% lowering in cardiovascular deaths can be achieved through CF intake at half the amount in elderly individuals free from CVD after a 3.6-year follow-up. The amount of dietary CFs given in our study is above the average intake in a typical European diet. Dietary extracts of CFs are generally considered safe in concentrations up to 2 g day⁻¹.⁵⁸ Our results for vascular function assessment serve for indirect validation of the CF effect. Importantly, we did not see any non-responder regarding CF effect on FMD in the intervention group. (–)-Epicatechin is likely the major bioactive in cocoa responsible for vascular effects.¹³ Our data indicate that 31 mg are required to acutely achieve half-maximal effects on FMD.¹⁵ The (–)-epicatechin content in commercial chocolates varies greatly, but can be zero and as high as 269 mg per 100 g chocolate.⁵⁹ The consumption of 60 g of a high (–)-epicatechin chocolate would achieve a similar amount as administered in the current study (160 mg).^{59–61}

Limitations

A few limitations of the present study are worth mentioning. First, we did not assess individual exercise behavior nor food diaries addressing estimates of habitual CF intake in the participants' regular diet. Second, we did not collect plasma nor urine levels of circulating flavanols and their structurally related or gut microbiome derived metabolites to ensure



regular intake and sufficient absorption. However, the observed selective increase in FMD all participants within the CF group with a 10% percent response rate argues for a high adherence within the entire study cohort. Further, we did not assess participants' physical activity status at baseline or throughout the study period and did not ask for caffeine intake prior to exercise testing. Further studies should consider activity trackers or questionnaires to meet this issue.

Conclusion

We here provide evidence, that intake of CFs at a dose efficient to improve endothelial function improves markers of cardiorespiratory fitness in healthy elderly humans highlighting their potential for supporting maintenance of cardiovascular health and fitness with increasing age. Future studies should evaluate whether these effects are sustained with long-term CF intake and translate into clinically relevant improvements in fitness, quality of life and lowering of cardiovascular events and mortality.

Author contributions

All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

M. G., R. S., C. H., M. K. and R. E. designed the study; M. G., N. O. and N. K. conducted the research; M. G., N. O., N. K., R. S. and R. E. analyzed the data; M. G., D. D., C. H., C. J., M. K. and R. E. wrote the manuscript.

Abbreviations

BMI	Body mass index
BP	Blood pressure
BSA	Body surface area
CAP	Central aortic pressure
CF	Cocoa flavanol
CPET	Cardiopulmonary exercise testing
CVD	Cardiovascular disease
FMD	Flow-mediated vasodilation
HR	Heart rate
PeakVO ₂	Maximum oxygen consumption
RA	Radial artery
RER	Respiratory exchange ratio
VO ₂	Volume flow of oxygen
VT	Ventilatory threshold

Conflicts of interest

There are no conflicts to declare.

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