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A single dose of dark chocolate increases parasympathetic modulation and heart rate variability in healthy subjects

Uma única dose de chocolate amargo aumenta a modulação parassimpática e a variabilidade da frequência cardíaca em indivíduos saudáveis

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ABSTRACT

Objective

The aim of this study was to investigate the acute effect of a single dose of dark chocolate (70% cocoa) on blood pressure and heart rate variability.

Methods

Thirty-one healthy subjects (aged 18-25 years; both sexes) were divided into two groups: 10 subjects in the white chocolate (7.4 g) group and 21 in the dark chocolate (10 g) group; measurements were performed at the university's physiology lab. An electrocardiogram measured the sympathovagal balance by spectral and symbolic analysis.

Results

A single dose of dark chocolate significantly reduced systolic blood pressure and heart rate. After consuming 10 g of dark chocolate, significant increases were observed for heart rate variability, standard deviation of RR intervals standard deviation of all NN intervals, square root of the mean squared differences between adjacent

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normal RR intervals root mean square of successive differences, and an increase in the high frequency component in absolute values, representing the parasympathetic modulation.

Conclusion

In conclusion the importance of our results lies in the magnitude of the response provoked by a single dose of cocoa. Just 10 g of cocoa triggered a significant increase in parasympathetic modulation and heart rate variability. These combined effects can potentially increase life expectancy because a reduction in heart rate variability is associated with several cardiovascular diseases and higher mortality.

Keywords: Autonomic nervous system. Cocoa. Flavonoids.

RESUMO

Objetivo

O objetivo deste estudo foi investigar o efeito agudo de uma única dose de chocolate amargo (70% cacau) sobre a pressão arterial e a variabilidade da frequência cardíaca.

Métodos

Trinta e um indivíduos saudáveis (com idade entre 18-25 anos; ambos os sexos) foram divididos em dois grupos: 10 indivíduos no grupo chocolate branco (7,4 g) e 21 no grupo chocolate amargo (10,0 g); as avaliações foram realizadas no laboratório de fisiologia da Universidade Federal de Ciências da Saúde de Porto Alegre.

Resultados

O eletrocardiograma foi realizado para analisar o balanço simpatovagal através da análise espectral e simbólica. Uma única dose de chocolate amargo reduziu significativamente a pressão arterial sistólica e a frequência cardíaca. Depois de consumir 10 g de chocolate amargo, observou-se aumento significativo na variabilidade da frequência cardíaca, o desvio padrão dos intervalos*, a raiz quadrada da média do quadrado das diferenças entre os intervalos RR normais adjacentes a raiz quadrada da média da soma dos quadrados das diferenças entre os intervalos NN normais adjacentes e um aumento na componente de alta frequência em valores absolutos, o que representa a modulação parassimpática.*

Conclusão

Em conclusão, a importância dos resultados aqui apresentados reside na magnitude da resposta provocada por uma dose única de cacau (10 g). Esta provocou um aumento significativo da modulação parassimpática e da variabilidade da frequência cardíaca no coração. Esses efeitos, quando combinados, podem, potencialmente, aumentar a esperança de vida, porque a redução da variabilidade da frequência cardíaca está associada a doenças cardiovasculares e maior mortalidade.

Palavras-chave: *Sistema nervoso autônomo. Cacau. Flavonoides.*

INTRODUCTION

Studies indicate that consumption of foods rich in polyphenols is associated with lower risk of disease[1,2] and consequently, with a decreased risk of morbidity and mortality[3]. Cocoa products, such as dark chocolate, which are rich in polyphenols, have demonstrated beneficial effects, particularly on the cardiovascular system[4,5].

Protection of the cardiovascular system by cocoa, present in dark chocolate, stems from several effects, such as antioxidant[6], antiatherosclerotic[7,8], anti-inflammatory[9,10] and platelet anti-aggregation effects[11], and reduced Angiotensin-Converting Enzyme (ACE) activity[12], among others. Improvements in nitric oxide bioavailability[13] and endothelial function[14] have also been reported, and ultimately, these changes lead to vascular relaxation and antihypertensive effects[15,16]. On the other hand, there is no published data associating cocoa consumption and autonomic nervous system modulation.

One of the mechanisms that improves Blood Pressure (BP) control could be associated

with the peripheral afferent nerve fibers that carry BP information to the central nervous system. This mechanism includes the sympathetic and/or parasympathetic activation that changes Heart Rate (HR) to promote BP stabilization[17]. Heart Rate Variability (HRV) has been studied for the last few decades[18] to assess the autonomic modulation of the cardiovascular system[19,20]. Good BP adaptation is an indication of high HRV, whereas lower HRV is frequently an indicator of abnormal and/or insufficient adaptation of cardiac Autonomic Nervous System (ANS) modulation[21].

In fact the cardiovascular and autonomic functions of hypercholesterolemic rabbits treated chronically with cocoa liquor were preserved. Additionally, baroreflex regulation improved. These authors also observed that parasympathetic modulation was preserved in the group that consumed cocoa liquor, differently from the control group, which experienced lower cardiac parasympathetic[22] modulation.

It is well described in the literature that there is an increase in sympathetic modulation in most diseases. Sympathetic modulation is associated with worse prognosis and increased mortality[23]. Thus, chocolate consumption is simple and easy to do, and its potential impact over autonomic modulation makes it a preventive and nonpharmacological strategy.

However, no study was found in the literature that described the effect of a single dose of dark chocolate on the modulation of the autonomic nervous system in experimental models or subjects. The result of this study highlights the potential effects of cocoa intake and draw attention to the importance of regular cocoa consumption in small doses. Thus, the aim of this study was to investigate the effect of dark chocolate intake on BP and HRV in healthy individuals.

METHODS

The protocol was approved by the Ethics Committee of *Universidade Federal de Ciências da Saúde de Porto Alegre* (UFCSPA, Federal University of Health Sciences of *Porto Alegre*) nº 075/05. The study was conducted in 2013 at the physiology laboratory of said university. Thirty-one UFCSPA students aged 18-25 years were invited to participate in the study. After signing the informed consent, the subjects were randomly allocated into one of the two groups. All subjects included were clinically healthy, non-obese, normotensive, and nonsmokers, but they did not exercise regularly. They were not taking any antioxidant supplements or medications, except oral contraceptives. In women all measurements were collected during the early follicular phase (days 1-3) of the menstrual cycle[24].

The subjects entered a further cocoa-free washout phase of 7 days, according to the protocol described by Taubert and coworkers[25]. For 24 hours before the experiment, the volunteers were instructed to abstain from drinking alcohol or caffeinated products, and exercising.

The dark chocolate group received 10 g of dark chocolate (Lugano®, *Gramado*, RS, Brazil) with 70% cocoa. The dark chocolate had the following composition (10g): energy=54.4 Kcal, carbohydrates=4 g, total fats=3.6 g, proteins=0.8 g, sodium=0 mg, dietary fibers=0.8 g, and total flavonoids/epicatechin=5.41 mg.

The white chocolate group received 7 g of white chocolate (Laka, Lacta® *Curitiba*, PR, Brazil) containing the same amount of sugar as the 10 g of dark chocolate. The white chocolate composition (7g) was as follows: energy=38.08 Kcal, carbohydrates=4.2 g, total fats=2.1 g, proteins=0.5 g, sodium=12.32 mg, dietary fibers=0 g, and total flavonoids/epicatechin=0 mg.

Experimental sequence were as follows: 1) BP, HR and HRV were measured at rest; 2) the subjects consumed chocolate (white or dark); 3) anthropometric data were collected immediately after the chocolate intake; 4) One hour after chocolate intake, a second set of BP, HR and HRV measurements were performed, also at rest.

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The subjects were seated in an upright position with back support and were asked to relax for 5 min. Blood pressure was measured by an automatic digital device (Omron®, IntelliSense, BP785, Bannockburn, Illinois, USA). The blood pressure cuff covered at least 80% of the upper arm. To confirm the data, BP was measured three times at 2- minute intervals. When a difference of more than 6 mmHg between measurements was detected, the measurements were repeated until the difference was less than 4 mmHg. The participants' BP, Mean BP (MBP), HR, and Pulse Pressure (PP) were given by averaging the three BP measurements.

Heart rate variability

An Electrocardiogram (ECG) signal was acquired at a 1 kHz sampling rate for a 10-minute period with the subject in the supine position and head elevation of 30°. The protocol used three ECG derivations. To assess the HRV, the temporal series of RR intervals were registered and processed by the MP150 system (Biopac, California, United States of America).

The temporal series were analyzed by the program Matlab, in which the ECG signal was processed to obtain the variables related to HRV in the time and frequency domains. The Standard Deviation of all NN (SDNN) intervals and the Root Mean Square of Successive Differences (RMSSD) between adjacent normal RR intervals and the square root of the mean squared differences between adjacent normal RR intervals (RMSSD) were chosen as the variables in the time domain, expressed in meter per second. In the frequency domain, analysis of HRV was performed by Fast Fourier Transform (FFT) in portions of 5 minutes with 4 Hz interpolation and an overlap of 50%. The low frequency (LF; 0.04 to 0.15 Hz; this component predominantly corresponds to sympathetic modulation) and high frequency (HF; 0.15 to 0.4 Hz; corresponds to parasympathetic modulation) components were presented in absolute (Low Frequency absolut (LFa) and High Frequency absolut (HFa) and normalized Low Frequency normalised units (LFnu) and High Frequency normalised units (HFnu). Normalized units were obtained by dividing the power of a given component by the total power (from which Very Low Frequency [VLF] was subtracted) and multiplying by 100. The *ratio* between absolute values (LF/HF) was used as an index of cardiac autonomic modulation[26].

Symbolic analysis is a powerful and validated tool to detect changes in autonomic modulation of cardiovascular variability[27], which transforms a time series into short, three-beat patterns. It was carried out according to the approach previously described and validated by Porta *et al*.[28]. However, the data were adjusted into three groups instead of four in accordance with Guzzetti *et al*.[29].

The sequences are spread on six levels, and all possible patterns are divided into four groups, consisting of patterns with: 1) No Variations (0V, three symbols equal); 2) One Variation (1V, two equal symbols and one different); 3) Two Like Variations (2LV); and 4) Two Unlike Variations (2UV)[29].

The data were analyzed by repeated measures Analysis of Variance (ANOVA) followed by the Mann-Whitney and Wilcoxon tests. The data are presented as mean ± Standard Deviation (SD), and $p<0.05$ was considered statistically significant.

RESULTS

The subjects were 22±2 years old, and the Body Mass Index (BMI) was 22.5±3 kg/m^2. Systolic Blood Pressure (SBP) and HR decreased significantly after dark chocolate intake. On the other hand, Diastolic Blood Pressure (DBP), MAP, and PP did not change in either group (Table 1).

In the time domain, spectral analyses showed that a single dose of dark chocolate significantly increased the total power of HRV, SDNN, and RMSSD. In addition, in the frequency

Table 1. Hemodynamic values before and after chocolate intake.

	White chocolate (n=10)				White chocolate (n=21)			
	Before		After		Before		After	
	M	SD	M	SD	M	SD	M	SD
SBP (mmHg)	102 ±	14	100 ±	11	106 ±	9	102 ±	10*
DBP (mmHg)	66 ±	6	66 ±	3	69 ±	6	68 ±	1
MBP (mmHg)	79 ±	8	78 ±	5	81 ±	6	79 ±	6
PP (mmHg)	36 ±	10	34 ±	9	37 ±	7	34 ±	8
HR (bpm)	81 ±	12	75 ±	11*	78 ±	8	74 ±	8*

Note: *$p<0.05$. Data expressed as means (M) ± Standard Deviation (SD).
SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; MBP: Mean Blood Pressure; PP: Pulse Pressure; HR: Heart Rate.

Table 2. Spectral and symbolic analysis data before and after chocolate intake.

	Spectral analysis							
	White chocolate (n=10)				White chocolate (n=21)			
	Before		After		Before		After	
	M	SD	M	SD	M	SD	M	SD
Time Domain								
HRV (ms^2)	2897 ±	331	3332 ±	1128	1896 ±	1026	4192 ±	874*
SDNN (ms)	56 ±	5	59 ±	8	42 ±	6	61 ±	6*
RMSSD (ms)	56 ±	9	48 ±	8	34 ±	7	55 ±	6*
Frequency Domain								
LFa (ms^2)	916 ±	117	864 ±	211	775 ±	152	976 ±	192
HFa (ms^2)	799 ±	474	989 ±	384	826 ±	135	1219 ±	206*
Lfnu (%)	50 ±	3	47 ±	5	47 ±	3	45 ±	4
Hfnu (%)	50 ±	3	53 ±	5	53 ±	3	55 ±	4
LF/HF *ratio*	1.0 ±	0.14	0.9 ±	0.18	1.1 ±	0.2	0.9 ±	0.14
Symbolic Analysis								
0V (%)	18 ±	1.9	20 ±	1.5	21 ±	3.1	15 ±	2.0
1V (%)	47 ±	1.5	46 ±	1.0	50 ±	0.9	48 ±	1.5
2LV (%)	14 ±	1.5	13 ±	1.0	15 ±	1.8	18 ±	2.0
2ULV(%)	20 ±	1.8	21 ±	1.7	14 ±	2.0	19 ±	2.0

Note: *$p<0.05$. Spectral analysis results.
HRV: Heart Rate Variability; SDNN: Standard Deviation of Normal RR Intervals; RMSSD: Square Root of the Mean Squared differences between consecutive RR intervals; LF: Low Frequency Component; HF: High Frequency Component; a: Absolute; nu: Normalized. LF/HF *ratio*: sympathovagal balance; V: Variation. Data expressed as Means (M) and ± Standard Deviation (SD); 0V: No Variations (three symbols equal and indicate sympathetic modulation); 1V: One Variation (two symbols equal and one different. Indicate both sympathetic and parasympathetic modulation); 2LV: Two Like Variations and indicate parasympathetic modulation; and 2ULV: Two Unlike Variations and indicate parasympathetic modulation.

domain, there was a significant increase only in the HFa component (ms^2; Table 2). Collectively, these results indicate a significant predominance of the parasympathetic system after dark chocolate intake. Table 2 shows the symbolic analysis results (%). Symbolic analysis found no significant difference between the groups after chocolate intake.

DISCUSSION

The most significant observation of this study is that a single dose of 10 g of dark chocolate improves cardiac Autonomic Nervous System (ANS) modulation in healthy subjects. After one hour, dark chocolate intake significantly increased (200%) HRV total power and improved parasympathetic modulation, seen by spectral

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analysis. On the other hand, no difference was observed after chocolate intake by symbolic analysis. Although the mechanism still remains under investigation, recent studies have suggested that chronic dark chocolate intake decreases BP[13,15,30, 31] and SBP[25,32], improves endothelial function in healthy subjects[32-36] , and reduces both systolic and DBP in soccer players[37]. The present study only found a reduction in the SBP of healthy individuals.

In addition, acute cocoa intake improves endothelial function and BP in smokers[38] and overweight subjects[39]. Moreover, it also improves flow-mediated dilatation in patients with peripheral artery disease[40].

According to Sudarma *et al.*[41], flavonols may explain BP improvement as they have been shown to stimulate endothelial nitric oxide production and thus cause blood vessel dilatation in prehypertensive subjects. This conclusion is also supported by Flammer *et al.*[42], who had shown that a single dose of dark chocolate improved coronary vascular function and decreased platelet adhesion and serum oxidative stress, probably correlated to serum epicatechin level.

In fact epidemiological studies indicate that, at least partially, the good effects of flavonoids are due to their antioxidant and anti-inflammatory properties. Consumption of flavonoids, also present in cocoa, is associated with better cognitive performance[43] and lower risk of neurodegenerative disorders in older adults[44]. Collectively, these findings enhance the beneficial effects of cocoa on the central nervous system and, as seen by our results, its strong impact on the autonomic nervous system and cardiovascular function.

The importance of our results lies in the magnitude of the response provoked by a single dose of cocoa. Even being a nonpharmacological intervention, less than 10 g of cocoa triggered a significant increase in cardiac parasympathetic modulation and HRV. These combined effects can potentially increase life expectancy[45] because a decrease in HRV is associated with several cardiovascular diseases[18], and increased cardiovascular risk and mortality[46-48]. Our results were surprising because single nonpharmacological interventions usually have little physiological effect.

Thus, although it is very well established in the literature that chronic dark chocolate intake is beneficial for BP control, our results are the first demonstrating the improvement in autonomic modulation. The antioxidant properties of cocoa are probably what improve BP control, centrally and peripherally. The magnitude of their effect can be demonstrated by the increase in heart rate variability.

Moreover, in the time domain, dark, but not white, chocolate intake increases all parameters: SDNN, RMSSD, and HRV. In the frequency domain, there was also an increase in parasympathetic modulation, seen by HF in absolute values; and the symbolic analysis showed that dark chocolate decreased sympathetic modulation.

Furthermore, sympathetic predominance increases plasma angiotensin II[49], which leads to an increase in Nicotinamide Adenine Dinucleotide Phosphate (NADPH), oxidase activity, and superoxide anion formation. The increase in superoxide anion induces oxidative stress[50], which, ultimately, decreases nitric oxide bioavailability[40], and its effects probably impair cardiac autonomic modulation and BP control.

Our results are supported also by Villarreal Calderon *et al.*[51], who showed a neuroprotective effect of chocolate. They found that, in mice subjected to urban air pollution, chocolate intake mitigated vagal inflammation, supporting the idea that cocoa may improve nervous system function.

CONCLUSION

These results demonstrate that a single dose of dark chocolate improves the HRV and reduces SBP and HR. Collectively, these results reinforce the importance of consuming dark chocolate to prevent cardiovascular diseases and

highlight the fact that a small dose can be of impact to induce benefits that are not seen by white chocolate intake. Improvement in the sympathovagal balance, seen by higher HRV after dark chocolate, but not white chocolate intake, was responsible for better cardiovascular system control and probably can be attributed to the flavonoids of the cocoa. Thus, it is reasonable to believe that dark chocolate represents a valuable option that might promote health and reduce cardiovascular risk and mortality. A randomized, controlled clinical trial of the population's life expectancy would be very useful to demonstrate the beneficial effects of daily dark chocolate intake on autonomic modulation, as seen from heart rate variability.

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CONTRIBUTORS

The authors C MOSTARDA and CM IRIGOYEN contributed to data analysis, and the authors AAM DUARTE and K RIGATTO contributed to study conception and design, and data analysis and interpretation. The article is based on a master's thesis.

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ERRATUM

On page 769, Table 1, "White chocolate (n=21)" **should read** "Dark chocolate (n=21)".

On page 769, Table 2, "White chocolate (n=21)" **should read** "Dark chocolate (n=21)".