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Ana Amélia Machado Duarte

Efeito Agudo da Ingestão de Chocolate Amargo na Modulação do Sistema Nervoso Simpático e Parassimpático

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Orientador: Dra. Katya Vianna Rigatto
Coorientadora: Dra. Erna Vogt

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“Nós devemos ser a mudança que desejamos ver”

Gandhi.

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LISTA DE ABREVIATURAS

ECA – Enzima conversora de angiotensina

EROS – Espécies reativas de oxigênio

EDHF – Fator hiperpolarizante derivado do endotélio

ET – Endotelina

ECG – Eletrocardiograma

FC – Freqüência Cardíaca

HAS – Hipertensão arterial Sistêmica

HF – High frequency – alta freqüência

LF – Low frequency – baixa freqüência

mmHg – Milímetros de mercúrio

NO – Óxido nítrico

PA – Pressão arterial

PAS – Pressão arterial sistólica

PAD – Pressão arterial diastólica

PP – Pressão de pulso

RMSSD - raiz quadrada da média do quadrado das diferenças entre intervalos RR

SDNN - desvio padrão do intervalo R-R

SNA – Sistema nervoso Autônomo

VFC – Variabilidade da freqüência cardíaca

SUMÁRIO

RESUMO

O cacau e o chocolate amargo têm demostrado efeitos cardioprotetores, como atividade antioxidante, antiinflamatória e vasodilatadora. As ações são atribuídas aos flavonóides, compostos bioativos, presentes no cacau e nos produtos derivados do cacau. Dessa forma, este estudo foi delineado para verificar o efeito agudo da ingestão de chocolate amargo, com 70% de cacau, sobre a pressão arterial (PA), a frequência cardíaca (FC) e sobre o balanço autonômico para o coração. Participaram do estudo 17 indivíduos saudáveis, com idades entre 18 e 25 anos de ambos os sexos. Após um período de 5 minutos de repouso, em ambiente calmo e silencioso, a PA (mmHg) e a FC (bpm) foram aferidas conforme as VI Diretrizes Brasileiras de Hipertensão. Logo após foi realizado um eletrocardiograma (ECG) por 10 minutos, para verificação do balanço simpatovagal pela análise espectral. Em seguida os participantes foram convidados a ingerir 10g de chocolate amargo (70% de cacau) e uma hora após a ingestão de chocolate, repetimos as medidas de PA, FC e o ECG com o objetivo de verificar os efeitos do chocolate amargo sobre os parâmetros hemodinâmicos e sobre a modulação autonômica para o coração, visando detectar diferenças entre o período Pré e Pós-ingestão. Na análise estatística o teste T de Student pareado foi utilizado para dados paramétricos e o teste de Wilcoxon para dados não paramétricos. Todas as análises foram realizadas utilizando o software SigmaPlot 12.0. Os dados são apresentados como média±desvio padrão e foram considerados estatisticamente significativo para valores de $P \leq 0.05$. A PA sistólica (pré 104±14XPAS e pós 98±13 $P \leq 0.001$), média (pré 83±9 e pós 78±6 $P \leq 0.05$) e a FC (pré 80±6 e pós 75±6 $P \leq 0.00$) foram significativamente menores após 1h da ingestão de chocolate amargo. No entanto, a PA diastólica (pré 69±6 e pós $P \leq 0.14$) não foi significativamente diferente. A variabilidade total da FC foi maior (pré 1991±895 pós 3622±2627 $P \leq 0.02$), após a ingestão de chocolate amargo, acompanhada de uma diminuição na modulação simpática representada pelo componente LF normalizado (%) (pré 44±14 e pós 39±16 $P \leq 0.03$) e aumento da modulação parassimpática (HFnu pré 55±14 x HFnu pós 60±16 $P \leq 0.03$). Houve uma melhora no balanço simpatovagal (LF/HF) após a ingestão de chocolate (pré 0.94±0.60 e pós 0.79±0.52 $P \leq 0.05$). Os resultados desse estudo sugerem que o

consumo de 10g de chocolate amargo podem aumentar a participação do sistema nervoso parassimpático. Esse resultado indica que houve uma melhora no controle da PA, uma vez que melhorou o balanço autonômico. Dessa forma, mais estudos devem ser realizados para demonstrar este benefício.

Palavras chaves: chocolate amargo, sistema nervoso autônomo, variabilidade da freqüência cardíaca, pressão arterial e flavonóides.

ABSTRACT

Cocoa and dark chocolate have demonstrated cardioprotective effects, such as antioxidant, anti-inflammatory and vasodilatory. The shares are attributed to flavonoids, bioactive compounds present in cocoa and cocoa products. Thus, this study was designed to investigate the effect of acute ingestion of dark chocolate with 70% cocoa on blood pressure (BP), heart rate (HR) and the autonomic balance to the heart. The study included 17 healthy subjects, aged 18 to 25 years for both sexes. After a period of rest, the BP (mmHg) and HR (bpm) were measured according to the VI Brazilian Guidelines on Hypertension. Similarly, we performed the electrocardiogram (EKG) to check sympathovagal balance for spectral analysis. Then participants were asked to eat 10g of dark chocolate (70% cocoa) and one hour after ingestion, measures of BP, HR and EKG were repeated. The statistical analysis performed was of the paired t test for parametric data and the Wilcoxon Signed Rank Test for nonparametric data. All analyses were conducted using SigmaPlot 12.0 software. Data are presented as the mean ± SD and the $P<0.05$ was considered statistically significant. The systolic BP (before 104±14 and after 98±13 $P\leq0.001$), mean (before 83±9 X after 78±6 $P\leq0.05$) and HR (before 80±6 X after 75±6 $P\leq0.00$) were significantly lower after 1 hour of intake dark chocolate. However, diastolic BP (before 69±6 and after $P\leq0.14$) were not significantly different. The total variability of HR was higher (before 1991±895 X after 3622±2627 $P\leq0.02$), after eating dark chocolate, accompanied by a decrease in sympathetic modulation represented by normalized LF component (%) (before 44±14 X after 39±16 $P\leq0.03$) and increased parasympathetic modulation (before HFnu 55±14 x after 60±16 HFnu $P\leq0.03$). There was an improvement in sympathovagal balance (LF/HF) after ingestion chocolate (before 0.94±0.60 X after 0.79±0.52 $P\leq0.05$). The results of this study suggest that consumption of 10g of dark chocolate may increase the involvement of the parasympathetic nervous system. This result indicates that there was an improvement in BP control, since improved autonomic balance. Thus, further studies should be conducted to demonstrate this benefit.

Keywords: Dark chocolate, autonomic nervous system, heart rate variability, blood pressure and flavonoids.

Capitulo I - Revisão da Literatura

1. INTRODUÇÃO

1.1 Aspectos Históricos sobre a ingestão de cacau e chocolate

Os alimentos e bebidas produzidos a partir dos frutos da árvore, *Theobroma cacao,* o cacau, são consumidos pelos humanos há muitos anos. Documentos históricos fazem referencia, ao valor medicinal do chocolate, consumido como um alimento para tratar uma série de distúrbios, incluindo angina e dor no coração (Keen, 2001).

Os povos indígenas, situados nas Américas faziam uso medicinal do cacau, porém somente a partir de 1500 ele se difundiu pela Europa. As principais utilizações do cacau incluíam na época estimulo ao sistema nervoso, ao ganho de peso e ainda melhora na digestão (Dillinger et al., 2000). Hollenberg *et al* (2009) estudando os índios Kuna das ilhas San Blas do Panamá, verificaram que eles consumiam em média três copos de uma bebida a base de cacau por dia , e que a prevalência da hipertensão entre os Kuna, era de apenas 2,2% e que a pressão arterial (PA) não aumentava com a idade (Hollenberg et al., 2009). Coincidentemente esta população também apresentava menores taxas de diabetes mellitus, infarto do miocárdio, acidente vascular cerebral e câncer (Hollenberg et al., 1997).

1.2 Componentes ativos do cacau e do chocolate

O cacau contêm flavonóides, uma classe de polifenóis mais abundante na natureza. Nas sementes de cacau, utilizadas para produção de chocolate e outros

produtos; o flavonóide que se destaca é o Flavan-3-ols ou Flavanol, principalmente nas formas monoméricas de catequinas e epicatequinas, e na forma oligomérica de procianidinas (Andujar et al., 2012, Katz et al.). Apesar da perda de flavonóides chegar a 90% após o processamento das sementes de cacau, os efeitos benéficos para a saúde ainda são observados nos produtos de cacau, como no chocolate amargo. As estruturas monoméricas, após a ingestão de cacau, são rapidamente absorvidas no intestino e aumentam sua concentração no plasma, principalmente de epicatequina (Spencer et al., 2001, Holt et al., 2002).

Os flavonóides se destacam por apresentar efeitos benéficos na prevenção de doenças, principalmente das doenças cardiovasculares. Devido aos flavonóides terem a capacidade de proteger as células contra o estresse oxidativo, são classificados como antioxidantes. A capacidade antioxidante dos compostos fenólicos se deve a facilidade com o qual um átomo de hidrogênio do grupo hidroxil aromático pode ser doado para um radical livre e a habilidade do grupo fenólico suportar um elétron não pareado (Burns et al., 2000).

O chocolate meio amargo segundo a ANVISA, deve conter no mínimo 35% de cacau em sua composição. Em nossa pesquisa utilizamos um chocolate amargo contendo 70% de cacau que acreditamos apresentar potencialmente os efeitos benéficos do chocolate capazes de alterar os valores de PA.

1.3 Efeitos fisiológicos do cacau e do chocolate

De fato, o estudo observacional “Zutphen Idosos”, uma coorte com 470 homens demostrou que a ingestão de cacau estava inversamente associada com a PA (Buijsse et al., 2006). Da mesma forma, um estudo transversal, realizado em

2011 com 4970 americanos demonstrou que o consumo de chocolate estava inversamente associado com a prevalência de doença arterial coronariana (Djousse, Hopkins, North *et al*, 2011). Além disso, neste mesmo ano, Djousse *et al* verificaram que a calcificação de artérias coronárias, avaliada por tomografia computadorizada em 2217 indivíduos, também estava inversamente associada com o consumo de chocolate ou de cacau (Djousse et al.). Finalmente, um estudo de coorte que acompanhou por seis anos mulheres suecas demonstrou que a ingestão moderada de chocolate, 19 a 30 g por semana, foi inversamente associado com a insuficiência cardíaca (Mostofsky, Levitan *et al*, 2010).

Dessa forma, está claro na literatura que os efeitos do cacau e do chocolate sobre o organismo, que também incluem: redução da resistência insulínica, proteção do sistema nervoso central, da função imune e do sistema cardiovascular, prevenção do câncer, entre outros (Katz et al.)(2011), podem ser verificados em praticamente todos os sistemas orgânicos. Por esta razão, o estudo dos efeitos da ingestão de cacau é muito provocativo, no sentido de buscar novas alternativas não farmacológicas que possam desempenhar um papel preventivo às doenças cardiovasculares.

Estão bem estabelecidos na literatura os efeitos cardioprotetores (Mehrinfar and Frishman, 2008) do cacau. Entre eles podemos citar: melhora na função endotelial (Vlachopoulos et al., 2005, Fisher et al., 2003, Heiss et al., 2003, Heiss et al., 2006, Schroeter et al., 2006, Heiss et al., 2007, Heiss et al., 2005), inibição da agregação plaquetária (Murphy et al., 2003, Pearson et al., 2002, Innes et al., 2003), efeitos antiinflamatórios (Selmi et al., 2008) e antiaterosclerótico com a diminuição da oxidação da LDLc (Steffen et al., 2005, Bordeaux et al., 2007, Kondo et al., 1996) e efeito anti-hipertensivo (Grassi et al., 2005a, Grassi et al., 2005b, Actis-Goretta et al.,

2006, Buijsse et al., 2006, Taubert et al., 2007b). No entanto, não está claro na literatura se a ingestao isolada de chocolate amargo seria capaz de alterar o controle da PA, em especial o controle autonômico cardiocirculatório.

Sabemos dos efeitos redutores da PA, em indivíduos normotensos (Grassi et al., 2005a, Fraga et al., 2005, Buijsse et al., 2010), pré-hipertensos e com hipertensão estágio I (Desch et al., 2010, Ried et al., 2012) quanto em outros estágios de hipertensão (Grassi et al., 2005b, Taubert et al., 2003).

Inúmeras pesquisas demonstram uma melhora na função endotelial (Engler et al., 2004, Vlachopoulos et al., 2005, Nogueira Lde et al.) acompanhada de uma maior biodisponibilidade de óxido nítrico (NO) (Fisher et al., 2003, Taubert et al., 2007a) após o consumo chocolate amargo ou de cacau (Corti et al., 2009). Estudos experimentais realizados com ratos espontaneamente hipertensos (*SHR)*, também demonstraram que os antioxidantes presentes no cacau reduzem a PA (Quinones, Muguerza *et al*., 2011; Sánchez, Quinones *et al*., 2010). Além disso, Pearson *et al* (2011) estudando o efeito do chocolate amargo *in vitro* e *in vivo*, sugerem uma inibição na atividade da enzima conversora da angiotensina (ECA)(Persson et al.). Esses resultados são confirmados por Guerrero *et al* (2012) que demonstraram a aplicação dos flavonóides como inibidores da ECA, podendo ser utilizados como suplementos nutricionais (Guerrero, Castillo *et al.* 2012). Essa importante ação dos flavonóides presentes no cacau é outro possível mecanismo de ação do chocolate amargo e de produtos derivados do cacau em reduzir a PA e, consequentemente, diminuir o risco cardiovascular (Actis-Goretta et al., 2003).

A figura 1 ilustra os mecanismos de ação responsáveis pela redução da PA induzido pelo cacau e encontrada nos estudos clínicos. O cacau aumenta as concentrações de L- arginina, o que em ultima instância aumenta o óxido nítrico (NO)

intracelular através da ação da enzima óxido nítrico sintase (eNos), causando o relaxamento da musculatura lisa dos vasos.

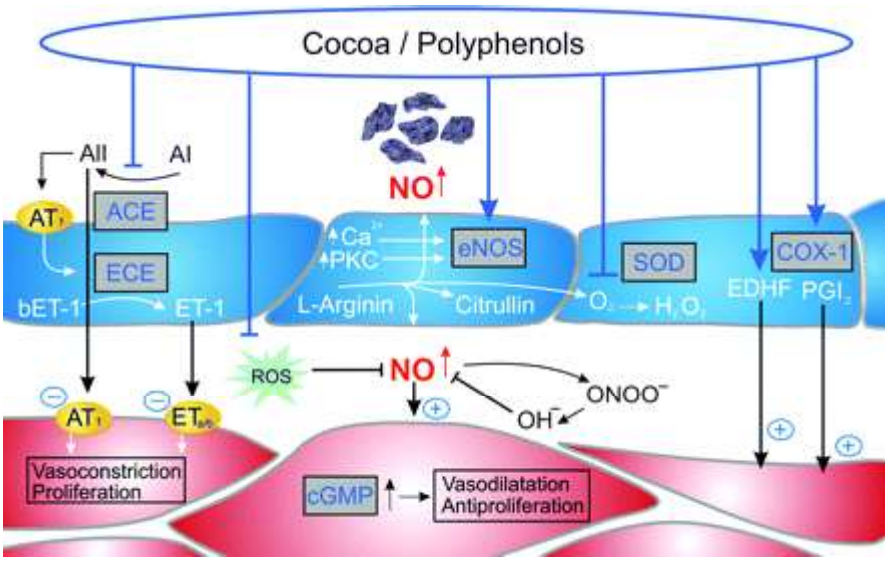

Figura 1: Efeito dos polifenóis do cacau no endotélio. Legenda com abreviaturas. Oxido Nítrico (NO); Fator hiperpolarizante derivado do endotélio (EDHF); Prostaciclina (PGI); Superóxido desmutase (SOD); enzima conversora da angiotensina (ACE); Receptor da Angiotensina (AT1) Endotelina (ET-1); espécies reativas oxigênio (ROS), enzima óxido nítrico sintetase (eNos), Angiotensina I (AI); Angiotensina II (AII); Proteína C quinase (PKC); Monofosfato cíclico de guanosina (GMPc); receptores de endotelina ETa/b. Fonte: Corti et al, (2009).

Além de aumentar a síntese de NO, outros efeitos podem ser observados, tais como efeitos antioxidantes podendo reduzir a produção de espécies reativas de oxigênio (EROS), contribuindo assim para uma melhor função endotelial por favorecer indiretamente o aumento da biodisponibilidade do NO. Além disso, os polifenóis contidos no cacau podem ativar o fator hiperpolarizante derivado do endotélio (EDHF) aumentando a liberação de prostaciclina endotelial ou inibindo a síntese de endotelina (ET). Os polifenóis podem também inibir a atividade da enzima conversora de angiotensina (ECA), o que foi demonstrado em um estudo *in*

vivo e *in vitro* e, conseqüentemente, reduziu a atividade da NADPH oxidase que produz EROS e é estimulada por este hormônio (Corti et al., 2009). De fato, uma significativa redução na ECA sanguínea de indivíduos foi observada 3 h após a ingestão de 75g de chocolate com 72% de cacau (Persson et al., 2011).

Nosso interesse no Sistema Nervoso Autônomo (SNA) se justifica pelo fato de ser um sistema que atua em diversas situações fisiológicas e interage com inúmeros sistemas de controle de funções corporais, como o sistema renina angiotensina, por exemplo, e qualquer modificação no balanço entre os sistemas nervoso simpático e parassimpático pode apresentar efeitos que vão além daqueles verificados sobre o sistema cardiovascular. Esse fato aumenta o desafio de estudar o SNA que potencialmente pode interferir na promoção da saúde de forma significativa e abrangente.

1.4 O Papel do Sistema Nervoso Autônomo sobre o sistema cardiovascular

O controle reflexo da PA, realizado pelo SNA, é realizado através de diferentes sensores periféricos (Michelini and Morris, 1999) e que atuam sobre as eferências nervosas simpáticas e parassimpáticas (Abboud e Thames, 1983) determinando alterações fisiológicas que contribuem para diminuir as variações da PA (Krieger, et al 1999). Essa regulação promove ajustes através da bradicardia e da taquicardia reflexa que são moduladas pelos pressorreceptores (Lopes et al., 2000). A atividade vagal diminui a frequência cardíaca (FC), enquanto as fibras pós-ganglionares simpáticas, que inervam quase todo o coração (Hainsworth, R.; 1995) aumentam a FC (Furnival, et al; 1973). Em seres humanos o SNA está tonicamente ativado com a dominância da regulação vagal para o coração que influencia fortemente a FC de cada indivíduo. Neste sentido, o controle autonômico é

fundamental para as adaptações da freqüência cardíaca que determinam a variabilidade da FC fundamental para os ajustes momento a momento da PA.

Além disso, nas últimas décadas, têm se observado uma associação positiva e significativa entre o funcionamento do SNA e a mortalidade por doença cardiovascular. Evidências experimentais mostraram uma associação entre arritmias letais e sinais de aumento, mesmo que pequeno da atividade simpática ou redução parassimpática. Isso tem incentivado o desenvolvimento de marcadores quantitativos da atividade autonômica e sua relação com parâmetros de saúde cardiovascular (Task Force, 1996).

Já está bem estabelecido na literatura que na Hipertensão Arterial Sistêmica (HAS) há um desbalanço simpatovagal, com um predomínio no componente simpático (Irigoyen, et al., 2001) e que as alterações do SNA são um importante indicador de prognóstico (Kleiger et al., 1987, Montano et al., 2009), de maneira que qualquer intervenção farmacológica ou não, como o consumo de chocolate, que contribua para a melhora do funcionamento nesse sistema representa uma ferramenta importante tanto para a manutenção da saúde como para tratar patologias.

Considerando a importância do estudo do SNA através da sua manifestação no controle da FC, o eletrocardiograma (ECG) constitui um método de baixo custo de diagnóstico que fornece informações essenciais sobre o intervalo Q-T (Molnar et al., 1997) e que nos permite avaliar o balanço simpatovagal para o coração. As oscilações que ocorrem entre os intervalos R-R, intervalo entre duas ondas R do eletrocardiograma ou entre dois ciclos cardíacos, tem sido descrito como a variabilidade da frequência cardíaca (VFC) (Lahiri et al., 2008).

As variações na modulação do sistema nervoso simpático e parassimpático alteram a FC e a PA para manter a adequada homeostasia celular. Neste sentido, o ECG, um método não invasivo que permite a médicos e pesquisadores coletar informações valiosas sobre a modulação do SNA, possibilita o estudo quantitativo do balanço simpatovagal. Estudos mostraram que há uma relação entre VFC e o avanço da idade, sugerindo um declínio dependente da idade. Tal afirmação tem aplicação clínica, por apresentar correlação inversa com o risco de morte por doenças cardiovasculares (Paolisso et al., 1999). Reis e colaboradores (1998) observaram que quanto menor a VFC, maiores eram os riscos de doenças cardíacas. Sendo assim a diminuição da VFC constitui importante fator prognóstico para o surgimento de doenças cardíacas tanto para indivíduos saudáveis como em indivíduos cardiopatas (Malliani et al., 1991).

Sabendo que as alterações no balanço autonômico estão associadas a diversas patologias, torna-se relevante estudar a modulação autonômica uma vez que essas alterações no balanço simpatovagal são fundamentais para o prognóstico das doenças. Da mesma forma, a melhora deste balanço assume um papel importante na sobrevida das populações e que, se confirmadas as hipóteses desse estudo, o chocolate talvez contribua para a prevenção/retardo no aparecimento de doenças cardiovasculares.

As oscilações nos intervalos R-R podem ser avaliadas pelo domínio do tempo e da freqüência. No domínio do tempo foram avaliadas as variáveis SDNN (desvio padrão do intervalo R-R) e RMSSD (raiz quadrada da média do quadrado das diferenças entre intervalos RR normais adjacentes, expresso em ms). A análise dos dados de VFC no domínio da frequência foi realizada pela Transformada Rápida de Fourier (FFT) em trechos de 5 minutos, com interpolação de 4 Hz, overlap de 50%.

As bandas de interesse da FC, no domínio da freqüência, foram as 2 faixas distintas: a faixa LF (Low Frequency) e a faixa HF (High Frequency) que representam a modulação simpática e parassimpática, respectivamente. A avaliação da VFC, que é composta pelo balanço entre os dois sistemas permite avaliar o controle autonômico através da relação dos componentes LF/HF (Malliani *et al*., 1991).

Pesquisando a literatura, somente um estudo foi encontrado envolvendo licor de cacau e o balanço autonômico. Neste estudo, realizado em coelhos hipercolesterolêmicos, tratados com o licor do cacau por 6 meses, os autores observaram uma redução da FC, da PA e da área das lesões ateroscleróticas na aorta no grupo tratado com cacau. Da mesma forma o componente HF (High Frequency), ou modulação vagal, se manteve inalterado no grupo tratado, enquanto esse componente estava reduzido no grupo controle (Akita et al., 2008).

No entanto, até a presente data não foram encontrados na literatura trabalhos que avaliem a ação aguda do chocolate amargo no controle do SNA em humanos. Dada à importância desse sistema no controle fisiopatológico da PA e de diversos sistemas corporais, o nosso trabalho tem o objetivo de investigar se uma única ingestão de chocolate (70% cacau) pode melhorar o balanço simpatovagal em adultos saudáveis e, assim, melhorar os mecanismos de controle da PA e FC para proteger o organismo do desenvolvimento de doenças do aparelho cardiocirculatório.

2. OBJETIVOS

2.1 Objetivo Geral:

O objetivo do estudo foi avaliar o efeito da ingestão aguda de chocolate amargo (70% cacau) sobre a modulação do Sistema Nervoso Autônomo cardíaco.

2.2 Objetivos Específicos:

Verificar em indivíduos saudáveis o efeito agudo da ingestão de 10g de chocolate amargo com 70% de cacau, sobre a pressão arterial (PA), freqüência cardíaca (FC), e variabilidade da freqüência cardíaca.

REFERÊNCIAS

Abboud, F. M., M.D. Thames. Interaction of cardiovacular reflexes in circulatory control. In: Sheperd J.T and F.M. Abboud (eds) – Handbook of Physiology. The cardiovascular system. Cardiovascular reflex and circulatory integration. Bethesda. American Physiological Society, v.3, p. 675-753, 1983.

Actis-Goretta, L., J. I. Ottaviani*, et al.* Inhibition of angiotensin converting enzyme activity by flavanol-rich foods. J Agric Food Chem, v.54, n.1, Jan 11, p.229-34. 2006.

_____. Inhibition of angiotensin converting enzyme (ACE) activity by flavan-3-ols and procyanidins. FEBS Lett, v.555, n.3, Dec 18, p.597-600. 2003.

Akita, M., M. Kuwahara*, et al.* Effects of cacao liquor polyphenols on cardiovascular and autonomic nervous functions in hypercholesterolaemic rabbits. Basic Clin Pharmacol Toxicol, v.103, n.6, Dec, p.581-7. 2008.

Andujar, I., M. C. Recio*, et al.* Cocoa polyphenols and their potential benefits for human health. Oxid Med Cell Longev, v.2012, p.906252. 2012.

Bordeaux, B., L. R. Yanek*, et al.* Casual chocolate consumption and inhibition of platelet function. Prev Cardiol, v.10, n.4, Fall, p.175-80. 2007.

Buijsse, B., E. J. Feskens*, et al.* Cocoa intake, blood pressure, and cardiovascular mortality: the Zutphen Elderly Study. Arch Intern Med, v.166, n.4, Feb 27, p.411-7. 2006.

Buijsse, B., C. Weikert*, et al.* Chocolate consumption in relation to blood pressure and risk of cardiovascular disease in German adults. Eur Heart J, v.31, n.13, Jul, p.1616-23. 2010.

Burns, J., P. T. Gardner*, et al.* Relationship among antioxidant activity, vasodilation capacity, and phenolic content of red wines. J Agric Food Chem, v.48, n.2, Feb, p.220-30. 2000.

Corti, R., A. J. Flammer*, et al.* Cocoa and cardiovascular health. Circulation, v.119, n.10, Mar 17, p.1433-41. 2009.

Desch, S., J. Schmidt*, et al.* Effect of cocoa products on blood pressure: systematic review and meta-analysis. Am J Hypertens, v.23, n.1, Jan, p.97-103. 2010.

Dillinger, T. L., P. Barriga*, et al.* Food of the gods: cure for humanity? A cultural history of the medicinal and ritual use of chocolate. J Nutr, v.130, n.8S Suppl, Aug, p.2057S-72S. 2000.

Djousse, L., P. N. Hopkins*, et al.* Chocolate consumption is inversely associated with calcified atherosclerotic plaque in the coronary arteries: the NHLBI Family Heart Study. Clin Nutr, v.30, n.1, Feb, p.38-43.

Engler, M. B., M. M. Engler*, et al.* Flavonoid-rich dark chocolate improves endothelial function and increases plasma epicatechin concentrations in healthy adults. J Am Coll Nutr, v.23, n.3, Jun, p.197-204. 2004.

Fisher, N. D., M. Hughes*, et al.* Flavanol-rich cocoa induces nitric-oxide-dependent vasodilation in healthy humans. J Hypertens, v.21, n.12, Dec, p.2281-6. 2003.

Fraga, C. G., L. Actis-Goretta*, et al.* Regular consumption of a flavanol-rich chocolate can improve oxidant stress in young soccer players. Clin Dev Immunol, v.12, n.1, Mar, p.11-7. 2005.

Grassi, D., C. Lippi*, et al.* Short-term administration of dark chocolate is followed by a significant increase in insulin sensitivity and a decrease in blood pressure in healthy persons. Am J Clin Nutr, v.81, n.3, Mar, p.611-4. 2005.

Grassi, D., S. Necozione*, et al.* Cocoa reduces blood pressure and insulin resistance and improves endothelium-dependent vasodilation in hypertensives. Hypertension, v.46, n.2, Aug, p.398-405. 2005.

Heiss, C., A. Dejam*, et al.* Vascular effects of cocoa rich in flavan-3-ols. JAMA, v.290, n.8, Aug 27, p.1030-1. 2003.

Heiss, C., D. Finis*, et al.* Sustained increase in flow-mediated dilation after daily intake of high-flavanol cocoa drink over 1 week. J Cardiovasc Pharmacol, v.49, n.2, Feb, p.74-80. 2007.

Heiss, C., P. Kleinbongard*, et al.* Acute consumption of flavanol-rich cocoa and the reversal of endothelial dysfunction in smokers. J Am Coll Cardiol, v.46, n.7, Oct 4, p.1276-83. 2005.

Heiss, C., H. Schroeter*, et al.* Endothelial function, nitric oxide, and cocoa flavanols. J Cardiovasc Pharmacol, v.47 Suppl 2, p.S128-35; discussion S172-6. 2006.

Hollenberg, N. K., N. D. Fisher*, et al.* Flavanols, the Kuna, cocoa consumption, and nitric oxide. J Am Soc Hypertens, v.3, n.2, Mar-Apr, p.105-12. 2009.

Hollenberg, N. K., G. Martinez*, et al.* Aging, acculturation, salt intake, and hypertension in the Kuna of Panama. Hypertension, v.29, n.1 Pt 2, Jan, p.171-6. 1997.

Holt, R. R., S. A. Lazarus*, et al.* Procyanidin dimer B2 [epicatechin-(4beta-8)-epicatechin] in human plasma after the consumption of a flavanol-rich cocoa. Am J Clin Nutr, v.76, n.4, Oct, p.798-804. 2002.

Innes, A. J., G. Kennedy*, et al.* Dark chocolate inhibits platelet aggregation in healthy volunteers. Platelets, v.14, n.5, Aug, p.325-7. 2003.

Katz, D. L., K. Doughty*, et al.* Cocoa and chocolate in human health and disease. Antioxid Redox Signal, v.15, n.10, Nov 15 2011
p.2779-811.

Keen, C. L. Chocolate: food as medicine/medicine as food. J Am Coll Nutr, v.20, n.5 Suppl, Oct, p.436S-439S; discussion 440S-442S. 2001.

Kleiger, R. E., J. P. Miller*, et al.* Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. Am J Cardiol, v.59, n.4, Feb 1, p.256-62. 1987.

Kondo, K., R. Hirano*, et al.* Inhibition of LDL oxidation by cocoa. Lancet, v.348, n.9040, Nov 30, p.1514. 1996.

Lahiri, M. K., P. J. Kannankeril*, et al.* Assessment of autonomic function in cardiovascular disease: physiological basis and prognostic implications. J Am Coll Cardiol, v.51, n.18, May 6, p.1725-33. 2008.

Lopes, H. F., F. M. Consolim-Colombo*, et al.* Hormonal and cardiovascular reflex assessment in a female patient with pure autonomic failure. Arq Bras Cardiol, v.75, n.3, Sep, p.235-42. 2000.

Malliani, A., M. Pagani*, et al.* Cardiovascular neural regulation explored in the frequency domain. Circulation, v.84, n.2, Aug, p.482-92. 1991.

Mehrinfar, R. e W. H. Frishman. Flavanol-rich cocoa: a cardioprotective nutraceutical. Cardiol Rev, v.16, n.3, May-Jun, p.109-15. 2008.

Michelini, L. C. e M. Morris. Endogenous vasopressin modulates the cardiovascular responses to exercise. Ann N Y Acad Sci, v.897, p.198-211. 1999.

Molnar, J., J. E. Rosenthal*, et al.* QT interval dispersion in healthy subjects and survivors of sudden cardiac death: circadian variation in a twenty-four-hour assessment. Am J Cardiol, v.79, n.9, May 1, p.1190-3. 1997.

Montano, N., A. Porta*, et al.* Heart rate variability explored in the frequency domain: a tool to investigate the link between heart and behavior. Neurosci Biobehav Rev, v.33, n.2, Feb, p.71-80. 2009.

Murphy, K. J., A. K. Chronopoulos*, et al.* Dietary flavanols and procyanidin oligomers from cocoa (Theobroma cacao) inhibit platelet function. Am J Clin Nutr, v.77, n.6, Jun, p.1466-73. 2003.

Nogueira Lde, P., M. P. Knibel*, et al.* Consumption of high-polyphenol dark chocolate improves endothelial function in individuals with stage 1 hypertension and excess body weight. Int J Hypertens, v.2012, p.147321.

Paolisso, G., D. Manzella*, et al.* Baseline heart rate variability in healthy centenarians: differences compared with aged subjects (>75 years old). Clin Sci (Lond), v.97, n.5, Nov, p.579-84. 1999.

Pearson, D. A., T. G. Paglieroni*, et al.* The effects of flavanol-rich cocoa and aspirin on ex vivo platelet function. Thromb Res, v.106, n.4-5, May 15, p.191-7. 2002.

Persson, I. A., K. Persson*, et al.* Effects of cocoa extract and dark chocolate on angiotensin-converting enzyme and nitric oxide in human endothelial cells and healthy volunteers--a nutrigenomics perspective. J Cardiovasc Pharmacol, v.57, n.1, Jan, p.44-50.

_____. Effects of cocoa extract and dark chocolate on angiotensin-converting enzyme and nitric oxide in human endothelial cells and healthy volunteers--a nutrigenomics perspective. J Cardiovasc Pharmacol, v.57, n.1, Jan, p.44-50. 2011.

Reis AF, Bastos BG, Mesquita ET, Romeu Filho LJM, Nóbrega, ACL. Disfunção parassimpática, variabilidade de frequência cardíaca e estimulação colinérgica após infarto agudo do miocárdio. Arq Bras Cardiol. 1998;70(3):193-7.

Ried, K., T. R. Sullivan*, et al.* Effect of cocoa on blood pressure. Cochrane Database Syst Rev, v.8, p.CD008893. 2012.

Schroeter, H., C. Heiss*, et al.* (-)-Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans. Proc Natl Acad Sci U S A, v.103, n.4, Jan 24, p.1024-9. 2006.

Selmi, C., C. A. Cocchi*, et al.* Chocolate at heart: the anti-inflammatory impact of cocoa flavanols. Mol Nutr Food Res, v.52, n.11, Nov, p.1340-8. 2008.

Spencer, J. P., H. Schroeter*, et al.* Epicatechin is the primary bioavailable form of the procyanidin dimers B2 and B5 after transfer across the small intestine. Biochem Biophys Res Commun, v.285, n.3, Jul 20, p.588-93. 2001.

Steffen, Y., T. Schewe*, et al.* Epicatechin protects endothelial cells against oxidized LDL and maintains NO synthase. Biochem Biophys Res Commun, v.331, n.4, Jun 17, p.1277-83. 2005.

Taubert, D., R. Berkels*, et al.* Chocolate and blood pressure in elderly individuals with isolated systolic hypertension. JAMA, v.290, n.8, Aug 27, p.1029-30. 2003.

Taubert, D., R. Roesen*, et al.* Effects of low habitual cocoa intake on blood pressure and bioactive nitric oxide: a randomized controlled trial. JAMA, v.298, n.1, Jul 4, p.49-60. 2007.

_____. Effect of cocoa and tea intake on blood pressure: a meta-analysis. Arch Intern Med, v.167, n.7, Apr 9, p.626-34. 2007.

Vlachopoulos, C., K. Aznaouridis*, et al.* Effect of dark chocolate on arterial function in healthy individuals. Am J Hypertens, v.18, n.6, Jun, p.785-91. 2005.

Capítulo II – Artigo em Inglês

Acute Dark chocolate intake improves heart rate variability in healthy subjects.

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Acute Dark chocolate intake improves heart rate variability in healthy subjects.

Ana Amélia Machado Duarte[1], Cristiano Mostardas[2], Denieli Bós[1], Maria Claudia Irigoyen[2], Erna Vogt[3], Katya Vianna Rigatto[1].

[1] Departamento de Ciências Básicas da Saúde,Universidade Federal de Ciências da Saúde de Porto Alegre, RS – Brazil

[2] Hospital das Clinicas da Faculdade de Medicina da Universidade de São Paulo, Unidade de Hipertensão, Instituto do Coração (InCor), São Paulo/SP, Brazil

[3] Departamento de Ciências dos Alimentos, Universidade Federal do Rio Grande do Sul. RS – Brazil

Corresponding Author: Katya Vianna Rigatto

Physiology Departament, Universidade Federal de Ciências da Saúde de Porto Alegre.

Sarmento Leite, 245, Porto Alegre, RS, Brazil. Zip Code: 90050-170

Telephone: 55 (51) 3330-8753 - Fax Number: 55 (51) 3330-8810

Email: krigatto@gmail.com

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Keys Words: dark chocolate, autonomic nervous system, heart hate variability, blood pressure

ABSTRACT

Cocoa and dark chocolate have demonstrated cardioprotective effects, such as antioxidant, anti-inflammatory and vasodilatory. The shares are attributed to flavonoids, bioactive compounds present in cocoa and cocoa products. Thus, this study was designed to investigate the effect of acute ingestion of dark chocolate with 70% cocoa on blood pressure (BP), heart rate (HR) and the autonomic balance to the heart. The study included 17 healthy subjects, aged 18 to 25 years for both sexes. After a period of rest, the BP (mmHg) and HR (bpm) were measured according to the VI Brazilian Guidelines on Hypertension. Similarly, we performed the electrocardiogram (EKG) to check sympathovagal balance for spectral analysis. Then participants were asked to eat 10g of dark chocolate (70% cocoa) and one hour after ingestion, measures of BP, HR and EKG were repeated. The statistical analysis performed was of the paired t test for parametric data and the Wilcoxon Signed Rank Test for nonparametric data. All analyses were conducted using SigmaPlot 12.0 software. Data are presented as the mean ± SD and the $P<0.05$ was considered statistically significant. The systolic BP (before 104±14 and after 98±13 $P\leq0.001$), mean (before 83±9 X after 78±6 $P\leq0.05$) and HR (before 80±6 X after 75±6 $P\leq0.00$) were significantly lower after 1 hour of intake dark chocolate. However, diastolic BP (before 69±6 and after $P\leq0.14$) were not significantly different. The total variability of HR was higher (before 1991±895 X after 3622±2627 $P\leq0.02$), after eating dark chocolate, accompanied by a decrease in sympathetic modulation represented by normalized LF component (%) (before 44±14 X after 39±16 $P\leq0.03$) and increased parasympathetic modulation (before HFnu 55±14 x after 60±16 HFnu $P\leq0.03$). There was an improvement in sympathovagal balance (LF/HF) after ingestion chocolate (before 0.94±0.60 X after 0.79±0.52 $P\leq0.05$). The results of this study suggest that

consumption of 10g of dark chocolate may increase the involvement of the parasympathetic nervous system. This result indicates that there was an improvement in BP control, since improved autonomic balance. Thus, further studies should be conducted to demonstrate this benefit.

Keywords: Dark chocolate, autonomic nervous system, heart rate variability, blood pressure and flavonoids.

INTRODUCTION

Epidemiological reports indicate that consumption of foods rich in polyphenols are associated with lower incidence of cardiovascular diseases (Hertog et al., 1993, Manach et al., 2005) and decreased risk for cardiovascular morbidity and mortality (Keli et al., 1996). The cacao products, such as dark chocolate, rich in polyphenols has demonstrated beneficial, particularly cardiovascular effects (Corti et al., 2009, Buijsse et al., 2010).

The cardioprotetion of cocoa and dark chocolate include, antioxidant (Serafini et al., 2003), antiatherosclerotic, by decreasing the LDL oxidation, (Kondo et al., 1996, Baba et al., 2007), and anti-inflammatory effects (Selmi et al., 2008, Mao et al., 2000), as well as, reduction in platelet aggregation (Bordeaux et al., 2007), and angiotensin converting enzyme (ACE) activity (Persson et al., 2011). It is documented an increased nitric oxide bioavailability (Taubert et al., 2007a), and improved endothelial function (Vlachopoulos et al., 2005) , that ultimately, induce vascular relaxation and antihypertensive effects (Desch et al., 2010, Mehrinfar and Frishman, 2008).

One of the mechanisms by which blood pressure (BP) control is improved is associated to the peripheral afferents that carry out BP information to the central nervous system. This mechanism include sympathetic and/or parasympathetic activation and thus changes heart rate (HR) to promote BP stabilization (Franchini and Cowley, 1996). The study of HR variability (HRV) has been used for the last few decades (TASK FORCE, 1996) to assess autonomic modulation of the cardiovascular system (Bertagnolli et al., 2008). Higher HRV is an indication of good BP adaptation, while lower HRV is frequently an indicator of abnormal and insufficient

adaptation of the autonomic nervous system (ANS) modulation (Pumprla et al., 2002).

Akita *et al.*, (2008), observing rabbits treated with liquor cocoa for 6 months, demonstrated that there was a decrease in heart rate, blood pressure and an improvement in the baroreflex function. In addition, these authors also observed that there was an improvement in parasympathetic modulation when compared to the rabbits feed with standard diet.

However, as far as we search in the area, we did not find any study describing the effect of the acute ingestion of dark chocolate over the autonomic nervous system modulation in healthy or hypertensive subjects. Thus, considering the importance of decreasing the risk for cardiovascular disease and the fact that dark chocolate may improve the ANS modulation. This study was conducted to investigate whether a single of dark chocolate, with 70% of cocoa, would be able to improve BP control of in healthy subjects.

MATERIALS and METHODS

Subjects

The protocol was approved by the Ethics Committee of Universidade Federal de Ciências da Saúde de Porto Alegre. A total of 17 subjects (10 women, 7 men), between 18 and 25 years old, voluntarily participated in the study after signing the informed consent. Subjects were clinically healthy, nonobese, normotensive, nonsmokers and were not taking any medications or antioxidant supplements, except oral contraceptives for women. To minimize the effects of hormones fluctuation on autonomic control of the cardiovascular system, all measurements in women were

colleted during the early follicular phase, 1° - 3° day, of the menstrual cycle (Minson et al., 2000).

Study design

The subjects entered a further cocoa-free washout phase of 7 days, according to the protocol described by (Taubert et al., 2003). 24 hours before the experiment, the volunteers were instructed not to: drink alcohol, caffeinated products and practicing physical exercise intense. The following measurements comprised the experimental sequence: 1. BP, RH and HRV were measured at rest; 2. 10g of dark chocolate with 70% cocoa (Nutrition composition on Table 1) was taken by the subjects; 3. The anthropometric data were obtained immediately after the chocolate intake; 4. One hour after the chocolate consumption, also at rest, a second BP, HR and HRV was performed.

Blood pressure measurement

The subjects were seated in upright position with back support, and were asked to relax for 5 min. BP was determined with the use of the automatic digital device (Omron® IntelliSense® BP785). The BP cuff covered at least 80% of the upper arm. To confirm the data, the BP measurement was repeated at least 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. An average of three measurements was used for the BP of each participant. From this, mean arterial pressure (MAP), HR and pulse pressure (PP) were determined.

Heart Rate Variability

Time and frequency domain

The electrocardiogram signal (EKG) acquisition was colleted at,1kHz sample rate, for a 10 minute period in the supine position, head elevation of 30°. It was done using a protocol with three derivations. To assess the HRV, the temporal series of RR intervals, were registered and processed by the MP150 system (Biopac, California, USA).

In text format, the temporal series were analyzed using the program Kubios HRV 2.0 (Biosignal Analysis and Medical Imaging Group, Kuopio, Finland) in which the EKG signal was processed to obtain the variables related to HRV in the time domain and frequency. In the time domain variables were chosen SDNN (standard deviation of RR intervals) and RMSSD (square root of the mean squared differences between adjacent normal RR intervals, expressed in ms). The analysis of HRV in the frequency domain raw state was performed by Fast Fourier Transform (FFT) in portions of 5 minutes with interpolation 4 Hz, overlap by 50%. The bands of interest were low frequency or LF (0:04 to 0:15 Hz and this component refers predominantly sympathetic modulation) and high frequency or HF (0.15 to 0.4 Hz, refers to parasympathetic modulation).

Normalized LF and HF components of R–R variability were considered, respectively, as markers of cardiac sympathetic and parasympathetic modulation, and the ratio between them (LF/HF) was considered as an index of the autonomic modulation of the heart (Montano et al., 2009). Normalized units (nu) were obtained by dividing the power of given component by the total power (from which VLF has

been subtracted) and multiplying by 100 (Montano et al., 2008). The sympatho-vagal index (LF/HF) was calculated based on the LF and HF absolute values.

Symbolic analysis

Symbolic analysis was used to detect changes in autonomic modulation of cardiovascular variability (Guzzetti et al., 2005 and Porta et al., 2007). 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 and indicate sympathetic modulation); 2) one variation (1V, two symbols equal and one different. Indicate both sympathetic and parasympathetic modulation); 3) two like variations (2LV) and indicate parasympathetic modulation; and 4) two unlike variations (2UV) and indicate parasympathetic modulation (Guzzetti et al., 2005). All recordings were performed in a sound attenuated room.

Statistical Analysis

The statistical analysis performed was of the paired t test for parametric data and the Wilcoxon Signed Rank Test for nonparametric data. The normality the data were assessed through the Shapiro-Wilk test. All analyses were conducted using SigmaPlot 12.0 software. Data are presented as the mean ± SD and the $P<0.05$ was considered statistically significant.

RESULTS

Hemodynamic and anthropometric data

Subjects were 22±2 years old and the body mass index (BMI) was 22.5±3 kg/m^2. SBP, mean arterial pressure (MAP), PP and HR were significantly decreased

after 1 hour of dark chocolate intake. However, there was no difference in DBP (Table 2).

Heart Rate Variability

A single dose of dark chocolate significantly increased the total power of HRV. Furthermore, in absolute values, the parasympathetic (HFa) and the sympathetic modulation (LFa) to the heart were, respectively, 85% and 45% higher after chocolate consumption. The spectral analysis data, also demonstrated that the proportion between sympathetic and parasympathetic was also changed after dark chocolate intake. The HFnu, SDNN and RMSSD that represent the parasympathetic modulation, were significantly increased. On the other hand, the LFnu, or the sympathetic modulation to the heart and LF/HF ratio were significantly decreased after the chocolate intake. Regarding to symbolic analysis, the 0V pattern (%) was significantly decreased after chocolate intake indicating a reduction of sympathetic modulation. On the other hand, the parasympathetic modulation was increased, as seen by increase in 2 LV and 2 UV pattern.

Collectively, our results of spectral and symbolic analysis strongly indicate that the ingestion of a single dose (10g) of dark chocolate increase the parasympathetic nervous system modulation to the heart (Table 3).

DISCUSSION

The most significant observation of this study is that a single dose of dark chocolate improves the autonomic nervous system (ANS) modulation to the heart in healthy subjects. After one hour, the dark chocolate intake significantly increased in

82% the total power of HRV. This improvement in the ANS modulation was associated to a decrease in SBP, PP and HR, without changing DBP.

Indeed, although the mechanism remains under investigation, recent studies have suggested that, chronically, the dark chocolate consumption may have blood pressure lowering effects (Egan et al., 2010, Taubert et al., 2007a, Desch et al., 2010, Zomer et al., 2012). According to Sudarma, *et al. (*2011), the possible explanation to this effect could be the flavanols that have been shown to stimulate the endothelial nitric oxide production causing blood vessels dilatation in pre-hypertensive subjects. Moreover, it was also demonstrated that chronic dark chocolate intake improves endothelial function in healthy subjects (Engler et al., 2004, Grassi et al., 2005a, Fisher et al., 2003, Ried et al., 2010, Heiss et al., 2006) and in smokers, who after an acute cocoa consumption, had not only an improvement in endothelial function but also in BP control(Heiss et al., 2003).

In addition, in agreement with our results, although using a different dose, Faridi et al (2008) also demonstrated that the single ingestion of 74g of dark chocolate (22g of cocoa) significantly decreased BP and improved endothelial function in overweight but healthy subjetcs (Faridi et al., 2008).

Although we have studied the acute ingestion of a single dose of dark chocolate, our results are consistent also with previous reports, involving subjects and chronic chocolate intake in subjects. Similarly to ours, those results, showed a decrease in systolic blood pressure but not in DBP (Sudarma *et al*., 2011; (Taubert et al., 2003, Grassi et al., 2005a). On the other hand, Fraga *et al* (2005) have also demonstrated, in soccer players, a decrease in DBP, after 2 weeks of 100g of dark chocolate intake (Fraga et al., 2005). Others and we did not find significant difference in DBP, probably due to the time and/or dose of treatments and also it is important to

considered that Fraga el al were observing athletes, while we had studied an heterogeneous group with no athletes in it.

Moreover, we also found that PP was significantly lower after chocolate ingestion. It is very well established in the literature that an improvement in endothelial function may result in a decrease in artery smooth muscle tone and so the vascular resistance (Schmitt et al., 2005, Wilkinson et al., 2002, McEniery et al., 2006). Indeed, McEniery M. *et al* (2006) demonstrated that, even in healthy individuals, there is an association between endothelial functions and central pulse pressure probably due to the momentarily improvement in endothelial function after dark chocolate intake (McEniery et al., 2006).

A possible limitation of our study is the fact that we did not investigate the mechanisms by which the single dose of dark chocolate may provoke these important effects over BP. In addition, we also did not demonstrate whether the improvement in those parameters were due to the cocoa, sugar or any other chocolate component. On the other hand, as far as we could search the literature, this is the first study to demonstrate that the dark chocolate might improve the ANS balance in healthy subjects.

Regarding to the component that could be inducing those efects, Brown *et all* (2008) studying healthy subjects, demonstrated an increase in SBP and in DBP after a single dose (60g) of fructose, but not after 60g of glucose (Brown et al., 2008). Thereby, it is not reasonable to believe that the decrease in SBP and MAP, seen in our results, are due to the ingestion of these sugars contained in the dark chocolate. In addition, the fructose has negligible effects on insulin release (Crapo et al., 1980, Pozza et al., 1958, Chong et al., 2007) that could would increase in BP probably mediated by an increase in cardiac output without compensatory peripheral

vasodilatation (Muniyappa et al., 2007). Thus collectively, these opposite findings might indicate that the decrease in SBP and MAP cannot be due to sugar intake or insulin release.

Moreover, our results also showed that dark chocolate improve the ANS modulation to the heart. In normalized units, which represent a percentage, there was an increase in parasympathetic (HFnu) and a decrease in sympathetic (LFnu) modulation to the heart. These results are in agreement with the decrease in LF/HFratio, and with the increase in HRV, SDNN and RMSSD, after chocolate intake. These results are an indication that the parasympathetic modulation is proportionally increased to the heart and that ANS modulation to the cardiovascular system is improved.

On the other hand, in absolute values there was an increase of both sympathetic (45%) and parasympathetic (100%) modulation, as seen by LFa and HFa, respectively. These results indicate that chocolate intake increase the ANS participation to the heart, in favor to parasympathetic modulation. In addition, the decrease in SBP, MAP, PP and HR cannot be attributed to the resting period effect over BP, since after chocolate intake there was an increase in LF in absolute values or sympathetic modulation. These results are also confirmed by symbolic analyses. There was a decrease in the 0V pattern indicating a reduction of sympathetic modulation and an increase in 2LV and 2UV pattern that represent the parasympathetic modulation.

Indeed, our findings are similar to that reported by Akita *et al, (2008)* who, studying the effects of cocoa liquor administration for 6 months, in rabbits, found a reduction in BP and HR. In this animals the parasympathetic modulation was not changed with age, when compared to animals feed with standard diet (Akita et al.,

2008). In addition, reinforcing the idea that chocolate has neuroprotective effect, Calderon-Garciduena et al (2010) found that, in mice under urban air pollution, the chocolate intake mitigated the vagal inflammation. This result reinforces beneficial effect of chocolate effect on parasympathetic function.

The clinical relevance of our result is based on the fact that an increase in HRV is associated, in subjects, to a decrease in cardiovascular risk and mortality. It is clear in the literature that impaired HRV induces an increase in BP or vice versa (TASK FORCE, 1996). Since that a small daily amount of chocolate is an easy intervention, with significant effects over BP, introduce it as a daily consumption habit could decrease cardiovascular risks and improve the subjects quality of life.

CONCLUSION

The dark chocolate, a non-pharmacological intervention, has beneficial effects on autonomic modulation to the cardiovascular system as seen by the increase in HRV and in HF nu. These effects may have strong impact on the reduction of cardiovascular events, and consequently mortality. More studies are necessary to confirm these hypotheses.

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REFERENCES

ACTIS-GORETTA, L., OTTAVIANI, J. I. & FRAGA, C. G. (2006) Inhibition of angiotensin converting enzyme activity by flavanol-rich foods. *J Agric Food Chem,* 54**,** 229-34.

ACTIS-GORETTA, L., OTTAVIANI, J. I., KEEN, C. L. & FRAGA, C. G. (2003) Inhibition of angiotensin converting enzyme (ACE) activity by flavan-3-ols and procyanidins. *FEBS Lett,* 555**,** 597-600.

AKITA, M., KUWAHARA, M., ITOH, F., NAKANO, Y., OSAKABE, N., KUROSAWA, T. & TSUBONE, H. (2008) Effects of cacao liquor polyphenols on cardiovascular and autonomic nervous functions in hypercholesterolaemic rabbits. *Basic Clin Pharmacol Toxicol,* 103**,** 581-7.

ANDUJAR, I., RECIO, M. C., GINER, R. M. & RIOS, J. L. (2012) Cocoa polyphenols and their potential benefits for human health. *Oxid Med Cell Longev,* 2012**,** 906252.

BABA, S., NATSUME, M., YASUDA, A., NAKAMURA, Y., TAMURA, T., OSAKABE, N., KANEGAE, M. & KONDO, K. (2007) Plasma LDL and HDL cholesterol and oxidized LDL concentrations are altered in normo- and hypercholesterolemic humans after intake of different levels of cocoa powder. *J Nutr,* 137**,** 1436-41.

BERTAGNOLLI, M., SCHENKEL, P. C., CAMPOS, C., MOSTARDA, C. T., CASARINI, D. E., BELLO-KLEIN, A., IRIGOYEN, M. C. & RIGATTO, K. (2008) Exercise training reduces sympathetic modulation on cardiovascular system and cardiac oxidative stress in spontaneously hypertensive rats. *Am J Hypertens,* 21**,** 1188-93.

BORDEAUX, B., YANEK, L. R., MOY, T. F., WHITE, L. W., BECKER, L. C., FARADAY, N. & BECKER, D. M. (2007) Casual chocolate consumption and inhibition of platelet function. *Prev Cardiol,* 10**,** 175-80.

BROWN, C. M., DULLOO, A. G., YEPURI, G. & MONTANI, J. P. (2008) Fructose ingestion acutely elevates blood pressure in healthy young humans. *Am J Physiol Regul Integr Comp Physiol,* 294**,** R730-7.

BUIJSSE, B., FESKENS, E. J., KOK, F. J. & KROMHOUT, D. (2006) Cocoa intake, blood pressure, and cardiovascular mortality: the Zutphen Elderly Study. *Arch Intern Med,* 166**,** 411-7.

BUIJSSE, B., WEIKERT, C., DROGAN, D., BERGMANN, M. & BOEING, H. (2010) Chocolate consumption in relation to blood pressure and risk of cardiovascular disease in German adults. *Eur Heart J,* 31**,** 1616-23.

BURNS, J., GARDNER, P. T., O'NEIL, J., CRAWFORD, S., MORECROFT, I., MCPHAIL, D. B., LISTER, C., MATTHEWS, D., MACLEAN, M. R., LEAN, M. E., DUTHIE, G. G. & CROZIER, A. (2000) Relationship among antioxidant activity, vasodilation capacity, and phenolic content of red wines. *J Agric Food Chem,* 48**,** 220-30.

CHONG, M. F., FIELDING, B. A. & FRAYN, K. N. (2007) Mechanisms for the acute effect of fructose on postprandial lipemia. *Am J Clin Nutr,* 85**,** 1511-20.

CORTI, R., FLAMMER, A. J., HOLLENBERG, N. K. & LUSCHER, T. F. (2009) Cocoa and cardiovascular health. *Circulation,* 119**,** 1433-41.

CRAPO, P. A., KOLTERMAN, O. G. & OLEFSKY, J. M. (1980) Effects of oral fructose in normal, diabetic, and impaired glucose tolerance subjects. *Diabetes Care,* 3**,** 575-82.

DESCH, S., SCHMIDT, J., KOBLER, D., SONNABEND, M., EITEL, I., SAREBAN, M., RAHIMI, K., SCHULER, G. & THIELE, H. (2010) Effect of cocoa products on blood pressure: systematic review and meta-analysis. *Am J Hypertens,* 23**,** 97-103.

DILLINGER, T. L., BARRIGA, P., ESCARCEGA, S., JIMENEZ, M., SALAZAR LOWE, D. & GRIVETTI, L. E. (2000) Food of the gods: cure for humanity? A cultural history of the medicinal and ritual use of chocolate. *J Nutr,* 130**,** 2057S-72S.

DJOUSSE, L., HOPKINS, P. N., ARNETT, D. K., PANKOW, J. S., BORECKI, I., NORTH, K. E. & CURTIS ELLISON, R. Chocolate consumption is inversely associated with calcified atherosclerotic plaque in the coronary arteries: the NHLBI Family Heart Study. *Clin Nutr,* 30**,** 38-43.

EGAN, B. M., LAKEN, M. A., DONOVAN, J. L. & WOOLSON, R. F. (2010) Does dark chocolate have a role in the prevention and management of hypertension?: commentary on the evidence. *Hypertension,* 55**,** 1289-95.

ENGLER, M. B., ENGLER, M. M., CHEN, C. Y., MALLOY, M. J., BROWNE, A., CHIU, E. Y., KWAK, H. K., MILBURY, P., PAUL, S. M., BLUMBERG, J. & MIETUS-SNYDER, M. L. (2004) Flavonoid-rich dark chocolate improves endothelial function and increases plasma epicatechin concentrations in healthy adults. *J Am Coll Nutr,* 23**,** 197-204.

FARIDI, Z., NJIKE, V. Y., DUTTA, S., ALI, A. & KATZ, D. L. (2008) Acute dark chocolate and cocoa ingestion and endothelial function: a randomized controlled crossover trial. *Am J Clin Nutr,* 88**,** 58-63.

FISHER, N. D., HUGHES, M., GERHARD-HERMAN, M. & HOLLENBERG, N. K. (2003) Flavanol-rich cocoa induces nitric-oxide-dependent vasodilation in healthy humans. *J Hypertens,* 21**,** 2281-6.

FRAGA, C. G., ACTIS-GORETTA, L., OTTAVIANI, J. I., CARRASQUEDO, F., LOTITO, S. B., LAZARUS, S., SCHMITZ, H. H. & KEEN, C. L. (2005) Regular consumption of a flavanol-rich chocolate can improve oxidant stress in young soccer players. *Clin Dev Immunol,* 12**,** 11-7.

FRANCHINI, K. G. & COWLEY, A. W., JR. (1996) Sensitivity of the renal medullary circulation to plasma vasopressin. *Am J Physiol,* 271**,** R647-53.

GRASSI, D., LIPPI, C., NECOZIONE, S., DESIDERI, G. & FERRI, C. (2005a) Short-term administration of dark chocolate is followed by a significant increase in insulin sensitivity and a decrease in blood pressure in healthy persons. *Am J Clin Nutr,* 81**,** 611-4.

GRASSI, D., NECOZIONE, S., LIPPI, C., CROCE, G., VALERI, L., PASQUALETTI, P., DESIDERI, G., BLUMBERG, J. B. & FERRI, C. (2005b) Cocoa reduces blood pressure and insulin resistance and improves endothelium-dependent vasodilation in hypertensives. *Hypertension,* 46**,** 398-405.

HEISS, C., DEJAM, A., KLEINBONGARD, P., SCHEWE, T., SIES, H. & KELM, M. (2003) Vascular effects of cocoa rich in flavan-3-ols. *JAMA,* 290**,** 1030-1.

HEISS, C., FINIS, D., KLEINBONGARD, P., HOFFMANN, A., RASSAF, T., KELM, M. & SIES, H. (2007) Sustained increase in flow-mediated dilation after daily intake of high-flavanol cocoa drink over 1 week. *J Cardiovasc Pharmacol,* 49**,** 74-80.

HEISS, C., KLEINBONGARD, P., DEJAM, A., PERRE, S., SCHROETER, H., SIES, H. & KELM, M. (2005) Acute consumption of flavanol-rich cocoa and the reversal of endothelial dysfunction in smokers. *J Am Coll Cardiol,* 46**,** 1276-83.

HEISS, C., SCHROETER, H., BALZER, J., KLEINBONGARD, P., MATERN, S., SIES, H. & KELM, M. (2006) Endothelial function, nitric oxide, and cocoa flavanols. *J Cardiovasc Pharmacol,* 47 Suppl 2**,** S128-35; discussion S172-6.

HERTOG, M. G., FESKENS, E. J., HOLLMAN, P. C., KATAN, M. B. & KROMHOUT, D. (1993) Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *Lancet,* 342**,** 1007-11.

HOLLENBERG, N. K., FISHER, N. D. & MCCULLOUGH, M. L. (2009) Flavanols, the Kuna, cocoa consumption, and nitric oxide. *J Am Soc Hypertens,* 3**,** 105-12.

HOLLENBERG, N. K., MARTINEZ, G., MCCULLOUGH, M., MEINKING, T., PASSAN, D., PRESTON, M., RIVERA, A., TAPLIN, D. & VICARIA-CLEMENT, M. (1997) Aging, acculturation, salt intake, and hypertension in the Kuna of Panama. *Hypertension,* 29**,** 171-6.

HOLT, R. R., LAZARUS, S. A., SULLARDS, M. C., ZHU, Q. Y., SCHRAMM, D. D., HAMMERSTONE, J. F., FRAGA, C. G., SCHMITZ, H. H. & KEEN, C. L. (2002) Procyanidin dimer B2 [epicatechin-(4beta-8)-epicatechin] in human plasma after the consumption of a flavanol-rich cocoa. *Am J Clin Nutr,* 76**,** 798-804.

INNES, A. J., KENNEDY, G., MCLAREN, M., BANCROFT, A. J. & BELCH, J. J. (2003) Dark chocolate inhibits platelet aggregation in healthy volunteers. *Platelets,* 14**,** 325-7.

KATZ, D. L., DOUGHTY, K. & ALI, A. Cocoa and chocolate in human health and disease. *Antioxid Redox Signal,* 15**,** 2779-811.

KEEN, C. L. (2001) Chocolate: food as medicine/medicine as food. *J Am Coll Nutr,* 20**,** 436S-439S; discussion 440S-442S.

KELI, S. O., HERTOG, M. G., FESKENS, E. J. & KROMHOUT, D. (1996) Dietary flavonoids, antioxidant vitamins, and incidence of stroke: the Zutphen study. *Arch Intern Med,* 156**,** 637-42.

KLEIGER, R. E., MILLER, J. P., BIGGER, J. T., JR. & MOSS, A. J. (1987) Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. *Am J Cardiol,* 59**,** 256-62.

KONDO, K., HIRANO, R., MATSUMOTO, A., IGARASHI, O. & ITAKURA, H. (1996) Inhibition of LDL oxidation by cocoa. *Lancet,* 348**,** 1514.

LAHIRI, M. K., KANNANKERIL, P. J. & GOLDBERGER, J. J. (2008) Assessment of autonomic function in cardiovascular disease: physiological basis and prognostic implications. *J Am Coll Cardiol,* 51**,** 1725-33.

LOPES, H. F., CONSOLIM-COLOMBO, F. M., HACHUL, D., CARVALHO, M. E., PILEGGI, F. & SILVA, H. B. (2000) Hormonal and cardiovascular reflex assessment in a female patient with pure autonomic failure. *Arq Bras Cardiol,* 75**,** 235-42.

MALLIANI, A., PAGANI, M., LOMBARDI, F. & CERUTTI, S. (1991) Cardiovascular neural regulation explored in the frequency domain. *Circulation,* 84**,** 482-92.

MANACH, C., MAZUR, A. & SCALBERT, A. (2005) Polyphenols and prevention of cardiovascular diseases. *Curr Opin Lipidol,* 16**,** 77-84.

MAO, T. K., POWELL, J., VAN DE WATER, J., KEEN, C. L., SCHMITZ, H. H., HAMMERSTONE, J. F. & GERSHWIN, M. E. (2000) The effect of cocoa procyanidins on the transcription and secretion of interleukin 1 beta in peripheral blood mononuclear cells. *Life Sci,* 66**,** 1377-86.

MCENIERY, C. M., WALLACE, S., MACKENZIE, I. S., MCDONNELL, B., YASMIN, NEWBY, D. E., COCKCROFT, J. R. & WILKINSON, I. B. (2006) Endothelial

function is associated with pulse pressure, pulse wave velocity, and augmentation index in healthy humans. *Hypertension,* 48**,** 602-8.

MEHRINFAR, R. & FRISHMAN, W. H. (2008) Flavanol-rich cocoa: a cardioprotective nutraceutical. *Cardiol Rev,* 16**,** 109-15.

MICHELINI, L. C. & MORRIS, M. (1999) Endogenous vasopressin modulates the cardiovascular responses to exercise. *Ann N Y Acad Sci,* 897**,** 198-211.

MINSON, C. T., HALLIWILL, J. R., YOUNG, T. M. & JOYNER, M. J. (2000) Influence of the menstrual cycle on sympathetic activity, baroreflex sensitivity, and vascular transduction in young women. *Circulation,* 101**,** 862-8.

MOLNAR, J., ROSENTHAL, J. E., WEISS, J. S. & SOMBERG, J. C. (1997) QT interval dispersion in healthy subjects and survivors of sudden cardiac death: circadian variation in a twenty-four-hour assessment. *Am J Cardiol,* 79**,** 1190-3.

MONTANO, N., PORTA, A., COGLIATI, C., COSTANTINO, G., TOBALDINI, E., CASALI, K. R. & IELLAMO, F. (2009) Heart rate variability explored in the frequency domain: a tool to investigate the link between heart and behavior. *Neurosci Biobehav Rev,* 33**,** 71-80.

MUNIYAPPA, R., MONTAGNANI, M., KOH, K. K. & QUON, M. J. (2007) Cardiovascular actions of insulin. *Endocr Rev,* 28**,** 463-91.

MURPHY, K. J., CHRONOPOULOS, A. K., SINGH, I., FRANCIS, M. A., MORIARTY, H., PIKE, M. J., TURNER, A. H., MANN, N. J. & SINCLAIR, A. J. (2003) Dietary flavanols and procyanidin oligomers from cocoa (Theobroma cacao) inhibit platelet function. *Am J Clin Nutr,* 77**,** 1466-73.

NOGUEIRA LDE, P., KNIBEL, M. P., TORRES, M. R., NOGUEIRA NETO, J. F. & SANJULIANI, A. F. Consumption of high-polyphenol dark chocolate improves

endothelial function in individuals with stage 1 hypertension and excess body weight. *Int J Hypertens,* 2012**,** 147321.

PAOLISSO, G., MANZELLA, D., BARBIERI, M., RIZZO, M. R., GAMBARDELLA, A. & VARRICCHIO, M. (1999) Baseline heart rate variability in healthy centenarians: differences compared with aged subjects (>75 years old). *Clin Sci (Lond),* 97**,** 579-84.

PEARSON, D. A., PAGLIERONI, T. G., REIN, D., WUN, T., SCHRAMM, D. D., WANG, J. F., HOLT, R. R., GOSSELIN, R., SCHMITZ, H. H. & KEEN, C. L. (2002) The effects of flavanol-rich cocoa and aspirin on ex vivo platelet function. *Thromb Res,* 106**,** 191-7.

PERSSON, I. A., PERSSON, K., HAGG, S. & ANDERSSON, R. G. Effects of cocoa extract and dark chocolate on angiotensin-converting enzyme and nitric oxide in human endothelial cells and healthy volunteers--a nutrigenomics perspective. *J Cardiovasc Pharmacol,* 57**,** 44-50.

PERSSON, I. A., PERSSON, K., HAGG, S. & ANDERSSON, R. G. (2011) Effects of cocoa extract and dark chocolate on angiotensin-converting enzyme and nitric oxide in human endothelial cells and healthy volunteers--a nutrigenomics perspective. *J Cardiovasc Pharmacol,* 57**,** 44-50.

POZZA, G., GALANSINO, G., HOFFELD, H. & FOA, P. P. (1958) Stimulation of insulin output by monosaccharides and monosaccharide derivatives. *Am J Physiol,* 192**,** 497-500.

PUMPRLA, J., HOWORKA, K., GROVES, D., CHESTER, M. & NOLAN, J. (2002) Functional assessment of heart rate variability: physiological basis and practical applications. *Int J Cardiol,* 84**,** 1-14.

RIED, K., SULLIVAN, T., FAKLER, P., FRANK, O. R. & STOCKS, N. P. (2010) Does chocolate reduce blood pressure? A meta-analysis. *BMC Med,* 8**,** 39.

RIED, K., SULLIVAN, T. R., FAKLER, P., FRANK, O. R. & STOCKS, N. P. (2012) Effect of cocoa on blood pressure. *Cochrane Database Syst Rev,* 8**,** CD008893.

SCHMITT, M., AVOLIO, A., QASEM, A., MCENIERY, C. M., BUTLIN, M., WILKINSON, I. B. & COCKCROFT, J. R. (2005) Basal NO locally modulates human iliac artery function in vivo. *Hypertension,* 46**,** 227-31.

SCHROETER, H., HEISS, C., BALZER, J., KLEINBONGARD, P., KEEN, C. L., HOLLENBERG, N. K., SIES, H., KWIK-URIBE, C., SCHMITZ, H. H. & KELM, M. (2006) (-)-Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans. *Proc Natl Acad Sci U S A,* 103**,** 1024-9.

SELMI, C., COCCHI, C. A., LANFREDINI, M., KEEN, C. L. & GERSHWIN, M. E. (2008) Chocolate at heart: the anti-inflammatory impact of cocoa flavanols. *Mol Nutr Food Res,* 52**,** 1340-8.

SERAFINI, M., BUGIANESI, R., MAIANI, G., VALTUENA, S., DE SANTIS, S. & CROZIER, A. (2003) Plasma antioxidants from chocolate. *Nature,* 424**,** 1013.

SPENCER, J. P., SCHROETER, H., SHENOY, B., SRAI, S. K., DEBNAM, E. S. & RICE-EVANS, C. (2001) Epicatechin is the primary bioavailable form of the procyanidin dimers B2 and B5 after transfer across the small intestine. *Biochem Biophys Res Commun,* 285**,** 588-93.

STEFFEN, Y., SCHEWE, T. & SIES, H. (2005) Epicatechin protects endothelial cells against oxidized LDL and maintains NO synthase. *Biochem Biophys Res Commun,* 331**,** 1277-83.

TAUBERT, D., BERKELS, R., ROESEN, R. & KLAUS, W. (2003) Chocolate and blood pressure in elderly individuals with isolated systolic hypertension. *JAMA,* 290**,** 1029-30.

TAUBERT, D., ROESEN, R., LEHMANN, C., JUNG, N. & SCHOMIG, E. (2007a) Effects of low habitual cocoa intake on blood pressure and bioactive nitric oxide: a randomized controlled trial. *JAMA,* 298**,** 49-60.

TAUBERT, D., ROESEN, R. & SCHOMIG, E. (2007b) Effect of cocoa and tea intake on blood pressure: a meta-analysis. *Arch Intern Med,* 167**,** 626-34.

VLACHOPOULOS, C., AZNAOURIDIS, K., ALEXOPOULOS, N., ECONOMOU, E., ANDREADOU, I. & STEFANADIS, C. (2005) Effect of dark chocolate on arterial function in healthy individuals. *Am J Hypertens,* 18**,** 785-91.

WILKINSON, I. B., WEBB, D. J. & COCKCROFT, J. R. (2002) Nitric oxide and regulation of arterial elasticity: right idea, wrong vascular bed? *Hypertension,* 40**,** e3-4; author reply e3-4.

ZOMER, E., OWEN, A., MAGLIANO, D. J., LIEW, D. & REID, C. M. (2012) The effectiveness and cost effectiveness of dark chocolate consumption as prevention therapy in people at high risk of cardiovascular disease: best case scenario analysis using a Markov model. *BMJ,* 344**,** e3657.

Table 1. Dark chocolate nutritional composition

Content per dose	Dark chocolate
Energy, Kcal	54.4
Carbohydrates, g	4
Total fat, g	3.6
Saturated fat, g	2
Trans fat, g	0
Protein, g	0.8
Dietary fiber, g	0.8
Sodium, mg	0
Total flavonoids (epicatechin) mg	5.41

Data provided by Lugano Chocolate.

Table 2: Hemodynamic values before and after chocolate ingestion

	Before	After	P
SBP(mmHg)	104±14	98±13	0.001
DBP (mmHg)	69±6	65±12	0.12
MAP (mmHg)	83±9	78±6	0.01
PP (mmHg)	38±6	33±7	0.004
HR (bpm)	80±6	75±6	0.00

Blood pressure (BP) and heart rate (HR) before and 1 hour after 10g of dark chocolate ingestion. SBP= systolic BP; DBP= diastolic BP; MAP= mean arterial pressure; PP=pulse pressure. The paired Student's t test was used to detect differences between measurements. Data represent means±standard deviation. $P<0.05$ was considered statistically significant.

Table 3: Spectral and symbolic analysis data before and after chocolate intake

	Spectral Analysis		
	Before	**After**	**P**
HRV (ms^2)	1991±895	3622±2627	0.00
SDNN (ms)	43±10	57±18	0.00
RMSSD (ms)	33±10	49±20	0.00
LFa (ms^2)	550±354	800±464	0.00
HFa (ms^2)	699±466	1396±996	0.00
Lfnu (%)	44±14	39±16	0.03
Hfnu (%)	55±14	60±16	0.03
LF/HF ratio	0.94±0.60	0.79±0.52	0.05
	Symbolic Analysis (%)		
	Before	**After**	**P**
0V pattern	21±9	15±8	0.00
1V pattern	50±3	48±6	0.24
2LV pattern	14±6	17±8	0.01
2UV pattern	14±6	17±8	0.02

Legend to Table 3: Spectral and symbolic analysis results. HRV=heart rate variability; SDNN=standard deviation of normal RR intervals, RMSSD=square root the mean squared differences between consecutive RR intervals; LF= low frequency component; HF= high frequency component; a= absolute; nu= normalized. LF/HF ratio= sympatho-vagal balance. A paired Student's t test was used to detect differences between measurements; Data represent means±standard deviation. $P<0.05$ was considered to be statistically significant. Symbolic analysis Patterns: 1) no variations (0V, indicate sympathetic modulation); 2) one variation (1V, indicate both sympathetic and parasympathetic modulation); 3) two like variations (2LV) and indicate parasympathetic modulation; and 4) two unlike variations (2UV) and indicate parasympathetic modulation (Guzzetti et al., 2005).

ANEXOS

Anexo 1

Guide for Authors

Types of Article
Full-length articles of original research; Short Communications; Review articles; Clinical Reports and Book Reviews. For Full-length articles, please note you will be required to select a classification when submitting to the online system. The Editor-in-Chief should be consulted whenever a review article is under consideration. Rapid Communications will be accepted if extremely concise, fully documented and dealing with a novel and important observation in a research area in rapid expansion. These will receive priority handling both in the reviewing and publishing processes such that their publication will be advanced.

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Papers describing experiments on living animals should provide (a) a full description of any anaesthetic and surgical procedure used, and (b) evidence that adequate steps were taken to ensure that animals did not suffer unnecessarily at any stage of the experiment, and comply with the existing national and international guidelines on the conduct of animal experiments.

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b. Line drawings should normally be about twice the final size. Symbols should be used sparingly and direct labelling with an explicative term or abbreviation is preferred. All symbols and lettering should be large enough to permit reduction.
c. Micrographs. These should be submitted in a form suitable for direct reproduction without reduction. The maximum space available for micrographs is 16x19 cm. Micrographs should by carefully cropped, to leave out areas of low information content, and they should be grouped and arranged to optimize the available space. They should be separated by gutters of 2-3 mm and be directly labelled. Micrographs must have a calibration bar. Illustrations and legends should not be placed sideways.

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Tables should be submitted online as a separate file and should bear a short descriptive title. Legends for each table should appear on the same page as the table. All tables must be numbered consecutively in Arabic numerals and cited in the text. Titles should be brief but descriptive. Tables should not have vertical lines, and horizontal lines must be kept to a minimum. Tables should be prepared for use in a single column (8.4 cm wide) or be of page width (17.6 cm).
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Each illustration should bear the author's name and be numbered in Arabic numerals (Fig. 1, Fig. 2, etc.), must be referred to in the text and should be accompanied by a legend (typed with double spacing on separate pages). An illustration, together with its legend, should be understandable with minimal reference to the text.
a. All illustrations should be designed to fit either a single column (7 cm) or the full text width (16 cm).
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c. *Micrographs*. These should be mounted on **thin** cardboard and submitted in a form suitable for direct reproduction without reduction. The maximum space available for

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Anexo 2

Termo de Consentimento Livre e Esclarecido

Você está sendo convidado a participar, como voluntário, em uma pesquisa, que se intitula: “EFEITO AGUDO DA INGESTAO DE CHOCOLATE AMARGO NA MODULAÇÃO DO SISTEMA NERVOSO SIMPÁTICO E PARASIMPÁTICO”.

Orientadora do projeto: Profa Katya Rigatto
Telefones para contato: (051) 3303-8753 ou 8115-0246
Mestranda: Ana Amélia Duarte
Telefones para contato: (051) 91085534 ou 85548558
Telefones do comitê de ética para contato: (051) 3303-8804

Antes de assinar o consentimento para a sua participação, por favor, leia com atenção as informações abaixo. Aproveite para esclarecer todas as dúvidas com o pesquisador que lhe apresentou o estudo. Queremos deixar claro que, caso você decida não participar, não haverá prejuízo no seu atendimento.

Esta pesquisa faz parte da dissertação de Mestrado do Programa de Pós Graduação de Ciências da Saúde da Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA), na qual você esta sendo convidado a participar desse estudo, que tem por objetivo avaliar o efeito da ingestão aguda de chocolate amargo no controle do sistema nervoso simpático e parassimpático de indivíduos saudáveis. Dessa forma estaremos conhecendo outros possíveis mecanismos de ação das substâncias presentes no chocolate amargo o que contribui para a saúde cardiovascular.

Se aceitar participar deste estudo, será realizado um exame de eletrocardiograma (ECG), para obtenção de informações que serão utilizadas para o nosso estudo. Este exame será realizado no laboratório de fisiologia da UFCSPA, o qual fica no prédio principal desta Universidade. Será realizado o ECG pré e outro ECG 1 hora após a ingestão do chocolate amargo. Verificaremos sua pressão arterial e seus dados para avaliação nutricional, como o peso, atura e circunferência abdominal.

O ECG é um exame não-invasivo que permite a avaliação elétrica do coração através de eletrodos conectados à pele do paciente. Ele não apresenta riscos à saúde.

Este estudo não trará nenhum tipo de risco ou prejuízo a sua saúde. Os participantes não serão lesados nem sofrerão de desconforto. Os procedimentos são simples e garantimos que os dados coletados serão mantidos em sigilo e sua identidade não será revelada. Ainda assim, você poderá retirar seu consentimento a qualquer momento, ligando para qualquer um dos telefones acima.
O Senhor (a) concorda em participar?

Declaro que me foram dadas às informações descritas acima e que concordo em participar do estudo.

---------- Local e Data: _____ _ / _ _ / _ _
Nome do Participante

---------- ----------
Fone para contato Assinatura do mestrando

---------- ----------
Testemunha Assinatura do Orientador

Anexo 3:

UFCSPA

COMISSÃO CIENTÍFICA E COMISSÃO DE PESQUISA E ÉTICA EM SAÚDE

COMITÊ DE ÉTICA EM PESQUISA - CEP
UFCSPA

O Comitê de Ética em Pesquisa da UFCSPA, registrado na Comissão Nacional de Ética em Pesquisa (CONEP) sob o nº 075/05 em 23/07/04, analisou o Projeto:

Projeto: 12-954 **Versão do Projeto:** **Versão do TCLE:**

Pesquisadores:
KATYA VIANNA RIGATTO
ANA AMÉLIA MACHADO DUARTE

Título: EFEITO AGUDO DA INGESTÃO DE CHOCOLATE AMARGO NA MODULAÇÃO DO SISTEMA NERVOSO SIMPÁTICO E PARASSIMPÁTICO.

Esse projeto foi aprovado em seus aspectos éticos e metodológicos conforme as Resoluções 196/09 e demais Resoluções complementares. Toda e qualquer alteração do projeto, assim como eventos adversos graves, deverão ser comunicados a este CEP. Os TCLE, quando necessários, somente poderão ser utilizados após prévia e explícita aprovação (carimbo) de sua redação por este CEP".

Porto Alegre, 17 de julho de 2012.

José Geraldo Vernet Taborda
Coordenador do CEP/UFCSPA