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Impact of chocolate on the cardiovascular health

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1. ABSTRACT

The antioxidants such as polyphenols, especially flavonols, present in large quantitites in cocoa, cause vasodilation, modulate inflammatory markers and cardiovascular health, and possess a range of protective cardiovascular effects. On the other hand, overconsumption of chocolate can lead to tachyarrhythmias, supraventricular tachycardia, atrial fibrillation, ventricular tachycardia and ventricular fibrillation due to its caffeine content. This review describes both the cardioprotective and adverse effects of chocolate and its constituents.

2. INTRODUCTION

Cardiovascular disease (CVD) represents an important worldwide public health issue, probably representing the main cause of mortality and morbidity in the Western world. Epidemiological studies suggest that cocoa-rich products could reduce the risk of CVD. In fact, the potential role of a number of cocoa polyphenolic compounds in modulating cardiovascular health and disease is currently under extensive study. Multiple cardiovascular risk factors, such as smoking, aging, hypercholesterolemia, hypertriglyceridemia, arterial hypertension, hyperglycemia, hyperhomocystinemia, family history of premature atherosclerotic disease, are associated with a loss of endothelium-dependent vasodilation. Different types of diet and numerous dietary components have been associated to cardiovascular disease and have long been thought to have a significant effect on endothelial function (1).

Indeed, epidemiologic evidence supports the hypothesis that diets rich in fruits and vegetables can promote health and delay the onset of various diseases, including CVD (2). In this context, the chemical structure of polyphenols, consisting of aromatic rings capable of quenching free radicals, makes them ideal candidates to protect against oxidative damage. A number of studies have shown that dietary polyphenols exert a protective effect on hypertension, dyslipidemia, inflammation, endothelial function and atherosclerosis. Studies indicate that by decreasing cholesterol absorption, polyphenols modulate hepatic cholesterol metabolism resulting in reduced plasma lipids and atherogenic lipoproteins; they have also been shown to decrease the activity of enzymes involved in the renin-angiotensin-aldosterone system, and to increase nitric oxide (NO) production, thus improving blood pressure and endothelial function.

Among polyphenols, flavonols (in particular flavonoids) are currently receiving considerable attention. The habitual consumption of dark (cocoa >55%) and extra-dark chocolate (cocoa >70%), both very rich in flavonoids, has been shown to improve endothelial function and reduce blood pressure, with a

Table 1. Amount (mg) of phenolics, flavonoids and metylxanthines in three variants of chocolate

Chocolate variant	Phenolics	Flavonoids	Theobromine	Caffeine	Theophylline
Cacao (raw ground paste)	6500	203-1233	2057	230	200
Dark chocolate	579	28	802	80	< limit of detection
Milk chocolate	160	13	125	20	< limit of detection
White chocolate	126	8	Not determined	Not determined	< limit of detection

Adapted from (10, 37, 94-98)

possible cardioprotective effect even in healthy subjects (3). Even if flavonols represent a promising option for primary cardiovascular prevention, cocoa-rich chocolate also contains several other substances, potentially beneficial but with poorly studied cardiovascular side effects. In fact, chocolate derives from the roasted seeds of the plant theobroma cacao, rich in methylxanthine alkaloids, such as theobromine, paraxanthine, pentoxifylline, theophylline and caffeine (4).

In humans, caffeine is metabolized by the cytochrome P4501A2 (CYP1A2) into three dimethylxanthines: theobromine, theophylline and paraxanthine. The latter represents the main metabolite: about 80% of caffeine is converted into paraxanthine (5). Methylxanthines are adenosine receptor antagonists, phosphodiesterase (PDE) inhibitors and histone deacetylase inducers, with documented anti-inflammatory effects. For example, a beneficial effect of caffeine on insulin resistance in type 2 diabetes and on hepatic damage in liver cirrhosis has been documented; theophylline is a powerful bronchodilator used against asthma attacks; pentoxifylline displays immuno-modulating effects against peripheral vascular diseases. On the other hand, it is still largely unknown whether these methylxanthines, in certain quantities and circumstances, may exert stimulant and arrhythmogenic effects on the cardiac muscle and conductive tissues. The competitive antagonism of the adenosine receptor (6) after normal methylxanthine consumption is not usually associated with increased risk of arrhythmias, such as atrial fibrillation. However, sympathomimetic effects mediated by circulating catecholamines, which are responsible for the cardiac manifestations of caffeine overdose/toxicity, can produce tachyarrhythmias, such as ventricular or supraventricular tachycardia, atrial fibrillation and even ventricular fibrillation (7); a case report of acute atrial fibrillation associated with chocolate abuse has recently been reported (8).

3. CHOCOLATE CONSTITUENTS: EFFECTS OF BIOACTIVE SUBSTRATES FROM THEOBROMA CACAO

Chocolate is produced from cabossides of the tropical cacao plant, which was firstly cultivated by the Maya people. In the past, many cocoa derivatives were widely appreciated for their invigorating and stimulating effects. Important chocolate components are unsaturated lipids (cocoa butter), flavonoids (a class of compounds with antioxidant activity found in almost all fruits and vegetables) and methylxanthines (psychoactive dopaminergic substances responsible for its pleasurable effects). Methylxanthines produced by the theobroma cacao plant (caffeine, theobromine, phenylethylamine, paraxanthine and theophylline) are reported to have antitumor and anti-inflammatory effects, partially mediated by inhibition of PDE found to be over-expressed in some brain tumors (9). In particular, chocolate contains caffeine (1,3,7-trimethylxanthine) and theobromine (3,7-dimethylxanthine) that may contribute to its stimulant psycoactive action. A single portion (20g) of dark chocolate (Table 1) contains more than 15 mg of caffeine and 100–150 mg of theobromine (10). Caffeine and theobromine metabolites are known to enhance the action of cAMP, which is involved in the intracellular signal transmission.

Dark chocolate has been shown to exert anti-inflammatory, cardioprotective and neuroprotective effects ; it also enhances nitric oxide bioavailability, thus improving both platelet function and blood pressure/fluidity (11). Besides caffeine, other cocoa costituents can increase sympathetic tone: all metylxanthines increase plasmatic levels of stress hormones cortisol and catecholamines, such as adrenaline and noradrenaline. Both the sympathetic adrenomedullary and the adrenocorticoid mechanisms of the neuroendocrine response to stress are stimulated (12): the ingestion of methylxanthines activates the sympathetic nervous system and leads to increased bodily temperature, arterial blood pressure, and even heart rate. These cardiovascular effects are due to a sympathetic nervous system activation, so that a high dose of dark chocolate or coffee may increase plasmatic adrenaline levels, with consequent tachycardia, thermogenic and lipolytic activities. In addition, chocolate consumption can also determine an increase in plasmatic renin levels, which regulate extracellular volume and arterial blood pressure (13).

In humans, the cardiovascular effects of methylxanthines are attributable to the antagonism of adenosine A1 and A2 receptors. Such effects can be evoked at lower methylxanthine concentrations

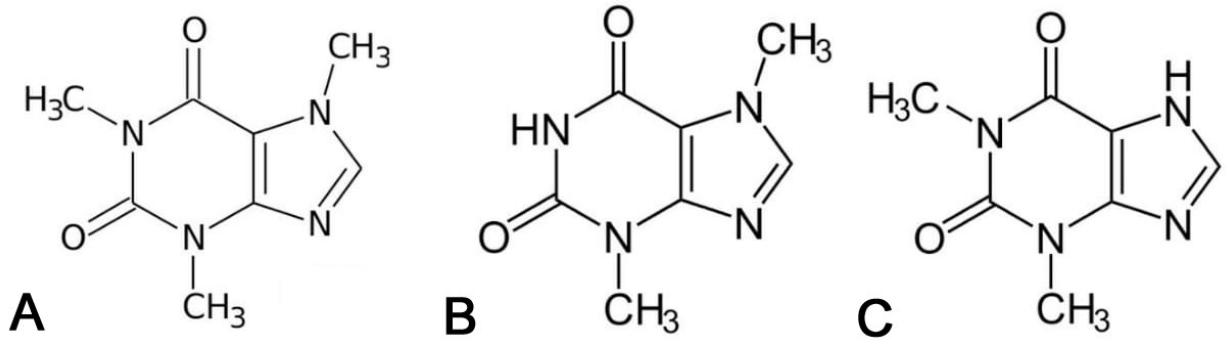

Figure 1. Molecular structure of metylxanthines; A: caffeine, B: theobromine, and C: theophylline.

resulting from the consumption of a small quantity of dark chocolate (15 g) or coffee (40 ml); higher concentrations are required for PDE inhibition or intracellular calcium mobilization. This possible hemodynamic responses are due to the direct effect of methylxanthines on vascular tone, on myocardial contractility, on rhythm conduction and on the sympathetic nervous system (14). Even if the association between chocolate or coffee consumption and cardiac arrhythmia has not been clearly reported, the possible acute hemodynamic and neurohumoral changes following an excessive methylxanthine consumption, namely an increase in total peripheral resistance and blood pressure coupled with slight increases in heart rate, could have an adverse cardiac effect in patients undergoing particular pharmacological treatments or with specific genetic profiles.

3.1. Caffeine

Caffeine (Figure 1a) is a methylxanthine alkaloid, found in some medications, beverages (coffee, cola or tea) and cocoa derived foods, very similar to theophylline in structure and biological function. Because of the combined consumption of coffee, tea, cola drink and chocolate, mean daily doses of caffeine in the general population are notable: approximately 2.4 mg/kg in adults and 0.7 mg/kg in children (13). Caffeine, which is metabolized into about 25 metabolites in humans, has a number of effects on vascular tissue. Caffeine metabolism consists of five main metabolic pathways, three of which require demethylization of N-3 to form paraxanthine or N-1 and N-7 to form theophylline. These three demethylization reactions, mediated by the hepatic cytochrome P-450 (CYP) isoenzyme, give an in vivo percentage of 85% paraxanthine, 10% theobromine, and 5% theophylline. The other metabolic pathways lead to degradation in uracil metabolites and renal elimination (14). At moderate doses, less than 2 mg/kg of body weight, caffeine increases alertness, attention and psychomotor performance. In fact, it increases intracellular calcium in endothelial cells, stimulating NO production through the expression of the endothelial NOS. It also acts as a central nervous system stimulant and modulator of cardiovascular function and may improve endurance in athletes such as runners (15). However, at higher doses (and in some individuals even at low-moderate doses) caffeine can also determine anxiety and adverse cardiovascular effects (16).

It is still controversial whether caffeine consumption could be associated with an increased risk for atrial fibrillation. Caffeine acts as an Autonomic Nervous System (ANS) stimulant: it blocks the adenosine receptors, determining a reflex activation of the sympathetic system. In habitual coffee consumers, the sympathetic system is also activated, but the increased sympathetic tone does not cause a significant increase in peripheral vascular resistance and in arterial pressure; however, such effects can be observed in non-habitual consumers (17). Excessive sympathomimetic effects due to catecholamine stimulation are responsible for the cardiac manifestations of caffeine abuse, which in toxic amounts may produce tachyarrhythmias such as supraventricular tachycardia, atrial fibrillation, ventricular tachycardia, and ventricular fibrillation (18). The potential arrhythmogenic risk following excessive consumption of coffee, tea, cola and chocolate does not seem to be increased at the ventricular level, but more at a supraventricular and specifically at the atrial level (19). In an animal study, atrial tachyarrhythmias, such as atrial fibrillation and atrial flutter, have been reported after intravenous administration of caffeine at a dose from 1 to 5 mg caffeine/kg of body weight (20). However, in the Swedish Multifactor Primary Prevention Study, consumption of 4 cups of coffee per day was associated with an age-adjusted odds ratio of atrial fibrillation of 1.24 (21). Both this study and the so called “Danish diet and Health Study” indicate that it is unlikely that habitual consumption of caffeine from coffee, tea, cola drinks and chocolate is associated with a risk of atrial fibrillation in humans (19). No effect of coffee and chocolate consumption was reported on

the P-wave, as evaluated by electrocardiograms (ECG) and as assessed by invasive electrophysiological testing, showing that caffeine has no effect on interatrial and intraatrial conduction intervals (22).

The Women's Health prospective Study also tried to assess the relation between caffeine intake and incidental atrial fibrillation: more than 33,000 initially healthy 45 years old women were recruited and provided information on caffeine intake through food-frequency questionnaires. This large cohort of subjects, with broad levels of caffeine consumption, was prospectively followed for atrial fibrillation for 16 years (22). The absence of increased atrial fibrillation risk associated with higher caffeine amounts confirmed the previous findings from the prospective "Danish, Diet, Cancer, and Health Study", which suggested that high caffeine intake is not associated with an increased hospitalization rate for atrial fibrillation (19). These results are further supported by experimental human and animal studies, where acute caffeine ingestion in healthy subjects did not affect ECG markers of atrial fibrillation risk, such as P-wave duration and dispersion (23). No influence of caffeine consumption on interatrial and intra-atrial conduction intervals was electrophysiologically evidenced (24); in an experimental dog model, the intravenous administration of caffeine did not result in a higher incidence of atrial fibrillation (25). In addition, the major sources of caffeine also contain polyphenols and other antioxidant ingredients (26) that may even be protective against atrial fibrillation (27). However, many patients with paroxysmal atrial fibrillation indicate caffeine intake as a triggering factor for arrhythmia (28). In a Swedish study, men who consumed daily 4 cups of coffee had a hazard ratio for atrial fibrillation of 1.24 compared with men who did not drink coffee (29).

Caffeine is quickly absorbed in the intestinal tract, becoming rapidly bioavailable, with maximum plasmatic concentrations reached about 30–45 minutes after ingestion, roughly when its effects begin to manifest (13). Large inter-individual differences in plasmatic concentrations of caffeine can be observed after administration of a standard dose, mainly due to variations in caffeine metabolism, depending on genetic polymorphisms, metabolic induction and inhibition of cytochrome P-450 isoenzyme, presence of hepatic disease, as well as other individual factors, such as weight or sex (30). Consequently, the cardiovascular response to caffeine and more in general to coffee and chocolate depends on a variety of factors: the total amount ingested, frequency and rapidity of consumption, degree of absorption and individual efficiency of hepatic metabolism. These numerous aspects determine widely variable response in different individuals. In addition, other active substances found in chocolate and cocoa, such as paraxanthine, theobromine and theophylline, could play a role in the extreme variability of physiologic responses.

3.2. Theophylline, theobromine and paraxanthine

Theophylline (Figure 1c) and its isomers theobromine and paraxanthine are alkaloids originally discovered in cocoa, but present in many other plants like cola and tea. These substances represent the natural metabolites of caffeine in the human body. In some human models, in vitro leukocyte treatment with 5 μM theophylline decreased production of the leukotrienes B4 and C4. Regarding in vivo effects, chronic obstructive pulmonary disease patients who were treated with 400 mg/d theophylline displayed decreased sputum levels of IL-8, TNF and neutrophil count (31). Pharmacological assays confirm that theobromine (Figure 1b) is less adrenergically active than caffeine, having a three-fold lower affinity for the A1 and A2 receptors (32) and seems to be a less powerful PDE inhibitor. They differ in pharmacokinetics also: caffeine is highly soluble in water, peaks in the blood about 30 minutes after ingestion, and has a half-life of 2.5–5 hours, while theobromine is fat soluble, peaks about 2–3 hours after ingestion, and has an estimated half-life of 7–12 hours (10). However, theobromine is a more potent cardiac stimulant and has been used in the past in humans as a coronary artery dilator at daily doses of 300 mg; at a high dose of 979 mg given daily for 3 weeks, theobromine decreased systolic blood pressure and increased heart rate (33). In addition, it has a less rapid diffusion across the blood-brain barrier compared to caffeine.Individuals vary in their sensitivity to bioactive compounds and drugs, including caffeine and theobromine, and this variability appears to be partially genetic (34). For example, individual experience of anxiety after moderate doses of caffeine is widely variable, and this anxiogenic response is associated with a single nucleotide polymorphism in the A2A receptor gene (35). Whether this polymorphism can contribute to differences in physiological response to theobromine has not been investigated.

Among methylxanthines, theobromine exerts growth inhibitory effects on the human malignant glioma cell line, whose growth is promoted by PDE4. Theobromine inhibits cell proliferation by increasing both intracellular cAMP levels and the activity of c-Jun N-terminal kinase and p38 mitogen-activated protein kinase. In addition, it attenuates nuclear factor-kappa B (NF-kB) signal pathways, p44/42 extracellular kinase and the Akt/mammalian target of rapamycin kinase, thus resulting also effective in human glioblastoma prevention (9). Previous clinical studies suggested that cocoa also could increase high-density lipoprotein cholesterol (HDL) concentrations: in particular, a daily intake of 850 mg theobromine significantly increases HDL concentrations by 0.16 mmol/L in healthy subjects,

suggesting that it is the major bioactive compound of cocoa. In addition, theobromine also determines a moderate reduction of low-density lipoprotein cholesterol (LDL) serum concentrations, further improving the blood lipid profile and, consequently, cardiovascular health (36). Chocolate represents the main source of theobromine in the Western diets. Unfortunately, more than 100 g of dark chocolate are needed for an intake of about 850 mg of theobromine (37), this amount also containing approximately 40 g of saturated fat. In addition, an acute post-intake increase in heart rate was observed in the same clinical study. This undesirable effect on 24-hour heart rate profiles was also reported in previous studies on theobromine or other methylxanthines (37, 38), albeit with a positive effect on blood pressure profiles. Cardiostimulatory effects were recently reported even on fetal heart rate: one hundred pregnant women with uncomplicated gestation underwent computerized fetal heart rate recording before and after consumption of 30 g dark chocolate (80% cocoa). Cardiotocography parameters, including contractions, fetal movements, baseline fetal heart rate, accelerations greater than 15 bpm for 15 seconds, number of decelerations, long and short term heart rate variability, were recorded. The percent change after chocolate intake for accelerations, fetal movements and short-term fetal heart rate variation was calculated, resulting significantly higher after maternal dark chocolate intake.

Thus, maternal consumption of dark chocolate has a stimulatory effect on fetal cardiac reactivity, most likely because of the pharmacological action of theobromine (39). Another study suggested that maternal intake of both caffeine and 70% cocoa stimulate fetal reactivity: the number of uterine contraction peaks, the number of small and large accelerations, the duration of episodes of high variations and the short-term fetal heart rate variations were significantly higher after maternal coffee intake, as well as after maternal consumption of chocolate (40). However, differently from caffeine, theobromine showed differential effects depending on dose: at 250 mg it showed limited positive effects on mood that became negative at higher doses; effects on heart rate follow a similar dose-dependent pattern. This indicates that theobromine, at normal intake levels, such as in a standard 40 g bar of dark chocolate, may promote the positive effects of cocoa consumption, but higher intakes may be associated to adverse events (10).

Paraxanthine is the main metabolite of caffeine and theobromine, structurally related to caffeine: about 84% of the daily ingested caffeine is eventually demethylated to paraxanthine. It is a psychoactive substance and a central nervous system stimulant, responsible for the sympathomimetic effects of caffeine (41). On animal studies, paraxanthine has shown anti-inflammatory effects, because of its competitive nonselective PDE inhibition, which raises intracellular cAMP and inhibits TNF-alpha (42) and leukotriene (43) synthesis. Furtheremore, it mediates the lipolytic properties of caffeine, determining an increase in serum free fatty acid concentrations. On the other side, even if it is believed to exhibit a lower toxicity than caffeine, paraxanthine is a nonselective adenosine receptor antagonist, which raises plasmatic epinephrine levels and, consequently, increases diastolic blood pressure (44).An additional mechanism, different from adenosine antagonism, which can be responsible for these stimulant effects, may be via cGMP-dependent PDE inhibition in contrast to caffeine (5). In addition, paraxanthine shows lower anxiogenic activity and toxicity in rodents, but it contributes to the stimulating properties of caffeine. Its metabolism is dose-dependent, with a consequent non linear accumulation: after repetitive and chronic caffeine consumption in experimental animals, paraxanthine plasmatic levels even surpass those of caffeine (45). CYP1A2 enzymatic activity, which is responsible for its metabolism, shows a pronounced inter-individual variability, depending on factors both genetic, such as polymorphisms, and environmental, such as exposure to smoking, drugs and dietary habits (46); further research is required in order to clarify the role of paraxanthine as a bioactive component of dark chocolate.

3.3. Phenylethylamine

Phenylethylamine is an organic compound and a natural monoamine alkaloid with stimulant and psychoactive effects responsible for the chocolate-related state of mood and pleasure. It results to be a neuromodulator in the mammalian central nervous system, and it is biosynthesized from the aminoacid L-phenylalnine by enzymatic decarb oxylation through the enzyme aromatic L-amino acid decarboxylase. Phenylethylamine is a primary amine and a neurotransmitter, present in many organisms and foods, such as chocolate, especially after microbial fermentation, and has been proposed as a dietary supplement for its mood and weight loss therapeutic benefits; however, orally ingested phenethylamine experiences extensive first-pass metabolism by monoamine oxidase B, which turns it into phenylacetic acid. This important first-pass metabolism prevents high concentrations from reaching the brain even when taken in significant doses (47).

Its pharmacologic action is quite similar to amphetamine: phenylethylamine releases norepine-prine and dopamine (48) and after dietetic oral consump-tion it is quickly metabolized by phenylethanolamine N-methyltransferase, monoamine oxidase A and mono-amine oxidase B, aldehyde dehydrogenase and do-pamine-beta-hydroxylase producing the amphetamine

isomer N-methylphenethylamine, when PEA is used as a substrate by phenylethanolamine N-methyltransferase. The so-called "trace amines", which include tyramine, tryptamine and beta-phenylethylamine, are biologically active amines mostly based on phenylethylamine, occurring in the body in small amounts. These sympathomimetic agents act on vascular system, determining vasoconstriction and increasing blood pressure, so that they are often components of nasal decongestant medications. They are considered indirectly-acting sympathomimetic amines, because their effect is mainly due to the release of noradrenaline from sympathetic neuronal cells. However, recent studies evidence that this is not their only mechanism of action but they could also exert direct vascular effects independent of a noradrenergic pathway: in this respect, a group of novel amine-associated receptors, called TAARs, have been identified in blood vessels, where trace amines can bind. This is why their vasoconstrictor effects can partially be due to this mechanism (49). Cardiology is recently recognizing their clinical and pharmacological relevance because these stimulant molecules result to be constituents of both medications and diet, being mostly found in chocolate and wine (50).

4. COCOA AND CARDIAC RHYTHM: CHOCOLATE ABUSE AND ARRHYTHMOGENIC RISK

The US Food and Drug Administration stated that caffeine is safe up to a level of 0.0.2% in cocoa-beverages or cola-beverages (51). However, the population shows a variable sensitivity to its stimulant effects: this different tolerance is partially heritable and may be linked to genetic polymorphisms (52). Conflicting studies have been published concerning the association between chocolate and CVD. Even if normal chocolate consumption is not usually associated with atrial fibrillation, some sympathomimetic effects, due to circulating catecholamines, may be able to cause the cardiac manifestations of caffeine overdose toxicity, thus producing tachyarrhythmias, such as supraventricular or ventricular tachycardia, and even atrial or ventricular fibrillation, ventricular tachycardia, and ventricular fibrillation (7).

Moreover some pathophysiological changes have been described about the increased oxidative stress in atrial fibrillation, such as changes in gene transcriptional profiles and mitochondrial DNA, increased activity of enzymes such as NADPH oxidase and xanthine oxidase, activation of the renin-angiotensin system and inflammatory processes (53). Few articles have described the potential arrhythmogenic risk related to chocolate intake. In this respect, a case of atrial fibrillation associated with chocolate intake abuse during salbutamol treatment, was recently reported (8). This case report displayed a correlation between excessive chocolate consumption and the sudden onset of an arrhythmia: a 19 years old woman was admitted to the Cardiology Unit because of palpitations. She reported an anamnestic history of asthma and chronic salbutamol inhalation in absence of CVD and other diseases. The ECG revealed an atrial fibrillation, while the echocardiography displayed a mild mitral regurgitation and an ejection fraction of 50%. The medical anamnesis revealed an excessive chocolate intake both on the same day and on the day before. Another ECG was performed just after the patient was treated, confirming the persistence of atrial fibrillation. After the restoration of sinusal rhythm a short PR tract was observed, thus revealing an occult accessory pathway at ECG performed the day after and also a week later.

Normal consumption of chocolate is not associated with risk of arrhythmias, at the same way, a normal therapeutic dose of inhaled salbutamol is not associated with arrhythmias. However, on the one side, a cronic salbutamol therapy shifts the cardiovascular autonomic regulation to a major sympathetic responsiveness and slight beta2-receptor tolerance; on the other side, sympathomimetic effects due to circulating catecholamines determine the cardiac manifestations of caffeine and theobromine overdose toxicity. This recent case report evidenced a link between prolonged inhaled salbutamol treatment and concurrent chocolate abuse, which may lead to atrial arrhythmia, postulating that methylxanthines in the chocolate, coupled with the short-acting beta agonist, could trigger this arrhythmia (8).

Also a case of paroxysmal supraventricular tachycardia after consumption of large quantity of chocolate was reported (54): an adult woman without significant medical history presented palpitations and shortness of breath after consuming a large amount of chocolate. ECG revealed a supraventricular tachycardia at 165 beats per minute, which was quickly restored to sinus rhythm after adenosine bolus injection. Electrophysiology studies displayed atrioventricular nodal reentry tachycardia, which was treated with radiofrequency ablation. This episode of tachycardia, precipitated by large amount of chocolate consumption in a patient with underlying substrate, happened because chocolate contains methylxanthines, which are competitive antagonists of adenosine and may have arrhythmogenic potential in particular conditions.

Food can directly or indirectly influence human health (55-57) often resulting helpful in prevention of several diseases (58), in particular from a cardiovascular point of view (59, 60). These clinical cases evidence a possible new relationship between some kinds of food and heart conduction (defining the very novel field of arrhythmogenic foods and of "nutri-arrhythmias") showing how chocolate intake abuse associated with chronic salbutamol can trigger

an arrhythmia recognizing cocoa-rich foods abuse as the substrate for atrial fibrillation.Exogenous causes of arrhythmias such as atrial fibrillation through a substrate such as chocolate derived metabolites have not been widely reported in the literature. Few studies focused on its pharmacology, toxicology, metabolism, and safety assessment, evidencing that cocoa has not attracted a great deal of scientific interest because of its long-term usage with no reported adverse effects to man. On the other hand, research has always been interested in understanding the pharmacological properties of its methylxanthines. Considering the lack of basic information on theobromine, paraxanthine and theophylline, a major research program should be undertaken to evaluate these methylxanthines and, subsequently, cocoa, chocolate, coffee, and tea "side effects": the complete elucidation of their mechanism of action will allow to assess their safety.

5. EFFECTS OF DARK CHOCOLATE CONSUMPTION ON CARDIOVASCULAR PHYSIOLOGY

The endothelium maintains vascular homeostasis through multiple intercellular interactions and regulates vasodilators and vasoconstrictors production, thus regulating vascular tone. In addition, the endothelium regulates both blood fluidity and coagulation (by secreting regulator factors in order to balance the cloth cascade, platelet activity and the fibrinolytic system) and inflammatory processes through cytokines, chemokines and adhesion molecules production (61). A decreased bioavailability of endothelial NO, which is a key determinant for vascular vasomotricity, is closely related to the severity of endothelial impairment and, consequently, to the risk for cardiovascular accidents.

In this context, arterial stiffness results to be an important cardiovascular risk biomarker (62), because the progressive loss of arterial compliance in endothelial dysfunction represents the initial step of atherosclerosis (63). In fact, as the vascular wall stiffens, the pulse wave velocity (PWV), which is a measure of arterial stiffness, increases. PWV has a strong correlation with cardiovascular events and all-cause mortality and it is integral to the diagnosis and treatment of hypertension (64): when PWV increases the reflected pressure wave reaches the heart earlier, causing an increased central systolic blood pressure and thus increasing the cardiac afterload. In this respect, dark chocolate may improve the endothelial function. The consumption of dark chocolate by healthy people during 28 days was shown to increase plasma concentrations of ascorbic acid, catechins and epicatechins and reduces platelet aggregation (65).Engler *et al*. (66) also described an increased concentration of plasmatic epicatechin after the consumption of dark chocolate during 2 weeks. This was associated with improved endothelial function. Faridi *et al*. (67), demonstrated that the acute consumption of dark chocolate or cocoa powder significantly lowered the brachial blood pressure: the magnitude of these changes was greater with the consumption of sugar-free cocoa. Balzer *et al*. (68) evidenced some beneficial effects of dark chocolate consumption in the vascular function of medicated diabetic patients: the fasting plasma levels of flavanols metabolites increased significantly, and endothelial function improved.

The probable mechanism underlying the improvement of the PWV, but also of the atherosclerotic score index (ASI), and of the augmentation index (AiX) after dark chocolate consumption may be the parietal relaxation of the large arteries, as well as a dilation of small and medium peripheral arteries; this is due to an increased concentration of plasma epicatechins, which increases endothelium-derived vasodilators and also increases the concentration of plasma procyanidins, which leads to a major NO production and bioavailability (69). In fact, arterial stiffness and pressure wave reflection are closely associated with cardiovascular risk and the AiX is the proportion of central aortic pulse pressure, that is attributed to the reflected pulse wave (70).

The concomitant finding of improved flow-mediated dilatation (FMD), which is an accepted technique to quantify endothelial function with prognostic value for future CVD, strongly suggests endothelium-dependent vascular relaxation as the reason for the vasomotor benefit found, translating into lower PWV, ASI and AiX and for a trend in blood pressure reduction.

Cocoa has a high content in antioxidants such as polyphenols, especially flavonols (Table 1), which exert their favourable effects on endothelium-derived vasodilation via the stimulation of NOS, the increased availability of l-arginine and the decreased degradation of NO. Cocoa may also have a beneficial effect via the decreased platelet aggregation, the decreased lipid oxidation and insulin resistance. These effects are associated with a modest decrease of blood pressure and a significant improvement in healthy adults coronary circulation, with a favourable trend towards a reduction in cardiovascular events and strokes (71). Flavanol-rich chocolate and cocoa products have been shown to increase the formation of endothelial NO, which promotes vasodilation and therefore blood pressure reduction, thus displaying a small but statistically significant effect in lowering blood pressure: about 3 mm Hg in the short term (72). The high polyphenols content of cocoa is of particular interest from both nutritional and pharmacological viewpoints: cocoa polyphenols possess a range of cardiovascular protective activities, and can play a meaningful role through modulating different inflammatory markers involved in atherosclerosis (73).

6. CHOCOLATE AND BLOOD PRESSURE

High blood pressure is an important risk factor for cardiovascular disease attributing to about 50% of cardiovascular events worldwide and 37% of cardiovascular deaths in Western populations (72). Hypertension can be treated through lifestyle changes and medications. These lifestyle modifications include physical activity, tobacco avoidance, limitation of alcohol consumption, dietary changes: they can help treat and often prevent hypertension. In particular, multiple dietary factors affect blood pressure (74): moderation of alcohol drinking, salt restriction and increased potassium intake, through a diet rich in fruits, vegetables and legumes (and low in snacks, sweets, meat and saturated fat) are helpful in the treatment of hypertension.

Over the past decade, the American Heart Association and the National Cancer Institute for prevention and management of hypertension promoted the adoption of the DASH (Dietary Approaches to Stop Hypertension) dietary pattern, focused on ingestion of more fruits, vegetables, low-fat dairy products, and complex carbohydrates (75). Other dietary factors that may reduce blood pressure include increased intakes of calcium, fish oil, fibers and vegetable-based protein (74).Consumption of dark chocolate is also associated with a drop in systolic blood pressure. In fact, flavanol-rich chocolate and cocoa products have attracted interest as non-pharmacological treatment options for hypertension (76, 77), because even small reductions in blood pressure substantially decrease cardiovascular risk. Current guidelines strongly recommend integration of lifestyle modification and complementary treatment with the use of conventional blood pressure medications (78).

Polyphenols, in particular flavanols in cocoa products, have been shown to increase the formation of endothelial NO, which promotes vasodilation and may consequently lower blood pressure (79, 80). Meta-analyses by Taubert *et al*.(81-83) and Desch *et al*. (84), including 10 trials (82, 83, 85-20), concluded that cocoa-rich foods may reduce blood pressure. A recent meta-analysis (78) of 15 trials suggests that dark chocolate and flavanol-rich cocoa products are superior to placebo in reducing systolic hypertension and diastolic prehypertension.Additional trials of hypertensive populations are needed to elucidate whether local dietary habits or genetic factors influence the blood pressure-lowering effect of cocoa.

7. CONCLUSIONS

Many reviews support a short-term effect of lowering blood pressure by consuming cocoa products. Long-term cardiovascular benefits should be investigated in depth, even if daily consumption of cocoa flavanols (minimum dose of 200 mg) appears to benefit platelet and vascular function (91). The effect of chocolate on body weight is unclear too: excessive consumption of chocolate may promote high calorie intake and weight gain, a risk factor for metabolic and cardiovascular diseases. Large quantities of dark chocolate in an attempt to protect against CVD is likely to add excessive calories and induce weight gain (92). On the other side, chocolate contains caffeine, theobromine and other mild stimulants, which may exert an excitatory effect on hearth in some susceptible backgrounds. At present, there are only occasional case reports describing association between chocolate, caffeine, and arrhythmias.

Given this possibility of cardiac consequences developing from chocolate use and abuse, further clinical trials examinating its physiological effects and arrhythmogenic potential should be pursued, expecially in adolescents and children (93). Considering the widespread consumption of chocolate and cocoa sweets and also the increasing popularity of energy drinks in pediatric/adolescent population physicians should be aware of the arrhythmogenic potential associated with their excessive consumption. Further studies could evaluate serum caffeine and theobromine levels in pediatric patients with tachyarrhythmias and concurrent chocolate consumption, in order to assess this correlation and quantify it into a risk model. Future studies should also include identification and quantification of the metabolites of caffeine, theophylline, and theobromine in chronic consumption, the respective pharmacokinetics and eventual dose-dependency, especially taking into account the influence of other drugs and dietary variables.

8. REFERENCES

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