Coffee and cardiovascular disease: In vitro, cellular, animal, and human studies

Jennifer Stella Bonita, Michael Mandarano, Donna Shuta, Joe Vinson*

Department of Chemistry, Loyola Hall, University of Scranton, Scranton, PA 18510, USA

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Abstract

Coffee is a commonly consumed beverage with potential health benefits. This review will focus on cardiovascular disease. There are three preparations of coffee that are commonly consumed and thus worthy of examination; boiled unfiltered coffee, filtered coffee, and decaffeinated coffee. Coffee has over a thousand chemicals, many formed during the roasting process. From a physiological point of view, the potential bioactives are caffeine, the diterpenes cafestol and kahweol found in the oil, and the polyphenols, most notably chlorogenic acid. We will examine coffee and its bioactives and their connection with and effect on the risk factors which are associated with heart disease such as lipids, blood pressure, inflammation, endothelial function, metabolic syndrome and potentially protective in vivo antioxidant activity. These will be critically examined by means of in vitro studies, cell experiments, animal supplementation, epidemiology, and the most definitive evidence, human trials.

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Keywords: Coffee; Cardiovascular disease; Antioxidant; Epidemiology; Cholesterol; Blood pressure

Contents

1. Introduction ................................................................. 188
2. In vitro studies .......................................................... 188
   2.1. Diterpenes ......................................................... 188
   2.2. Caffeine ............................................................ 189
   2.3. Polyphenols ....................................................... 189
3. Cell studies ............................................................... 190
   3.1. Caffeine ............................................................ 190
   3.2. Polyphenols ....................................................... 190
4. Animal studies ........................................................... 191
   4.1. Caffeine ............................................................ 191
   4.2. Polyphenols ....................................................... 191
   4.3. Coffee ............................................................. 191
5. Human epidemiology studies ........................................... 192
   5.1. Uric acid ......................................................... 192
   5.2. Hypertension ................................................... 193
   5.3. Inflammation and endothelial function ....................... 193
   5.4. Cardiovascular disease ........................................ 193
6. Human supplementation and clinical trials ......................... 194
   6.1. Polyphenol absorption and antioxidant activity ................ 194
   6.2. Lipids ............................................................. 195

* Corresponding author. Tel.: +1 570 941 7551; fax: +1 570 941 7510.
E-mail address: vinson@scranton.edu (J. Vinson).

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1. Introduction

Coffee is among the most widely consumed pharmacologically active beverages in the world. Caffeine is the most widely consumed psychoactive substance. Since drinking coffee is very common in Western society, it is important that it be investigated. For example 52% of all persons in the US over 10 years of age consume coffee [1]. The latest consumption data for coffee importing countries from the International Coffee Organization are from 2004 and found in Fig. 1 [2]. Finland consumes the most coffee and the United Kingdom the least. The average for the European Community is 5.1 kg/year and is similar to the US. Coffee has recently been recommended by a US review panel to be consumed along with tea in greater quantities than all other beverages save water. These include caloric beverages such as milk, non-calorically sweetened beverages, fruit and vegetable juices, alcohol, sports drinks and calorically sweetened, nutrient-poor beverages [3]. But is coffee good or bad for the heart? That is the question that needs to be answered since heart disease is the number one cause of death in the developed world.

There are two species of coffee trees of commercial importance, Coffea arabica and Coffea robusta. The two species differ in chemical composition of the green coffee bean. Arabica contains more lipids and robusta contains more caffeine and sucrose as well as the polyphenols antioxidant chlorogenic acid and its derivatives [4]. Due to the fact that arabica has a more desirable flavor, this variety constitutes 80% of the world trade. Robusta is often used in instant coffee and as fillers in roast and ground blends [4]. The roasting process causes a loss of water from the green bean and degradation of many of the compounds including the antioxidant polyphenols; however, there is very little difference in total antioxidants between the different roasts of a bean [5]. There are three main methods of coffee preparation; boiled unfiltered coffee, filtered coffee, and decaffeinated coffee, the latter primarily consumed as instant coffee.

Before examination of the evidence, it is wise to appreciate what are probably the bioactives in coffee. There are over a thousand compounds, many formed during the roasting process, which produce the unique taste and smell of coffee [4]. However, from the point of view of concentration in coffee, prior detection of the parent compound or metabolites in the body, and physiological effects, there are essentially only three ingredients that are important; caffeine, the diterpene alcohols cafestol and kahweol, and chlorogenic acid and other polyphenols. Their structures are displayed in Fig. 2. Although caffeine is a major component of coffee, the caffeine content is highly variable. A cup of home prepared coffee (150 ml) contains between 30 and 175 mg [6]. In specialty coffees consumed outside the home the range is 18–80 mg/cup and decaffeinated coffees averaged 5 mg/cup [7]. Coffee is an important source of caffeine; it provides 71% of the caffeine in the US diet [8]. The diterpenoid alcohols are the oils in coffee and their concentration depends on the how the coffee is prepared. Filtered coffee has less than 0.1 mg/100 ml, i.e. essentially none, and unfiltered coffee can have between 0.2 and 18 mg/100 ml depending on the method. The order of decreasing amounts is the following: Scandinavian boiled > Turkish/Greek > French press > Espresso > Filter [9]. Boiled coffee has a higher concentration of coffee oils because of the higher temperature used during its preparation and a longer contact time between the coffee grounds and water [10, 11].

We hypothesize that the bioactive ingredients in coffee relating to heart disease are the polyphenols which in the body may be acting as protective antioxidants but also could have other beneficial mechanisms. However, the most well-known ingredient in coffee is the alkaloid caffeine. This compound has a variety of pharmacological effects with respect to mood, cognitive performance, and motor activity. At present there is no evidence that caffeine can have any benefit for the heart; on the contrary some results indicate that it is detrimental in certain conditions. Thus, there is a Jekyll and Hyde or yin and yang aspect to coffee. We will attempt to separate the effects of caffeine from the rest of the compounds in coffee as much as possible.

This work will examine coffee and its ingredients, in particular the polyphenols, diterpenes, and caffeine, for potential protective cardiovascular effects on risk factors of heart disease by means of in vitro experiments, cell and animal studies, epidemiology, and lastly human trials.

2. In vitro studies

2.1. Diterpenes

The two diterpene compounds cafestol and kahweol are suspected to be the coffee substances which cause the elevation of serum cholesterol. One mechanism may be the LDL receptor which is involved in the endocytic process of apoB- and apoE-containing lipoproteins. Cholesterol content of the cell is regulated via feedback repression of the gene for the LDL receptor. When the cell is depleted of cholesterol, the LDL receptor gene is actively transcribed and LDL is removed from the plasma to replenish the cellular cholesterol. On the other hand when cholesterol accumulates in the cell, the number of LDL receptors is down regulated and LDL continues to circulate, eventually making its way below the endothelial surface, becoming oxidized and ultimately producing foam cells and subsequent atherosclerosis [12]. In an elegant in vitro cellular study Rustan et al. showed the diterpenes reduced the activity of LDL receptors thus causing extra cellular accumulation of LDL [13]. Although there is a positive side to the coffee oils in that they have been shown to have anticarcinogenic properties mediated by induction of phase II enzymes in cell culture studies [14].
2.2. Caffeine

Certainly caffeine is a major component in coffee and perhaps the most often used rationale for drinking it. However, it is our contention that in order to be beneficial to heart disease caffeine or any bioactive must improve one of more of the major risk factors such as lipids or blood pressure or else have a direct or indirect in vivo antioxidant effect at physiological levels. There is nothing in the literature to indicate a lipid effect of caffeine in humans although if it induced a weight loss this could indirectly decrease lipids. Then the question is whether caffeine is an in vivo antioxidant, in which case it might be bioactive. It has been shown to be an in vitro scavenger of hydroxyl radicals at millimolar concentrations using electron spin resonance spectroscopy [15]. However, human studies have shown a maximum plasma concentration of 46 μM with four cups of coffee and caffeine equivalent to 5 mg/kg of body weight [16]. Caffeine itself has no LDL antioxidant activity. However, some of the metabolites of caffeine, namely 1-methylnxanthine and 1-methyluric acid are as effective at preventing LDL oxidation as ascorbic acid at 40 μM [17]. Unfortunately these water-soluble demethylated metabolites have not been detected in plasma using UV detection although they are seen in urine [18]. This finding is probably indicative of sub-micromolar plasma levels of the metabolites which are probably not bioactive although this needs to be explored further.

2.3. Polyphenols

The major polyphenol in coffee is chlorogenic acid (CGA) an ester of caffeic acid and quinic acid as depicted in Fig. 2. CGA is an in vitro antioxidant with two phenolic groups for radical scavenging via proton transfer. It has been studied in a number of models. With two antioxidant assays it was the poorest antioxidant among seven phenolic acids tested [19]. Coffee has also been extensively studied for antioxidant activity. A Norwegian group found that coffee had the highest concentration of polyphenols among the beverages [20]. We used our Folin–Cocicalteu assay with catechin as the standard and coffee prepared according to the manufacturer’s instructions. Coffee was then hydrolyzed with base to liberate some bound phenolic groups. We calculate that the average 180 ml cup of brewed coffee provides 396 mg of polyphenols and instant coffee 316 mg. Combining this data and US per capita consumption it is apparent that coffee is the number one source of antioxidants in the US diet [21]. Coffee was also found using other antioxidant assay methods to be the number one source of antioxidants in Norway and Spain [22,23].

A literature search indicated that there have been studies of the effect of CGA on LDL oxidation [24]. Also caffeic acid, a putative metabolite of CGA, was investigated and found to have activity [25]. We have compared a number of polyphenols and flavonoids in an in vitro model of atherosclerosis, namely the

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Fig. 1. Per capita coffee consumption in 2004 for the EU countries, UK, Japan and the US.

Fig. 2. Chemical structures of proposed bioactive compounds in coffee.
Fig. 3. Concentration of pure compounds and coffee (antioxidants measured as catechin equivalents) to inhibit the oxidation of LDL + VLDL 50% compared with the control.

Fig. 4. Plasma spiking with antioxidants followed by isolating LDL + VLDL and oxidizing under standard conditions to determine the lag time of oxidation (lipoprotein-bound antioxidant activity) vs. a control.

oxidation of LDL + VLDL, two atherogenic lipoproteins. LDL is the major carrier of cholesterol and VLDL is the major carrier of triglycerides. They are isolated from plasma by an affinity column method and oxidized with cupric ion under physiological conditions. The polyphenols concentration to inhibit the oxidation 50% (IC$_{50}$) is used for comparison after a dose–response assay [26]. A comparison of some antioxidant vitamins and other polyphenols is shown in Fig. 3 [27]. The lower IC$_{50}$ concentration indicates stronger antioxidant activity. CGA is moderate in activity for polyphenols and is much better as an antioxidant than gallic acid, a black tea component and plasma and urine metabolite [28]. CGA is much more potent than the vitamin antioxidants which are poor protectors of lipoprotein oxidation in this model. The CGA IC$_{50}$ concentration is certainly physiologically possible but of course the metabolism of CGA must be taken into account. Surprisingly coffee has the best quality antioxidants in Fig. 3. Perhaps there is synergism between the polyphenols in coffee as was found between CGA and catechin for LDL oxidation [29].

There are two areas where LDL is oxidized, in the plasma and below the endothelial surface. In the plasma the endogenous proteins, enzymes and antioxidant vitamins inhibit LDL oxidation effectively. However, oxidation in the sub-endothelial space is more critical since that is where atherosclerosis begins [30]. It is our hypothesis that LDL is best protected there by antioxidants which can bind to the LDL and be carried with it below the endothelium. We have measured LDL + VLDL lipoprotein-bound antioxidant activity by ex vivo spiking of human plasma with polyphenols at different concentrations and subsequent isolation of bound compounds by affinity column chromatography. The bound antioxidant activity is then determined by our standard oxidation protocol and determining the lag time of conjugated diene oxidation. This time is defined as the intersection of the initial slow propagation reaction and the rapid oxidation reaction when all the antioxidants in the lipoprotein are oxidized and can no longer protect the lipoproteins from oxidation [26]. For mixtures such as coffee we determine the total antioxidants by the Folin–Cocialteu method using catechin as the standard. Results are shown for α-tocopherol, CGA and brewed coffee in Fig. 4. For comparative purposes the concentration to increase the lag time 50% or CLT$_{50}$ is calculated from the linear concentration–% lag time increase curve. For tocopherol it is 50 μM, CGA 108 μM and coffee 100 μM [31]. Tocopherol is the best antioxidant probably due to its highly lipophilic nature compared with the more water-soluble phenolic acid CGA and the coffee polyphenols. However, this experiment demonstrates that the coffee polyphenols can bind to lower density lipoproteins, protect them from oxidation and thus be in vivo heart-protective antioxidants.

3. Cell studies

3.1. Caffeine

Cellular studies can be a simple way to determine the metabolism of coffee compounds and to discover possible mechanisms for bioactivity in vivo. Caffeine metabolites are inhibitors of the enzyme poly(ADP-ribose)polymerase-1 in hydrogen peroxide-treated epithelial cells at physiological concentrations [32]. This is a potential in vivo anti-inflammatory caffeine function in humans and possibly beneficial to the heart.

3.2. Polyphenols

CGA is categorized as a phenolic acid, specifically a hydroxycinnamic acid (see Fig. 2). Metabolism of CGA and other phenolic acids has been accomplished in human endothelial cells [33] and most recently in human liver cells [34]. Metabolites were formed by methylation, sulfation and glucuronidation pathways. CGA was found to be part of the most powerful antioxidant fraction from brewed coffee [35,36]. CGA was effective in preventing oxidative damage to human epithelial cells [37]. Of interest for heart health is the study with rat cardiomyocytes. CGA and other hydroxycinnamic acids proved non-cytotoxic, and they both stabilized membranes and improved the energetic status of cardiomyocytes [38].
The phenolic acid ferulic acid has been found in coffee [39]. The bioactivity of ferulic acid was investigated after uptake by cultured human cells and found to be an antioxidant with equivalent potency to the spice polyphenol curcumin [40]. Pre-incubation of CGA and other phenolic acids with neuronal cells showed subsequent protection from perox radicals or hydrogen peroxide by inhibition glutathione depletion, lipid peroxidation and reactive oxygen substance formation. [41] Such pre-incubation cellular studies demonstrate that CGA and other polyphenols and/or their metabolites have potential antioxidant activity in vivo.

Another important cellular study was done by Huang et al. [42]. Dihydrocaffeic acid (DHCA) is a metabolite of caffeic acid (found in coffee) with potent antioxidant properties. Since DHCA has been detected in human plasma following coffee ingestion, they tested the hypothesis that DHCA protects the endothelium from oxidative stress in a model using human-derived endothelial cells. During culture for 16–24 h, the cells accumulated DHCA against a concentration gradient to low millimolar concentrations. In α-tocopherol-loaded cells, DHCA spared α-tocopherol during overnight culture in a dose-dependent manner. In response to oxidant stress induced by a water-soluble free radical initiator, both α-tocopherol and DHCA diminished oxidation of cis-parinaric acid that had been incorporated into the cells. This suggests that the protective effects of DHCA were caused by scavenging of intracellular reactive oxygen species. DHCA also increased nitric oxide synthase activity in a dose-dependent manner in cultured cells, which was associated with a comparable increase in endothelial nitric oxide synthase protein (eNOS). This mechanism implies that coffee may prove to protect the heart by improving endothelial function which is mediated by a decrease in eNOS. This mechanism has been shown to occur with several foods and beverages containing polyphenols [43]. Although the DHCA concentrations required for these effects are higher than those likely to be present in plasma or the interstitial space, these results indicate that a coffee metabolite can function as an intracellular antioxidant.

4. Animal studies

4.1. Caffeine

Animal studies can provide valuable information on the mechanism of cardiovascular benefit with the proviso that doses of bioactives and coffee close to that consumed by humans are used. However, no animal study can with certainty predict the effect on humans. Chronic caffeine supplementation to pregnant monkeys produced no change in cholesterol or triglycerides which were depressed during pregnancy [44]. In a recent article, ovariectomized female rats were investigated as a model of post-menopausal cholesterol elevation in humans. Catheter-infused caffeine equivalent to a human dose of two cups of coffee significantly decreased cholesterol absorption in this animal model [45].

Although not a heart disease model, the obese diabetic rat exhibits elevated cholesterol and triglycerides. Caffeine was present at 0.1% in the water which produces plasma caffeine levels similar to consumption of 300 mg of caffeine for the average human [46]. This regimen was given for 30 weeks to the rats. The animals experienced an increase in plasma cholesterol and an adverse effect on renal function although glucose and insulin resistance were improved. Caffeine in this model proved to be harmful for kidney structure and function.

4.2. Polyphenols

The most definitive study of animal CGA metabolites was done in rats by a French group [47]. Four groups of rats (n=8) were fed a diet supplemented with chlorogenic, caffeic or quinic acids (250 μmol/day) or an unsupplemented diet for 8 days. The recovery of CGA in urine was low (0.8%, mol/mol), and the total urinary excretion of caffeic acid liberated by hydrolysis of CGA and its methylated metabolites (ferulic and isoferrulic acids) did not account for >0.5% (mol/mol) of the dose ingested. On the other hand, the metabolites of microbial origin, namely, m-coumaric acid and derivatives of phenylpropionic, benzoic and hippocuric acids, represented the major compounds in both urine and plasma. Hippuric acid largely originated from the transformation of the quinic acid moiety, and all other metabolites from the caffeic acid moiety. These microbial metabolites accounted for 57.4 mole% of the CGA intake. This paper shows that the bioavailability of CGA depends largely on its metabolism by the gut microflora in the rat.

A Japanese group investigated the effect of CGA on spontaneously hypertensive rats [48]. A single ingestion of CGA (30–600 mg/kg) reduced blood pressure in spontaneously hypertensive rats, an effect that was blocked by administration of a nitric oxide synthase inhibitor, N(Gamma)-nitro-L-arginine methyl ester. The lower dose is still much higher than a common human dose of 5 mg/kg. When spontaneously hypertensive rats were fed diets containing 0.5% CGA for 8 weeks (approximately 300 mg/kg per day), the development of hypertension was inhibited compared with the control diet group. Dietary CGA reduced oxidative stress and improved nitric oxide bioavailability by inhibiting excessive production of reactive oxygen species in the vasculature, and led to the attenuation of endothelial dysfunction. At a more relevant dose, CGA given to mice at 10 mg/kg activated calcineurin and enhanced macrophage functions in normal mice, a possible cardiac benefit [49].

Caffeic acid, the initial metabolite of CGA, was given to the type 2 diabetic mouse (C57BL/KsJ-db/db) to examine its glucose-lowering and antioxidant effect. Although this is a diabetic mouse model, hyperglycemia and hypercholesterolemia are oxidative stress conditions. Caffeic acid significantly increased superoxide dismutase, catalase and glutathione peroxidase and lowered glucose. It also decreased lipid peroxidation products thus indicating an in vivo antioxidant activity [50].

4.3. Coffee

The following describes the first animal study on the relationship of coffee consumption and atherosclerosis [51]. In
experimental animals the authors investigated the relationship of coffee consumption with risk factors of atherosclerosis such as cholesterol, homocysteine, oxidative stress and inflammatory cytokines. Male Wistar rats were assigned to three treatment groups (a control diet group, 0.62% coffee diet group, and 1.36% coffee diet group), and animals were kept on the experimental diets for 140 days. Coffee diets increased serum caffeine in a dose–response manner, although caffeine in serum was not detected in rats fed the control diet. It also led to slightly increased total serum levels of homocysteine and cholesterol, but no significant differences were found between the control and coffee groups. Coffee intake did not affect the production of IL-6 and TNF-alpha induced by LPS, which contributes to the atheroma-promoting effect of recurrent bacterial infection. Biomarkers of oxidative stress, the serum level of 15-isoprostane, which was significantly increased by LPS injection, was not altered by coffee intake. In contrast, urinary 8-hydroxy-2-deoxyguanosine was significantly increased in the coffee groups (p < 0.05). From these results, the authors concluded that moderate coffee intake is not a risk factor for atherogenesis.

Our group conducted experiments with hamsters which are a better model than rats for atherosclerosis. Hamsters, when given a diet of cholesterol and saturated fat, have a lipid profile similar to humans. In a short-term study male adult hamsters (five per group) were given normal rodent food mixed with 0.2% cholesterol and 10% coconut oil for 2 weeks. The controls were given water and the experimental group brewed and filtered coffee at 1/10 the human recipe (described above) was used for the experimental groups. For this study commercial instant coffee at 1/10 the human recipe (described above) was used for the low decaffeinated and 1/2 the recipe for the high decaffeinated group. The caffeinated group was given the high dose caffeinated coffee. There was no significant effect of any dose of coffee, caffeinated or decaffeinated, on weight gain or food consumption. Also there was no significant effect on lipids due to large within group variations (data not shown). Although there was a dose–response decrease in cholesterol with decaffeinated coffee, the caffeinated coffee was equivalent to the decaffeinated coffee at the high dose. There was no effect of any dose of coffee on plasma lipid peroxides or atherosclerosis. In confirmation of our study a French group did not find that CGA or caffeic acid had any effect on lipids or atherosclerosis in this same hamster model. These phenolic acids did increase plasma antioxidant capacity and thus had an in vivo antioxidant effect [52].

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Cholesterol (mM)</th>
<th>HDL (mM)</th>
<th>LDL + VLDL (mM)</th>
<th>Triglycerides (mM)</th>
<th>Lipid peroxidation (µM)</th>
<th>Lag time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.72 ± 0.49</td>
<td>0.96 ± 0.16</td>
<td>2.76 ± 0.49</td>
<td>0.89 ± 0.51</td>
<td>0.92 ± 0.02</td>
<td>19</td>
</tr>
<tr>
<td>Coffee</td>
<td>3.54 ± 0.96</td>
<td>0.90 ± 0.16</td>
<td>2.64 ± 0.96</td>
<td>1.57 ± 1.40</td>
<td>0.63 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>56</td>
</tr>
</tbody>
</table>

Results are mean ± standard deviation.

<sup>a</sup> Significantly different from control group.

5. **Human epidemiology studies**

Epidemiological evidence can never prove cause and effect for any regimen such as coffee drinking since it cannot account for all the variables. For instance coffee drinkers may have a significantly less healthy lifestyle than tea drinkers. In a European study of men women aged 25–64 years, associations of lifestyle factors with coffee and tea consumption were analyzed [53]. Coffee drinkers smoked more, ate more meat and less fruits than tea drinkers and were more sedentary. The authors concluded that drinking coffee is positively associated with factors that promote coronary heart disease, while drinking tea is associated with a preventive lifestyle. Also there is a genetic component which may be significant. It is important to distinguish between filtered and non-filtered (boiled) coffee since the oils are hypercholesterolemic in humans. Also caffeinated and decaffeinated coffee may have different physiological effects. Since decaffeinated coffee is not popular in Japan and in Europe, only US studies can properly examine this variable.

#### 5.1. Uric acid

Elevated serum uric acid, although a well-known antioxidant, is a risk factor for CHD. Uric acid is positively correlated with aortal intimal media thickness, a measure of atherosclerosis, in humans [54]. One Japanese epidemiological study is significant as it is the only one to examine the relationship of coffee drinking and serum uric acid [55]. There was a clear inverse relationship between coffee consumption and serum uric acid but none with green tea, another important source of caffeine in Japan. In a Polish population of 2000 men and women it was concluded that coffee drinkers had lower serum uric acid than non-drinkers and there was a negative correlation between them. Elevated serum uric acid concentration was positively correlated with elevated blood pressure and increased body mass index [56]. Clearly further examination of coffee and uric acid needs to be done by means of supplementation trials.
5.2. Hypertension

Hypertension is a major risk factor for CHD, stroke and congestive heart failure [57,58]. Any long-term effect on hypertension of caffeine intake in the form of coffee would be of major news in the healthcare and coffee industries. Caffeine can raise plasma levels of stress hormones such as epinephrine, norepinephrine and cortisol, all of which can lead to an increase in blood pressure [59,60]. The literature on coffee and hypertension has recently been critically reviewed [61]. Interestingly no association of caffeine with risk of hypertension was found in the 10 year Nurses Health Study I and II. [62]. Nor were there any effects of coffee on hypertension. However, there was an association of consumption of diet and sugared colas and hypertension, \( p < 0.001 \). The risk was 20% higher for \( >4 \) cans of cola/day versus \(<1\) can/day. In a letter to the Editor we attributed this difference between coffee with no hypertension risk and cola with risk to the lack of polyphenols in the colas and the presence of them in the coffee [63]. Thus high consumption of caffeine with no antioxidants can lead to hypertension at least in women.

5.3. Inflammation and endothelial function

Boiled coffee consumed at moderate to high doses was also related to increases in C-reactive protein, an inflammatory marker, in a Greek study [64]. Another risk factor for heart disease is homocysteine. In a Norwegian study 12,000 heavy coffee-drinking men were examined [65]. Coffee consumption was found to be a major lifestyle determinant of plasma homocysteine in a healthy population. A similar study with men and women in Greece reached the same conclusion [66].

The US Nurses Health Study examined coffee drinking and markers of inflammation and endothelial function in healthy women and women with type 2 diabetes [67]. In healthy women, no appreciable differences in plasma concentrations of the markers were found across categories of caffeinated coffee intake. In women with type 2 diabetes, higher caffeinated coffee consumption was significantly associated with lower plasma concentrations of E-selectin and C-reactive protein. Higher decaffeinated coffee consumption was associated with lower plasma concentrations of E-selectin and C-reactive protein only in healthy women. These results indicated that neither caffeinated nor decaffeinated filtered coffee has a detrimental effect on endothelial function. On the contrary, the results suggest that filtered coffee consumption is inversely associated with markers of inflammation and endothelial dysfunction. Intervention studies are needed to definitively conclude the benefits of coffee in healthy and diabetic men and women.

5.4. Cardiovascular disease

The type of relationship in European studies examining coffee consumption and risk of developing acute coronary events is a J-curve such as seen in Fig. 5 for a case–controlled study of a Greek population [68]. The staple coffee is unfiltered and the mode of preparation is Greek/Turkish (boiled). After controlling for the usual risk factors, moderate coffee drinking (<300 ml/day) reduced the risk 31% relative to no consumption and higher amounts significantly increased the risk. A similar J-curve was seen in a Finnish study of middle aged men [69]. In the latter study there was a dose–response increase in LDL. Both studies agree that heavy drinking of boiled coffee (>600 ml/day) is very detrimental to heart health. A Swedish case–control study elegantly compared boiled unfiltered coffee with filtered coffee for the risk of the first MI [70]. Men consuming >1000 ml/day were over two-fold more likely to have an MI whether the coffee was filtered or not. Men and women consuming boiled coffee, after adjusting for the amount consumed, had a 40–60% greater risk of MI than those consuming filtered coffee.

In the largest epidemiological study done to date, long-term habitual coffee consumption was followed for 16–20 years in over 44,000 men and 85,000 women free of CHD to assess heart disease risk in the US Physician’s and Nurses Health Study [71]. There was no effect of coffee on risk even at \( >6\) cups/day. Regrettably there was no separation of caffeinated and decaffeinated coffee drinkers. The data does not provide any evidence that coffee consumption increases the risk of CHD. The most recent meta-analysis is a compilation of thirteen case-control and ten cohort studies followed from 3–44 years [72]. Decaffeinated coffee was not associated with CHD in four case–control and three cohort studies. There were no significant differences when considering the regions studied, or the type of coffee, filtered or unfiltered. The authors conclude that “despite a significant association between high consumption of coffee and CHD reported among case–control studies, no significant association between daily coffee consumption and CHD emerged from long-term follow-up prospective cohort studies”.

A study of over 40,000 post-menopausal US women was recently conducted which examined healthy subjects for 15 years [73]. In the fully adjusted model, similar to the relation of coffee intake to total mortality, the hazard ratio of death attributed to CVD was 0.76 for consumption of 1–3 cups/day, 0.81 for 4–5 cups/day, and 0.87 for \( \geq 6\) cups/day.
The hazard ratio for death from other inflammatory diseases was 0.72 for consumption of 1–3 cups/day, 0.67 for 4–5 cups/day, and 0.68 for ≥6 cups/day. Consumption of coffee may inhibit inflammation and thus reduce the risk of cardiovascular and other inflammatory diseases in postmenopausal women.

Very recently there have been two epidemiological investigations of coffee consumption and myocardial infarction (MI). In a study of subjects in Costa Rica it was found that the relative risk of MI in the first hour after coffee drinking was 1.49 [74]. Occasional coffee drinkers (≤1 cup/day) had a very high relative risk of 4.14 and the risk decreased with increasing coffee consumption. Heavy drinkers (≥4 cups/day) had a relative risk of 1.06. When the hazard examination period was extended to 2 or 3 h there was no increase in risk from coffee consumption. This implicates the acute sympathetic nerve activation of caffeine and the development of tolerance to caffeine from heavy coffee drinking. The Costa Rican population has a very high intake of saturated fats from tropical oils, an overall low HDL and an increased risk for MI [75]. The second study was in the US and investigated almost 2000 subjects who had an acute MI and survived [76]. There was no association of coffee with post-infarction mortality, even among the heaviest coffee drinkers. However, in the first 90 days following an MI there was an inverse association with mortality from another MI and coffee consumption, i.e., coffee was protective. The authors had no explanation for this surprising result. It is our hypothesis that there was a coffee polyphenol-induced improvement in endothelial function of the MI survivors which had been even more compromised by the initial MI. Also the fact that there was no relationship between caffeinated cola and mortality rate indicating that caffeine was probably not responsible for the coffee benefit.

Most people, laypersons and scientists including this author, believe that consumption of tea is more beneficial than coffee. However, a recent study in Japan may change our opinion. For the Japanese, metabolic syndrome is becoming more prevalent due to the more Western lifestyle adopted by the Japanese, especially the young. People who have metabolic syndrome are at considerably elevated risk for CHD [77,78]. The components of this syndrome are waist circumference, systolic BP (SBP), diastolic BP (DBP), HDL, triglycerides and fasting blood glucose. In a study of 2000 men and women who were studied for 6 years there was a strong inverse relationship between coffee consumption and the number of components of the syndrome for the subjects (see Fig. 6). In contrast, there was no correlation for green tea drinking [79]. This is certainly paradoxical since coffee consumption in the West is usually associated with an unhealthy lifestyle. The effect of coffee on metabolic syndrome should be examined in randomized controlled clinical trials.

On a lighter note, a Boston group examined the Ben Franklin hypothesis, namely “early to bed, early to rise, makes a man healthy, wealthy and wise” and the James Thurber hypothesis, or “healthy, wealthy and dead”. They found that survivors of MI were no more likely to die whether they went to bed early or late or whether they were rich or poor. Nor did sleeping habits have any relationship with wealth or wisdom (surrogate measure-

Fig. 6. Mean coffee consumption stratified by the number of components of the metabolic syndrome in Japanese men and women.

6. Human supplementation and clinical trials

6.1. Polyphenol absorption and antioxidant activity

A Dutch study examined the human absorption of CGA and caffeic acid using ileostomy subjects who lack a colon [81]. Study of this group eliminates bacterial degradation of the polyphenols which is extensive in normal humans. By urine analysis they found that CGA and caffeic acid were absorbed 33 and 95% in the body, respectively. Another research team found that the major metabolites from coffee consumption in normal human subjects, after hydrolysis of the conjugated metabolites, were CGA and caffeic acid [82]. An Italian group was the first to examine polyphenols in human plasma due to coffee. After drinking 200 ml of coffee, caffeic acid was found in the plasma and it persisted for 2 h [83]. This same group also found an increase in plasma antioxidant capacity of 7%, i.e., an in vivo antioxidant effect, after coffee drinking [84]. This result was not due to uric acid but to other antioxidants, presumably the polyphenols from coffee.

In a short-term study with 22 subjects and a control group it was found that 5 cups/day of unfiltered Italian-style coffee produced a significant 16% increase in plasma glutathione, a major in vivo antioxidant. No difference in plasma hydroperoxides or homocysteine (both pro-oxidants) was noted in this intervention study with a coffee intake which was average for the Italian population [85]. Another study of healthy Finnish men consuming 3 or 6 cups of filtered coffee for 3 weeks produced no significant changes in homocysteine, lipid peroxidation or antioxidant enzymes. Urinary excretion of ferulic and caffeic acid was increased [86]. A one-week study with healthy male Japanese subjects given three cups of coffee/day showed that LDL oxidizability was significantly decreased 30% and returned to baseline after cessation of coffee [87]. This result corroborates our ex vivo study and short-term animal study in Sections 2.3
and 4.3 and is indicative of a heart protective mechanism that should be substantiated in a longer human trial.

6.2. Lipids

Coffee oils from arabica but not robusta raised cholesterol and triglycerides in 36 normal subjects [88]. Because only arabica oil has kahweol and arabica coffee contains more cafestol than does robusta, this implicates the diterpenes as the lipid-raising ingredients. They were also implicated in the in vitro studies mentioned in Section 2.1.

The first human trial to convincingly show the different effects of boiled and filtered coffee on lipids occurred in 1985 [89]. In a 10-week trial 33 hypercholesterolemic men were randomly assigned; (a) to continue with their usual coffee intake; (b) stop drinking coffee altogether; or (c) stop drinking coffee for five weeks and thereafter drink either boiled or filter coffee. Cholesterol concentrations fell significantly in all subjects abstaining for the first 5 weeks compared with subjects consuming coffee, and continued to fall in those abstaining for 10 weeks. Cholesterol concentrations rose again in subjects returning to boiled coffee but remained the same in those returning to filtered coffee. Thus boiled coffee raises cholesterol in humans compared with filtered coffee.

Some but not all observational studies have demonstrated a positive association of coffee drinking and higher levels of serum cholesterol. A total of 14/23 published trials prior to 1989 were deemed to be well conducted and these were subjected to a meta-analysis [90]. The authors found that increases in serum lipids were significantly greater in studies of hyperlipidemic subjects and in trials of caffeinated or boiled coffee compared with the few studies with decaffeinated coffee. Trials with filtered coffee showed very little increase in cholesterol. This is most reassuring for those who drink filtered coffee. The authors concluded that “consumption of unfiltered, but not filtered, coffee increases serum levels of total and LDL cholesterol”.

6.3. Blood pressure and vasoreactivity

CGA was examined in human studies for blood pressure and vasoreactivity effects. There were 28 mild hypertensive subjects who were randomized in a double-blind placebo-controlled study to receive either 140 mg of CGA (in a green coffee extract) or placebo for 12 weeks followed by a 2-week washout period [91]. The CGA regimen had no effect on normal serum or cell biochemistry. Compared with the placebo group, the CGA group experienced a significant lowering of SBP and DBP, 10 and 7 mm Hg, respectively. During washout the values increased to the baseline value in the CGA group. The authors speculate that the mechanism of CGA effect is an increase in the level of nitric oxide (NO). There was no effect on serum magnesium by CGA, thus excluding magnesium from being a bioactive as often mentioned in epidemiological studies of hypertension. Serum magnesium is inversely related to hypertension risk [92]. Normotensive subjects with reduced vasoreactivity were also studied with the CGA green coffee bean extract. The same dose of CGA as in ref 91 was given for 4 months and there was a placebo group [93]. There were mild decreases in DBP and SBP in the CGA group but they were not significant. There was a significant decrease in plasma homocysteine compared with the baseline value for the CGA group. This indicates a decreased risk of CHD. Also a positive benefit was noted in the vasodilation responses to ischemic reactive hyperemia in the CGA group indicating improved vasoreactivity, a surrogate for endothelial function.

The effect of acute administration of caffeine on vascular function was examined in healthy young men give 300 mg of caffeine or a placebo in a double-blind protocol. Blood pressure was increased by the caffeine but heart rate and forearm blood flow were not changed. Caffeine was found to improve endothelium-dependent vasodilation by a mechanism which increased the production of nitric oxide [94]. In normotensive subjects a single dose of caffeine has been shown to cause an increase in systolic BP of 3–14 mmHg and diastolic BP 4–13 mmHg [95]. A meta-analysis of well conducted clinical trials of coffee or caffeine before 1997 was undertaken. The median trial duration was 56 days. Systolic BP increased an average 2.4 and diastolic BP increased by 1.2 mmHg with coffee compared with control [96]. They found a greater effect on younger trial subjects. Trials where the control group consumed no coffee or the small number that consumed decaffeinated coffee gave the same results. This indicated that the BP-raising effects are due to caffeine rather than other ingredients. A more recent meta-analysis from 1966 to 2003 found that regular caffeine consumption significantly increases BP [97]. When ingested in coffee, however the BP effect is small. Coffee intake with a median of 725 ml/day produced only an increase of systolic BP 1.2 mm Hg and diastolic 0.5 mm Hg [95]. In this study caffeine given as tablets produced BP elevations four times higher than the same dose of caffeinated coffee. Thus it appears that moderate caffeinated coffee intake would have no effect on BP.

One of the mechanisms for which coffee or its ingredients may be detrimental to CV health is mental stress-induced BP increase. The sympathetic nervous system has an import role in the regulation of the CV system. Vasomotor sympathetic nerve activity to skeletal muscle typically increases in response to mental stress. In patients with borderline hypertension, which is a large segment of many populations, sympathetic nerve activity is already increased under basal conditions and even in normotensive offspring of hypertensive parents during mental stress conditions [98]. When given acutely caffeine or coffee, at a dose which gives the same plasma caffeine, increases sympathetic nerve activity and BP in non-habitual coffee drinkers. On the other hand, habitual coffee drinkers experienced a lack of BP increase despite an increased nerve activity from mental stress. Decaffeinated coffee also increases BP and nerve activity in non-habitual drinkers. These results point to coffee ingredients other than caffeine that activate the CV system [99]. It is our contention that coffee contains substances, presumably polyphenols, able to reduce the mental stress-related BP increases.

There has been only one long-term human study with decaffeinated coffee and BP which lasted 12 weeks [100]. The Dutch group gave 45 male and female subjects with a habitual intake of 4–6 cups of coffee per day a regimen of five cups of caf-
decaffeinated coffee (445 mg dose of caffeine) for 6 weeks or five cups of decaffeinated coffee (40 mg dose of caffeine) for 6 weeks then switched to the other form of coffee for 6 weeks. Use of decaffeinated coffee led to a significant increase in systolic and diastolic BP, 1.5 and 1 mmHg, respectively. They conclude that normotensive subjects switching from caffeinated to decaffeinated coffee would experience a small but real decrease. However as seen in the epidemiology discussion Section 5.2, consumption of decaffeinated coffee does not lead to a greater risk of hypertension. It is known that chronic alcohol consumption increases in BP [101]. In fact, 4 weeks of moderate coffee consumption for Japan hypertensive or pre-hypertensive subjects who also were heavy alcohol drinkers (>60 ml/day) caused a significant decrease of 7–10 mmHg and 3–7 mmHg in systolic and diastolic BP, respectively [102]. Thus coffee blunts the BP increase due to alcohol consumption.

7. Conclusions

Although there have been numerous in vitro studies, cell studies, animal studies and epidemiology investigations with coffee and its bioactive components, there has been a paucity of human trials as shown in this review. This is probably due to the early evidence that boiled coffee increased lipids in humans. The initial negative finding was an obstacle to a scientific assessment of the potential benefits of coffee. From the data presented here, it is concluded that only heavy consumption (>6 cups/day) of boiled unfiltered coffee is harmful to the heart as a result of the dose-related plasma cholesterol and LDL increase due to the diterpene oils. Although epidemiological studies show that moderate consumption of this coffee appears to confer some cardiovascular benefit. The CGA effect on blood pressure and endothelial function is intriguing. Polyphenols are the components in filtered and unfiltered coffee that have potential cardiovascular benefits via antioxidant mechanisms related to LDL oxidation as well as NO bioavailability and blood pressure lowering. However, their benefit is less obvious when consuming unfiltered coffee. Polyphenols seem to be countering many of the negative effects of caffeine and diterpenes in the coffee studies. More long-term interventional studies need to be done with caffeinated and decaffeinated coffees in different human populations and disease states. In the meantime moderate filtered coffee consumption, which is the usual pattern of the many of the subjects in the populations studied, is recommended.

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