Genetic Modifiers of Caffeine Consumption and Risk of Myocardial Infarction

by

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Abstract

The variability in caffeine consumption and inconsistencies among studies linking caffeine to heart disease may be explained by genetic variation. Caffeine antagonizes adenosine receptors with downstream effects on dopamine and serotonin. The objectives of this thesis were to determine whether the DRD2 957C>T or HTR2A 102C>T polymorphisms are associated with caffeine consumption or modify the association between coffee consumption and risk of myocardial infarction (MI). DRD2 genotype was associated with caffeine consumption among non-smokers and CYP1A2 -163C allele carriers. HTR2A genotype was associated with caffeine consumption among non-smokers and subjects with the ADORA2A TT genotype. Neither polymorphism modified the association between coffee consumption and risk of MI; however, a significant coffee x HTR2A interaction was seen among subjects with the CYP1A2 -163C allele. The results suggest caffeine’s reinforcing effects may be mediated by the dopamine and serotonin receptors and implicate serotonin in caffeine’s effect on risk of MI.
Acknowledgments

“Where coffee is served, there is grace and splendor and friendship and happiness”
Sheikh Abd-al-Kadir, 1857

Coming across this quote while lunching at a small café in Halifax with my lab-mates, I knew it would have to find a way into my thesis. In my mind, the quote not only applies to where coffee is served, but also to where coffee is studied. During my time at U of T, the El-Sohemy lab has always been full of the most supportive and brilliant individuals. There was no obstacle I faced, no laboratory technique or statistical calculation I tackled, for which I was not offered encouragement, guidance, or assistance. I am so appreciative of all of the El-Sohemy students, past and present, who offered aide whenever it was needed. My success is yours.

To my good friends in the department, I would like to extend a large amount of gratitude for your friendship and support. We shared amazing adventures, stories, laughs, jogs, and of course the most delicious foods. I will cherish those moments and I hope for many more in the years to come. My graduate experience would not have been the same without you all.

I would like to thank all of my family and friends, but especially my parents and significant other. Your love and support have been the foundation and mortar from which all the achievements in my life have been built. From your kind words of encouragement to your unwavering confidence in my success, I could not have done it without you.

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Chapter 1: Introduction and Literature Review

1.1 Introduction

All across the globe, caffeine (1,3,7-trimethylxanthine) is consumed regularly by individuals of almost all ages. It is found naturally in a variety of regularly consumed products such as coffee, tea, and chocolate but it is also added to medications, cola beverages, and energy drinks. Regular consumption of these products makes caffeine the most commonly used stimulant and psychoactive substance; however, consumption varies both within and between populations (1). Some of this variation may be explained by the differences in subjective effects reported with consumption. Caffeine has been found to produce a variety of positive and negative effects in the body which may affect motivation for consumption and also disease risk (2-4). Coronary heart disease (CHD), one of the leading causes of death worldwide, has been associated with coffee consumption; however, there are inconsistencies between studies (4, 5). Potential explanations for these inconsistencies have included a variety of methodological issues involving study design, measurement of intake, and adjustment for confounding (4, 5). Recent studies support the role of genetic differences as a potential contributor to these inconsistencies. By comparing subjects with genetic differences in caffeine metabolism, these studies show the caffeine in coffee may be contributing to CHD risk while other components in coffee such as antioxidants may be protective (6, 7). Although implicating caffeine, these studies cannot elucidate the specific mechanism by which caffeine increases disease risk.

Caffeine’s mechanism of action in the body has been attributed to its ability to antagonize adenosine receptors and oppose the actions of adenosine (8-10). As adenosine has many functions in the body, it is not known how this antagonism may specifically contribute to CHD risk. Caffeine’s antagonism of adenosine receptors has secondary effects on neurotransmitters, which may be responsible for some of caffeine’s subjective and cardiovascular effects (11). Therefore, variation in the components of these neurotransmitter systems may explain some of the variation seen in caffeine’s effects and may modify the association between caffeine consumption and CHD risk.
1.2 Coronary Heart Disease

Heart disease accounted for 22% of deaths in Canada and 25% of deaths in the United States in 2007 (12, 13). While deaths related to CHD have been on the decline, it is still one of the leading causes of death around the world (14). CHD is characterized by blockage of the coronary arteries, which supply oxygenated blood to the heart. Atherosclerosis is believed to be the primary underlying cause of all CHD including the most common and serious form, myocardial infarction (MI) (14).

Atherosclerosis begins in susceptible blood vessels as a result of endothelial damage or injury, inflammation, increased levels of pro-atherogenic substances, and/or decreased levels of protective mediators. The resulting changes in the endothelium result in accumulation of lipids and inflammatory cells in the arterial wall to form “fatty streaks”. Fat accumulated and trapped in the artery wall undergoes oxidation and other modifications which induce adhesion molecule expression and cytokine release, bringing inflammatory monocytes into the arterial wall. Monocytes proliferate, differentiate into macrophages, take up oxidized-low-density lipoprotein (LDL), and ultimately become foam cells. These cells produce cytokines and growth factors, which cause smooth muscle cells to migrate into the intima, proliferate, and produce a matrix of collagen and extracellular materials that forms a fibrous cap. As the plaques begin to develop, the endothelium becomes disrupted and causes platelet activation and aggregation, which further promotes lesion development. Macrophages and foam cells undergo apoptosis which produces a lipid-rich core in the centre of atherosclerotic plaques. When plaques become disrupted, the lipid-rich core containing pro-coagulants becomes exposed and promotes thrombus formation. A partial occlusion of the artery may result producing ischemia, which may be chronic in nature. The body has natural anti-thrombotic mechanisms that may result in lysis and healing, however, repeated episodes of minor plaque rupture and thrombus formation also contribute to atherosclerosis progression. Prolonged ischemia or a complete occlusion of an artery may result in infarction and death of the tissues supplied blood by the artery. In acute MI, rupture of a vulnerable plaque results in occlusion of the coronary artery and myocardial cell death. Vulnerable plaques which lead to acute MI tend to have a large amount of macrophages and necrotic lipid at the core and thinner fibrous caps, making them more susceptible to rupture. Fibrous caps can become weakened as a result of metalloprotease enzyme digestion of the extracellular matrix, which is expressed due to cytokine release. In combination with
mechanical strain and weakening of the endothelium, the result is plaque rupture and thrombus formation (15-18).

Atherosclerosis generally develops gradually over time; however, the presence of chronic risk factors may enhance its development. Many factors have been associated with increased risk of CHD and the development of atherosclerosis including dyslipidemia, cigarette smoking, high blood pressure, diabetes, abdominal obesity, psychosocial factors, low fruit and vegetable consumption, physical inactivity, male gender, infectious agents, family history of early CHD, haemostatic factors, and elevated homocysteine (14, 15, 18). These risk factors may explain a large portion of the global risk of CHD, and indeed a landmark investigation of MI in 52 countries revealed 90% of the population attributable risk for MI could be explained by smoking, history of hypertension, history of diabetes, waist-to-hip ratio, fruit and vegetable consumption, physical activity, alcohol consumption, blood apolipoproteins, and psychosocial factors (19). Although risk factors such as smoking and physical activity can be modified directly by an individual, the physiological risk factors such as dyslipidemia, hypertension, and diabetes are not environmental factors that one can simply ‘remove’. These risk factors, like CHD itself, are multifactorial in origin and may result from a combination of genetic susceptibility and environmental influences. Therefore, continued investigation of risk factors for CHD including gene-environment interactions and their mechanisms is needed in order to improve prevention and treatment strategies.

In addition to factors contributing to the development of atherosclerosis, acute risk factors may also increase risk of triggering acute coronary events such as MI in vulnerable individuals. These risk factors include internal and external triggers, which activate the sympathetic and parasympathetic nervous system producing vasoconstrictive, prothrombotic, and biomechanical forces (20). Internal triggers such as circadian rhythm and external triggers such as eating, heat exposure, physical or mental stress, sexual activity, and caffeine intake, do not always result in pathological consequences and depend upon the complex internal response effected by environmental and genetic factors unique to each individual (20). This is emphasized in the highly inconsistent findings relating coffee intake and risk of CHD, which may in part be explained by differences in genetic vulnerability to this external trigger.
1.2.1 Coffee Consumption and CHD

Many epidemiological studies have examined the relationship between coffee consumption and CHD; however, findings have been highly inconsistent. Associations between coffee consumption and cardiovascular disease (CVD) have shown increased risk (21-25), decreased risk (26-29), no association (30-34), U- and J-shaped associations (35-37), and sex-specific associations (38-42). The most recent meta-analysis analysed 21 prospective cohort studies published between 1966 and 2008 (43). The authors concluded that coffee consumption was not associated with risk of CHD over the long-term and in fact moderate consumption was associated with a significantly decreased long-term risk in women (43). These results are similar to the findings of two previously published meta-analyses that showed no effect of coffee consumption on CHD risk when examining prospective cohort studies (44, 45). In contrast, both of these previous meta-analyses also examined case-control data, which showed a significantly increased risk of CHD in the highest intake groups compared to the lowest (44, 45). These results emphasize the tendency for case-control but not cohort studies to report increased risk of CHD with coffee consumption. Traditionally, inconsistencies in these epidemiological studies have been attributed to inadequate adjustment for confounding or lack of information on coffee preparation methods, which can affect coffee composition (46). For example, boiled coffee contains the diterpenes kahweol and cafestol, which may increase cholesterol levels and consequently risk of CHD (47). These compounds are largely trapped by paper filters and appear at very low levels in filtered coffee (46, 48). However, studies have shown that both boiled and filtered coffee can increase CHD risk (23, 41) and most studies sufficiently adjust for confounding factors (5, 49). Inconsistencies in case-control and cohort studies may be explained by time differences between exposure and outcome. In cohort studies, the lag time between assessment of exposure and the outcome may mask the acute effects of coffee consumption on CHD risk (5, 45). Indeed, in two cohort studies, the positive association between coffee consumption and CHD was stronger when shorter follow-up times were considered (22, 50). These differences between case-control and cohort studies support the hypothesis that coffee may have acute rather than chronic adverse effects on the cardiovascular system (4). Several studies have shown that coffee consumption may act to trigger an acute cardiovascular event (21, 51, 52), whereas coffee consumption does not affect more chronic CHD risk factors such as the development of atherosclerosis (30, 42) and diabetes (53-55). In
fact, there is strong evidence to suggest that coffee consumption protects against the development of diabetes (56, 57). Therefore, the effect of coffee on risk of CHD may be acute rather than chronic in nature and not captured by cohort studies with long lag times between measurement of exposure and outcome. This may explain some of the tendency for case-control but not cohort studies to find positive associations between coffee consumption and CHD.

Another potential explanation for the inconsistencies between studies is the role of genetic variation. A large case-control study in a Costa Rican population has shown that variation in the gene that codes for the caffeine metabolizing cytochrome P450 1A2 (CYP1A2) enzyme modified the association between coffee consumption and MI (7). In that study, subjects who were genetically predisposed to slower caffeine metabolism showed a significantly increased risk of MI with consumption of 2 or more cups of coffee per day, whereas subjects who metabolized caffeine more rapidly showed protective effects with moderate consumption (7). The results suggest that it is the caffeine in coffee that increases risk of MI and that fast metabolizers may benefit from other compounds in coffee such as antioxidants (58, 59). These results were recently supported by a prospective study which found subjects with the same genetically determined slow caffeine metabolism were at significantly increased risk of developing hypertension with coffee consumption compared to abstainers, whereas subjects with fast caffeine metabolism had a significantly lower risk (6). As high blood pressure is a well established risk factor for CHD (60, 61), these results together support a detrimental role of caffeine on CHD risk.

1.3 Caffeine

1.3.1 Caffeine in the Diet

Caffeine occurs naturally in the fruit, leaves, and seeds of a variety of plants and can thus be found in the food products derived from them such as coffee, tea, and chocolate. Caffeine is also added to products and can be found in medications, soft drinks, energy drinks, and an increasingly diverse list of other products including water, gum, and candy (62). The average
The caffeine content of these sources can be quite variable depending on the source, brand, and preparation methods. The caffeine content of a cup of coffee (150 ml) is approximately 100 mg, but can be as much as 200 mg (63, 64). Table 1-1 shows the range of caffeine amounts reported for various sources of caffeine.

Coffee is the largest contributor of caffeine intake among adults in most countries (64-66), accounting for up to 75% of the North American adult consumption of caffeine (67). Soft drinks have traditionally been the major source of caffeine for children and adolescents (65, 66), however, energy drinks are becoming increasingly popular among youth (62, 68).

Global consumption of caffeine is estimated to be 70-76 mg/person/day but varies by country and has been noted to be 210-238 mg/day in the United States and Canada and exceeds 400 mg/person/day in Finland and Sweden (63, 64). Data from a large American sample found approximately 90% of subjects surveyed consumed caffeine daily including 76% of children (65). An extensive review of the health effects of caffeine reported consumption of up to 400 mg/day was not associated with adverse effects in healthy adults, while pregnant women and children 12 years of age and under are recommended to limit caffeine intake to less than 300 mg/day and 2.5 mg/kg of body weight, respectively (67). These findings have translated into the current recommendations for caffeine intake by Health Canada.
Table 1-1: Caffeine Sources and Content Ranges (1, 64, 68-70)

<table>
<thead>
<tr>
<th>Product</th>
<th>Serving Size</th>
<th>Caffeine Range (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roasted and ground coffee</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brewed</td>
<td>150 mL</td>
<td>53-90</td>
</tr>
<tr>
<td>Percolated</td>
<td>150 mL</td>
<td>40-170</td>
</tr>
<tr>
<td>Drip</td>
<td>150 mL</td>
<td>60-70</td>
</tr>
<tr>
<td>Decaffeinated coffee</td>
<td>150 mL</td>
<td>2-4</td>
</tr>
<tr>
<td>Instant coffee</td>
<td>150 mL</td>
<td>25-108</td>
</tr>
<tr>
<td>Espresso</td>
<td>59 mL</td>
<td>70-100</td>
</tr>
<tr>
<td>Tea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf or bag</td>
<td>150 mL</td>
<td>28-48</td>
</tr>
<tr>
<td>Green</td>
<td>150 mL</td>
<td>20</td>
</tr>
<tr>
<td>Iced</td>
<td>355 mL</td>
<td>66-76</td>
</tr>
<tr>
<td>Chocolate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hot chocolate</td>
<td>150 mL</td>
<td>2-7</td>
</tr>
<tr>
<td>Chocolate chips</td>
<td>43 g</td>
<td>13-15</td>
</tr>
<tr>
<td>Colas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr. Pepper</td>
<td>335 mL</td>
<td>41</td>
</tr>
<tr>
<td>Pepsi</td>
<td>355 mL</td>
<td>38</td>
</tr>
<tr>
<td>Energy Drinks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red Bull</td>
<td>248 mL</td>
<td>80</td>
</tr>
<tr>
<td>Full Throttle</td>
<td>473 mL</td>
<td>144</td>
</tr>
<tr>
<td>Rockstar</td>
<td>473 mL</td>
<td>160</td>
</tr>
</tbody>
</table>
1.3.2 Caffeine Pharmacokinetics

1.3.2.1 Absorption & Distribution

The bioavailability of caffeine is considered to be nearly 100% as it is rapidly and completely absorbed from the gastrointestinal tract into the bloodstream (71, 72). By 45 minutes post-ingestion, absorption from the gastrointestinal tract reaches 99% (71-74). However, absorption properties can differ by caffeine source (73, 74). Caffeine concentration peaks in the plasma at approximately 0.5-1.5 hours, but can peak as quickly as 15 minutes post-ingestion (71, 72, 75). Peak plasma concentrations with consumption of a single cup of coffee (0.4-2.5 mg/kg) can range from 1-10 μM (1), which is well below toxic levels of greater than 200 μM (76).

Caffeine distributes to all areas of the body as it is sufficiently hydrophobic to pass through all biological membranes including the blood-brain barrier and the placenta (77, 78). Low to moderate doses (70-100 mg) of caffeine tend to show linear (74) while higher doses (250-500 mg) show non-linear pharmacokinetics (75), suggesting a saturable metabolism in humans (1).

1.3.2.2 Metabolism

The liver is the main site of caffeine metabolism where 99% of ingested caffeine is metabolized (74). The most important metabolic pathway, representing 95% of caffeine metabolism, involves demethylation into 3 metabolites: paraxanthine (~80%), theobromine (~11%), and theophylline (~4%) (79, 80). The remaining 5% represent C8-oxidation and elimination of unchanged caffeine in the urine (71, 74). The cytochrome P450 1A2 (CYP1A2) enzyme is responsible for more than 95% of caffeine metabolism in the liver while CYP2E1 plays a role in metabolism of caffeine at much higher doses (79, 81, 82). Other CYP isoforms as well as N-acetyltransferase and xanthine oxidase are involved in the further breakdown of caffeine metabolites (83, 84).
The elimination half-life of caffeine can range 2-12 hours with averages of 4-6 hours and plasma clearance rates of approximately 1-3 mL/kg/min after low doses (71, 75, 85). Therefore, for most individuals, caffeine is cleared from the body after overnight abstinence while it can still be detected after 24 hours abstinence in others (86). Differences in dose may partially explain variability in caffeine clearance; however, it is also attributed to variability in metabolism by CYP1A2. Indeed, CYP1A2 activity has been reported to vary 5-60 fold (82, 87-90) and reflects 15-40 fold variations noted in human liver mRNA levels (91, 92).

1.3.3 Cytochrome P450 1A2

1.3.3.1 CYP1A2 Activity

The CYP1A2 enzyme is a part of the superfamily of heme-containing proteins that metabolize a variety of endogenous and exogenous substances and is located exclusively in the liver (93, 94). CYP1A2 substrates tend to be planar molecules with polyaromatic rings that fit the active site of this enzyme (95, 96). Substrates include a variety of drugs such as clozapine and olanzapine (93) as well as endogenous compounds like melatonin (97) and estradiol (98). CYP1A2 also plays a role in the bioactivation of procarcinogens such as heterocyclic amines and polycyclic aromatic hydrocarbons (99, 100).

CYP1A2 activity is affected by a variety of factors including age, sex, oral contraceptive use, pregnancy, ethnicity, diet, and smoking. Caffeine half-life is particularly long in new born infants due to decreased CYP1A2 activity, exceeding 100 hours in premature infants and reaching adult levels by about 6 months of age (101, 102). Females tend to have a lower CYP1A2 activity than males (88, 103-106) and women taking oral contraceptives have lower activity than women who are not (104, 105, 107, 108). The half-life of caffeine also increases with pregnancy, particularly in the third trimester (109-111). Ethnicity also affects CYP1A2 activity and Chinese females, Koreans, and individuals of African descent have been shown to have lower activities than Caucasians (106, 112, 113). Dietary intake of vegetables may also affect CYP1A2 activity; in particular, brassica vegetables have been shown to increase activity.
while apiaceous vegetables have been shown to decrease CYP1A2 activity (107, 114, 115). Polycyclic aromatic hydrocarbons in tobacco smoke are known inducers of CYP1A2 and therefore smokers have increased caffeine metabolism in comparison to non-smokers (89, 103-105, 116). Although some of the factors listed above are modifiable aspects of the individual’s environment, twin studies suggest approximately 70% of the variability in CYP1A2 activity is attributable to genetics (104).

1.3.3.2 Genetic Variation in CYP1A2

The CYP1A2 gene is located on chromosome 15q24.1 and has been found to contain several polymorphisms that may alter enzyme expression and activity (117-121). Among these is a synonymous polymorphism in intron 1, upstream of the first transcribed nucleotide (nucleotide position -163, rs762551), that results in an adenine (A) to cytosine (C) substitution and occurs in about 30% of Caucasians (121). This polymorphism was associated with a 1.6-fold lower caffeine metabolism among carriers of the C allele only among smokers suggesting the AA genotype represents a high inducibility genotype (121). These results were later supported in populations made up of smokers and non-smokers (122, 123). Although these results suggest the differences in CYP1A2 activity by genotype may only be seen in the presence of an inducer such as cigarette smoking, several dietary components including coffee itself may be inducers of the CYP1A2 enzyme (124, 125). Therefore, this polymorphism has been used to classify individuals with the AA genotype as “fast” and those with the AC or CC genotypes as “slow” caffeine metabolizers (7). This variant has been shown to be an important modifier of the relationship between coffee consumption and risk of MI (7) and hypertension (6).
1.3.4 Caffeine Pharmacology

1.3.4.1 Acute Effects of Caffeine

Caffeine is a behavioural stimulant with mild reinforcing properties (126). Indeed, a large range of consumption levels (128-595 mg/day) have been associated with reinforcement in as few as 45% and as many as 100% of subjects (63). Caffeine has been shown to have biphasic effects. Consumption of low to moderate doses (200-300 mg) has been associated with positive effects on well-being, concentration, arousal, and energy, while higher doses (>400 mg) have been associated with increased anxiety, jitteriness, nervousness, and tension (2, 3). Positive effects on mood may be particularly evident in subjects who are in a lower state of baseline arousal such as during sleep deprivation or illness (1, 67, 127). In addition, the anxiogenic effects of higher doses may be particularly heightened in subjects with underlying panic or anxiety disorders (128-133). The negative effects associated with heavier consumption or caffeine sensitivity may be self-limiting and negatively reinforce caffeine consumption (67).

Although there is strong evidence for the subjective experience of positive effects with caffeine consumption, some argue these effects are limited to the reversal of withdrawal symptoms (134). Caffeine withdrawal can occur 12-24 hours after abstinence and is reliably associated with headache, fatigue, decreased energy, decreased contentedness, depressed mood, difficulty concentrating, irritability, and being not clear headed (135). Other symptoms which may be associated with withdrawal include flu-like symptoms such as nausea, vomiting, and muscle stiffness (135). Symptoms appear to be more severe among heavier consumers while even modest consumption of 100 mg/day has been associated with withdrawal (135). The relief of withdrawal effects occurs with caffeine ingestion and therefore the positive effects of caffeine consumption are thought to be simply the reversal of negative withdrawal symptoms (134, 135). However, this cannot explain the positive effects reported in caffeine naive and non-withdrawn subjects or those who do not experience withdrawal (62, 127). Therefore, a combination of modest reinforcing effects and reversal of withdrawal is likely to explain caffeine consumption behaviours.
Other effects attributed to caffeine intake have included increased respiratory rate and bronchodilation, increased gastric section, cerebrovascular constriction, diuresis, increased lipolysis, and enhanced athletic performance (3, 136, 137). Caffeine may also have negative effects on sleep including increased sleep latency and daytime sleepiness and decreased sleep quality (127, 136, 138). Large individual differences in sensitivity to caffeine dose and effects have been noted and may be explained, in part, by differences in pharmacokinetics (139) and genetics (140).

1.3.4.2 Cardiovascular Effects of Caffeine

Caffeine may have acute and long-term effects on the cardiovascular system. Blood pressure constitutes one of the most widely investigated cardiovascular effects in response to caffeine and coffee intake. A recent meta-analysis of randomized controlled trials of coffee and caffeine intake (≥7 days in duration) and blood pressure reported increases in systolic and diastolic blood pressures of 2.04 and 0.73 mmHg, respectively (141). The increases in blood pressure were attenuated when examining coffee only suggesting other compounds in coffee may have beneficial effects. In an earlier meta-analysis of controlled trials examining coffee’s effects on blood pressure, increases of 2.4 and 1.2 mmHg for systolic and diastolic blood pressure, respectively, were reported (142). Therefore, there is strong evidence from experimental investigations that caffeine increases blood pressure, possibly resulting from caffeine’s antagonism of adenosine receptors producing increased vasoconstriction and total peripheral resistance (143-145). However, findings from epidemiological studies are less consistent (49, 146). Prospective studies have shown no association of coffee consumption with the development of hypertension (147, 148), while others support an increased risk with moderate consumption of coffee (149, 150) and caffeine (148). Although there has been some evidence of development of tolerance to caffeine’s pressor effects with chronic consumption, only about 50% of subjects appear to develop tolerance while the rest remain sensitive to caffeine’s pressor effects (151, 152). Overall, caffeine appears to have modest pressor effects, which may contribute to CVD risk (49).

Coffee consumption has also been associated with elevated serum total and LDL cholesterol levels, however, this effect appears to be largely mediated by the diterpenes kahweol
and cafestol found in unfiltered coffee (46-48). Coffee consumption has also been associated with elevated homocysteine in several investigations (153-155), which appears to be mediated by chlorogenic acids and caffeine in coffee (156, 157). Other potential cardiovascular effects of caffeine and/or coffee consumption include associations with endothelial dysfunction, inflammation, and fibrinolysis (158, 159). However, results are conflicting (160) and limited and require further investigation.

Coffee consumption may also have beneficial effects on the cardiovascular system through decreased risk of diabetes (56, 57), which is a strong risk factor for CVD (161, 162). Several mechanisms have been proposed to explain this relationship including delayed glucose absorption, reduced hepatic glucose output, and increased insulin sensitivity; however, these effects may be mediated by constituents in coffee, other than caffeine, such as antioxidants and minerals (163-165). Further research including well-controlled human studies will be required to clarify the mechanism by which long-term coffee intake reduces risk of diabetes and its subsequent effect on cardiovascular risk (163).

### 1.4 Neurotransmission

Neurons are the cells within the nervous system capable of transmitting and receiving information. Through changes in electrical potential, neurons transmit and receive information using chemical messengers known as neurotransmitters. The classical definition of a neurotransmitter is a neuroactive chemical messenger capable of initiating synaptic transmission in the nervous system. Neurotransmitters are defined by synthesis and storage within neurons, selective release upon electrical signal in a calcium-dependent manner, activation of pre- or post-synaptic receptors, and inactivation by enzymatic degradation or reuptake into the neuron (166, 167).

Transmission begins with the arrival of an action potential in the pre-synaptic neuron which opens voltage-dependent calcium channels and leads to the release of neurotransmitters into the synaptic cleft. The neurotransmitters diffuse through the synaptic cleft and bind to receptors on the post-synaptic neurons. This results in the opening of ion channels and a change in electrical potential at the post-synaptic neuron. The specific results of the transmission
process will depend on the neurotransmitter and receptor to which it binds. Neurotransmitter receptors which gate ion channels are ionotropic whereas others involve a cascade of biochemical events involving second messengers and are metabotropic. Ionotropic receptors generally produce rapid responses while metabotropic receptors produce slower and prolonged effects. These processes are not limited to the central nervous system and also occur in the periphery (166, 167).

Neurotransmitters include amino acids (gamma-aminobutyric acid (GABA), glutamate, and glycine) and biogenic amines (acetylcholine, histamine, and monoamines). Monoamines can be further subdivided into the indolamine serotonin and the catecholamines (epinephrine, norepinephrine, and dopamine) (166, 167).

1.5 The Adenosinergic System

Adenosine is an endogenous purine nucleoside that modulates a variety of physiological processes in the body including pacemaker activity in the heart, tubuloglomerular filtration rate in the kidney, suppression of inflammation, promotion of wound healing, vasodilation, sleep promotion and maintenance, neuroprotection, and angiogenesis (168-171). Adenosine does not meet the classical definition of a neurotransmitter as it is not produced and released from vesicles in response to the firing of neurons; however, it is an important modulator of other neurotransmitter systems (168). Production of adenosine may occur both intracellularly and extracellularly in a variety of cells and accumulate in the extracellular space in response to cellular stress that utilizes adenosine triphosphate (ATP) (172). There are four adenosine receptors to which adenosine binds, which are called A₁, A₂A, A₂B, and A₃ (173). All four receptor types are G protein-coupled receptors where A₁ and A₃ inhibit adenylate cyclase and A₂A and A₂B activate adenylate cyclase (171). Adenosine has the highest affinity for A₁ and A₂A receptors and much lower affinities for A₂B and A₃ receptors, which are not highly expressed in the brain and whose roles have yet to be clearly elucidated (168, 170).

Under normal conditions, adenosine continuously activates A₁ receptors which provides a general inhibitory effect on synaptic transmission and neurotransmitter release (171). However, in the presence of a high-frequency stimulus, adenosine produced from large amounts
of released ATP selectively stimulates $A_{2A}$ receptors in the activated synapse (174). Stimulation of $A_{2A}$ receptors also decreases inhibition by $A_1$ receptors thereby further allowing change in synaptic transmission of the activated synapse while depression in the surrounding synapses remains by $A_1$ stimulation (175). Therefore, the adenosine system can be seen as a way of enhancing signalling and decreasing noise in synaptic transmission (169, 174).

$A_1$ receptors are located both pre- and post-synaptically where they inhibit neurotransmitter release and cause hyperpolarization, respectively (174). $A_1$ receptors can be found in almost all regions of the brain whereas $A_{2A}$ receptors tend to be highly concentrated in the basal ganglia (170, 176). The $A_1$ and $A_{2A}$ receptors have also been localized to a variety of different cell and tissue types including the heart and vasculature (177, 178).

1.5.1 Caffeine Antagonizes Adenosine Receptors

Caffeine, which has a comparable double bond ring structure to adenosine, acts as a non-selective adenosine receptor antagonist (8-10). It is this antagonism of the actions of adenosine that is thought to be responsible for the physiological effects associated with caffeine intake. Other mechanisms such as release of intracellular calcium and inhibition of cyclic nucleotide phosphodiesterases have been proposed but require caffeine concentrations that greatly exceed those seen with regular consumption (1). In contrast, antagonism of adenosine $A_1$ and $A_{2A}$ receptors by caffeine occurs at levels normally consumed in the diet (1, 10, 179). As described above, adenosine has important functions in modulating neurotransmission and therefore, through antagonism of adenosine receptors, caffeine has downstream effects on transmission of a variety of neurotransmitters including serotonin and dopamine. Indeed, studies have shown that caffeine can increase central dopamine (180-185) and serotonin (186-188) release in experimental animals. Caffeine’s effects on adenosine receptors and the secondary neurotransmitter targets of adenosine receptor action are proposed to play a role in caffeine’s physiological effects (11, 189).
Figure 1-1: Chemical structure of A) caffeine and B) adenosine
Evidence for the role of adenosine receptor antagonism comes from extensive research in knockout mice. Administration of caffeine to A<sub>2A</sub> receptor knockout mice fail to produce psychomotor stimulation suggesting a role for this receptor in caffeine’s stimulating effects (190-193). Similarly, administration of selective A<sub>2A</sub> antagonists, but not A<sub>1</sub> antagonists, mimics the effects of caffeine in animals (194). Other lines of research suggest caffeine’s antagonism of both receptors are involved in the motor activating properties of caffeine (195). In addition to effects on locomotion, A<sub>1</sub> and A<sub>2A</sub> receptor knockout mice also show increased anxiety in comparison to their wild-type littermates, which suggests an important role for these receptors in the regulation of anxiety (196-198). Therefore, caffeine’s ability to increase anxiety is likely also attributed to adenosine receptor antagonism and this has been supported by examination of variation in the adenosine receptors and caffeine-induced anxiety in humans (199-201).

1.5.2 Variation in ADORA2A

The A<sub>2A</sub> receptor (ADORA2A) gene is located on chromosome 22q11.23 and contains 2 coding exons (202-204). A common polymorphism occurs at nucleotide position 1083 (rs5751876) resulting in a C to thymine (T) substitution, which was initially associated with panic disorder (205). The association of the TT genotype to panic disorder has since been confirmed in other investigations (206, 207). The TT genotype has also been associated with caffeine-induced anxiety (199-201). In the first double-blind placebo-controlled study of 94 infrequent consumers of caffeine, subjects with the TT genotype reported greater increases in anxiety than those with the CT or CC genotype (201). This was followed by a second study that examined different doses of caffeine in 102 subjects that also found similar differences in self-reported anxiety by the ADORA2A polymorphism after a 150 mg caffeine dose (200). The effects of this polymorphism on caffeine-induced anxiety may negatively reinforce caffeine consumption as a significantly higher frequency of subjects with the TT genotype were found to limit their caffeine intake to less than 100 mg per day in comparison to subjects with the CT or CC genotype in a large Costa Rican population (208). Although another study that examined the effect of this polymorphism also found the TT genotype to be associated with higher caffeine-induced anxiety among non- and low-consumers, there were no differences in caffeine intakes and a higher consumption of coffee among subjects with the TT genotype (199). The authors
suggest this paradoxical finding may be explained by other positive effects that outweigh the reported anxiety or interpretation of the anxiety-related feelings in a positive manner such as excitement (199). In addition, the two populations compared vary in age, smoking habits, and ethnic background which may complicate direct comparisons of the findings. Despite these inconsistencies, together these studies support an important role for the A\textsubscript{2A} receptor in caffeine’s acute effects and consumption behaviours. However, caffeine’s antagonism of adenosine receptors has secondary consequences on neurotransmitters such as dopamine and serotonin which may also play a role in caffeine’s effects. While there is some evidence to suggest genetic variation in these downstream targets may be associated with caffeine’s effects (200), associations with consumption behaviours and cardiovascular effects have not been examined.

1.6 Dopamine

Dopamine is synthesized in the brain and sympathetic ganglia from the essential amino acid tyrosine. In the rate-limiting step of dopamine synthesis, tyrosine hydroxylase forms 3,4-dihydroxy-L-phenylalanine (L-DOPA) from tyrosine which is then converted to dopamine by cytosolic aromatic amino acid decarboxylase (167, 209). Dopamine released into the synapse acts on dopamine receptors which can be located post-synaptically and pre-synaptically in the form of autoreceptors (210). Dopamine receptors are metabotropic G protein-coupled receptors and are classified as D\textsubscript{1}-like (D\textsubscript{1} and D\textsubscript{5}) or D\textsubscript{2}-like (D\textsubscript{2}, D\textsubscript{3}, and D\textsubscript{4}), which activate and inhibit adenylate cyclase, respectively (167, 211). Autoreceptors, which belong to the D\textsubscript{2}-like class of receptors, act to inhibit dopamine synthesis and dopaminergic neuron firing, increase uptake of dopamine by the dopamine transporter (DAT), and enhance DAT expression (212-214). Actions of dopamine are terminated by reuptake into the pre-synaptic terminals by DAT as well as degradation by monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT) (167, 169).

Dopamine neurons are categorized into the nigro-striatal, mesolimbic, and mesocortical systems. The nigro-striatal region is involved in motor behaviour while the mesolimbic and mesocortical systems have been implicated in reward-mediated and motivation-dependent
behaviour. Dopamine has been implicated in a variety of other important processes in the body including arterial blood-flow, cognition, learning, mood, reinforcement, hormone secretion, sodium excretion, vascular tone, and anxiety-related behaviour (167, 211).

1.6.1 Dopamine D₂ Receptor

The dopamine D₂ receptor can be found in the brain, spinal cord, as well as the systemic arteries (167, 215-217). A single human D₂ receptor gene codes two isoforms by alternative splicing producing a long (D₂L) and short (D₂S) form (218). Evidence suggests D₂L and D₂S receptors may function as post-synaptic receptors and pre-synaptic autoreceptors, respectively (219, 220). In addition, the D₂ receptor can appear in monomeric and dimeric forms with other receptors such as the adenosine A₂A receptor. Specifically, the adenosine A₂A receptors co-localize with the dopamine D₂ receptors in the striatum where they interact at both the receptor level and the second messenger level (221, 222). Indeed, activation of the A₂A receptor decreases agonist affinity at the D₂ receptor while activation of the D₂ receptor is capable of counteracting activation of adenylate cyclase by the A₂A receptor (223-226). Because of these receptor interactions, caffeine antagonizing the adenosine A₂A receptor results in potentiation of dopaminergic signalling via the D₂ receptor (189, 227, 228). These types of interactions are thought to explain much of the motor stimulant effects of caffeine (228), however, dopamine also has a well established role in mood and reward and may be involved in the reinforcing effects of caffeine (189). Indeed, administration of a D₂ receptor antagonist to healthy volunteers produces a negative impact on measures of contentment (229). In addition, research in animals suggests a role for the D₂ receptor in blood pressure regulation (230-232) and, therefore, caffeine’s effects on the D₂ receptor may also have cardiovascular consequences.

1.6.2 Variation in DRD2

The D₂ receptor (DRD2) gene is located on chromosome 11q22-23 and contains 7 exons (233). Many polymorphisms in DRD2 have been identified and investigated, however, few
show strong candidacy for association studies related to caffeine consumption and cardiovascular effects.

A double-blind placebo controlled study investigated the effect of caffeine capsules on subjective anxiety in 102 male and female infrequent caffeine consumers (200). A significant association was identified between caffeine induced anxiety and a polymorphism in intron 6 of the DRD2 gene (rs1110976) after administration of 150 mg of caffeine (200). This polymorphism has only been assessed in one study which showed it to be associated with lower binding potential in post-mortem brain samples and linked to other polymorphisms which also affected receptor binding potential (234). In a sample of 284 older men, a DRD2 NcoI restriction fragment length polymorphism in exon 6 (rs6275) was associated with elevated systolic and diastolic blood pressure independent of obesity and endocrine abnormalities (235). However, there is no evidence of a functional effect of this polymorphism (236). In two samples of Chinese subjects, the TaqIA polymorphism (rs1800497) was associated with increased blood pressure among normoglycaemic subjects (237, 238). Although this polymorphism has been associated with reduced dopaminergic functioning in some studies (239), it actually resides in the ankyrin repeat and kinase domain 1 (ANKK1) gene, which is adjacent to DRD2 (240). However, it has been found to be in strong linkage disequilibrium (LD) with polymorphisms in DRD2 such as C957T (rs6277) in some populations (241-244) and may be a marker of variation in DRD2. The C957T polymorphism is located in exon 7 of DRD2 and has been associated with altered functionality of the D2 receptor. The T allele has been associated with altered mRNA folding, decreased mRNA stability and translation, and reduced receptor up-regulation in response to dopamine in vitro (244). These results were supported by in vivo imaging in men, which showed lower receptor availability, indexed by binding potential, in extra-striatal tissues associated with the TT genotype (245). In contrast, a similar study noted the TT genotype had a higher receptor affinity in the striatum, which the authors hypothesize may be associated with a lower dopamine tone (246). However, a higher functioning of the C allele and CC genotype is also supported by associations with schizophrenia (242, 247-250) and higher reward sensitivity (251). Therefore, the C957T polymorphism in DRD2 is a strong candidate for association with reinforcing behaviours and cardiovascular outcomes such as caffeine intake and MI.
1.7 Serotonin

Serotonin (5-hydroxytryptamine) was discovered and named for its vasoactive properties in the serum (252). Serotonin neurons are concentrated in the raphe nuclei of the brain stem but innervate virtually all brain areas and the spinal cord (253). Neurons produce serotonin in the central nervous system from the essential amino acid tryptophan which competes with other neutral amino acids for transport across the blood-brain barrier via the active amino acid transporter. Tryptophan is converted to 5-hydroxytryptophan by the tryptophan hydroxylase 2 enzyme followed by decarboxylation by amino acid decarboxylase. Serotonin released in the synapse is inactivated by re-uptake via the serotonin transporter and is metabolized by MAO and aldehyde dehydrogenase to form 5-hydroxyindolic acid which is transported out of the brain and excreted in the urine (166, 254).

Peripheral serotonin is primarily produced by action of tryptophan hydroxylase 1 in the enterochromaffin cells of the gastrointestinal tract where it functions in peristalsis and secretory reflexes (255). In addition to uptake of serotonin released in the blood from the gastrointestinal tract, platelets are capable of producing and storing serotonin (256).

There are 7 classes of serotonin receptors (5-HT$_1$-5-HT$_7$) each of which are further subdivided based on their structure and pharmacological properties producing a total of 14 different receptors. All of the serotonin receptors are metabotropic G protein-coupled receptors with the exception of 5-HT$_3$ which activates a ligand-gated ion channel (257). Because of the large number of serotonin receptors that are located throughout the brain and periphery, serotonin has been implicated in a variety of physiological processes including cognition, mood, reward, aggression, nociception, sexual and feeding behaviours, sleep, cardiovascular and temperature regulation, and hormone release (169, 258, 259).

1.7.1 The 5-HT$_{2A}$ Receptor

The serotonin receptor 2A (5-HT$_{2A}$) is of particular interest as it is widely distributed in the brain and has been implicated in the pathophysiology and treatment of several psychiatric disorders (260). The 5-HT$_{2A}$ receptors trigger a cascade of intracellular second messengers that
may result in activation of phospholipases, protein kinases, transcription pathways, and ion channels (261). In the nervous system, 5-HT$_{2A}$ receptors mediate a neuronal response that is generally considered excitatory (262). Activation of these receptors may also modulate the activity of other neurotransmitters such as dopamine, glutamate, and GABA (263, 264).

As caffeine has been shown to increase serotonin release (186-188) and may up-regulate the expression of 5-HT$_2$ receptors in the brain (265, 266), serotonin receptors are possible downstream targets of caffeine action that may mediate effects on mood and the cardiovascular system. In the periphery, the 5-HT$_2A$ receptors are located on coronary smooth muscle cells where they mediate constriction and on platelets where activation enhances the process of platelet aggregation and thrombus formation (267, 268). Indeed, polymorphisms in the gene coding the 5-HT$_2A$ receptor (HTR2A) have already been associated with mood disorders (269, 270) and cardiovascular disease (271, 272); however, they have yet to be investigated in relation to caffeine’s effects and consumption behaviours.

1.7.2 Genetic Variation in HTR2A

The 5-HT$_2A$ receptor is located on chromosome 13q14-21 and contains 3 exons (273, 274). Although several non-synonymous polymorphisms in HTR2A have been identified, these occur at very low frequencies in the populations investigated making them less than ideal for genetic association studies (275). One of the most commonly investigated variants is a synonymous polymorphism which occurs at nucleotide position 102 in the HTR2A gene that results in a C to T substitution (rs6313). The polymorphism was first identified in association with schizophrenia (276, 277). This polymorphism has since been extensively investigated in the psychiatric literature and a meta-analysis has confirmed association of the C allele with schizophrenia (278). In the initial investigations which examined 5-HT$_2A$ receptor expression in small groups of schizophrenic patients and controls, no differences by genotype were found (279, 280). A larger sample of post-mortem frontal cortex tissues of 63 schizophrenic patients and 63 controls also showed no difference in receptor density by genotype (281). However, in another analysis of 35 post-mortem temporal cortex brain samples, a lower expression of the C allele was noted in heterozygotes in comparison to the T allele (282). A similar study examining 23 cortical brain samples showed no differences in expression by genotype (283). Most recently,
an examination of binding kinetics in 63 healthy subjects showed significantly greater receptor binding for the TT genotype (284). Although this polymorphism has been found to be in strong LD with an A to guanine (G) substitution in the promoter region of the gene (rs6311) (280, 283, 285), there is very little evidence the promoter polymorphism has a functional effect on promoter activity (286).

Despite the somewhat controversial findings on the functionality of the T102C polymorphism, there is some evidence this polymorphism may affect cardiovascular risk. Two investigations have found significant associations with the TT genotype and higher platelet aggregation (285, 287). These results were also supported by other investigations which have found significant associations with the TT genotype and MI (272) and stroke (288). These studies support the functional findings which suggest a higher receptor expression and binding associated with the TT genotype (282, 284), which may thus be associated with increased platelet aggregation and risk of cardiovascular events. Since this polymorphism has been associated with cardiovascular events as well as mood disorders (269, 270, 289, 290), it is a prime candidate for assessment of caffeine’s effects. However, no investigations to date have examined this polymorphism in relation to caffeine consumption and caffeine’s cardiovascular effects.

1.8 Hypothesis and Organization of Thesis

This research aims to expand upon the current understanding of caffeine consumption behaviours and caffeine’s cardiovascular effects. As current evidence suggests caffeine’s antagonism of adenosine receptors mediates its subjective effects that may contribute to consumption behaviours, the present studies aim to elucidate downstream targets of adenosine receptor antagonism that may be involved. In addition, recent evidence implicates caffeine in coffee as contributing to increased risk of MI among vulnerable individuals. By investigating downstream targets of caffeine action it may be possible to elucidate potential mechanisms by which caffeine increases MI risk. As dopamine and serotonin are modulated by adenosine and implicated in mood and cardiovascular regulation, receptors in these systems were chosen as targets to investigate. Therefore, investigating polymorphisms in the genes coding these receptors may help clarify these pathways and explain the large amount of variability seen in
caffeine consumption behaviours and studies examining caffeine’s effect on disease risk. Overall, it is hypothesized that genetic variation in the dopamine D₂ and 5-HT₂A receptors may be associated with caffeine consumption behaviours and may modify the association between coffee consumption and MI.

**Objective 1** (Chapter 2): To determine whether the *DRD2* (rs6277) or *HTR2A* (rs6313) polymorphisms affect habitual caffeine intake.

**Objective 2** (Chapter 3): To determine whether the *DRD2* (rs6277) or *HTR2A* (rs6313) polymorphisms modify the association between coffee consumption and risk of MI.
Chapter 2: Effect of Dopamine D$_2$ Receptor (DRD2) and Serotonin Receptor 2A (HTR2A) Genotypes on Habitual Caffeine Consumption

2.1 Abstract

The large variability in caffeine intake and strong heritability of caffeine consumption behaviours has led to the investigation of genetic variants associated with caffeine intake. Caffeine’s acute effects are attributed primarily to antagonism of adenosine receptors which may have downstream consequences on neurotransmitters such as dopamine and serotonin. Therefore, variation in the genes coding for dopamine and serotonin receptors may play a role in caffeine consumption behaviours. The objective of the current study was to determine if the C957T genetic variant in the dopamine D$_2$ receptor (DRD2) or the T102C genetic variant in the serotonin receptor 2A (HTR2A) are associated with caffeine consumption. Subjects are from a Costa Rican case-control study of acute non-fatal myocardial infarction (MI). Caffeine intake was assessed using a food frequency questionnaire. Data and genotype information was available for 2,595 subjects who did not report a history of hypertension. The association between genotype and caffeine consumption categories was examined using the Pearson’s chi-square test. In the entire sample, there was no significant association between DRD2 or HTR2A genotypes and caffeine consumption ($P=0.19$ and $P=0.07$, respectively). The DRD2 polymorphism was significantly associated with caffeine consumption among non-smokers ($P=0.04$) and subjects with the CYP1A2 -163C allele ($P=0.01$). In these groups, there was a higher frequency of the DRD2 CC genotype and a lower frequency of the CT genotype in the higher caffeine consumption categories. The HTR2A polymorphism was significantly associated with caffeine consumption among non-smokers ($P=0.03$) and subjects with the TT genotype for the 1083 C>T polymorphism of ADORA2A ($P=0.005$). In these subgroups, a higher frequency of subjects with the HTR2A C allele and a lower frequency of the TT genotype were observed among the higher caffeine consumption categories. In conclusion, variants in DRD2 and HTR2A are associated with caffeine consumption behaviours in certain subgroups. These results suggest that dopamine and serotonin may play a role in caffeine’s reinforcing effects to influence habitual consumption.
2.2 Introduction

For many individuals caffeine is consumed daily from sources such as coffee, tea, soft drinks, chocolate, energy drinks, and medications. Despite its widespread use, there is a large amount of variability in caffeine consumption behaviours (1, 11). It is generally believed that caffeine is consumed for its psychoactive and stimulating properties and that an individual will adjust consumption to attain the desired effects while avoiding the adverse effects (1, 127). Desirable effects attributed to low or moderate intake levels include effects on mood, energy, alertness, and vigour, which may be mildly reinforcing for some individuals (1-3, 63). Higher intake levels of caffeine have been reported to produce adverse effects such as anxiety, jitteriness, and nervousness (1-3, 136), which may discourage further intake. There is disagreement as to whether there are positive reinforcing effects with habitual consumption or whether these effects are limited to the reversal of negative withdrawal effects that have been reported such as headache, fatigue, depressed mood, and drowsiness (134, 135). In either case, there seems to be a large amount of variability in caffeine’s effects which may be, in part, explained by genetic differences. Twin studies have revealed a strong heritability for caffeine and coffee consumption behaviours in the range of 30-77% (291-298). Therefore, genetic variation may play a role in the experience of caffeine’s effects and motivation for consumption.

Caffeine antagonizes adenosine A_1 and A_2A receptors (8-10), thereby inhibiting the modulating function of adenosine on neurotransmitters such as dopamine and serotonin (1, 11). The resulting changes in neurotransmitters may be responsible for some of caffeine’s acute effects. Therefore, variation in the genes coding for the adenosine receptors have been examined in relation to caffeine’s effects and consumption behaviours.

In a Costa Rican population, it was shown that a cytosine (C) to thymine (T) change at position 1083 in the adenosine A_2A receptor (ADORA2A) gene was associated with habitual caffeine consumption (208). Specifically, subjects consuming >200 mg caffeine per day were significantly less likely to have the variant TT genotype in comparison to subjects consuming <100 mg per day. These results are supported by two double-blind placebo-controlled studies in which subjects with the TT genotype reported greater anxiety after administration of 150 mg of caffeine (200, 201). In a more recent study, subjects with the TT genotype also reported significantly greater caffeine-induced anxiety, but only those who were non- or infrequent-
consumers of caffeine (199). However, there was no significant difference in caffeine consumption by genotype, but subjects with the TT genotype consumed significantly more coffee (199). These results do not support previous findings on ADORA2A genotype and caffeine consumption (208); however, smoking habits and mean age, in addition to the frequency of the ADORA2A TT genotype were different between these study populations (199, 208). As smoking and age are independently associated with caffeine consumption (299-301), they may also contribute to differences in response to caffeine and motivation for consumption. For example, Cornelis et al reported stronger associations between ADORA2A genotype and caffeine consumption among smokers, whereas Rogers et al excluded individuals smoking more than 5 cigarettes per day from their study (199, 208). Therefore, these differences in the populations examined may explain the inconsistent findings between these two studies. Overall, these results support a role for genetic variation in adenosine receptors in the variability of caffeine’s acute effects and caffeine consumption. However, caffeine’s antagonism of adenosine receptors has downstream effects on several neurotransmitters including serotonin and dopamine, which may contribute to the subjective effects of caffeine and thus may also affect consumption. Indeed, variants in the dopamine D2 receptor (DRD2) gene have also been associated with caffeine induced anxiety independently and in combination with ADORA2A variants (200).

The present investigation seeks to expand upon the current knowledge of genetic variation and caffeine consumption behaviours by examining variants in the dopaminergic and serotonergic genes. A common variant in DRD2 at position 957 results in a C to T change in exon 7, which has been shown to reduce receptor production in vitro (244) and receptor availability in extrastriatal tissues in vivo (245). A common variant in the serotonin 2A (5-HT2A) receptor (HTR2A) gene results in a C to T change at position 102 of exon 1 and has been associated with higher receptor density, expression, and protein levels (282, 284). No published study to date has examined the role of these polymorphisms, or any polymorphism in the DRD2 and HTR2A genes, on habitual caffeine consumption. The DRD2 957 C>T and HTR2A 102 C>T polymorphisms will be examined in relation to caffeine intake independently and in analyses stratified by smoking status, a cytochrome P450 1A2 (CYP1A2) polymorphism affecting caffeine metabolism, and the ADORA2A 1083 C>T polymorphism.
2.3 Methods

2.3.1 Study Design and Participants

Details of methods and study design have been reported previously (7, 208). Data come from a Costa Rican case-control study of acute non-fatal myocardial infarction (MI). Subjects were recruited from 6 local hospitals in the Central Valley of Costa Rica. The catchment area included 36 counties representing rural, periurban, and urban lifestyles and a wide range of socioeconomic levels. Study fieldworkers visited hospitals daily for maximum ascertainment of eligible MI cases. Eligibility for study participation included diagnosis of a first acute MI by 2 independent cardiologists. MI was defined according to the World Health Organization criteria of typical symptoms with elevations in cardiac enzymes or changes in the electrocardiogram. Cases were ineligible if they were physically or mentally unable to complete the study protocol, died during hospitalization, or had a previous hospital admission related to cardiovascular disease (CVD). A population based control was randomly selected for each case using National Census information from the Statistics Bureau of Costa Rica. Controls were matched for age (± 5 years), sex, and county of residence and selected within 1 week of cases. Eligible controls were physically and mentally able to complete the study protocol and had no previous hospital admission related to CVD. Because of the comprehensive social services available in Costa Rica, all subjects have access to medical care making it unlikely for there to be a high degree of undiagnosed CVD in the control population.

Data were collected between 1994 and 2004 for cases and controls. Participation rate of eligible cases was 98% and for eligible controls was 88%. All data were collected in the homes of the participants by trained study fieldworkers and included collection of biological specimens, medical history, diet, and anthropometric measurements. General questionnaires used by study fieldworkers included questions concerning age, gender, education level, and annual household income. Questionnaires also queried smoking status (never smoker, ex-smoker, and current smoker), number of cigarettes smoked per day, age when began smoking, and age when quit smoking (for ex-smokers). Only questions related to cigarette smoking were included in questionnaires because use of other tobacco products is not common in this
population. Subjects were asked the average frequency and time spent on several occupational and leisure time physical activities over the past year. All activities were grouped into 6 categories based on intensity or metabolic equivalents (METS). One METS is considered the energy expenditure during quiet sitting or approximately 1 kcal per kg body weight per hour (302). For each activity, frequency, time, and intensity (METS) were multiplied to determine energy expenditure. The physical activity component of the questionnaires was validated by comparing its ability to predict fitness measured by the Harvard step test, plasma lipids, and obesity in another group of subjects from Puriscal, Costa Rica (303, 304). Subjects were also asked to self-report history of diabetes and hypertension. Validation of self-report against standard diagnostic criteria in this population showed the reliability of self-report to be high (305). All anthropometric measurements were taken in duplicate with subjects wearing light clothing and without shoes. The average of the two measurements was used in analyses. Measurement of weight was taken on a Detecto bathroom scale that was calibrated biweekly. Non-stretching fibreglass metal tapes were used to measure waist circumference (smallest horizontal trunk circumference) and hip circumference (largest horizontal circumference around the buttocks and hip). A 135-item semi-quantitative food frequency questionnaire (FFQ) was used to collect information on dietary intake over the past year (year prior to MI for cases). This FFQ was modified from the Willett and developed and validated for the Costa Rican population (306). The United States Department of Agriculture (USDA) food composition data file supplemented with data from publications and personal communications with Costa Rican institutions were used to calculate nutrient intakes including caffeine consumption. Subjects reported intakes of all sources of caffeine including coffee, tea, chocolate, and colas. Information on coffee consumption for example, was reported as one of 9 frequencies of consumption of a standard portion of 1 cup of coffee (250 mL). Subjects were also asked to report coffee preparation method. Assessment of caffeine intake was found to be both reliable and valid when comparing administration of the FFQ twice 1 year apart (correlation coefficient, r=0.77) and comparing the average of 2 FFQ interviews to seven 24-hour recalls (correlation coefficient, r=0.83) (306).

All subjects gave written informed consent. The study received ethics approval from the Harvard School of Public Health, the University of Costa Rica, the University of Toronto, and the Office of Protection from Research Risk at the National Institutes of Health.
2.3.2 Genotyping

Subjects provided a fasting blood sample when visited in their homes by study fieldworkers. DNA was isolated from buffy-coats using standard procedures. All subjects were genotyped using real-time polymerase chain reaction (PCR) for the \textit{DRD2} (rs6277) and \textit{HTR2A} (rs6313) variants. Real-time PCR TaqMan® SNP Genotyping Assays for both polymorphisms were purchased from Applied Biosystems. Of all subjects with DNA available, 96% and 94% were successfully genotyped for the \textit{DRD2} and \textit{HTR2A} polymorphism, respectively. The distribution of the genotypes did not deviate from Hardy-Weinberg equilibrium among cases or controls (Pearson $\chi^2$, 1 df, $P>0.05$). The \textit{CYP1A2} -163 A>C (rs762551) and \textit{ADORA2A} 1083 C>T (rs5751876) polymorphisms were previously assessed in this population using restriction fragment length polymorphism-polymerase chain reaction and are described elsewhere (7, 208, 307).

2.3.3 Statistical Analysis

A total of 4,369 subjects had DNA available for analysis. Subjects were excluded if they did not have complete information on caffeine intake (n=62, 1%), were not successfully genotyped for the polymorphisms in \textit{DRD2} (n=104, 2%), \textit{HTR2A} (n=110, 3%), \textit{CYP1A2} (n=108, 3%), or \textit{ADORA2A} (n=36, 1%), or were missing information on variables of interest (smoking status and history of high hypertension, n=13, 0.3%). Subjects who reported a history of hypertension (n=1,341, 31%) were excluded from the analysis because they were likely advised to limit their caffeine intake (208). Indeed, subjects with a history of hypertension were significantly less likely to report caffeine intakes >400 mg/day (208). Subjects were not excluded based on case status as caffeine intake collected via FFQ reflects intake in the year preceding MI. Therefore, a total of 2,595 subjects were utilized in the current analyses.

Differences in subject characteristics by genotype were examined using the Pearson’s chi-square test for categorical variables and analysis of variance (ANOVA) for continuous variables. Caffeine consumption was categorized into 4 groups of intake based on FFQ data for all dietary sources of caffeine (<100, 100-200, >200-400, or >400 mg/day). Caffeine consumption categories were compared by \textit{DRD2} and \textit{HTR2A} genotypes using the Pearson’s
chi-square test. Analyses were stratified by variants in \textit{CYP1A2} (AA versus AC & CC genotypes) and \textit{ADORA2A} (CC & CT versus TT genotype) and smoking status (never smoker & past-smoker versus current smoker).

2.4 Results

Subject characteristics by \textit{DRD2} and \textit{HTR2A} genotype are reported in Table 2-1. There was a significant association between the \textit{DRD2} genotypes and education status (Pearson’s chi-square $P=0.004$). There were no other significant differences in subject characteristics by \textit{DRD2} genotype or \textit{HTR2A} genotype.
Table 2-1: Subject Characteristics by *DRD2* and *HTR2A* Genotype

<table>
<thead>
<tr>
<th>Subject Characteristic</th>
<th><em>DRD2 957 C&gt;T</em></th>
<th><em>HTR2A 102 C&gt;T</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC n=1,242</td>
<td>CT n=1,106</td>
</tr>
<tr>
<td><strong>Socio-demographics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y), mean ± SD</td>
<td>56.8 ± 11.1</td>
<td>57.1 ± 11.6</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>1002 (81)</td>
<td>893 (81)</td>
</tr>
<tr>
<td>Urban Residence, n (%)</td>
<td>904 (73)</td>
<td>811 (73)</td>
</tr>
<tr>
<td>Income (US$/mo), mean ± SD</td>
<td>529 ± 392</td>
<td>551 ± 414</td>
</tr>
<tr>
<td>Secondary education or greater, n (%)</td>
<td>474 (38)</td>
<td>451 (41)</td>
</tr>
<tr>
<td><strong>Medical History</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI, mean ± SD</td>
<td>25.5 ± 3.7</td>
<td>25.6 ± 3.9</td>
</tr>
<tr>
<td>Current Smoker, n (%)</td>
<td>427 (34)</td>
<td>402 (36)</td>
</tr>
<tr>
<td>Current alcohol consumption, n (%)</td>
<td>527 (42)</td>
<td>468 (42)</td>
</tr>
<tr>
<td>Physical Activity (METs), mean ± SD</td>
<td>1.59 ± 0.73</td>
<td>1.61 ± 0.75</td>
</tr>
<tr>
<td>History of Diabetes, n (%)</td>
<td>155 (12)</td>
<td>138 (12)</td>
</tr>
<tr>
<td>Total Energy (kcal), mean ± SD</td>
<td>2603 ± 834</td>
<td>2597 ± 877</td>
</tr>
<tr>
<td><strong>Genotypes, n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>ADOR2A</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC &amp; CT</td>
<td>881 (71)</td>
<td>759 (69)</td>
</tr>
<tr>
<td>TT</td>
<td>361 (29)</td>
<td>347 (31)</td>
</tr>
<tr>
<td><em>CYP1A2</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>550 (44)</td>
<td>522 (47)</td>
</tr>
<tr>
<td>AC &amp; CC</td>
<td>692 (56)</td>
<td>584 (53)</td>
</tr>
<tr>
<td><em>HTR2A</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>447 (36)</td>
<td>383 (35)</td>
</tr>
<tr>
<td>CT</td>
<td>578 (47)</td>
<td>525 (47)</td>
</tr>
<tr>
<td>TT</td>
<td>217 (17)</td>
<td>198 (18)</td>
</tr>
</tbody>
</table>

Abbreviations: METs, metabolic equivalent tasks; SD, standard deviation.
† Pearson’s chi-square P=0.004.
The frequency of DRD2 genotypes by caffeine consumption category is presented in Table 2-2. There was no association between DRD2 genotype and caffeine consumption in the entire sample or in analyses stratified by education status (data not shown). There was a significant association when examining non-smokers and subjects with the CYP1A2 AC & CC genotypes only who are considered ‘slow metabolizers’ of caffeine. In both sub-groups, the frequency of the DRD2 CC genotype increases while the frequency of the CT genotype decreases with increasing caffeine consumption. This pattern can be seen in Figure 2-1 where the frequency of DRD2 genotypes by caffeine consumption category is shown for non-smokers with the CYP1A2 AC & CC genotypes. The pattern among subjects with the TT genotype is less clear. Analysis stratified by ADORA2A genotype showed no significant association between DRD2 genotype and caffeine consumption category.

The frequency of HTR2A genotypes by caffeine consumption category is presented in Table 2-3. There was no association between HTR2A genotypes and caffeine consumption in the entire sample. However, there was a significant association among non-smokers and subjects with the ADORA2A TT genotype. Within each of these subgroups, the frequency of the HTR2A CC and CT genotypes increases while the frequency of the TT genotype decreases with increasing caffeine consumption. The distribution of the HTR2A genotypes by caffeine consumption category among non-smokers with the ADORA2A TT genotype is shown in Figure 2-2. There was no significant association between HTR2A genotypes and caffeine consumption in analyses stratified by CYP1A2 genotype.
Table 2-2: Frequency of *DRD2* Genotype by Caffeine Consumption Category

<table>
<thead>
<tr>
<th>Caffeine Consumption (mg/day)</th>
<th><em>DRD2</em> Genotype</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC</td>
<td>CT</td>
</tr>
<tr>
<td>Total Sample</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td>98 (41)</td>
<td>229 (50)</td>
</tr>
<tr>
<td>100-200</td>
<td>172 (46)</td>
<td>157 (42)</td>
</tr>
<tr>
<td>&gt;200-400</td>
<td>698 (49)</td>
<td>606 (42)</td>
</tr>
<tr>
<td>&gt;400</td>
<td>274 (50)</td>
<td>224 (41)</td>
</tr>
<tr>
<td>n=2,595, <em>P</em>=0.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smokers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td>79 (41)</td>
<td>99 (51)</td>
</tr>
<tr>
<td>100-200</td>
<td>135 (46)</td>
<td>124 (42)</td>
</tr>
<tr>
<td>&gt;200-400</td>
<td>479 (50)</td>
<td>397 (41)</td>
</tr>
<tr>
<td>&gt;400</td>
<td>122 (53)</td>
<td>84 (37)</td>
</tr>
<tr>
<td>n=1,681, <em>P</em>=0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smokers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td>19 (43)</td>
<td>20 (45)</td>
</tr>
<tr>
<td>100-200</td>
<td>37 (49)</td>
<td>33 (43)</td>
</tr>
<tr>
<td>&gt;200-400</td>
<td>219 (46)</td>
<td>209 (44)</td>
</tr>
<tr>
<td>&gt;400</td>
<td>152 (47)</td>
<td>140 (43)</td>
</tr>
<tr>
<td>n=914, <em>P</em>=0.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>ADORA2A</em> CC &amp; CT</td>
<td></td>
<td></td>
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<tr>
<td>&lt;100</td>
<td>61 (42)</td>
<td>71 (48)</td>
</tr>
<tr>
<td>100-200</td>
<td>117 (47)</td>
<td>109 (43)</td>
</tr>
<tr>
<td>&gt;200-400</td>
<td>500 (49)</td>
<td>422 (41)</td>
</tr>
<tr>
<td>&gt;400</td>
<td>203 (51)</td>
<td>157 (39)</td>
</tr>
<tr>
<td>n=1,816, <em>P</em>=0.57</td>
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<td></td>
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<tr>
<td><em>ADORA2A</em> TT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td>37 (41)</td>
<td>48 (53)</td>
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<td>100-200</td>
<td>55 (44)</td>
<td>48 (40)</td>
</tr>
<tr>
<td>&gt;200-400</td>
<td>198 (47)</td>
<td>184 (44)</td>
</tr>
<tr>
<td>&gt;400</td>
<td>71 (47)</td>
<td>67 (45)</td>
</tr>
<tr>
<td>n=779, <em>P</em>=0.22</td>
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<tr>
<td><em>CYP1A2</em> AA</td>
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</tr>
<tr>
<td>&lt;100</td>
<td>44 (43)</td>
<td>55 (53)</td>
</tr>
<tr>
<td>100-200</td>
<td>83 (47)</td>
<td>81 (46)</td>
</tr>
<tr>
<td>&gt;200-400</td>
<td>324 (49)</td>
<td>277 (42)</td>
</tr>
<tr>
<td>&gt;400</td>
<td>99 (42)</td>
<td>109 (47)</td>
</tr>
<tr>
<td>n=1,173, <em>P</em>=0.13</td>
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</tr>
<tr>
<td><em>CYP1A2</em> AC &amp; CC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td>54 (40)</td>
<td>64 (47)</td>
</tr>
<tr>
<td>100-200</td>
<td>89 (46)</td>
<td>76 (39)</td>
</tr>
<tr>
<td>&gt;200-400</td>
<td>374 (48)</td>
<td>329 (42)</td>
</tr>
<tr>
<td>&gt;400</td>
<td>175 (55)</td>
<td>115 (36)</td>
</tr>
<tr>
<td>n=1,422, <em>P</em>=0.01</td>
<td></td>
<td></td>
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</tbody>
</table>
Table 2-3: Frequency of HTR2A Genotype by Caffeine Consumption Category

<table>
<thead>
<tr>
<th>Caffeine Consumption (mg/day)</th>
<th>HTR2A Genotype</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC</td>
<td>CT n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Sample</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td>78 (33)</td>
<td>103 (43)</td>
<td>57 (24)</td>
<td></td>
</tr>
<tr>
<td>100-200</td>
<td>132 (35)</td>
<td>178 (48)</td>
<td>62 (17)</td>
<td></td>
</tr>
<tr>
<td>&gt;200-400</td>
<td>525 (37)</td>
<td>667 (47)</td>
<td>242 (17)</td>
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</tr>
<tr>
<td>&gt;400</td>
<td>182 (33)</td>
<td>282 (51)</td>
<td>87 (16)</td>
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<td>n=2,595, P=0.07</td>
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<tr>
<td>Non-smokers</td>
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<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td>62 (32)</td>
<td>83 (43)</td>
<td>49 (25)</td>
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<tr>
<td>100-200</td>
<td>104 (35)</td>
<td>143 (48)</td>
<td>49 (17)</td>
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</tr>
<tr>
<td>&gt;200-400</td>
<td>365 (38)</td>
<td>446 (46)</td>
<td>151 (16)</td>
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<td>85 (37)</td>
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<td>30 (13)</td>
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<td>n=1,681, P=0.03</td>
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<tr>
<td>Smokers</td>
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<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td>16 (36)</td>
<td>20 (45)</td>
<td>8 (18)</td>
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</tr>
<tr>
<td>100-200</td>
<td>28 (37)</td>
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<td>13 (17)</td>
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</tr>
<tr>
<td>&gt;200-400</td>
<td>160 (34)</td>
<td>221 (47)</td>
<td>91 (19)</td>
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<tr>
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<td>168 (52)</td>
<td>57 (18)</td>
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<td>n=914, P=0.80</td>
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</tr>
<tr>
<td>ADORA2A CC &amp; CT</td>
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<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td>55 (37)</td>
<td>60 (41)</td>
<td>32 (22)</td>
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</tr>
<tr>
<td>100-200</td>
<td>83 (33)</td>
<td>122 (49)</td>
<td>46 (18)</td>
<td></td>
</tr>
<tr>
<td>&gt;200-400</td>
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<td>471 (46)</td>
<td>184 (18)</td>
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</tr>
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<td>199 (50)</td>
<td>71 (18)</td>
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</tr>
<tr>
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<td>ADORA2A TT</td>
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</tr>
<tr>
<td>&lt;100</td>
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<td>43 (47)</td>
<td>25 (27)</td>
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<tr>
<td>100-200</td>
<td>49 (41)</td>
<td>56 (46)</td>
<td>16 (13)</td>
<td></td>
</tr>
<tr>
<td>&gt;200-400</td>
<td>163 (39)</td>
<td>196 (47)</td>
<td>58 (14)</td>
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</tr>
<tr>
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<td>51 (34)</td>
<td>83 (55)</td>
<td>16 (11)</td>
<td></td>
</tr>
<tr>
<td>n=779, P=0.005</td>
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<tr>
<td>CYP1A2 AA</td>
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<tr>
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<td>48 (47)</td>
<td>24 (23)</td>
<td></td>
</tr>
<tr>
<td>100-200</td>
<td>55 (31)</td>
<td>89 (50)</td>
<td>33 (19)</td>
<td></td>
</tr>
<tr>
<td>&gt;200-400</td>
<td>238 (36)</td>
<td>306 (46)</td>
<td>115 (17)</td>
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<td>80 (34)</td>
<td>122 (52)</td>
<td>32 (14)</td>
<td></td>
</tr>
<tr>
<td>n=1,173, P=0.31</td>
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<td></td>
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</tr>
<tr>
<td>CYP1A2 AC &amp; CC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td>47 (35)</td>
<td>55 (41)</td>
<td>33 (24)</td>
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<tr>
<td>100-200</td>
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</tr>
<tr>
<td>&gt;200-400</td>
<td>287 (37)</td>
<td>361 (47)</td>
<td>127 (16)</td>
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<tr>
<td>&gt;400</td>
<td>102 (32)</td>
<td>160 (50)</td>
<td>55 (17)</td>
<td></td>
</tr>
<tr>
<td>n=1,422, P=0.16</td>
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</tbody>
</table>
Figure 2-1: Frequency of DRD2 genotype by caffeine consumption category among non-smokers with the CYP1A2 AC & CC genotype (n=916, Pearson’s chi-square $P=0.005$).
Figure 2-2: Frequency of HTR2A genotype by caffeine consumption category among non-smokers with the ADORA2A TT genotype (n=502, Pearson’s chi-square P<0.0001).
2.5 Discussion

In the present study, significant associations between DRD2 and HTR2A genotypes and caffeine consumption were seen only among subgroups in the population. The DRD2 genotype was significantly associated with caffeine consumption among non-smokers and subjects with slow caffeine metabolism (CYP1A2 AC & CC genotypes) only. The results showed a higher frequency of subjects with the DRD2 CC genotype and a lower frequency of subjects with the DRD2 CT genotype among the higher caffeine consumption categories. The patterns in the data for the TT genotype are less clear; however, there was a low frequency of this genotype in the population. As the T allele has been associated with reduced receptor production (244) and availability (245), these subjects may be less sensitive while subjects with the CC genotype may be more sensitive to caffeine’s effects. Since there were more subjects with the CC genotype in the higher caffeine consumption categories, it appears that some of caffeine’s reinforcing effects may be mediated through the dopamine D2 receptor. In an investigation of reward sensitivity, DRD2 and binge eating disorder, normal weight controls (n=59) with the CC genotype had significantly higher reward sensitivity as measured by a validated self-report questionnaire compared to those with CT and TT genotypes (251). Therefore, subjects with the CC genotype in the present study may be more sensitive to caffeine’s rewarding and reinforcing effects thereby consuming larger amounts of caffeine. This could explain the higher proportion of CC genotypes in the highest category of caffeine consumption.

A significant association between DRD2 genotype and caffeine consumption was limited to non-smokers and subjects with the CYP1A2 AC & CC genotypes. Interestingly, both these subgroups have relatively slower rates of caffeine metabolism compared to smokers and those with the CYP1A2 AA genotype. Indeed, tobacco smoking induces the CYP1A2 enzyme and results in a faster rate of caffeine metabolism compared to non-smokers (104, 116). The CYP1A2 AC & CC genotypes have also been associated with a slower rate of caffeine metabolism compared to the AA genotype (121-123). Therefore, these subgroups may represent a prolonged exposure to caffeine in comparison to smokers and subjects with the CYP1A2 AA genotype. As such, the prolonged level of exposure to caffeine may be required for significant reinforcing effects to be mediated by the dopamine D2 receptor, thereby unmasking the effect of this polymorphism.
It is important to note that education status was significantly associated with \textit{DRD2} genotype and higher education is associated with lower caffeine consumption in this population (data not shown). However, if education were driving the association between \textit{DRD2} genotype and caffeine consumption then we would expect to see similar associations between \textit{DRD2} genotype and caffeine consumption as there are for education and caffeine consumption. However, \textit{DRD2} genotype was not significantly associated with caffeine consumption in the entire population. In addition, the association between \textit{DRD2} genotype and education status appears to be driven by large differences in the TT genotype group whereas different patterns in consumption are seen between the CC and CT genotype groups. Therefore, it is not likely significant associations seen among non-smokers and subjects with the \textit{ADORa2A} TT genotype are being confounded by education status.

Although no significant association between \textit{HTR2A} genotype and caffeine consumption was found in the entire sample, significant associations were identified among non-smokers and subjects with the \textit{ADORa2A} TT genotype. There was a higher frequency of subjects with the C allele, and a lower frequency for those with the TT genotype, for \textit{HTR2A} among the higher caffeine consumption categories. Examination of the functionality of this polymorphism has associated the C allele in some studies with reduced receptor production (282, 284). Therefore, these subjects may be less sensitive to caffeine’s effects on the serotonergic system and may seek larger doses to achieve the desired effects. The C allele has been associated with panic disorder (269, 289) as well as the severity of panic disorder symptoms (308), although several other studies have found no associations (309-312). Therefore, the C allele may represent a greater susceptibility to caffeine-induced anxiety, which may negatively reinforce caffeine intake. However, in the present study, subjects with the C allele tended to be heavier caffeine consumers. Similarly, Rogers and colleagues found subjects with the \textit{ADORa2A} TT genotype reported higher caffeine-induced anxiety, but surprisingly, also heavier coffee consumption (199). The authors suggest that other unidentified benefits may outweigh the reported anxiety or alternatively the reported anxiety may be interpreted positively as excitement, for example (199). Further research will be needed to determine how caffeine’s effects are altered by the \textit{HTR2A} polymorphism.

Associations limited to the \textit{ADORa2A} TT genotype are not surprising as it has been associated with consumption (208) and heightened sensitivity to caffeine’s effects (199-201). In
addition, caffeine’s effects on the serotonergic system are likely a downstream effect of adenosine receptor antagonism and, therefore, interactions between ADORA2A and HTR2A would be anticipated. However, it was previously found that the association between caffeine consumption and the ADORA2A genotype was stronger among current smokers as opposed to non-smokers, although trends within each group were similar and the caffeine x smoking interaction was not significant (208). Here, however, results were limited to non-smokers and is not likely explained by a faster caffeine metabolism as stratification by the CYP1A2 genotype did not yield any significant associations. Instead, this may be a result of smokers’ caffeine consumption behaviours being less motivated by caffeine’s effects and possibly more motivated by conditioned associations between use of the two substances (313). Therefore, caffeine consumption by non-smokers may be more motivated by the reinforcing effects of the substance than among smokers. Also, it is interesting that both non-smokers and subjects with the ADORA2A TT genotype consume less caffeine than smokers and subjects with the ADORA2A C allele, respectively (208), and therefore may be more sensitive to caffeine’s effects at lower doses. It is perhaps only among these individuals that the reinforcing effects of caffeine mediated through the 5-HT2A receptor, which is encoded by the HTR2A gene, are unmasked.

Overall, significant associations were found between caffeine intake and HTR2A and DRD2 genotype in subgroup analyses. The results suggest that each of these receptors play a role in the reinforcing properties of caffeine consumption among certain susceptible groups in the population. There is currently no published data on the role these polymorphisms play in caffeine consumption behaviours; however, the current results suggest that these polymorphisms could be potential genetic confounders in observational studies which associate caffeine intake to disease. Similarly, studies which have examined the role of these polymorphisms on development of disease may be confounded by their association to caffeine consumption. Replication and further investigation will be needed to confirm these findings in other populations and to identify the specific role each of these polymorphisms play in caffeine’s effects.
3.1 Abstract

Cytochrome P450 1A2 (CYP1A2) has been shown to modify the relationship between coffee consumption and myocardial infarction (MI), which implicates caffeine in this association. Caffeine may exert some of its physiological effects through neurotransmitters such as dopamine and serotonin. The objective of the current study was to determine if the C957T or T102C variants in the genes coding the dopamine D2 receptor (DRD2) or serotonin receptor 2A (HTR2A), respectively, modify the association between coffee consumption and MI, particularly among subjects with slow caffeine metabolism (carriers of the -163C allele of CYP1A2). Subjects are from a Costa Rican matched case-control study of acute non-fatal MI. Cases were matched to controls for age, sex, and area of residence. Dietary information including coffee consumption was collected using a 135-item semi-quantitative food frequency questionnaire. Data and genotype information was available for 1,824 matched pairs. Multivariate conditional and unconditional logistic regression was used to assess the relationship between coffee consumption and MI in the whole sample and in stratified analyses, respectively. In the entire sample, the odds ratio (OR) and 95% confidence interval (CI) for consumption of 1, 2-3, and ≥4 cups/d of coffee compared to <1 cup/d were 0.92 (0.68-1.24), 1.10 (0.86-1.40), and 1.33 (0.99-1.80), respectively. No coffee x gene interaction was observed for either DRD2 (P=0.97) or HTR2A (P=0.46). When the population was stratified by CYP1A2 genotype, there was a significant coffee x HTR2A interaction (P=0.001) among subjects with slow caffeine metabolism. Compared to <1 cup/d of coffee, the OR (95%CI) for 1, 2-3 and ≥4 cups/d was 1.24 (0.61-2.51), 2.02 (1.16-3.52), and 3.13 (1.55-6.31), respectively, for subjects with the combined -163C allele for CYP1A2 and CC genotype for HTR2A. In conclusion, only subjects with slow caffeine metabolism and the HTR2A T102C CC genotype are at significantly increased risk of MI associated with coffee consumption. These results suggest that caffeine in coffee may trigger an MI through the serotonergic system.
3.2 Introduction

Although caffeine and its primary dietary source, coffee, have been the subject of extensive research, it is still unclear what effect coffee has on cardiovascular diseases such as myocardial infarction (MI). Indeed, the relationship may be much more complex as J- (36, 37) and U-shaped (22, 27, 35) associations have been described. Genetic differences between the populations studied could also explain some of the inconsistencies reported (7, 314). Consumption of 4 or more cups of coffee per day has been associated with a significantly increased risk of MI only among individuals who are carriers of a variant in the cytochrome P450 1A2 (CYP1A2) gene (7). CYP1A2 is the primary enzyme responsible for caffeine metabolism in the body and an A>C polymorphism at nucleotide position -163 results in lower enzyme inducibility and impaired caffeine metabolism (121-123). The modifying effect of this polymorphism was later supported by a prospective study of incident physician-diagnosed hypertension in an Italian population (6). Moderate and heavy coffee consumers with the variant CYP1A2 -163C allele showed significantly increased risk of developing hypertension in comparison to abstainers, whereas subjects with the CYP1A2 AA genotype were significantly protected (6). Because caffeine is the only major compound in coffee known to be detoxified by the CYP1A2 enzyme (5), these studies implicate caffeine and suggest only subjects with “slow” caffeine metabolism are at increased risk (7).

At levels normally consumed in the diet, caffeine’s main mechanism of action involves antagonism of adenosine receptors (8-10). As adenosine is an important neuromodulator that can inhibit synaptic transmission and release of neurotransmitters, caffeine has indirect effects on neurotransmitters such as dopamine and serotonin (1, 11). It is possible that the effects of caffeine on neurotransmitters may be responsible for some of the cardiovascular outcomes associated with caffeine consumption.

Dopamine and serotonin are both monoamine neurotransmitters that have important roles in cardiovascular regulation. Dopamine may play a role in blood pressure regulation (315) and experiments in animal models suggest an important role of the dopamine D2 receptor (230-232). These receptors have been localized to various regions of the cardiovascular system and brain (216, 217). A C>T polymorphism at position 957 in the dopamine D2 receptor (DRD2) gene has been associated with reduced mRNA stability and receptor production in vitro (244)
and reduced extra-striatal binding potential \textit{in vivo} (245). This polymorphism might, therefore, modify the effects of caffeine on cardiovascular disease.

The serotonin receptor 2A (5-HT\textsubscript{2A}) plays an important role in the cardiovascular system as it is expressed in platelets (316) and coronary arteries (267, 317). Activation of the 5-HT\textsubscript{2A} receptors on platelets enhances coagulation (318) while activation of 5-HT\textsubscript{2A} receptors in the coronary arteries results in contraction (267). The 5-HT\textsubscript{2A} receptor gene (HTR2A) has a common variant at position 102 that results in a C>T substitution in which the T allele has been associated with higher receptor density on platelets of healthy controls (284) and increased expression and mRNA and protein levels in post-mortem brain samples of the temporal cortex (282). Although other studies have reported no differences in receptor expression and density by the T102C polymorphism, the TT genotype has been associated with platelet aggregation (285, 287), MI (272), and stroke (288), supporting the higher expression and density of the T allele.

There is currently a very limited understanding of caffeine’s effects on risk of MI. A previous investigation suggests genetic variation that affects rate of caffeine metabolism may modify the association between coffee consumption and risk of MI (7); however, the mechanism by which caffeine contributes risk remains unknown. An improved understanding of the genetic susceptibility to risk of MI associated with coffee consumption may help elucidate mechanisms as well as the development of more targeted interventions and advice for prevention. The objective of this study was to determine whether genetic variation in \textit{DRD2} or \textit{HTR2A} modifies the association between coffee consumption and risk of MI, particularly among subjects with slow caffeine metabolism.

### 3.3 Material and Methods

#### 3.3.1 Study Design and Participants

Refer to Chapter 2 (pages 28 – 39).
3.3.2 Genotyping

Refer to Chapter 2 (page 30).

3.3.3. Statistical Analysis

All statistical analyses were conducted in SAS version 9.2 (SAS Institute Inc, Cary, NC). A total of 2,113 cases and 2,256 controls had DNA available for analysis (n=4,369). Subjects were excluded from the analysis if they could not be genotyped for the DRD2, HTR2A, or CYP1A2 polymorphism (n=376, 9%), were missing data on confounding variables (n=50, 1%), or became unmatched as a result of the above exclusions (n=295, 7%). Therefore, 1,824 matched pairs were available for analyses. Nutrient intakes of subjects were adjusted for energy using the residual method as previously applied to this population (7, 306). To assess differences in subject characteristics between matched pairs, the McNemar test was used for categorical variables and the paired t-test or Wilcoxon signed rank test for parametric and non-parametric continuous variables, respectively. Conditional logistic regression was used to assess risk of MI associated with coffee consumption in the entire sample. All stratified analyses were performed using unconditional logistic regression with matching variables (age, sex, and area of residence) in the model to maximize the number of subjects retained in the analyses. Risk of MI is represented by odds ratios (ORs) and 95% confidence intervals (CIs). A multivariate logistic regression model of risk of MI associated with coffee consumption was previously constructed for this sample using the likelihood ratio test (7). The same model is used here which includes adjustment for smoking status (never, past, 1-19 cigarettes/day, and ≥20 cigarettes/day), alcohol consumption (never, past, and tertiles of intake among current consumers), dichotomous variables representing history of diabetes and history of hypertension, quintiles of waist-to-hip ratio, physical activity, income, and total energy intake, and quintiles of energy-adjusted folate, sucrose, saturated fat, polyunsaturated fat, and trans fat intake (7). Analyses were stratified by DRD2 and HTR2A genotype using an additive model (CC, CT, and TT genotype groups). Gene x coffee interaction was tested using the –2 log (likelihood) ratio test, comparing a multivariate logistic model with main effects of genotype and coffee and a model with the addition of their multiplicative interaction term. CYP1A2 genotype was analyzed using a dominant C allele
model (AA versus AC & CC) as carriers of the C allele have been reported to have a comparable rate of caffeine metabolism (121-123). This allows the comparison of subjects with “fast” versus “slow” caffeine metabolism as previously examined (7). Gene x coffee interaction was tested within each of the two CYP1A2 genotype groups. A $P$ value <0.05 was considered significant for all interactions.

3.4 Results

Subject characteristics were compared between matched pairs and are presented in Table 3-1. All characteristics considered were significantly different between matched cases and controls with the exception of genotypes ($P=0.6$, $P=0.4$, and $P=0.6$ for DRD2, HTR2A and CYP1A2, respectively) and monounsaturated fat intake represented as a percent of energy ($P=0.07$). The frequency of the DRD2 CC, CT, and TT genotypes was 48%, 43%, and 9% among cases and 48%, 42%, and 10% among controls, respectively. The frequency of the HTR2A CC, CT, and TT genotypes was 36%, 47%, and 17% among cases and 35%, 48%, and 18% among controls, respectively. The frequency of the CYP1A2 AA and AC & CC genotypes was 45% and 55% among cases and 46% and 54% among controls, respectively.
### Table 3-1: Subject Characteristics by Case-Control Status

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases  n = 1,824</th>
<th>Controls  n = 1,824</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Matching Variables</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, mean (SD)</td>
<td>58.5 (11.0)</td>
<td>58.2 (11.3)</td>
<td></td>
</tr>
<tr>
<td>Males, n (%)</td>
<td>1344 (74)</td>
<td>1344 (74)</td>
<td></td>
</tr>
<tr>
<td>Urban residence, n (%)</td>
<td>1337 (73)</td>
<td>1337 (73)</td>
<td></td>
</tr>
<tr>
<td><strong>Genotypes, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>DRD2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>871 (48)</td>
<td>872 (48)</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>787 (43)</td>
<td>771 (42)</td>
<td>0.6</td>
</tr>
<tr>
<td>TT</td>
<td>166 (9)</td>
<td>181 (10)</td>
<td></td>
</tr>
<tr>
<td><strong>HTR2A</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>653 (36)</td>
<td>634 (35)</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>856 (47)</td>
<td>868 (48)</td>
<td>0.4</td>
</tr>
<tr>
<td>TT</td>
<td>315 (17)</td>
<td>322 (18)</td>
<td></td>
</tr>
<tr>
<td><strong>CYPIA2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>817 (45)</td>
<td>835 (46)</td>
<td>0.6</td>
</tr>
<tr>
<td>AC + CC</td>
<td>1007 (55)</td>
<td>989 (54)</td>
<td></td>
</tr>
<tr>
<td><strong>Demographics &amp; Medical History</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary education or higher, n (%)</td>
<td>659 (36)</td>
<td>729 (40)</td>
<td>0.008</td>
</tr>
<tr>
<td>Household income (US$/month), mean (SD)</td>
<td>506 (388)</td>
<td>572 (420)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Waist-hip ratio, mean (SD)</td>
<td>0.97 (0.07)</td>
<td>0.95 (0.07)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Physical Activity (METs), mean (SD)</td>
<td>1.51 (0.67)</td>
<td>1.57 (0.68)</td>
<td>0.0009</td>
</tr>
<tr>
<td>History of hypertension</td>
<td>701 (38)</td>
<td>543 (30)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>History of diabetes</td>
<td>444 (24)</td>
<td>259 (14)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Current smoker</td>
<td>726 (40)</td>
<td>383 (21)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Current alcohol drinker</td>
<td>894 (49)</td>
<td>950 (52)</td>
<td>0.05</td>
</tr>
<tr>
<td><strong>Nutrient Intakes, mean (SD)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total energy (kcal)</td>
<td>2702 (937)</td>
<td>2445 (760)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Carbohydrate (% of energy)</td>
<td>54.3 (7.6)</td>
<td>55.4 (7.3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Protein (% of energy)</td>
<td>13.2 (2.2)</td>
<td>12.9 (2.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fat (% of energy)</td>
<td>32.4 (5.9)</td>
<td>31.9 (5.9)</td>
<td>0.003</td>
</tr>
<tr>
<td>Saturated fat (% of energy)</td>
<td>11.1 (2.9)</td>
<td>10.4 (2.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Polyunsaturated fat (% of energy)</td>
<td>6.0 (2.0)</td>
<td>6.2 (2.0)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Monounsaturated fat (% of energy)</td>
<td>11.9 (3.5)</td>
<td>11.8 (3.9)</td>
<td>0.07</td>
</tr>
<tr>
<td>Trans fat (% of energy)</td>
<td>1.2 (0.6)</td>
<td>1.2 (0.6)</td>
<td>0.02</td>
</tr>
<tr>
<td>Cholesterol (mg/1000 kcal)</td>
<td>127 (58)</td>
<td>118 (53)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sucrose (g/d)</td>
<td>79.2 (50.0)</td>
<td>74.9 (43.2)</td>
<td>0.04</td>
</tr>
<tr>
<td>Fiber (g/1000kcal)</td>
<td>9.5 (2.4)</td>
<td>10.0 (2.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Folate (µg/1000kcal)</td>
<td>171 (47)</td>
<td>177 (47)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Abbreviations: METs, metabolic equivalent tasks; SD, standard deviation.
The DRD2 and HTR2A polymorphisms were not independently associated with risk of MI in this population in conditional logistic regression models with and without multivariate adjustments (data not shown). In comparison to <1 cup/d of coffee, the multivariate adjusted OR (95% CI) for risk of MI was 0.92 (0.68-1.24), 1.10 (0.86-1.40), and 1.33 (0.99-1.80) for 1, 2-3, and ≥4 cups/d of coffee, respectively (Table 3-2).

In analyses stratified by DRD2 genotype, there was no significant risk of MI at any level of coffee consumption (Table 3-2). There was no significant DRD2 x coffee interaction (P=0.97). Stratifying by CYP1A2 genotype did not materially alter the results with DRD2 genotype.

Stratification by HTR2A genotype showed no significantly increased risk of MI associated with coffee consumption (Table 3-2). There was no significant HTR2A x coffee interaction in the entire sample (P=0.46) or among subjects with the CYP1A2 AA genotype (P=0.86), however, a significant interaction was observed among subjects with the CYP1A2 AC & CC genotypes (P=0.001). Among subjects with the CYP1A2 AC & CC genotypes, the ORs (95% CIs) were 1.24 (0.61-2.51), 2.02 (1.16-3.52), and 3.13 (1.55-6.31) for consumption of 1, 2-3 and ≥4 cups/d of coffee, respectively, compared to <1 cup/d for subjects with the HTR2A CC genotype (Table 3-3).
### Table 3-2: Coffee Intake and Risk of MI in the Total Sample and by DRD2 and HTR2A Genotype

<table>
<thead>
<tr>
<th>Coffee Intake, cups/d</th>
<th>Cases n (%)</th>
<th>Controls n (%)</th>
<th>OR (95% CI)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Population</strong></td>
<td>n=1824</td>
<td>n=1824</td>
<td>Model 1</td>
<td>Model 2</td>
</tr>
<tr>
<td>&lt;1</td>
<td>183 (10)</td>
<td>237 (13)</td>
<td>1.00*</td>
<td>1.00†</td>
</tr>
<tr>
<td>1</td>
<td>217 (12)</td>
<td>306 (17)</td>
<td>0.91 (0.70-1.18)</td>
<td>0.92 (0.68-1.24)</td>
</tr>
<tr>
<td>2-3</td>
<td>1035 (57)</td>
<td>1033 (57)</td>
<td>1.27 (1.03-1.57)</td>
<td>1.10 (0.86-1.40)</td>
</tr>
<tr>
<td>≥4</td>
<td>389 (21)</td>
<td>248 (14)</td>
<td>2.02 (1.57-2.61)</td>
<td>1.33 (0.99-1.80)</td>
</tr>
</tbody>
</table>

**DRD2**

<table>
<thead>
<tr>
<th>CC</th>
<th>n=871</th>
<th>n=872</th>
<th>OR (95% CI)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>72 (8)</td>
<td>101 (12)</td>
<td>1.00‡</td>
<td>1.00§</td>
</tr>
<tr>
<td>1</td>
<td>102 (12)</td>
<td>142 (16)</td>
<td>0.99 (0.67-1.47)</td>
<td>0.92 (0.60-1.41)</td>
</tr>
<tr>
<td>2-3</td>
<td>502 (58)</td>
<td>502 (58)</td>
<td>1.39 (1.01-1.93)</td>
<td>1.13 (0.79-1.61)</td>
</tr>
<tr>
<td>≥4</td>
<td>195 (22)</td>
<td>127 (15)</td>
<td>2.17 (1.49-3.16)</td>
<td>1.29 (0.85-1.96)</td>
</tr>
</tbody>
</table>

**CT**

<table>
<thead>
<tr>
<th>n=787</th>
<th>n=771</th>
<th>OR (95% CI)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>91 (12)</td>
<td>104 (13)</td>
<td>1.00‡</td>
</tr>
<tr>
<td>1</td>
<td>92 (12)</td>
<td>130 (17)</td>
<td>0.79 (0.54-1.17)</td>
</tr>
<tr>
<td>2-3</td>
<td>448 (57)</td>
<td>438 (57)</td>
<td>1.15 (0.84-1.57)</td>
</tr>
<tr>
<td>≥4</td>
<td>156 (20)</td>
<td>99 (13)</td>
<td>1.82 (1.24-2.66)</td>
</tr>
</tbody>
</table>

**TT**

<table>
<thead>
<tr>
<th>n=166</th>
<th>n=181</th>
<th>OR (95% CI)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>20 (12)</td>
<td>32 (18)</td>
<td>1.00‡</td>
</tr>
<tr>
<td>1</td>
<td>23 (14)</td>
<td>34 (19)</td>
<td>1.09 (0.50-2.38)</td>
</tr>
<tr>
<td>2-3</td>
<td>85 (51)</td>
<td>93 (51)</td>
<td>1.49 (0.79-2.83)</td>
</tr>
<tr>
<td>≥4</td>
<td>38 (23)</td>
<td>22 (12)</td>
<td>2.85 (1.32-6.16)</td>
</tr>
</tbody>
</table>

**HTR2A**

<table>
<thead>
<tr>
<th>CC</th>
<th>n=653</th>
<th>n=634</th>
<th>OR (95% CI)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>61 (9)</td>
<td>93 (15)</td>
<td>1.00‡</td>
<td>1.00§</td>
</tr>
<tr>
<td>1</td>
<td>68 (10)</td>
<td>92 (15)</td>
<td>1.13 (0.72-1.77)</td>
<td>1.12 (0.69-1.83)</td>
</tr>
<tr>
<td>2-3</td>
<td>394 (60)</td>
<td>370 (58)</td>
<td>1.62 (1.13-2.30)</td>
<td>1.43 (0.97-2.13)</td>
</tr>
<tr>
<td>≥4</td>
<td>130 (20)</td>
<td>79 (12)</td>
<td>2.49 (1.62-3.82)</td>
<td>1.60 (0.99-2.61)</td>
</tr>
</tbody>
</table>

**CT**

<table>
<thead>
<tr>
<th>n=856</th>
<th>n=868</th>
<th>OR (95% CI)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>88 (10)</td>
<td>98 (11)</td>
<td>1.00‡</td>
</tr>
<tr>
<td>1</td>
<td>108 (13)</td>
<td>163 (19)</td>
<td>0.73 (0.50-1.07)</td>
</tr>
<tr>
<td>2-3</td>
<td>464 (54)</td>
<td>481 (55)</td>
<td>1.06 (0.77-1.46)</td>
</tr>
<tr>
<td>≥4</td>
<td>196 (23)</td>
<td>126 (15)</td>
<td>1.75 (1.22-2.53)</td>
</tr>
</tbody>
</table>

**TT**

<table>
<thead>
<tr>
<th>n=315</th>
<th>n=322</th>
<th>OR (95% CI)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>34 (11)</td>
<td>46 (14)</td>
<td>1.00‡</td>
</tr>
<tr>
<td>1</td>
<td>41 (13)</td>
<td>51 (16)</td>
<td>1.01 (0.55-1.86)</td>
</tr>
<tr>
<td>2-3</td>
<td>177 (56)</td>
<td>182 (57)</td>
<td>1.31 (0.80-2.14)</td>
</tr>
<tr>
<td>≥4</td>
<td>63 (20)</td>
<td>43 (13)</td>
<td>2.06 (1.14-3.72)</td>
</tr>
</tbody>
</table>

* Conditional logistic regression model (unadjusted).
† Conditional logistic regression model adjusted for smoking (never, past, 1-19 cig/d, ≥20 cig/d), waist-hip ratio, income, physical activity, history of diabetes, history of hypertension, and intakes of alcohol, total energy, and energy-adjusted saturated fat, polyunsaturated fat, trans fat, folate, and sucrose.
‡ Unconditional logistic regression model that included matching variables (age, sex, and area of residence).
§ Unconditional logistic regression model that included matching variables and the confounders listed above for model 2.

DRD2 x coffee interaction, P=0.97. HTR2A x coffee interaction, P=0.46.
Table 3-3: Coffee Intake and Risk of M1 by CYP1A2 and HTR2A Genotype

<table>
<thead>
<tr>
<th>Coffee Intake, Cups/d</th>
<th>Cases</th>
<th>Controls</th>
<th>Model 1*</th>
<th>Model 2†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CYP1A2 AA (Fast)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HTR2A CC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1</td>
<td>32 (11)</td>
<td>33 (12)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>1</td>
<td>37 (12)</td>
<td>37 (13)</td>
<td>1.00 (0.51-1.95)</td>
<td>0.83 (0.39-1.79)</td>
</tr>
<tr>
<td>2-3</td>
<td>174 (58)</td>
<td>165 (60)</td>
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* Unconditional logistic regression model that included matching variables (age, sex, and area of residence).
† Unconditional logistic regression model that included matching variables (age, sex, and area of residence), smoking (never, past, 1-19 cg/d, ≥20 cig/d), waist-hip ratio, income, physical activity, history of diabetes, history of hypertension, and intakes of alcohol, total energy, and energy-adjusted saturated fat, polyunsaturated fat, trans fat, folate and sucrose.
Gene x coffee interaction, $P=0.86$ for CYP1A2 AA genotype (fast) and $P=0.001$ for CYP1A2 AC & CC genotypes (slow).
3.5 Discussion

Numerous studies have examined the association between coffee consumption and risk of MI, but the findings have been equivocal. We recently showed that the risk of MI was observed only among carriers of the -163C allele for CYPIA2 who have impaired caffeine metabolism (7). Subjects with the AA genotype were not at increased risk, but may be protected with moderate consumption. Because caffeine is the only major compound in coffee known to be detoxified by the CYPIA2 enzyme, this study implicated caffeine in the relationship between coffee consumption and MI (7). The precise mechanism by which caffeine might trigger an MI, however, remains unknown. Caffeine acts primarily by antagonizing the adenosine receptors which may result in dopamine and serotonin release in the brain (11). In this study we sought to examine whether caffeine’s effects on the serotonergic or dopaminergic systems contribute to cardiovascular risk by examination of a common polymorphism in DRD2 and HTR2A.

The DRD2 C957T polymorphism did not modify the association between coffee consumption and MI in the entire sample or in analyses stratified by CYPIA2 genotype. These results suggest that the dopamine D2 receptor does not play an important role in the relationship between coffee consumption and MI. Caffeine alters dopaminergic neurotransmission in the brain (319), however, the consequences may be limited to motorstimulant effects or may result in the development of tolerance over time (183). Genetic association studies have found two DRD2 polymorphisms to be associated with hypertension in different populations (237, 238, 320), which may contribute to risk of MI (60, 61). The role of chronic caffeine consumption in the development of hypertension and its subsequent contribution to cardiovascular outcomes, however, is still unclear (4, 146, 321). This too may be explained, in part, by genetic variation as the CYPIA2 -163 A>C polymorphism has also been shown to modify the association between coffee consumption and the development of hypertension (6). Nevertheless, in this population the DRD2 C957T variant was not associated with MI and did not modify the association between coffee consumption and MI. It should be noted, however, that the frequency of the DRD2 TT genotype was very low in this population (n=166 cases and n=181 controls), which may have affected the power to detect associations in this group. It is also possible that other DRD2 polymorphisms or haplotypes may play a role in this relationship.
In the present study, subjects with slow caffeine metabolism (CYP1A2 AC & CC genotype) showed a significantly increased risk of MI associated with consumption of 2-3 and 4 or more cups of coffee per day for those with the HTR2A CC genotype only. Studies report the 102C allele of the HTR2A polymorphism is associated with lower receptor density and expression (282, 284), although no differences were found in other studies (280, 281, 283). This polymorphism has been shown to be in strong linkage disequilibrium (LD) with a polymorphism in the promoter region of the gene (280, 283, 288). A functional study of that -1438 A>G HTR2A promoter polymorphism suggests altered promoter activity (286) while others do not support this conclusion (280, 283, 322). Despite the unclear role of this polymorphism on the 5-HT2A receptor, the T102C variant has been associated with several cardiovascular outcomes. Interestingly, in most of these studies it is the TT genotype which imparts cardiovascular risk. In two studies, the TT genotype was associated with higher platelet aggregation in response to serotonin (285, 287). In a Japanese case-control study of acute non-fatal MI, there was a higher frequency of the TT genotype among cases (272). These results were not supported by a Spanish case-control study of male MI patients in which no association with the T102C polymorphism was found (323). In the present study, the T102C polymorphism was not independently associated with MI, however, the CC genotype was associated with an increased risk of MI among coffee drinkers (2-3 and ≥4 cups/day) with slow caffeine metabolism. The association of the TT genotype and not the CC genotype with enhanced platelet aggregation in other studies suggests this may not be the mechanism involved here. This hypothesis is supported by experimental studies which have shown that caffeine decreases platelet aggregating response to agonists (324, 325). Another suggests coffee, independently of caffeine, may have anti-coagulating effects due to its high concentration of phenolic acids which become incorporated into platelets (326).

The CC genotype has, however, been associated with hypertension among elderly females in a UK population (327), but not in a smaller Chinese sample (328). Additionally, in an American population of European ancestry, the GG genotype of the -1438 HTR2A A>G polymorphism was associated with elevated blood pressure (271). This supports the findings of Liolitsa and colleagues as the polymorphisms have been found to be in strong LD with CC co-occurring with GG (280, 283, 288). The data linking coffee or caffeine consumption to hypertension is unclear; however, one could speculate that subjects with slow caffeine
metabolism and the *HTR2A* CC genotype may be more sensitive to caffeine’s pressor effects. This is supported by the finding that the *CYP1A2* polymorphism also modified the association between coffee consumption and the development of hypertension (6). In that investigation, 553 young individuals with stage 1 hypertension were followed prospectively for a median follow-up of 8.3 years for incident physician-diagnosed hypertension requiring treatment (6). Carriers of the *CYP1A2* slow allele were at significantly increased risk consuming moderate and heavy amounts of coffee compared to abstainers, while subjects with two copies of the fast allele were significantly protected (6). Therefore, subjects with slow caffeine metabolism may be at increased risk of MI through mechanisms involving increases in blood pressure and the serotonergic system.

In summary, we observed a significant *HTR2A* x coffee interaction among subjects with slow caffeine metabolism. In this large Costa Rican case-control study of acute non-fatal MI, slow caffeine metabolism and the *HTR2A* 102 CC genotype were associated with increased risk of MI with heavier coffee consumption. These polymorphisms may be acting in concert to affect risk of MI through blood pressure related mechanisms; however, replication in other populations and further study will be required to confirm this.
Chapter 4: Overview and General Discussion

4.1 Overview

There is presently a limited understanding of the role of genetics in caffeine’s subjective and cardiovascular effects. It is generally accepted that caffeine’s antagonism of adenosine receptors is responsible for most of caffeine’s effects in the body. This antagonism has downstream effects on neurotransmitters such as dopamine and serotonin, which may mediate the reinforcing properties of caffeine or be responsible for some of the cardiovascular effects associated with caffeine consumption. Therefore, it was hypothesized that genetic variation in the dopamine $D_2$ receptor ($DRD_2$) and serotonin receptor 2A ($5-HT_2A$) may be associated with caffeine consumption behaviours. In addition, variation in the genes coding these receptors may modify the association between coffee consumption and cardiovascular diseases (CVDs) such as myocardial infarction (MI). These hypotheses were investigated by examining a single nucleotide polymorphism in the $DRD_2$ ($DRD2$) and $5-HT_2A$ receptor ($HTR2A$) genes in a large Costa Rican case-control study of acute non-fatal MI. The summary of the findings for each of the experimental chapters is as follows:

**Objective 1** (Chapter 2): To determine whether the $DRD2$ (rs6277) or $HTR2A$ (rs6313) polymorphisms affect habitual caffeine intake.

**Results:** $DRD2$ genotype was significantly associated with caffeine consumption among non-smokers and carriers of the -163C allele for $CYP1A2$, subjects with relatively slow caffeine metabolism. $HTR2A$ genotype was significantly associated with caffeine consumption among non-smokers and subjects with the $ADOR_2A$ TT genotype ($ADOR_2A$ 1083 C>T polymorphism).
Objective 2 (Chapter 3): To determine whether the \textit{DRD2} (rs6277) or \textit{HTR2A} (rs6313) polymorphisms modify the association between coffee consumption and risk of MI.

Results: The \textit{DRD2} polymorphism did not modify the association between caffeine consumption and risk of MI. The \textit{HTR2A} polymorphism modified the association between coffee consumption and risk of MI among subjects with slow caffeine metabolism (\textit{CYP1A2} -163C carriers).

The present findings suggest that genetic variation plays a role in caffeine consumption behaviours in certain subgroups in the population. In particular, variation in \textit{DRD2} and \textit{HTR2A} was associated with caffeine consumption, and as both dopamine and serotonin are implicated in mood (229, 329, 330) and reward (331-334), this suggests that these receptors may play a role in caffeine’s reinforcing effects. In addition, the \textit{HTR2A} 102 C>T polymorphism modified the association between coffee consumption and risk of MI among subjects with slow caffeine metabolism (carriers of the \textit{CYP1A2} -163C allele). The \textit{HTR2A} CC genotype was associated with a significantly increased risk of MI with consumption of 2 or more cups of coffee per day among subjects with the \textit{CYP1A2} AC & CC genotypes. As slow metabolizers are at increased risk of hypertension associated with coffee consumption (6) and the \textit{HTR2A} CC genotype has been independently associated with increased blood pressure (271, 327), these results suggest that caffeine in coffee may be acting through blood pressure mechanisms related to the serotonergic system to affect risk of MI. The \textit{DRD2} 957 C>T polymorphism did not modify the association between coffee consumption and risk of MI in the total population or in stratified analyses; however, this does not rule out the dopaminergic system in the relationship between coffee consumption and MI as other \textit{DRD2} polymorphisms or variations in other dopamine receptors may be involved.
4.2 Limitations

There are several limitations that should be considered in the interpretation of the current findings. Firstly, there was a low frequency of the DRD2 TT genotype in this population, which may have limited the statistical power to detect associations, particularly in subgroup analyses. The data assessing the impact of this polymorphism on binding kinetics in the brain supports an additive model for this polymorphism (245, 335) and investigations have reported heterozygote effects (heterosis) for this polymorphism (336). Therefore, no model assumptions were made and the CT and TT genotype groups were not combined in these analyses. Other polymorphisms in DRD2 should be examined for a more definitive understanding of the role of this receptor in caffeine’s cardiovascular effects.

In terms of the case-control design, this study is limited in its ability to assess the effect of chronic consumption of coffee on MI risk as the food frequency questionnaire used assessed intakes over the previous year. Nevertheless, a previous analysis from this case-control study showed an association between coffee, CYP1A2 genotype and risk of MI (7) that was consistent with a prospective study on coffee, CYP1A2 genotype and risk of hypertension (6). Although, coffee intake likely imparts acute rather than chronic risk (21, 51, 52), caffeine is essentially consumed daily and throughout life for many individuals and therefore it is also important to understand the impact of chronic coffee consumption on MI risk and the role of genetics in this relationship. In addition, the results can only be applied to non-fatal MI as the retrospective nature of the case-control design is limited to assessment of non-fatal MI cases. Replication in studies using a prospective design will be needed to determine if the results would apply differently to fatal and non-fatal MI cases. Recall bias is another potential limitation of the retrospective nature of the case-control study design. However, there is no biologically plausible explanation for differential recall bias between genotype groups and therefore this is unlikely to explain the present findings.

Lastly, the lack of information on mental illness may be a limitation of the present study. Indeed, several conditions have been associated with caffeine consumption and cardiovascular outcomes. For example, subjects with panic disorder and generalized anxiety disorders may be more sensitive to caffeine’s anxiogenic effects and limit their intake (128, 130-133, 337, 338). In addition, depression is a well recognized independent risk factor for CVD, and is not only
common among individuals with CVD but may also increase risk of mortality (339-342). Interestingly, caffeine and coffee consumption have also recently been associated with decreased risk of depression (343, 344). Although an understanding of the physiology linking these conditions is not known, depression (260), CVD (345), and now caffeine consumption, have all been associated with changes and dysregulation in neurotransmitter systems such as the serotonergic system. As subjects in the current investigation were not screened for mental illness, we cannot rule out the possibility that the polymorphisms analyzed are markers of mental illness and potentially confounding the interpretation of the findings. However, in the present study, the HTR2A 102C allele which has been associated with panic disorder (269, 289), was associated with higher caffeine consumption and not lower caffeine consumption as an excess of anxiogenic conditions among subjects with this allele would predict. Although both the C (270) and T alleles (290) of the HTR2A T102C polymorphism have been associated with depression, most studies have reported no association with this polymorphism and mood disorders (284, 309-312, 346-361). Additionally, neither the HTR2A 102 C>T nor the DRD2 957 C>T was independently associated with risk of MI in this investigation. Also, higher caffeine and coffee intakes have been associated with reduced risk of depression (343, 344) whereas our results suggest higher coffee consumption is associated with increased risk of MI among vulnerable individuals. Therefore, it is unlikely that direct causal relationships between these polymorphisms and mental illness are solely responsible for the present findings. Nevertheless, caution must be used in interpreting these results and future studies linking neurotransmitter systems to caffeine consumption and cardiovascular outcomes should include measures of mental health.

4.3 Future Directions

The present investigation has improved our understanding of the genetic basis for caffeine consumption and elucidated a potential mechanism by which caffeine in coffee could increase risk of MI. Caffeine’s mechanism of action is mainly attributed to antagonism of adenosine receptors which affects downstream targets of adenosine modulation such as the neurotransmitters dopamine and serotonin. A polymorphism in the DRD2 and HTR2A genes was examined and associated with caffeine consumption among subgroups in the population,
possibly implicating the dopaminergic and serotonergic systems in the reinforcing properties of caffeine. However, adenosine modulates other neurotransmitters such as glutamate and gamma-aminobutyric acid and indeed other receptors within the serotonergic and dopaminergic systems may be involved in caffeine’s effects. In addition, neurotransmitter transporters and degradation enzymes play an important role in the functioning of these systems and may also be involved in caffeine’s effects. Therefore, in addition to replication of the current findings, future investigations should consider other polymorphisms in the serotonergic and dopaminergic system as well as other neurotransmitter systems and how they may influence caffeine consumption behaviours and caffeine’s cardiovascular effects.

Because of serotonin’s role in mood and reward, it was hypothesized that variation in the genes that affect caffeine consumption are likely contributing differences in the reinforcing properties of caffeine. However, the specific role and the specific effect of these receptors on caffeine’s effects will need to be further examined. Double blind placebo-controlled trials as used to investigate the ADORA2A 1083 C>T polymorphism on caffeine’s effects described earlier (199-201) provide the opportunity to determine specifically which of caffeine’s effects these polymorphisms may influence. This will enhance the understanding of how genetic variation contributes to differences in behaviour. These types of studies could also be used to assess genetic differences in the acute hemodynamic effects of caffeine such as blood pressure. For example, these studies could be used to examine the sensitivity to the acute blood pressure raising effects of caffeine in subjects with the HTR2A CC genotype and CYP1A2 -163C carriers, shown in this investigation to be associated with increased risk of MI. This could help elucidate the specific modifying role of this polymorphism between coffee consumption and risk of MI among subjects with slow caffeine metabolism. Replication of the current findings in other populations and in other types of investigations will help confirm and improve the understanding of the present results.
In conclusion, the present study has shown that polymorphisms in the \textit{DRD2} and \textit{HTR2A} genes are associated with caffeine consumption among subgroups in the population including non-smokers. The role these receptors play in mood and reward support the reinforcing properties of caffeine on individuals with a particular genotype, which may explain some of the variation in caffeine intake between individuals. The results also emphasize the importance of including genetic determinants of caffeine consumption as potential confounders in studies that examine caffeine consumption and disease, in addition to including caffeine consumption as a potential confounder in associations between polymorphisms in these genes and disease. The results also emphasize the importance of investigating gene-environment and gene-gene interactions as the \textit{HTR2A} polymorphism also modified the association between coffee consumption and risk of MI among subjects with the \textit{CYP1A2} -163C allele (“slow” metabolizers). These findings improve our understanding of the mechanisms relating caffeine to coronary heart disease (CHD) by implicating a potential role for the serotonergic system. As CHD is one of the leading causes of death around the world, an improved understanding of its aetiology and potentially targeted recommendations for prevention and treatment can have an important impact. The findings also have important implications in a time when an increasing number of products are being formulated with added caffeine; particularly in the case of energy drinks that contain large amounts of added caffeine and are targeted to youth (362).
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