EFFECTS OF COFFEE CONSUMPTION ON CHOLESTEROL, GLUCOSE, LACTATE AND TRIGLYCERIDE LEVELS DURING EXERCISE IN THE HEAT AMONG FEMALE UNIVERSITY STUDENTS

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SCHOOL OF HEALTH SCIENCES

UNIVERSITI SAINS MALAYSIA

2021

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by

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Dissertation submitted in partial fulfilment of the requirements

for the degree of Bachelor of Health Science (Honours)

(Exercise and Sports Science)

June 2021

CERTIFICATE

This is to certify that the dissertation entitled

EFFECTS OF COFFEE CONSUMPTION ON CHOLESTEROL, GLUCOSE, LACTATE AND TRIGLYCERIDE LEVELS DURING EXERCISE IN THE HEAT AMONG FEMALE UNIVERSITY STUDENTS

is bona fide record of research work done by

NOOR IMANI BINTI MOHD ZAKI

during the period from September 2020 to June 2021 under my supervision.

I have read this dissertation and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation to be submitted in partial fulfillment for the degree of Bachelor of Health Science (Honours) (Exercise and Sports Science)

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DECLARATION

I hereby declare that this dissertation is the result of my own investigation, except where otherwise stated and duty acknowleged. I also declared that is has not been previously or concurrently submitted as whole for any other degrees at Universiti Sains Malaysia or other institutions. I grant Universiti Sains Malaysia the right to use the dissertation for teaching, research and promotional proposes.

Signature,

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ACKNOWLEDGEMENTS

First of all, thanks to the Almighty God for giving me the strength and courage to complete this thesis. This project was a team effort and my thanks goes to everyone involved in it in one way or the other. My utmost gratitude to my supervisor Dr. Marilyn Ong Li Yin and co-supervisor Dr. Ayu Suzailiana Muhamed for their support, guidance and faith in me as their research student. The advice, encouragement and astute views shared were invaluable. They really showed me what research is all about – patience, creativity and diligence.

I thank every lecturer for all the lessons, knowledge and practical experience. The ever helpful laboratory staffs were indispensable in my data collection throughout the study. Special thanks to a few of my friends who shared their knowledge and experience in preparing the thesis. Last but not least, my gratitude to my family and to my loved ones for their kindness, tolerance and forgiveness when I had been selfish with my time and attention. Having a loving and caring family gave me the security to continue my studies and research work. I hope I have made you proud.

I know that what goes around comes around. So, my deepest apologies if I have erred in anyway or hurt anyone unintentionally.

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ABSTRAK

Pengenalan : Kopi adalah campuran bahan kimia yang kompleks dan merupakan sumber utama kafein. Pengambilan kopi telah diamalkan sejak seribu tahun kebelakangan ini dan sepanjang sejarahnya, pelbagai teori telah menemukan kesannya terhadap manfaat kesihatan, termasuk mengatur metabolisma dan termoregulasi.

Objektif : Untuk mengkaji kesan pengambilan kopi terhadap termoregulasi dan metabolisme profil darah yang dipilih semasa melakukan senaman dalam keadaan panas di kalangan pelajar universiti wanita.

Kaedah : Tujuh belas pelajar universiti wanita yang sederhana aktif dan berumur antara 19 hingga 40 tahun diagihkan secara rawak untuk memulakan sama ada percubaan kopi berkafein atau kopi tanpa kafein. Peserta diberi sarapan standard (dua keping roti putih, 300 ml kopi berkafein atau kopi tanpa kafein dan 300 ml air kosong) untuk diminum/dimakan 30 minit sebelum aktiviti berjalan kaki. Berat badan peserta diukur sebelum dan selepas latihan untuk mengira peratusan penurunan berat badan. Sampel darah juga diambil sebelum kopi diminum, 10-minit setelah kopi diminum, selepas berjalan dan selepas waktu pemulihan. Osmolaliti air kencing sebelum dan selepas latihan juga diperiksa dengan menggunakan Urin Specific Gravity (USG). Air kencing sebelum senaman diperiksa sebelum mereka mengambil sarapan pagi, dan air kencing selepas senaman diperiksa setelah mereka menyelesaikan semua prosedur. Para peserta berjalan di atas treadmill selama 30 minit dengan kelajuan yang akan menghasilkan simpanan denyut jantung (HRR) peserta dalam intensiti sederhana 60% dalam keadaan persekitaran 30°C dan kelembapan relatif 70%. Denyutan jantung dan suhu timpanik

dicatatkan pada selang 5 minit. Kadar aktiviti yang dirasakan (RPE) dicatatkan pada selang 10 min. Selama 10 minit masa pemulihan, degupan jantung, dan suhu badan teras dicatatkan pada selang 5 minit. Darah diambil pada empat waktu: sebelum senaman iaitu selepas 8 jam puasa semalaman, selepas 10 minit setelah minum kopi, segera setelah senaman selesai, dan selepas 10 minit setelah selesai senaman. Sampel darah dianalisis untuk kadar kolesterol, glukosa, laktat, dan trigliserida.

Keputusan : Tidak ada kesan yang signifikan dari kopi berkafein dan kopi tanpa kafein pada kadar jantung, status penghidratan, suhu teras badan, kadar aktiviti yang dirasakan (RPE), tahap kolesterol darah, dan tahap glukosa darah semasa percubaan senaman dalam keadaan panas di kalangan pelajar universiti wanita ($p > 0.05$). Terdapat kesan yang signifikan pada tahap laktat darah ($p = 0.014$) dan tahap trigliserida darah ($p = 0.002$) semasa percubaan senaman dalam keadaan panas dengan pengambilan kopi berkafein di kalangan pelajar universiti wanita. Tahap laktat darah dan trigliserida darah meningkat semasa bersenam dalam keadaan panas dengan pengambilan kafein.

Kesimpulan : Tidak ada pengaruh yang signifikan kopi berkafein dan kopi tanpa kafein pada kadar denyutan jantung, status penghidratan, kadar suhu teras badan yang dirasakan sebagai latihan (RPE), tahap kolesterol darah dan tahap glukosa darah. Walau bagaimanapun, terdapat pengaruh yang signifikan dari kopi berkafein dan kopi tanpa kafein terhadap kadar laktat darah dan trigliserida darah. Tahap laktat darah dan trigliserida darah meningkat semasa bersenam dalam keadaan panas dengan pengambilan kafein.

EFFECTS OF COFFEE CONSUMPTION ON CHOLESTEROL, GLUCOSE, LACTATE AND TRIGLYCERIDE LEVELS DURING EXERCISE IN THE HEAT AMONG FEMALE UNIVERSITY STUDENTS

ABSTRACT

Introduction : Coffee is a complex mixture of chemicals and is the main source of caffeine. People have been drinking coffee for the last thousand years and throughout its history, many theories have been reported on its possible health benefits, including regulating metabolism and thermoregulation.

Objective : To investigate the effect of coffee consumption on thermoregulation and selected metabolic blood profile during exercise in the heat among female university students.

Methods : Seventeen moderately active female university students aged between 19 to 40 years old were randomly assigned to start with either caffeinated coffee or decaffeinated coffee trials. Participants were given a standardised breakfast (two slices of white bread, 300 ml of caffeinated coffee or decaffeinated coffee and 300 ml of plain water) to be consumed 30 mins prior to the walking exercise. Participants' body weight was measured before and after exercise to calculate percentage of body weight loss. Blood samples were also collected before the coffee was drunk, 10-mins after coffee was drunk, post 30-min walking exercise and post 10-min recovery time. Pre- and post-exercise urine were also checked for osmolality using the Urine Specific Gravity (USG). Pre-exercise urine was checked before they took their breakfast, and post-exercise urine was checked after they finished the experiment. The participants walked on the treadmill for 30 min at a speed that would elicit the participant's heart rate reserve (HRR) in the moderate intensity of 60% with environmental conditions of 30^0C and 70% relative humidity. Heart rate and tympanic temperature were recorded at 5 minutes intervals. The rate of perceived exertion (RPE) was recorded at 10 min intervals. During 10

min recovery, heart rate, and core body temperature were recorded at 5 min intervals. Finger-pricked blood was collected at four time-points: pre-exercise after 8 hours of overnight fasting, at post-10 min coffee drinking, immediately after the exercise trial completion, and post 10 min after exercise completion. Blood samples were analysed for cholesterol, glucose, lactate, and triglyceride levels.

Results : There were no significant effect of caffeinated coffee on the heart rate, hydration status, body core temperature, rate of perceived exertion (RPE), blood cholesterol levels, and blood glucose levels during exercise trials in the heat among female university students ($p > 0.05$). There were significant effects of caffeinated coffee on blood lactate ($p = 0.014$) and blood triglyceride levels ($p = 0.002$) during exercise between trials in the heat among female university students. Blood lactate and blood triglyceride levels increased during exercise in the heat with caffeine ingestion.

Conclusion : There were no significant effects of caffeinated coffee on heart rate, hydration status, body core temperature rate of perceived exertion (RPE), blood cholesterol and blood glucose levels. However, consumption of caffeinated coffee 30 min prior to exercise had a significant effect on blood lactate and blood triglyceride levels. Blood lactate and blood triglyceride levels increased during exercise in the heat with caffeine ingestion.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Coffee is not only one of the most important commodities in international trade but also one of the world's most widely consumed beverage (Farah, 2009). People have been drinking coffee for the last thousand years and throughout its history all sorts of theories have been reported on its possible effects. Only in the last 10 to 15 years, however, have any rigorous scientific conclusions been reached. Coffee is a complex mixture of chemicals, and is the main source of caffeine in many populations. However, it also contains thousands of different chemicals. Although caffeine is a major component of coffee, the content is highly variable. Caffeine affects the body in more ways than one, but it is probably most famous for interacting with a group of receptors, called adenosine receptors. It's through its activation of adenosine receptors that caffeine affects brain functions, which include functions such as sleep, cognition, learning, and memory (Ribeiro & Sebastião, 2010).

High-cholesterol diet can lead to hypercholesterolemia which promotes inflammatory responses (Tall & Yvan-Charvet, 2015). Inflammation is a vital part of the immune system's response to injury and infection. It is the body's way of signaling the immune system to heal and repair damaged tissue, as well as defend itself against foreign invaders, such as viruses and bacteria. Chronic, low-grade inflammation often does not have symptoms, but doctors can test for specific markers for inflammation in the blood. For example, high levels of C-reactive protein (CRP) have been linked with an increased risk of heart disease (Tracy *et al.*, 1997). When we start exercising and moving our muscles, it is shown that exercise can improve

cholesterol levels (Leon & Sanchez, 2001). Thus, reducing cholesterol can prevent inflammation from occurring (Tsoupras, Lordan & Zabetakis, 2018).

A meta analysis highlighted the possible role of coffee consumption in lowering the risk of metabolic syndrome, a condition that affects more than one billion people worldwide (Townsend *et al.*, 2016). The risk of cardiovascular disorders, such as coronary heart disease and stroke, increases if the person have metabolic syndrome. A study concludes that moderate coffee consumption is inversely associated with risk of heart failure, with the largest inverse association observed for consumption of 4 servings per day. (Mostofsky *et al.*, 2012). A meta analysis scientific research on the association between coffee consumption and metabolic syndrome in Polish and Italian cohorts explored the potential mechanistic perspectives behind the inverse association (Grosso *et al.*, 2015). According to the findings, polyphenols found in coffee, notably phenolic acids and flavonoids, may have a role in the inverse relationship (Malerba *et al.*, 2013). Research also suggests that moderate coffee consumption is associated with a reduction of cardiovascular disease (CVD), cancer, all-cause mortality and type 2 diabetes (Voskoboinik, Koh & Kistler, 2019). According to the National Health and Morbidity Survey (NHMS) 2019, four out of ten Malaysians, or eight million adults, have high cholesterol (38.1 per cent prevalence). One in every four people is completely unaware of their situation. This was a decrease from 2015 figures, which showed that 47.7% of adults, or 9.6 million people, had high cholesterol (NHMS, 2020).

Cholesterol level is an indicator of our heart health to determine the risk of heart disease. Blood cholesterol level is a measure of low-density lipoprotein (LDL), high-density lipoprotein (HDL), and other lipid components. Cholesterol levels vary by age, weight, and gender (Weatherspoon, 2020). Over time, a person's body tends to produce more cholesterol, meaning that all adults should check their cholesterol levels regularly, ideally about every 4 to 6 years. High cholesterol at any age puts a person at risk for heart disease, heart attack, and strokes.

These risks only increase over time, especially for adults who are not taking action to reduce their cholesterol buildup.

The relationship of coffee with health has been featured in more than 8 000 professional medical studies during the past 40 years (Bae *et al.*, 2014). The association of caffeinated coffee consumption and exercise are limited. However, the effect of caffeine, the compound contained in coffee shows aerobic training combined with caffeine supplementation significantly decreased serum low density lipoprotein (LDL), cholesterol and triglycerides levels in the post-test versus pre-test compared to the control and caffeine supplementation groups (Aghajari, Khajehlandi & Mohammadi, 2020). In the same study, serum high density lipoprotein (HDL) levels were significantly higher in the post-test than in the pre-test and in comparison with the control and caffeine supplementation groups. Conversely, one study investigated the differences in the metabolic effect of coffee and caffeine during exercise that found significant increases of plasma glycerol in caffeine and coffee at the beginning of exercise (Hodgson et al. 2013). Hence, from these previous data of lipid profile, there is a need to understand the metabolic changes as a result of coffee supplementation during exercise.

Therefore, due to the limited and contradictory findings of the literature, the purpose of this study was to examine the effects of coffee consumption on cholesterol levels during exercise in the heat among female university students. We hypothesised that caffeinated coffee will reduce cholesterol and triglyceride levels during exercise in the heat among recreational female university students.

1.2 Problem Statement

One of the most common concerns regarding coffee consumption during exercise is the rate of caffeine intake among populations potentially vulnerable to its negative effects. Caffeine

is the most widely consumed psychoactive drug in the world (Nehlig, 1999). Caffeine is a constituent of many over-the-counter pain relievers and prescription drugs because of the vasoconstriction and anti-inflammatory effects of caffeine.

Trends in caffeine consumption have been stable among adults for the past two decades (Fulgoni, Keast & Lieberman, 2015). Caffeine intake usually begins in childhood, most often in the form of chocolate, soda, and chocolate milk (Ahluwalia & Herrick, 2015). Average caffeine intakes increase from about 50 mg/day in childhood (aged 2–11 years) to 180 mg/day in adulthood (Fulgoni *et al*., 2015). However, there is limited data regarding the doses of caffeine intake that are safe to be taken by the moderately active populations especially those who are involved in sports. This is because caffeine consumption among athletes should always be referred to the World Anti-Doping Agency (WADA) as it has been included in the 2019 Monitoring Programme (WADA, 2019).

Previous research has shown that coffee can increase cholesterol levels due to its compounds which is diterpenes (Urgert & Katan, 1997). The first human trial to convincingly show the different effects of boiled and filtered coffee on lipids occurred in 1985 (Thelle, Arnesen & Førde, 1983). Regular exercise or physical activity has also been shown to increase HDL cholesterol while maintaining, and theoretically offsetting increases in LDL (Mann, Beedie & Jimenez, 2014a). However, it is unknown whether the consumption of coffee and exercise in the hot and humid weather in Malaysia can change cholesterol and triglyceride levels by maintaining or reducing it. There is also limited data on how exercise in hot condition may change these levels.

1.3 Objectives of The Study

General : To investigate the effect of coffee consumption on thermoregulation and selected metabolic blood profile during exercise in the heat among female university students.

Specific:

1. To investigate the effect of caffeinated coffee and decaffeinated coffee during exercise in the heat on cholesterol levels among female university students.
2. To investigate the effect of caffeinated coffee and decaffeinated coffee during exercise in the heat on glucose levels among female university students.
3. To investigate the effect of caffeinated coffee and decaffeinated coffee during exercise in the heat on lactate levels among female university students.
4. To investigate the effect of caffeinated coffee and decaffeinated coffee during exercise in the heat on triglycerides levels among female university students.
5. To investigate the effect of caffeinated coffee and decaffeinated coffee during exercise trials in the heat on heart rate among female university students.
6. To investigate the effect of caffeinated coffee and decaffeinated coffee during exercise trials in the heat on body core temperature among female university students.
7. To investigate the effect of caffeinated coffee and decaffeinated coffee during exercise trials in the heat on hydration status among female university students.
8. To investigate the effect of caffeinated coffee and decaffeinated coffee during exercise trials in the heat on sweat rate among female university students.

1.4 Research Hypotheses

Null hypothesis (H_{O1}) : There is no significant difference in the cholesterol levels during exercise in the heat between caffeinated coffee and decaffeinated coffee trials among female university students.

Alternative hypothesis (H_{A1}) : There is a significant difference in the cholesterol levels during exercise in the heat between caffeinated coffee and decaffeinated coffee trials among female university students.

H_{O2} : There is no significant difference in the glucose levels during exercise in the heat between caffeinated coffee and decaffeinated coffee trials among female university students.

H_{A2} : There is a significant difference in the glucose levels during exercise in the heat between caffeinated coffee and decaffeinated coffee trials among female university students.

H_{O3} : There is no significant difference in the lactate levels during exercise in the heat between caffeinated coffee and decaffeinated coffee trials among female university students.

H_{A3} : There is a significant difference in the lactate levels during exercise in the heat between caffeinated coffee and decaffeinated coffee trials among female university students.

H_{O4} : There is no significant difference in the triglyceride levels during exercise in the heat between caffeinated coffee and decaffeinated coffee trials among female university students.

H_{A4} : There is a significant difference in the triglyceride levels during exercise in the heat between caffeinated coffee and decaffeinated coffee trials among female university students.

H_{O5} : There is no significant difference in the heart rate during exercise in the heat between caffeinated coffee and decaffeinated coffee trials among female university students.

H_{A5} : There is a significant difference in the heart rate during exercise in the heat between caffeinated coffee and decaffeinated coffee trials among female university students.

H_{O6} : There is no significant difference in the body core temperature during exercise in the heat between caffeinated coffee and decaffeinated coffee trials among female university students.

H_{A6} : There is a significant difference in the body core temperature during exercise in the heat between caffeinated coffee and decaffeinated coffee trials among female university students.

H_{O7} : There is no significant difference in the hydration status during exercise in the heat between caffeinated coffee and decaffeinated coffee trials among female university students.

H_{A7} : There is a significant difference in the hydration status during exercise in the heat between caffeinated coffee and decaffeinated coffee trials among female university students.

H_{O8} : There is no significant difference in the sweat rate during exercise in the heat between caffeinated coffee and decaffeinated coffee trials among female university students.

H_{A8} : There is a significant difference in the sweat rate during exercise in the heat between caffeinated coffee and decaffeinated coffee trials among female university students.

1.5 Research Questions

What are the effects of caffeinated and decaffeinated coffee consumption on thermoregulation and selected metabolic blood profile during exercise in the heat among female university students?

1.6 Significance of The Study

This study would help us understand more in-depth knowledge of coffee consumption and its effect on physiological properties that can provide a major contribution to overcome the deficiency of data on the prevalence and effect on physiological properties during exercise in the heat of moderately active female subjects, especially in Malaysia. This experimental study may help to provide the information on the acute positive effect of coffee consumption of female populations by determining and studying their physiological properties.

This could contribute towards building a proper dietary plan of coffee for female populations and coffee in combination with exercise may be used as a nutritional and exercise strategy to reduce cholesterol levels, blood glucose levels, lactate levels and triglycerides levels during exercise in the heat especially among athletes. Thus, this will increase the optimal sports performance.

1.7 Operational Definitions

Cholesterol : A waxy, fat-like substance that is found in all the cells in the body.

Glucose : Main type of sugar in the blood and is the major source of energy for the body's cells.

Lactate : Organic molecule produced by most tissues in the human body, with the highest production found in muscle.

Triglycerides : A type of fat (lipid) found in the blood.

Heart rate : The number of heart beats per minute.

Core temperature : Temperature of the internal environment of the body.

Hydration : Process of replacing fluid in the body.

Sweat rate : The rate of fluid lost through sweating during exercise.

CHAPTER 2

LITERATURE REVIEW

2.1 Coffee Components

The coffee tree is the origin of a genus of plants known as *Coffea*. The two most commercially important species grown are sorts of Coffea arabica (Arabicas) and Coffea canephora (Robustas). Arabica coffee is descended from the original coffee trees discovered in Ethiopia grown in mild environmental temperatures, ideally between 15 - 24 degrees Celsius (ISIC, 2020). Robusta coffee is mostly grown in Central and Western Africa, parts of Southeast Asia, including Indonesia and Vietnam, and Brazil. Robusta is primarily utilised in blends and for instant coffees. Robusta has the advantage of having the ability to withstand warmer climates, preferring constant temperatures between 24 and 29 degrees Celsius, which enables it to grow at far lower altitudes than Arabica. Compared with Arabica, Robusta beans produce a coffee that features a distinctive taste and about 50-60% more caffeine (NCA, 2020).

Coffee contains approximately 43% carbohydrates, 7.5–10% proteins, other nitrogenous compounds (caffeine, trigonelline and nicotinic acid), 10–15% lipids, 25% melanoidins, 3.7–5% minerals and ~6% organic and inorganic acids, and esters (chlorogenic acids and other phenolic compounds, aliphatic acids and quinic acid and inorganic acids) (Farah, 2012; Speer & Kölling-Speer, 2006). Coffee is rich in many bioactive compounds, and its consumption has been associated with many beneficial health effects. The main bioactive compounds of coffee are caffeine, chlorogenic acids, trigonelline, diterpenes, and melanoidins. The polysaccharides, galactomannans and type II arabinogalactans, and β-carbolines are amongst the emerging bioactive compounds for which there is still insufficient information to substantiate any health effects, and the coffee amines are referred to as bioactive amines (Farah, 2018). Some of the compounds will be introduced later.

Caffeine is a xanthine alkaloid that functions in the body as a stimulant. Caffeine in coffee is important due to its stimulating properties . There is no significant loss in terms of caffeine during coffee bean roasting. A typical cup of regular coffee contains 70 to 140 mg of caffeine, depending on the preparation, blend, and cup size. The caffeine content in foods and drinks are classified as low caffeine dose (3 mg/kg), moderate dose (6 mg/kg), and high dose (9 mg/ kg) (Spriet, 2014). In 2015, the European Food Safety Authority (EFSA) published their Scientific Opinion on the Safety of Caffeine, advising that caffeine intakes from all sources up to 400 mg per day and single doses of 200 mg do not raise safety concerns for adults in the general population. Therefore, from this information, the dosage of caffeine has been classified as stated above. The presence of caffeine stimulant in coffee may not be tolerated by some people. This necessitates the demand for decaffeinated coffee (Tuomilehto, 2013). The decaffeination process of coffee using methylene chloride or ethyl acetate solvents removes nearly all the caffeine from the coffee beans (Pietsch, 2017). This is the greatest challenge in decaffeination since coffee contains chemicals that are important to the taste and aroma of the coffee. Based on European law, decaffeinated coffee must contain 0.1%, or less, caffeine in roasted coffee beans and up to 0.3% or less in soluble/instant coffee (NCA, 2020).

Chlorogenic acids are the main phenolic compounds in coffee. The total amount of chlorogenic acids in Robusta beans is almost double than that found in Arabica beans, and because chlorogenic acids are partly degraded or transformed during roasting, dark roasted coffees contain lower amounts of these compounds. In roasted products, the difference between species is significantly reduced. These compounds are frequently referred to as powerful antioxidants and anti-inflammatory compounds due to the results of *in vitro* and animal studies, as well as a few human studies (Torres & Farah, 2017; dos Santos *et al.,* 2006; Folmer *et al.,* 2017). Chlorogenic acids demonstrate potent antioxidant effects, which means they neutralise free radicals that can potentially damage your body tissues.

Cafestol and kahweol are diterpenes present in coffee, mainly in the form of salts or esters of (predominantly) saturated and unsaturated fatty acids. They represent approximately 20% of the lipid fraction of coffee, with cafestol being more abundant. Higher levels of diterpenes are found in Arabica than in the Robusta species. Coffee diterpenes exhibit strong anticarcinogenic and hepatoprotective properties *in vitro* (Farah, 2012). An anticarcinogen is a substance that inhibits the development of cancer, while hepatoprotection is the ability of the diterpenes to prevent damage to the liver. Diterpene levels in the coffee cup vary significantly based on the natural variations in green coffee beans, roasting conditions and preparation methods.

The understanding of the health benefits of coffee is challenging as coffee is a complex mixture of bioactive substances. Studies have also shown that the components in coffee may act together to help prevent diseases when consumed appropriately.

2.2 Coffee and Cardiovascular Health

Cardiovascular disease (CVD) is a term used to describe all diseases of the heart and blood vessels, including coronary heart disease, cerebrovascular disease, rheumatic heart disease and other conditions. Coronary heart disease and stroke are common forms of CVD. CVD is a major cause of disability and premature death throughout the world and contributes substantially to the escalating costs of health care. Globally, CVD is the number one cause of death, and they are projected to remain so. It is estimated the disease will take 17.9 million lives each year (WHO, 2017).

According to the Department of Statistics Malaysia (2019), ischaemic heart disease is the main cause of CVD deaths in Malaysia, with a total of 18,267 deaths or 15.6% of total deaths from various causes. CVD is Malaysia's number one killer, with 50 people dying from the health condition daily. The deaths from the disease increase every year, and it is the leading cause of 'sudden death' in Malaysia (Statistics Malaysia, 2019). Ischaemic heart diseases occur when there is insufficient blood flow to a part or area of the heart muscle due to a blockage in the blood vessels leading to the area. If the flow of oxygen-rich blood to the heart muscle is reduced or blocked, angina or a heart attack may occur (Michigan Medicine, 2019).

According to Rebello and van Dam (2013), one of the misinterpretations linking coffee and health is the belief that CVD risk is increased by drinking coffee. This belief is supported by the fact that caffeine increases blood pressure and acutely reduces insulin sensitivity after coffee consumption. However, it is now known that most acute caffeine effects cease to exist with regular coffee consumption due to adaptation mechanisms and that other components of coffee, mainly chlorogenic acids and trigonelline, have compensatory effects on endothelial dysfunction and insulin resistance. (Rebello & van Dam, 2013). Improving endothelial dysfunction and reducing insulin resistance are key mechanisms for cardiovascular protection.

The association between coffee consumption and CVD has been widely researched with conflicting results. A meta-analysis was carried out by Ding *et al*. (2014), which included data from 36 studies with more than 1 million participants and more than 36,000 CVD cases that demonstrated a nonlinear relationship between chronic coffee consumption and CVD risk. Compared with the lowest category of coffee intake (median, 0 cups per day), the relative risk of CVD was 0.89 for a median of 1.5 cups per day, 0.85 for a median of 3.5 cups per day, and 0.95 for a median intake of 5 cups per day. The review concluded that coffee consumption is nonlinearly associated with both coronary heart disease and stroke.

Moderate coffee consumption lessens the risk of clogged arteries and heart attacks. People consuming three to five cups of coffee a day have a lower risk of clogging arteries, and those drinking a moderate daily amount of coffee are subordinate to develop clogged arteries that could lead to heart attacks (Kawachi, Colditz & Stone, 1994). Those who drank several cups of coffee a day had a lesser calcium buildups in the coronary arteries. Although these deposits are considered early warning signs of heart disease, the results do not mean that if the individual starts drinking coffee, he or she will be protected against this condition (Kawachi, Colditz & Stone, 1994). More research is needed to understand the protective effect of coffee.

2.3 Coffee and Cholesterol Levels

Several studies to investigate the effects of coffee on cholesterol level have been conducted. Heavy consumption of coffee has long been suspected of having a cholesterol-raising effect. One prospective study has found a lipid-raising effect for habitual coffee consumption (Wei *et al.*, 1995). In that investigation, drinking one cup of regular coffee a day was associated with a 2 mg/dl increase in total cholesterol over 16.7 months of follow-up after adjusting for age and changes in other potential confounders. Intervention trials have shown that each 10 mg of cafestol per day for four weeks increases serum cholesterol by 0.13 mmol/l, an 8–10% increase. Approximately 80% of the rise in serum cholesterol is due to an elevation in low-density lipoprotein. High-density lipoprotein is not affected or showed a slight decrease. Serum triglycerides are also increased by 0.08 mmol/l over the 4-week period. In another 6-month intervention trial, it was found that unfiltered coffee per day raised serum triglycerides by 26% in the first month, but the effect is reduced to 7% after 6 months of daily consumption (Urgert & Katan, 1996).

A meta-analysis of a set of 18 clinical intervention trials on coffee consumption and cholesterol and serum lipids was performed by Jee *et al*. (2001). The authors corroborated the dose-response relationship between coffee consumption and cholesterol. They observed a strong increase in the consumption of 6 or more cups of boiled coffee per day, which is not observed when a paper filter was used. Several human intervention trials have been performed and have shown that each 10 mg of cafestol per day for 4 weeks increases serum cholesterol by 0.13 mmol/l, an 8–10% increase (Urgert & Katan, 1997). Approximately 80% of the rise in serum cholesterol is due to an elevation in low-density lipoprotein. High-density lipoprotein is not affected or showed a slight decrease. Serum triglycerides are also increased by 0.08mmol/l over the 4-week period (Urgert & Katan, 1997).

The lipid raising effects of coffee drinking have been reported to be primarily due to cafestol that increases the synthesis of cholesterol. This relationship was found to be linear with increasing cafestol consumption (Urgert and Katan, 1997). Cafestol is the most potent cholesterol-elevating compound identified in the human diet. Cafestol elevates serum cholesterol levels by activating two receptors in an intestinal pathway critical to cholesterol regulation. Activation of the farnesoid X receptor and pregnane X receptor by cafestol in the intestine misleads the body into sending a signal to the liver to stop the breakdown of cholesterol. When the breakdown of cholesterol is prevented, it has nowhere to go except into the serum, thereby increasing serum cholesterol levels. The high consumption of diterpenes (cafestol and kahweol) has been associated with elevated homocysteine and low-density lipoprotein levels in human plasma, which may indirectly increase the risk of cardiovascular diseases (Farah, 2012).

The concentration of these diterpenes depends on how the coffee is prepared. Boiled coffee has higher concentrations because diterpenes are extracted from the coffee beans by prolonged contact with hot water. By comparison, brewed/filtered coffee, because of the much shorter contact with hot water and retention of diterpenes by filter paper, has a much lower concentration of cafestol and kahweol. The effect of coffee on serum lipid levels is studied in 107 young adults with normal cholesterol levels followed for 12 weeks. Coffee is brewed by two common methods, filtering and boiling, and the participants are assigned to 1 of 3 groups: drinking 4 to 6 cups of boiled coffee per day, 4 to 6 cups of filtered coffee per day, or no coffee, for a period of 9 weeks. A significant increase in total cholesterol and a non-significant increase in low-density lipoprotein (LDL) cholesterol were observed in participants consuming boiled coffee. On the other hand, there was no significant difference in serum total or LDL cholesterol levels between the filtered coffee group and the group who drank no coffee

(Bak & Grobbee, 1989). The summary of the literature review of coffee consumption on the effect of cholesterol levels is shown in Table 1.

Table 1 Summary review of coffee consumption on the effects of cholesterol levels

	STUDY (YEAR)	STUDY DESIGN	PARTICIPANTS	TYPE OF DRINKS	EXERCISE PROTOCOL	EFFECT OUTCOME
						CHOLESTEROL LEVEL
1	Yamashita *et al.*, (2012)	Cross-sectional study	N : 2554 M & 763 F Japanese workers Age : 35 - 69 YO	Coffee	None	Significantly increased ($p = 0.019$)
2	Wedick et al. (2006)	Randomised parallel-arm intervention (8 weeks)	N : 16 M & 29 F healthy overweight, nonsmokers & regular coffee consumers Mean age : 40.6 YO	5 cups (177 mL each) per day of instant caffeinated coffee, decaffeinated coffee, or no coffee (i.e., water)	None	No significant changes
3	Kempf *et al.*, (2010)	Single-blind, 3-stage clinical trial (12 weeks)	N : 47 habitual coffee drinkers Age : <65 YO	1) 4 cups (150 mL each) of filtered coffee/d (50 g coffee/d) 2) 8 cups (150 mL each) of filtered coffee/d (83.33 g coffee/d)	None	4 cups : No significant changes 8 cups : Significantly increased $p < 0.01$

4	Basu et al. (2011)	Randomised controlled trial (8 weeks)	N : 35 subjects obese Mean age : 42.5 YO	Decaffeinated green tea	None	Significantly decreased p = 0.16
5	Cheung, Gupta & Ito, (2005)	Randomized cross-over study	N : 26 M & 14 F Mean age : 45 YO	6-oz cup of coffee	None	Significantly increased (p = 0.019)
6	Williams *et al.*, (2010)	Randomised cross-over study	N : 77 M sedentary Age : 30 – 55 YO	>2-3 cups/day	None	LDL : significantly increased (p < 0.06)
7	D'Amicis *et al.*, (1996)	Randomised trial (9 weeks)	N : 84 M young adult Age : not mentioned	Italian style brewed coffee	None	No significant changes
8	Karabudak, Turkozu & Koksal, (2015)	Cross-sectional study	N : 48 M & 74 F Healthy Age : 18 – 75 YO	Turkish coffee & instant coffee	None	No significant changes
9	Corrêa *et al.*, (2013)	Randomised cross-over clinical trial (9 weeks)	N : 6 M & 14 F Healthy & habitual coffee drinkers Age : 20 – 65 YO	Medium light roast (MLR) & medium roast (MR) paper-filtered coffee	None	MLR TC : significantly increased (p < 0.01) HDL : significantly increased (p = 0.04) LDL : significantly increased (p < 0.01) MR TC : significantly increased (p < 0.001) HDL : significantly increased (p = 0.003)

						LDL : significantly increased ($p < 0.001$)
10	Lopez-Garcia *et al.*, (2006)	Cohort study	N : 44005 M & 84488 F	Filtered coffee	None	No significant changes
11	Bahorun *et al.*, (2012)	Randomised controlled clinical trial (15 weeks)	N : 42 M & 35 F non-smoker Age : 25 – 60 YO	Black tea	None	TC M : no significant changes F : significantly increased ($p < 0.05$) HDL significantly increased M & F : $p < 0.05$ LDL no significant changes (M & F)
12	Balk, Hoekstra & Twisk, (2009)	Observational longitudinal study	N : 123 M & 160 F Mean age : 13.1 YO	Coffee	None	HDL significantly decrease >2 & <4 cups/day : $p = 0.87$ >4 & <6 cups/day : $p = 0.62$ >6 cups/day : $p = 0.61$
13	Strandhagen & Thelle, (2003)	Prospective controlled study (7 weeks)	N : 121 & M & F Healthy , non-smoking Age : 30 – 65 YO	600 ml filtered brewed coffee/day	None	TC : significantly decreased ($p = 0.26$ HDL : significantly decreased ($p = 0.12$)
14	Riedel *et al.*, (2014)	Randomised double-blinded cross-over	N : 46 M & 38 F Healthy, non-smokers, habitual coffee drinkers	7.5 g market blend (MB) & study blend (SB) coffee/day	None	SB coffee HDL : significantly increased ($p < 0.001$)

		human intervention study (20 weeks)	Mean age : 25.6 YO			LDL: no significant changes MB coffee HDL : significantly increased ($p < 0.001$) LDL : no significant changes
15	Hochkogler *et al.*, (2019)	Randomised prospective controlled parallel study (12 weeks)	N : 48 M & 50 F Healthy Mean age : 24.3 YO	7.5 g dark-roast coffee/day	None	LDL significantly increases ($p < 0.05$)

N, numbers; M, male; F, female; YO, years old; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TC, total cholesterol

2.4 Mechanism of Action of Cafestol on Cholesterol

Cafestol is the most potent cholesterol-elevating compound identified in coffee. Several human and animal studies previously investigated its effect on lipoprotein metabolism. A transgenic mouse model is established to study hyperlipidemia and atherosclerosis which used *ApoE3Leiden* mice (van den Maagdenberg *et al.*, 1993; van Vlijmen et al., 1994). The effect of cafestol on *APOE3Leiden* mice display a lipoprotein profile comparable to that of patients with dysbetalipoproteinemia; that is, plasma cholesterol and triglyceride levels are increased.

Cholesterol homeostasis is achieved through the coordinated regulation of its dietary uptake, endogenous biosynthesis and disposal in the form of bile acids. Bile acids are not only metabolic byproducts but are essential for the appropriate absorption of dietary lipids and fat-soluble vitamins. Approximately 95% of bile acids are recycled in the small intestine by enterocytes and returned to the liver via the enterohepatic circulation on a daily basis. The rate-limiting enzyme in bile acid biosynthesis is cholesterol 7α-hydroxylase, encoded by the gene *CYP7A1* (Edwards, Kast & Anisfeld, 2002). In humans, a disabling mutation in the CYP7A1 gene is associated with increased plasma triglycerides and LDL cholesterol levels (Pullinger *et al.*, 2002). When bile acid synthesis decreases, less cholesterol is catabolised in the liver, and, as a consequence, plasma cholesterol increases. Cafestol has been shown to suppress bile acid synthesis in APOE3Leiden mice by down-regulation of CYP7A1, with a concomitant increase in serum lipids similar to that observed in humans (Post *et al.*, 2000; Ricketts *et al.*, 2007).

Cafestol can also directly regulate the expression of genes involved in cholesterol metabolism by activating the nuclear hormone receptors, known as the farnesoid X receptor (FXR) and pregnane X receptor (PXR). Nuclear hormone receptors are ligand-

activated transcription factors that regulate gene expression by interacting with specific DNA sequences upstream of their target genes. These two newer members of the family have been identified as key regulators in metabolic pathways. The direct regulation of FXR and PXR target genes in the intestine combines with indirect effects in the liver to contribute to the cholesterol-raising effect of cafestol in humans (Lu *et al.*, 2000; Goodwin *et al.*, 2000).

A study by Cheung, Gupta and Ito in 2005 has shown that acute coffee ingestion affects cholesterol levels. The level of total cholesterol and high-density lipoprotein cholesterol has increased significantly, while the low-density lipoprotein cholesterol has no significant changes. The increase in HDL-C may be related to the concomitant decrease in triglycerides. This research shows that in the black coffee arm, most lipid indices, except for triglycerides, increased after coffee consumption. The change in the primary endpoint, LDL-C, was not statistically significant, although the increases in TC and HDL-C were significant. The mean TC increased with $p = 0.019$, and the mean HDL-C also increased with $p < 0.001$). The mean elapsed time from the end of coffee consumption to the blood draw was 40.7 ± 14.5 minutes.

2.5 Effects of Caffeine on Glucose Levels

The most essential carbohydrate fuel in the body is glucose. The majority of circulating glucose comes from the diet in the fed state; in the fasting state, gluconeogenesis and glycogenolysis keep glucose levels stable. The majority of glucose in the diet is found in more complex carbohydrates, which are broken down into monosaccharides throughout the digestive process. Polysaccharides account for around half of total carbohydrates in the diet, with simpler sugars responsible for the rest. Sucrose, a disaccharide comprising glucose and fructose, accounts for around two-thirds of the sugar in the diet (Khowala, Verma & Banik, 2008).

Coffee has gained special attention in relation to its beneficial effects on several chronic diseases, specially type 2 diabetes mellitus (T2DM) (Cano-Marquina, Tarín & Cano, 2013). Studies suggest that drinking 3-4 cups of coffee per day is associated with an approximately 25% lower risk of developing T2DM compared to consuming none or less than 2 cups per day (Ding *et al.*, 2014). People who increase their consumption of coffee (+1 cup/day) had a decrease of 11% on T2DM risk in the subsequent 4 years. While people who decreased their coffee intake by more than 1 cup/day had a 17% higher risk for T2DM in 4 years (Bhupathiraju *et al.*, 2014). It seems that intake of caffeinated coffee can decrease T2DM risk.

Over the period of 7.6 years, the Strong Heart Study followed 1141 Native American subjects, a group renowned for having a high incidence and prevalence of diabetes. Coffee consumption at a high level (12 cups per day) lowered the risk of diabetes by 67% (Zhang *et al.*, 2011). Another prospective observational study, the Puerto Rico Heart Health Program, discovered that protection from diabetes requires a