HETEROZYGOUS *MTHFR* C677T POLYMORPHISM (RS1801133) IS ASSOCIATED WITH HIGHER TOTAL CHOLESTEROL/ HIGH DENSITY LIPOPROTEIN CHOLESTEROL RATIO IN HABITUAL COFFEE DRINKERS

By

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ABSTRACT

HETEROZYGOUS *MTHFR* C677T POLYMORPHISM (RS1801133) IS ASSOCIATED WITH HIGHER TOTAL CHOLESTEROL/ HIGH DENSITY LIPOPROTEIN CHOLESTEROL RATIO IN HABITUAL COFFEE DRINKERS

ONG XUAN

Coffee consumption and its effect on cardiometabolic health have been studied for decades. Yet, the epigenetic effects of coffee on blood pressure, pulse and lipid profile have not been extensively known. Increased prevalence of cardiometabolic disease and mortality is now an emerging issue globally. While *MTHFR* C667T is widely known as a risk factor for cardiometabolic diseases, the impact of coffee and this polymorphism on cardiometabolic risk is unknown. Convenient sampling was applied and a total of 298 (110 males and 188 females) and 106 (37 males and 69 females) participants at UTAR Kampar were recruited for the cross-sectional and experimental study. Questionnaire were distributed. Genotype identification, blood pressure, pulse and lipid profile measurement were performed in the experimental study. SPSS Version 27.0 was used to analyze the data. The prevalence of coffee consumption was 34.9% in the study cohort. Significantly, male habitual coffee drinkers are higher than female (χ^2=19.671, $p < 0.001$). Genotype distribution were 49.1%, 50% and 0.94% for CC, CT, and TT genotype. Interestingly, *MTHFR* C677T polymorphism was associated with habitual coffee consumption (χ^2=8.0381, $p < 0.05$). Significant

difference in the ratio of TC to HDL-C were found between habitual and non-habitual coffee drinkers among the T allele carriers ($p < 0.05$). Among regular coffee drinkers, SBP ($p < 0.05$) and DBP ($p < 0.05$) increased significantly while pulse rate ($p < 0.05$) decreased after coffee consumption. Regular coffee intake increased cardiovascular risk particularly in heterozygous *MTHFR* C677T by increasing the ratio of TC to HDL-C. Acutely, the intake of coffee increased the SBP and DBP but decreased pulse rate of habitual coffee drinkers. In conclusion, epigenetics modifications can potentially mediate the effects of coffee on gene expression and physiological process. Future studies should consider gender differences, dietary factors, physical activity and larger sample size.

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DECLARATION

I hereby declare that this final year project report is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

Ong Xuan

APPROVAL SHEET

The final year project entitled "**HETEROZYGOUS *MTHFR* C677T POLYMORPHISM (RS1801133) IS ASSOCIATED WITH HIGHER TOTAL CHOLESTEROL/ HIGH DENSITY LIPOPROTEIN CHOLESTEROL RATIO IN HABITUAL COFFEE DRINKERS**" was prepared by ONG XUAN and submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Hons) Dietetics at Universiti Tunku Abdul Rahman.

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PERMISSION SHEET

It is hereby certified that **ONG XUAN** (ID No. **20ADB05665**) has completed this final year project thesis entitled "**HETEROZYGOUS *MTHFR* C677T POLYMORPHISM (RS1801133) IS ASSOCIATED WITH HIGHER TOTAL CHOLESTEROL/ HIGH DENSITY LIPOPROTEIN CHOLESTEROL RATIO IN HABITUAL COFFEE DRINKERS**" under the supervision of Dr. Phoon Lee Quen from the Department of Allied Health Sciences, Faculty of Science.

I hereby give permission to the University to upload the soft copy of my final year project thesis in pdf format into the UTAR Institutional Repository, which may be made accessible to the UTAR community and public.

Yours truly,

(ONG XUAN)

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
ARMS-PCR	Amplification-refractory mutation system polymerase chain reaction
BMI	Body mass index
bp	Base pair
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DNA	Deoxyribonucleic acid
HDL-C	High-density lipoprotein cholesterol
IUPAC	International Union of Pure and Applied Chemistry
LDL-C	Low-density lipoprotein cholesterol
MTHF	Methylenetetrahydrofolate
MTHFR	Methylenetetrahydrofolate reductase
NHMS	National Health and Morbidity Survey
PCR	Polymerase chain reaction
PCR-RFLP	Polymerase chain reaction-restriction fragment length polymorphism
RNI	Recommended Nutrient Intake
SBP	Systolic blood pressure
SNP	Single nucleotide polymorphism
TC	Total cholesterol

TC/HDL-C	Ratio of total cholesterol to high-density lipoprotein cholesterol
TG	Triglyceride
UTAR	Universiti Tunku Abdul Rahman
Val	Valine
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

The term "cardiometabolic disease" is primarily used to describe type 2 diabetes and a number of cardiovascular events, which are currently the most prevalent diseases in the world. Cardiometabolic diseases are closely related to metabolic syndrome, despite the fact that the precise cause and mechanism of their occurrence are unknown. According to World Health Organization (2021a), 32% of global deaths were caused by cardiovascular disease (CVD) in 2019, mainly due to heart attack and stroke (85%). In low- and middle-income nations, CVD is a grave issue that requires attention (Wurie and Cappuccio, 2012). The poor in those nations are unable to access treatment or preventive services, which has increased CVD mortality. As a result, it causes a decline in the labour force, countries' productivity and an increase in preventable fatalities. Additionally, the inability to prevent diseases results in higher socioeconomic costs. All of these repercussions place the national healthcare systems and the struggle for policy development in a critical and difficult situation. If these nations cannot stop the CVD epidemic, their poverty will get worse. Therefore, it is important to prioritise and achieve CVD prevention in order to promote socioeconomic advancement and reduce poverty (Wurie and Cappuccio, 2012).

CVD is one of the leading causes of death in Malaysia, accounts for approximately 22% of total death in 2020 (Department of Statistics, Malaysia,

2021). Although individuals are not commonly being diagnosed with CVD in their young adulthood, the risk factor can be developed in this stage of life. According to Bucholz, Gooding and de Ferranti (2018), the prevalence of hypertension, diabetes and hypercholesterolemia is 7.3% and 2.6% and 8.8%, among the young adults in the United States. As compared to this, in Malaysia, the prevalence of hypertension and diabetes among young adults is 8.6% and 3.16%, while the dyslipidemia was 38.1% among the overall adults (Institute for Public Health, 2020; Soo, et al., 2020; Akhtar, et al., 2022). Meanwhile, the prevalence of the risk factors shows increasing trend over years and Bernama (2018) claimed that Malaysian tends to develop CVD at younger age. Although the prevalence of physical inactivity was similar between adults in the global (27%) and Malaysia (25.1%) as stated in World Health Organization (n.d.) and Institute for Public Health (2020), the obesity rate in Malaysia (17.7%) is higher than the global rate (13%) (World Health Organization, 2021b; Kyaw, et al., 2022).

Undoubtedly, as a developing nation, the rising incidence of CVD and CVD mortality will impact on the nation's productivity and socioeconomic burden (Khan, 2022). In addition, it will increase the cost of healthcare. Based on the World Health Organization (2022), there was an increase in the cost of non-communicable diseases like cancer, diabetes, and CVD between 2017 and 2022. In addition, the National Health and Morbidity Survey (NHMS) 2019 done by Ministry of Health, Malaysia (2020) suggested that diabetes, hypertension and high cholesterol level are the major risk factors that contribute to CVD. Three

out of ten Malaysians, according to the survey, have hypertension, and four out of ten are affected by high cholesterol level.

Metabolic syndrome (MetS) represents a collection of five metabolic issues, including high blood pressure, abdominal obesity, insulin resistance (high blood sugar), low levels of high-density lipoprotein cholesterol (HDL-C), and high levels of triglycerides (TG). A person is diagnosed with metabolic syndrome if they exhibit three or more of these symptoms, which raises their risk of developing type 2 diabetes and CVD (American Heart Association, 2021). According to the American Heart Association (2021), the fatty streaks that accumulate in the artery walls of people with metabolic syndrome make them more likely to develop type 2 diabetes and CVD. Although overweight, obesity and insulin resistance are widely known as the underlying causes of metabolic syndrome, genetic factors do play a significant role in predisposing a person to have the syndrome. Furthermore, the interaction between genes, diet, lifestyle, and environment is also thought to contribute to obesity and insulin resistance. Marc (2007) believed that each genetic factor can have different impact on the syndrome as a whole and/or its individual components.

One of the most popular and widely consumed beverages worldwide is coffee (Tim, 2018). The consumption of 800,000 60 kg bags of coffee by Malaysians in 2021 and 2022 demonstrates the country's popularity with coffee (Ahmad, 2022). In fact, coffee's origins can be traced to the British colonial era (Ahmad,

2022). Recently, modern coffee speciality shops and cafés as well as traditional "kopitiam" coffee shops are now widely available in Malaysia. As claimed by Ahmad (2022), the busy lifestyle and urbanisation have both contributed to Malaysians' habit of drinking coffee. Furthermore, in the local market, there are many different types of coffee. Instant coffee dominates the other types, perhaps as a result of its practicality and ease of preparation. According to Statista (2022), the revenue from instant coffee is anticipated to grow by 4.48% yearly (CAGR 2023–2025). In addition, aside from convenience, another factor that contributes to the popularity of instant coffee is the varieties of specialty instant coffee available now, allowing the consumers to sample a wider range of flavours and aromas.

1.1 Problem statements

According to Bae (2014), coffee beans are one of the world's most complex compounds because they are a complex synthesis of more than thousands different chemicals. Numerous studies have been conducted to determine how coffee affects cardiovascular health. According to the research done by Sarriá, et al. (2018) and Watanabe, et al. (2019), regular consumption of a coffee blend that includes green and roasted coffee has positive effects on blood pressure, TG levels and abdominal obesity, thus, preventing from metabolic syndrome. Nevertheless, due to the diterpene compounds found in unfiltered coffee, it has been suggested that coffee raises cholesterol levels and may even increase the risk of developing CVD (Jee, et al., 2015).

Coronary artery disease has been found associated with methylenetetrahydrofolate reductase (*MTHFR*) gene. It has recently been suggested that gene-diet interaction is crucial in determining the likelihood of developing CVD. A study conducted by McCarthy, et al. (2003) showed that eight gene polymorphisms have been linked to the risk of metabolic syndrome, the main risk factor for CVD. Other than that, a systematic review of observational and clinical trials on the gene-diet interaction for CVD, despite the paucity of the evidence, suggested that the interaction between genes and diet determines the likelihood of CVD (Roa-Daz, et al., 2022). In addition, Sadeghi, et al. (2019) calls for more research on how coffee affects people with different *CYP1A2* gene polymorphism in order to advance the development of personalised diets for disease management and prevention. However, there are not enough studies looking into the relationship between cardiometabolic risk factors, *MTHFR* gene polymorphism, and habitual coffee consumption.

1.2 Aims of the study

One's susceptibility to develop CVD depends on how genes interact with environmental factors, particularly diet. It is still unclear whether instant coffee has a good or bad effect on cardiovascular health. However, the local market is now dominated by instant coffee. The purpose of the current study is to examine the effects of *MTHFR* C677T polymorphism and instant black coffee on blood pressure, pulse and lipid profiles in individuals with different coffee consumption patterns (habitual and non-habitual coffee drinkers) and genetic background. When giving dietary recommendations on coffee intake for the

general healthy population, it is important to be aware of how different coffee consumption patterns will affect individuals with different genetic risk factors.

The objectives of the study include:

I. To identify the prevalence of habitual coffee consumption among the students at Universiti Tunku Abdul Rahman (UTAR) Kampar Campus.
II. To identify the *MTHFR* (rs1801133) genotypic distribution and allelic frequency among habitual and non-habitual coffee drinkers.
III. To investigate the association between *MTHFR* C677T polymorphism and habitual coffee drinking.
IV. To compare the blood pressure, pulse and lipid profile among habitual and non-habitual coffee drinkers of different genotypes.
V. To evaluate the effect of instant black coffee consumption on blood pressure, pulse and lipid profile among habitual coffee drinkers.
VI. To study the differences in the changes of blood pressure, pulse and lipid profile after coffee consumption between CC and CT genotypes.

CHAPTER 2

LITERATURE REVIEW

2.1 Cardiometabolic disease

Cardiometabolic disease describes a series of cardiovascular events (coronary heart disease, cerebral vascular disease, ischemic heart disease, etc.) and type 2 diabetes. CVD, together with type 2 diabetes, are the emerging health issue nowadays. Although the pathogenesis and exact mechanism are unclear. Modern civilization, sedentary lifestyle, the transition of socio-environment and dietary patterns believed to play important role in the development of cardiometabolic diseases, in which they are the outcome of unhealthy lifestyle and dietary pattern. Moreover, with the ever-changing and advanced technological development in the scientific field, the idea of genetic predisposition has been proposed to play a crucial role in the disease development, in which the mutation of some candidate genes are related to the occurrence or onset of diseases. In this research, we are going to examine how a specific food item can impact the risk factor of cardiometabolic disease by interacting with gene polymorphism.

CVD and type-2 diabetes are the major components under cardiometabolic disease. Together with hypertension, they negatively impact on the global healthcare system. In 2019, 32% of the global death were contributed by CVD mortality (World Health Organization, 2021). According to Baeradeh, et al.

(2022), the prevalence of CVD showed increasing trend, with the number of cases increased from 257 million in 1990 to 550 million in 2019. From population-representative studies that carried out from 1990 to 2019, among adults and elderly aged between 30 and 79 years old, the prevalence of hypertension has doubled, with the global age-standardized prevalence of hypertension recorded as 32% in women and 34% in men (NCD Risk Factor Collaboration (NCD-RisC), 2021).

In the United States, 49.64% of the population suffered from hypertension between 2017 and 2018 (Chobufo, et al., 2020). Besides that, the prevalence was found to be 35.5% among the Indians in 2019 to 2020 (Perappadan, 2023). Among the HOPE Asia Network countries, Japan (50%) accounted for the highest prevalence of hypertension, followed by Pakistan (46.2%) while Singapore (21.5%) recorded the lowest (Turana, et al., 2021). Besides that, the NHMS 2019 has revealed that the prevalence of hypertension among Malaysia population was 30%.

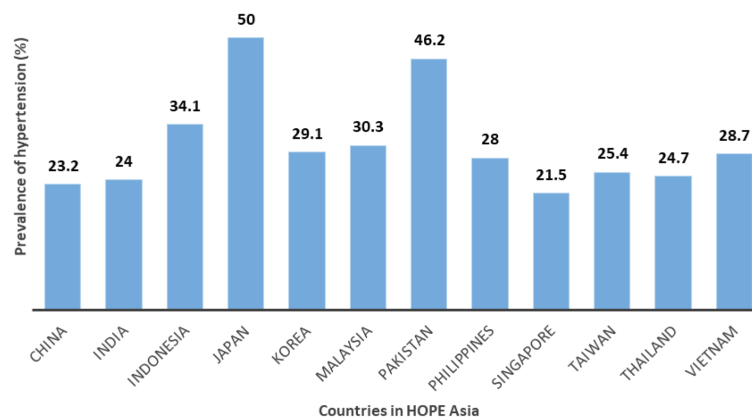

Figure 2.1: Prevalence of hypertension across the countries in HOPE Asia (Turana, et al., 2021)

Figure 2.2 shows the geographical distribution of diabetes around the world, with a total of 537 million adults aged between 20 and 79 years old living with diabetes (International Diabetes Federation, n.d.). As update from 2021, the worldwide prevelance of diabetes was 10%, however, 75% of the adults from low- and middle-incomes countries were diagnosed with diabetes (International Diabetes Federation, n.d). Surprisingly, Saeedi, et al. (2019) suggested that higher prevalence is observed in high-income (10.4%) or urban area (10.8%) than low-income (4.0%) or rural area (7.2%). Between 2019 to 2021, 11% of the Indians were having diabetes (Perappadan, 2023). In addition, 13% of the U.S. population were having diabetes, with 4.2% accounted for those aged between 18 to 44 (Centers for Disease Control and Prevention, 2020). In China, among the population aged 20 to 79 years old, 8% were diagnosed with diabetes in 2019, which increased by 4.7% in 1990. The NHMS 2019 has reported that among Malaysian, 18% of the residents had diabetes. Differences in geographical location and ethnicity does contribute to different prevalence of diabetes worldwide. According to Narayan and Kanaya (2020), Asian is more susceptible to be affected by insulin resistance and tends to be centrally obese at a lower BMI. In Malaysia, as reported by the Ministry of Health, highest prevalence of diabetes is found among Malays (59.15%), followed by Chinese (19.62%), Indian (13.17%) and indegenous population (8.05%) (Disease Control Division, 2020).

Figure 2.2: Diabetes around the world in 2021 (International Diabetes Federation, n.d.)

2.2 Cardiometabolic disease risk factor

Up to the current knowledge, the exact culprit that leads to cardiometabolic diseases are not known, however, metabolic syndrome is the most blamed reason. Metabolic syndrome, also known as cardiometabolic syndrome, is a collection of complex metabolic disorders. As stated by Kirk and Klein (2009), the clinical criteria that are widely used to diagnose metabolic syndrome includes insulin resistance, central obesity, dyslipidemia and hypertension, as proposed by the World Health Organization and the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III). These four metabolic abnormalities are inter-related, however, Kirk and Klein (2009) believed that insulin resistance is the main factor that contributes to the others. Although dietary pattern and physical activity serve as a modifiable risk factor, in this study, we are going to focus on the contribution of metabolic syndrome to disease outcomes.

2.2.1 Insulin resistance

Cardiometabolic syndrome, or metabolic syndrome are mainly caused by hyperglycemia and dyslipidemia whereby these two abnormalities are related to insulin resistance, which is the condition when body's cells respond to the hormone insulin ineffectively or with a decreased capacity to "sense" it and carry out the "command" that it issues, in this case, to perform glucose uptake and lipolysis inhibition. As mentioned by Kirk and Klein (2009), the major factor that contributes to dyslipidemia is the alternation in fatty acids metabolism whereby insulin resistance could be blamed. This is due to the fact that although insulin inhibits the lipolysis of adipose tissue, insulin resistance causes increased lipolytic activity, which raises the blood levels of free fatty acids. When excessive fatty acids being sent to the liver, it will increase the synthesis of hepatic very low-density lipoprotein (VLDL) cholesterol and the concentration of plasma TG. As a result, elevated plasma TG causes the reduction of HDL-C by increasing its clearance (Kirk and Klein, 2009).

On the other hand, it is well known that insulin resistance can lead to hyperglycemia. It should be highlighted that in fact, fatty acids also play a role in this mechanism, in which an immediate plasma free fatty acid concentrations increment will lead to impaired glucose uptake and the synthesis of hepatic glucose by interfering the signaling pathway of insulin to the receptor on the cells (Kirk and Klein, 2009). Therefore, glucose remains high in the blood and overtime, develop into diabetes.

To date, approximately 88 loci were found to be associated with the development of type 2 diabetes by the genome-wide association studies (GWAS). The relationship between *NAT2* gene and insulin resistance has been identified in the 3T3-L1 adipocytes of mouse, whereby the silence of this gene decreases the glucose uptake that stimulated by insulin, thus, reducing the insulin sensitivity. In addition, *PPARγ*, *KLF14*, *IRS1* and *GCKR* gene were found to be associated with insulin resistance in the expanded study that involved 14 cohorts, 29,084 non-diabetic individuals (Dimas, et al., 2014). For example, the Pro12Ala variant of *PPARγ* gene was found to be linked with a decreased risk of type 2 diabetes (Deeb, et al., 1998). Moreover, defective insulin action, and a reduction in the gene expression in the muscle and adipose tissue among type 3 diabetes patients were related to the rs972283 near *KLF14* gene (Yang, et al., 2015).

2.2.2 Dyslipidemia

Dyslipidemia in metabolic syndrome mainly refers to high level of TG and low level of HDL-C in the serum. As mentioned before, insulin resistance causes an increment in the adipose tissue's lipolytic activity, results in excessive TG to be delivered to the liver and consequently, elevated VLDL synthesis and therefore, leading to HDL-C level decrement. Other than the HDL-C, low-density lipoprotein cholesterol (LDL-C) also plays an important role in predicting the risk of getting CVD. According to Eckel, Grundy and Zimmet (2005), it was found that alteration in the composition of LDL-C serves as an independent risk factor to CVD. With the present of hypertriglyceridemia, LDL-C available in

the form of small dense LDL while this kind of LDL has higher atherogenicity than the buoyant type. This is due to the reason that small dense LDL has higher ability to penetrate the basement membrane of endothelium and it is more toxic to the inner layer of the vessels. Besides that, it is more resistant to oxidation, binds well to the glycosaminoglycans and more able to "protect" itself from the clearance by selectively binding to the macrophages, thus, threatening the cardiovascular health (Eckel, Grundy and Zimmet, 2005).

As far as we are aware, not only dietary and lifestyle habit, but genetic polymorphism also associates with the incidence of dyslipidemia. Individual with the respective gene variants is more susceptible to develop dyslipidemia as compared to those who are not. As stated by García-Giustiniani, and Stein (2016), *LDLR*, *ApoB*, *PCSK9* and *LDLRAP1* are the genes that their variants were thought to associate with elevated LDL-C level. Nevertheless, some polymorphisms in *ApoB* and *PCSK9* genes may result in lowered LDL-C level. For the alteration in TG, the variants of *APOA5*, *LPL*, *APOC2* and *APOE* genes were related to elevated TG level. Amongst, the gene variants of *APOA5* gene causes the carriers to have 3.3-fold higher risk to develop the acute myocardial infraction (García-Giustiniani, and Stein, 2016). Interestingly, reduction in TG level and 40% reduction in the coronary artery disease was associated with an uncommon polymorphism in the *APOC3* gene (García-Giustiniani, and Stein, 2016). Lastly, the variant of *APOA1*, *ABCA1*, *LCAT*, *SAR1B* and *ABCG1* genes were related to decreased HDL-C level. Despite the variation of *CETP*, *LIPC*, *PLTP* and *SCARB1* genes were thought to be linked with increase HDL-C level,

the protective effect against atherosclerosis remained unclear (García-Giustiniani, and Stein, 2016).

2.2.3 Hypertension

Hypertension is another important criterion for the diagnosis of metabolic syndrome, and it is thought to be resulted from the insulin resistance. Based on Eckel, Grundy and Zimmet (2005), insulin is a vasodilator, however, promotes the reabsorption of sodium in the kidneys. Having insulin resistance already set, Grundy and Zimmet (2005) explained that the effect of vasodilation is no longer available, however, sodium reabsorption remains. Other than that, since fatty acids mediate the vasoconstriction, together with the loss of vasodilating-effect and the increment in sodium reabsorption, it will result in elevated blood pressure. According to Mayo Foundation for Medical Education and Research (MFMER) (2022), prolonged elevated blood pressure burdens the heart by making it to "pump" harder. Chronically, it induces changes in the structure and function of the heart. Most of the time, the part that affected are the atrium and ventricle of the left side of the heart, as well as the coronary artery. Consequently, left ventricular hypertrophy is developed and serves as the contributor to CVD (Tackling and Borhade, 2022).

Apart from that, the vascular system also influenced by the elevated blood pressure. The most inner layer of the blood vessel, namely the endothelium is very smooth and elastic, however, due to complex mechanisms that

characterised by high blood pressure, insulin resistance and chronic inflammation, overtime, the vessels become fragile and may easily break. In this case, when excessive plasma free fatty acids present in the blood stream due to excessive fat and carbohydrate intake, the vessels are easily damaged and fatty streaks will adhere and stick to the endothelium, forming a plaque. Therefore, blood flow will be restricted due to decreased vessels' diameter, overtime, when the plaque develops in size, it will lead to insufficient oxygen supply to the tissue. Other than that, aneurysm, which is a bulge-like structure that normally found at the enlarged part of endothelium, is one of the consequences. The formation of aneurysm is dangerous as it can burst anytime, causing internal bleeding which is life-threatening (Mayo Foundation for Medical Education and Research (MFMER), 2022).

The variation of some candidate genes has been identified by the study conducted among Japan, in which the *SORBS1* A682G, *GCK* G30A and *WISP1* G2364A polymorphisms were significantly associated with both genders (Yamada, Ando and Shimokata, 2009). Besides that, another study investigated the susceptibility of blood pressure to salt intake as mediated by the gene variants has determine eleven single nucleotide polymorphisms (SNPs) that related to the phenotypes of salt sensitivity of blood pressure. The SNPs are within the eight genes, namely the *PRKG1*, *CYBA*, *BCAT1*, *SLC8A1*, *AGTR1*, *SELE*, *CYP4A11*, and *VSNL1* (Xie, et al., 2021). Currently, studies are focusing on investigating the variant of genes that responsible in the pathophysiology of primary hypertension. Several examples of the candidate genes and their

polymorphisms including the *MTHFR* (rs484604911 and rs17367504), *CAPZA1* (rs17030613), *SLC16A1* (rs2932538) and *PDE1A* (rs16823124) (Rodriguez-Iturbe and Johnson, 2018).

2.2.4 Central obesity

Furthermore, Rochlani, et al. (2017) suggested that central obesity, which is also known as abdominal obesity serves as the initiator that kick starts the other pathways involved in the metabolic syndrome. Unlike as mentioned by Kirk and Klein (2009), Rochlani, et al. (2017) stated that visceral fat contributes to the development of insulin resistance instead of the other way round. In addition, Rochlani, et al. (2017) further explained that, instead of the subcutaneous fat, visceral fat contributes more to insulin resistance and metabolic abnormality since the elevated free fatty acids that sent to the liver are mostly derived from the lipolysis of visceral fat. Other than a BMI of higher or equal to 30kg/m^2, waist circumference is another useful indicator to predict the risk of developing chronic disease in which waist circumference more than 80cm (female) or 90cm (male) indicating higher risk of disease development (British Heart Foundation, n.d.).

The development of obesity is resulted from the interaction between individual genetics and lifestyle factors (such as diet and physical activity). *FTO* gene has long been identify and its polymorphism is regarded as a strong predictor for the occurrence of obesity. As stated by Antonio, et al. (2018), in the rs1421085

as one of the *FTO* SNP, possess at least one copy of the mutant allele (C allele) can increase individual's risk of getting obesity. Besides that, a study carried out among the children population in Korea has identify 68 SNPs in 32 genes that associated with obesity. Homozygous mutant genotype of the *CNR1* (rs1049353), *HHEX* (rs1111875), *PPY* (rs231472) and heterozygote of the *LEP* (rs7799039), *LRP5* (rs4988300) and *UCP2* (rs660339) were believed to have higher obesity risk as compared to the wildtype genotype.

2.2.5 Genetic factors

Genetic factor is another important risk factors that contributes to the incidence of metabolic syndrome and study showed that a bundle of genes does associate with the syndrome. According to Ziki and Mani (2016), individual was genetically predisposed to metabolic syndrome whereby each of the traits of metabolic syndrome has more than 50% of heritability. Nowadays, with advanced genetic approaches, a collection of candidate genes has been identified for individual components that make up the metabolic syndrome (Ziki and Mani, 2016). Amongst, instead of insulin resistance, Ziki and Mani (2016) believed that obesity played the most important role in causing metabolic syndrome. *MC4R*, *FTO*, *NPC1* and *POMC* are several candidate genes that related to obesity, in which their mutation predisposes an individual to obese in the future (Ziki and Mani, 2016).

Other than obesity, *MTHFR* gene is one of the candidate genes that highly associated with blood pressure and serum lipid profile, with both these components are under metabolic syndrome and serve as important risk factors for the development of CVD (Ziki and Mani, 2016). Recently, *MTHFR* gene becomes an emergingly studied gene when investigating the incidence of CVD development, hyperhomocysteinemia and impaired folate metabolism. Numerous studies have been done, yet conclusion cannot be drawn while mix results remain for the effect of the interaction between coffee and *MTHFR* C677T polymorphism on serum lipid profile and blood pressure.

2.2.6 Age and gender

Age and gender are the non-modifiable factors that affect the risk of getting cardiometabolic disease. Aging is associated with an increased cardiometabolic risk. In the United States, the prevalence of CVD increased with age, with 90% (the highest) of those aged above 80 years old diagnosed with at least one of the CVDs (Sinclair and Abdelhafiz, 2020). Curtis, et al. (2018) explained that it is due to chronic inflammation, enhanced oxidative stress, degeneration and deterioration of the myocardium resulted from aging, and the cell apoptosis. Meanwhile, sarcopenia and frailty has recently be found to cause cardiometabolic abnormalities whereby these are the two common health issue that encountered in aging (Sinclair and Abdelhafiz, 2020).

Gender is known to affect individuals' risk of getting cardiometabolic disease as well. Differences in the cardiometabolic risk between genders are associated with different sex hormone and fat distribution among the genders. Although testosterone and estrogen both show cardioprotective effect, aging lead to the reduction in sex hormone. At the same time, the impact seen more obviously among women as estrogen drops dramatically after menopause (Bots, Peters and Woodward, 2017; Rodgers, et al., 2019). In men, the accumulation of adipose favours the abdominal region. This is because testosterone plays a role in regulating the visceral adiposity in men yet decrease in testosterone promotes visceral adiposity (Rotter, et al., 2018). On the other hand, women tend to have higher subcutaneous fat due to the effect of estrogen.

2.2.7 Diet, physical activity and smoking behavior

Dietary fat, salt intake, fiber intake and alcohol consumption are most concerned with the incidence of CVD. Diet high in saturated fat, trans-fat and cholesterol increase one's risk of getting CVD as it promotes the atherosclerosis. Besides that, excessive saturated fat intake led to elevated LDL-C level (Briggs, Petersen and Kris-Etherton, 2017). Manco, Calvani and Mingrone (2004) also proposed that by reducing the binding affinity, high saturated fat intake inhibits the response of cells to the action mediated by insulin, thus, contributes to insulin resistance. Although the exact action of mechanism and molecular pathway is unclear, trans-fat is believed to negatively impact the metabolism and signaling pathways occurs in the liver, adipose tissue, monocytes and endothelial cells (Iwata, et al., 2011). The consumption of trans-fat also led to chronic

inflammation, promote the formation of thrombus and the occurrence of endothelial dysfunction (Lopez-Garcia, et al., 2005).

Excessive salt intake is a widely known CVD risk factor that associated with hypertension. As recommended by the RNI 2017, the daily allowable salt intake is 5g, which is equivalent to 2300mg of sodium. However, 79% of the Malaysian has been found to have excessive salt intake, with an average of 3167mg, as revealed by the Malaysian Community Salt Survey (Institute for Public Health, 2019). Based on Jaques, Wuerzner and Ponte (2021), excessive salt intake is related to increased inflammation and oxidative stress while sodium was proven to exert pro-fibrotic effect directly on glomeruli in animal studies by Yu, et al. (1998).

The chemical constituents in tobacco smoke causes inflammation in the endothelial cells of blood vessels. As a result, the endothelium swells and narrows down. Although the complex mechanism of thrombosis are not well studied, tobacco smoking is related to atherosclerosis, which serve as the fundamentals of the CVD (Centers for Disease Control and Prevention, n.d.). The prevalence of tobacco use is 21.3% and 22.3% in Malaysia and worldwide. On the other hand, 24.6 % of the Malaysian was physically inactive as revealed by the NHMS 2019. As stated by Nik-Nasir, et al. (2022), this prevalence is slightly higher than other Asian population, including China, India, and Hong Kong. Physical inactivity is an important risk factor for a number of non-

communicable diseases and premature mortality (Katzmarzyk, et al., 2022). Regular exercise helps in enhancing the insulin sensitivity for normal and insulin resistance individuals (Lin, et al, 2022). As claimed by Lippi, Henry and Sanchis-Gomar (2020) individuals who are physically inactive are thought to have 24% enhanced risk of getting coronary heart disease (24%), stroke (16%) and diabetes (42%).

2.3 Coffee consumption in global world and Malaysia

2.3.1 Coffee consumption and cardiovascular disease

Over decades, coffee has been widely studied and the relationship between coffee and the risk of CVDs were investigated. However, the results are controversial that no conclusion can be drawn about whether coffee gives adverse or positive effect towards the cardiovascular health. In 2012, a study done among the United States population supported that the consumption of coffee was inversely related to CVD mortality, together with the total mortality (Freedman, et al., 2012). In addition, O'Keefe, et al. (2013) believed that coffee consumption can be considered healthy for individual who have CVD or high risk of getting CVD.

Afterwards, the role of the dosage was focused as it did play a crucial role in determining the effect exerted by coffee. In fact, early in 2007, Mattioli (2007) had proposed that high coffee consumption was associated with an increased risk of developing coronary artery disease while no adverse effect had been

found with moderate coffee consumption. Mattioli (2007), Larsson and Orsini (2011) and Klatsky, et al. (n.d.) further prove that moderate coffee intake was inversely linked with the risk of stroke and arrhythmias hospitalization while a J-shaped relationship was found between the intake of coffee and the probability to develop heart failure, in which 4 servings of coffee were thought to be associated with the lowest risk of getting heart failure (Mostofsky, et al., 2012). Nevertheless, in 2014, Lopez-Garcia, et al. (n.d.) stated that there was no relationship between regular coffee intake and increased mortality.

However, recently, in 2022, an ecological study carried out from 1990 to 2018 discovered that the relationship between the consumption of coffee and ischemic heart disease altered overtime due to social and environmental factors. Other than that, Renda, et al. (2012) further explained genetic factors play a role, not only in the variability of coffee metabolism, but also in affecting the risk factors of cardiometabolic disease. As a result of the interaction between gene variants and environmental factors such as diet, individual may have different risks of developing diseases. For example, adenosine A2A receptors and a2-adrenergic receptors gene polymorphism is associated with the differences of the acute effect of blood pressure after coffee consumption (Renda, et al., 2012). This idea has led to the development of the nutrigenomic and nutrigenetics study related to the relationship between the polymorphism of some candidate genes and the risk factors of cardiometabolic disease.

2.3.2 Coffee consumption and hypertension

Controversial findings have been found for the association between coffee consumption and the risk of hypertension as well as the effect of coffee on blood pressure. Back in 20th century and early 21st century, most of the studies claimed that coffee may increase blood pressure and exert adverse effect to the individual with hypertension (Jee, et al., 1999; Nurminen, et al., 1999; Noordzij, et al. (n.d.). Nevertheless, in 2011, Mesas, et al. (2011) found that despite blood pressure increased immediately after coffee consumption, no relationship between long-term coffee intake and elevated blood pressure among hypertensive patients was found and habitual coffee intake was not associated with increased CVD risk as well. Additionally, Steffen, et al. (2012) also clarified that the ingestion of coffee would not cause changes in systolic and diastolic blood pressure.

However, coffee was found to positively influence blood pressure as more research being carried out continuously. Grosso, et al. (2017) suggested moderate reduction in the incidence of hypertension was linked with higher consumption of coffee in prospective cohort studies. Xie, et al. (2018) further emphasised that one cup increment in the coffee intake decreased hypertension risk by 2 %. According to Surma and Oparil (2021), regularly consuming 2 to 3 cups of coffee daily did not change the risk of arterial hypertension development, or even reduced the risk. On the other hand, irregular coffee consumption pattern was related to an increment in blood pressure and the incidence of arterial hypertension as tolerance to caffeine only develop among

habitual coffee drinkers due to the lack of pressor effect (Godos, et al., 2014; Surma and Oparil, 2021).

Although the latest study suggested coffee drinking habit is appropriate with a balanced and healthy lifestyle, more studies are encouraged to discover the relationship between coffee and the candidate gene variants (Borghi, 2022). This is because not all are affected by coffee equally due to genetic variability (De Giuseppe, et al., 2019). In fact, other than the dosage and consumption frequency, De Giuseppe et al. (2019) proposed that factors, such as age, gender, lifestyle and genetic variability make it hard to determine the effect of coffee and caffeine on blood pressure and hypertension. Particularly, the genetic variability can lead to different coffee metabolism.

2.3.2.1 Acute effects of coffee on blood pressure

Experimental studies carried out among 16 healthy individuals aged between 24 to 38 years old showed that after 60 min and 90 min of regular (caffeinated) coffee intake, both central SBP and DBP increased significantly by 4mmHg among the habitual coffee drinkers (Karatzis, et al., 2005). In addition, the effect of coffee ingestion with different duration was investigated. Among the adults, blood pressure elevated 2.67mmHg (SBP) and 2.55 mmHg (DBP) within four weeks of coffee intervention. Besides that, 5.23/2.14 mmHg and 2.62/2.66 mmHg increment in blood pressure were observed for caffeine intake less than one week and more than one week, respectively (Xu, et al., 2021).

2.3.3 Coffee consumption and lipid metabolism

Unfiltered coffee has been widely known to increase cholesterol level and lead to undesirable lipid profile due to the diterpene compounds (cafestol and kahweol) found in coffee. Several researchers acknowledged that strong association was found between unfiltered coffee and elevated TC level (Rodrigues and Klein, 2006; Cai, et al., 2012; Jee, et al., 2001). Additionally, Cai, et al. (2012) indicated that unfiltered coffee also significantly increased TG and LDL-C. However, inconsistent results remained while people started to focus on the health risk of taking filtered coffee.

In fact, back in 2005, Christensen, et al. (2001) had proposed that abstention from filtered coffee was associated with decreased TC and homocysteine level This finding was supported by later research which claimed that moderate ingested of filtered coffee provided an undesirable effect on cholesterol among the Brazilian population (Corrêa, et al., 2013). However, Naidoo, et al. (2011) found that coffee low in the diterpene compounds was not associated with increased serum lipids. In addition, Karabudak, Türközü and Köksal (2015) also supported that consumption of instant coffee did not have significant effect on serum lipid levels among the Turkish population. As the studies develop overtime, the blend of green and roasted coffee has been thought to improve TG levels among healthy and hypercholesterolaemia individuals (Sarriá, et al., 2018). Conversely, Sarriá, et al. (2020) claimed that the cholesterol-reducing effect of the blend of green and roasted coffee can only be observed among the hypercholesterolaemia individuals but not the healthy population. Moreover,

one of the latest studies itself has reported controversial result in the study, in which a negative association was found between increased coffee consumption and some CVD risk factors such as HDL-C and DBP, however, increasing coffee intake leads to elevated TG levels (Ghavami, et al., 2021).

2.3.3.1 Acute effects of coffee on lipid profile

Two meta-analysis of randomized control trial conducted by Jee, et al. (2001) and Cai, et al. (2012) showed that significant increase in TC (11.8 mg/dl and 8.1 mg/dl), TG (5.9 mg/dl and 12.6 mg/dl) and LDL-C (6.5 mg/dl and 5.4 mg/dl) were found while no significant changes were observed for HDL-C level. The latter study further explained that the coffee intervention was lasted for 45 days. Furthermore, for experimental study that used the blend of Arabica and Robusta, significant increases in TC, HDL-C and LDL-C by 0.47 mmol/L, 0.1 mmol/L and 0.34 mmol/L were found. However, no significant difference in TG were recorded after the consumption of the coffee types (Surma, et al., 2023).

2.4 *MTHFR* gene and its C677T polymorphism

2.4.1 *MTHFR* C677T polymorphism and cardiometabolic disease

Given that the *MTHFR* C677T polymorphism raises plasma homocysteine levels, and that hyperhomocysteinemia is a significant risk factor for atherosclerosis, the relationship between *MTHFR* C677T polymorphism and the risk of developing CVD has received extensive research. Most of the studies revealed that there is an association between this polymorphism and CVD risk.

Among the Turkish population, being a homozygous polymorphic TT genotype or a T allele carrier increased the risk of developing essential hypertension (Er, et al., 2022). A meta-analysis concluded that among Chinese population, significant relationship between the *MTHFR* C677T polymorphism and coronary artery disease was discovered (Li, et al., 2022). In addition, Peng, et al. (2020) stated that among the Chinese Han population in Chongqing, China, this SNP serves as a risk factor for coronary artery spasm since the individual carries mutant allele is more prone to carotid plaque development.

Besides that, Yang, et al. (2015) indicated the prevalence of hyperhomocysteine is high in China, especially in the northern populations and the prevalence has risen these years. It has become a health issue as stated by Yang, et al. (2015) since hyperhomocysteinemia is related to elevated risk of many non-communicable diseases. Momin (2017) claimed that hyperhomocysteinemia contribute to hypertriglyceridemia and decreased levels of HDL-C in the blood while this rs1801133 polymorphism of *MTHFR* gene was also related to increased TG and LDL-C as well as higher risk of getting coronary artery disease (Luo, et al., 2018).

2.4.2 *MTHFR* C677T polymorphism and homocysteine

Methylenetetrahydrofolate reductase (*MTHFR*) gene, located at the short arm of chromosome 1 (1p36.3) is the gene that responsible in the production of 5-10-Methylenetetrahydrofolate reductase, the enzyme important in the

metabolism of folate and homocysteine. Folate, which is also known as vitamin B9, is the important micronutrient needed for the normal physiological function, however, it presents in its inactive form, 5-10-MTHF in food. Therefore, it is essential for the *MTHFR* enzyme to catalyse it into 5-methylTHF, which is the active form of folate. Before looking into how important this function is, we should know that homocysteine is a by-product produced in the metabolic pathway of methionine, one of the essential amino acids. This by-product should be metabolized as elevated homocysteine level serves as an independent risk factor for CVD. The clearance of homocysteine is achieved by remethylating it to generate methionine. A methyl group is needed in the re-methylation pathway of homocysteine, whereby it is donated by the 5-methylTHF. Hence, it is crucial to have proper enzyme function of 5-10-methylTHF reductase (Raghubeer and Matsha, 2021).

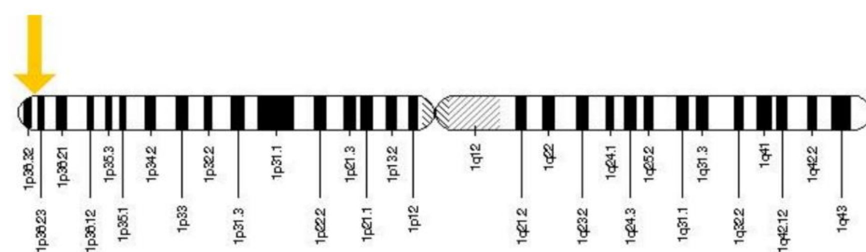

Figure 2.3: Gene location of *MTHFR* gene in chromosome 1 (Genetics Home Reference, 2014).

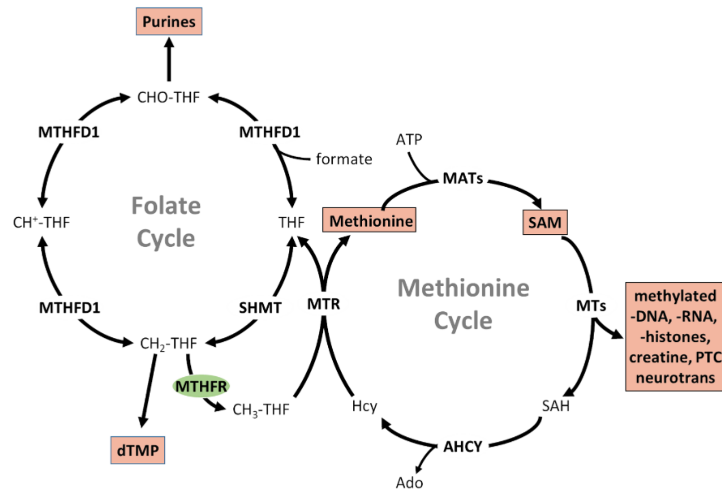

Figure 2.4: *MTHFR* at the intersection of the folate cycle (orange) and methionine cycle (blue) in one-carbon metabolism (Structural Genomics Consortium, 2019).

C677T polymorphism (rs1801133) is one of the most common gene variants of *MTHFR* gene. It is a single nucleotide polymorphism (SNP) that the nucleic acid cysteine is changed to thymine at the 677th position of the DNA sequence. Thus, results in the alteration of alanine to valine at 222th and 263th positions of the amino acid sequence (Er, et al., 2022). This missense mutation results in three genotypes, CC (homozygous wildtype), CT (heterozygote), and TT (homozygous mutant). According to Raghubeer and Matsha (2021), this SNP was associated with decreased enzyme activity as characterised by the thermolability of the enzyme encoded. Based on Er, et al. (2022), CT genotype has 70% residual enzyme activity while TT genotype has 30% of the enzyme activity. Enzyme deficiency or defective enzyme leads to reduction in the re-methylation of homocysteine into methionine, results in mild-to-moderate hyperhomocysteinemia (Raghubeer and Matsha, 2021). According to Bouzidi, et al. (2020), *MTHFR* C677T polymorphism influenced the level of circulating

homocysteine in the blood while the highest level was thought to be related to the TT genotype.

2.4.3 The association between *MTHFR* C677T polymorphism and blood pressure

As one of the indicator and risk factor of cardiometabolic disease, the association between rs1801133 polymorphism, homocysteine and the incidence of hypertension was studied. Homocysteine level was found to be significantly associated with an increased blood pressure as well as the risk of hypertension (Sengwayo, Moraba and Motaung, 2013). This finding was in agreement with a later study which believed that individuals with T allele was at higher risk of developing hypertension as compared to the case of homozygosity for the C allele (Ward, et al., 2020). Furthermore, a meta-analysis suggested that among the overall population, particularly the population of East Asia and Caucasian, *MTHFR* C677T polymorphism can be considered as a risk factor for the essential hypertension (Meng, et al., 2021). Similarly, Er, et al. (2022) reported that among the Turkish male population, homozygous polymorphic TT genotype and variant T allele was linked with higher incidence of essential hypertension.

2.4.4 The association between *MTHFR* C677T polymorphism and serum lipid profile

Serum lipid profile functions as an indicator to forecast the risk of developing CVD, much like blood pressure does. However, contradictory results remain for the association between *MTHFR* C677T polymorphism and serum lipid levels. Sengwayo, Moraba and Motaung (2013) suggested there was no significant association between plasma homocysteine and dyslipidemia. Besides that, although higher level of plasma homocysteine was found among 677T allele carriers, especially the TT genotype, neither the polymorphism nor hyperhomocysteinemia was associated with hyperlipidemia.

However, Chen, et al. (2014) found that women with 677T allele in the Chinese population in Bama, China had higher TC and LDL-C levels whereas Zhou, et al. (2022) proposed that in Beijing, North China, individuals with hyperhomocysteinemia had 0.18 ($p = 0.002$), 0.76 ($p < 0.001$) and 1.26 ($p < 0.001$) times higher prevalence of having elevated LDL-C, TG, and lower HDL-C than those with normal homocysteine levels before cofounding factors were adjusted. In addition, Luo, et al. (2018) claimed that the C677T variant of *MTHFR* gene was linked with higher TG and LDL-C levels while Momin, et al. (2017) found that hyperhomocysteinemia serve as an independent risk factor of low HDL-C level and hypertriglycemia among Chinese population in China. In animal study, it has been observed that decreased mRNA for ApoA-I and ApoA-IV were shown in the mice with one copy of the mutant allele, meanwhile, the mRNA for cholesterol 7α hydroxylase (*CYP7A1*) increased (Mikael, Genest,

and Rozen, 2006). ApoA-I plays a role in the removal of cholesterol whereas ApoA-IV is responsible in the modulation of TG-rich lipoprotein (Mangaraj, Nanda and Panda, 2016; Qu, et al., 2019). Furthermore, cholesterol 7α hydroxylase, the enzyme encoded by the *CYP7A1* gene plays a crucial role in regulating the conversion of cholesterol into bile acid and its deficiency may facilitate the formation of cholesterol gallstones (Pullinger, et al., 2002). Thus, we can conclude that defective *MTHFR* enzyme may disrupt the pathway of lipid metabolism, lead to undesirable lipid profile and lipid-related problem such as the gallstone.

2.5 Epigenetics effect of coffee

Numerous studies have been carried out to prove the epigenetics effect of coffee, in which the components inside coffee (caffeine, phenolic compounds, alkaloids and diterpenes) act like the bioactive compound that can interact with the gene, regulating the gene expression (de Melo Pereira, et al., 2020). For instance, based on Cornelis, et al. (2011), coffee was found to regulate the expression of *CYP1A2* and *AHR* gene. Specific loci on these two genes were believed to be associated with the consumption of caffeine, hence, further studies are required to discover the beneficial or toxic effect of coffee. In addition, Cornelis, et al. (2015) proposed that among the adult population of European and African American, the effect of coffee on health becomes different due to their genetic variability. This finding was in accordance with the result from Pourshahidi, et al. (2016), which claimed that regarding whether beneficial or adverse effect will be derived from the consumption of coffee, conclusion cannot be made

generally since genetic variability as well as the patterns (frequency, dose, types) of coffee consumption do play a role in deciding the effect. Moreover, another study done by Sadeghi, et al. (2019) suggested that disease outcome or the effect of certain food on health will be influenced by the interaction between diet and genetic variability whereby this study strengthened the statement that everyone is affected by coffee differently.

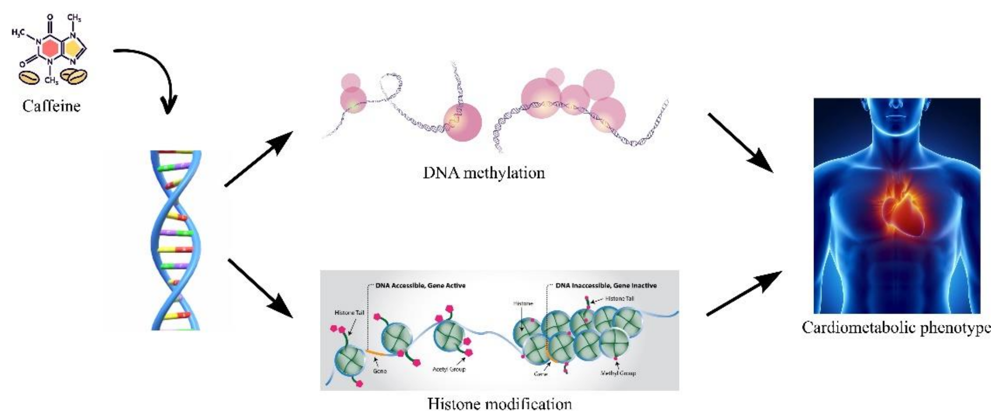

Figure 2.5: Epigenetics effect of caffeine on the impact of cardiometabolic disease via DNA methylation and histone modification (Active Motif, n.d.; bsd_studio, n.d.; EpigenTek, n.d.; Inspirit, n.d.; MetaCardis, n.d.).

2.5.1 Interaction between coffee, *MTHFR* C677T polymorphism and homocysteine level

As stated by Strandhagen, et al. (2004), elevated homocysteine level due to coffee was seen more obviously among the *MTHFR* 677TT polymorphism as compared to the C allele carriers. In accordance with it, Thelle and Strandhagen (2005) stated that homocysteine-increasing effect of coffee was mainly observed among the homozygous 677TT genotype. Furthermore, Thelle and

Strandhagen (2005) emphasised on the role of genetic variant on the effect of coffee by indicating that the heterogeneity in the impacts of coffee on serum lipid and plasma homocysteine level can be explained by the genetic susceptibility.

Up to date, numerous studies have been conducted to investigate the connection between coffee and cardiometabolic risk factors (blood pressure and serum lipid profile), but the findings are still ambiguous. As demonstrated by a number of studies that suggested an association between this SNP and homocysteine level, blood pressure, and serum lipid profile, it can be explained by genetic variability, such as the C677T polymorphism of the *MTHFR* gene. Despite the suggestion that coffee may act as a regulator for this gene variant, little is known about the interaction between instant black coffee and the *MTHFR* C677T polymorphism and how it affects blood pressure, pulse and lipid profiles. Therefore, present study aims to determine how instant black coffee affects the *MTHFR* C677T polymorphism (rs1801133) and the impact of their interaction on blood pressure, pulse and lipid profile.

CHAPTER 3

MATERIALS AND METHODS

3.1 Ethical approval

Ethical approval was granted from UTAR Scientific and Ethnical Review Committee (SERC) (code number: U/SERC/251/2022). The approval letter was attached in Appendix A.

3.2 Overview of the research methodology

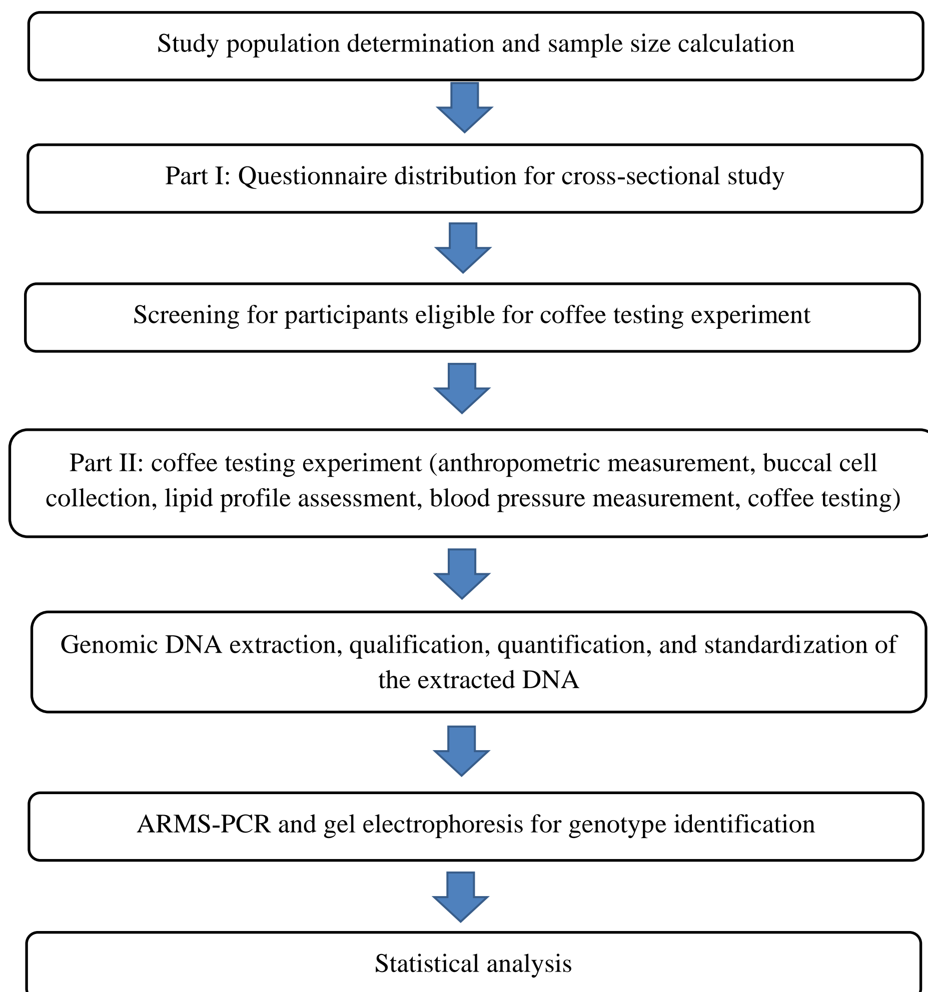

Figure 3.1: Overview of the research methodology

3.3 Study design

This study consisted of two parts, the Part I was a cross-sectional study whereas the Part II was an experimental study (coffee testing experiment). In the coffee testing experiment, the participants were classified into two groups (habitual and non-habitual coffee drinker).

3.4 Sample size

The sample size needed was calculated as the following:

$n= [t^2 \times p(1-p)]/ m^2]$

$n = 1.96^2 \times (0.25 \times 0.75) /0.05^2$

$= 288$

n = required sample size

t = confidence level at 95% (standard value of 1.96)

p = estimated prevalence of C677T gene polymorphism was 25% (Tan, 2015)

m = margin of error at 5%

*To allow 20% drop out, a total of 325 respondents will be targeted

3.5 Study population

3.5.1 Cross-sectional study

This study was conducted among the population of Universiti Tunku Abdul Rahman (UTAR) Kampar Campus. Besides that, convenient sampling was applied to collect the samples and the duration for sample recruitment lasted for six months (from January 2023 to June 2023). A booth was set up at Block D,

UTAR Kampar Campus to collect the samples. The inclusion criteria for the cross-sectional study were students or staffs in UTAR Kampar Campus. A total of 298 respondents were recruited for Part I.

3.5.2 Experimental study

Participants were further screened for taking part into the Part II, experimental study. The inclusion criteria were UTAR students aged between 18 to 30 years old. The exclusion criteria for the experimental study were as followed: obese, smoker, habitual alcohol drinker, in pregnancy or lactation, having unbalanced dietary pattern (high-fat and high-salt intake), having any disease and/or currently on medication. Questionnaire was utilized to screen for the participants eligible for coffee testing experiment, at the same time, to classify them into habitual and non-habitual coffee consumer according to their reported frequency of coffee consumption in the questionnaire. Participants who drank at least three or more than three times of any types of the coffee in a week were classified as habitual coffee drinker while the others were considered as non-habitual coffee drinker (not consume coffee or consume less than three times a week). Finally, a total of 106 (59 habitual coffee drinker and 47 non-habitual coffee drinker) participants were chosen for the experimental study.

3.6 Questionnaire

The consent form was attached in the first page of the questionnaire (Appendix B). It consisted of the project title, procedure, confidential details that would be

asked, possible hazard during buccal cell collection and finger pricking. Only the participants who agreed and signed the consent form would be required to join the study. This questionnaire (attached as Appendix C) was used to assess the sociodemographic characteristics, personal and family disease history, coffee consumption status and preferences, knowledge, perception, attitude and practices towards coffee consumption, dietary intake information, supplement and food supplement intake, physical activity status as well as time spent on sitting.

3.7 Coffee testing experiment

Participant recruitment was a major challenge in this study. Eligible participants were recruited and briefed the significance of the study on evaluating the influences of *MTHFR* C677T polymorphism and the effect of coffee on blood pressure, pulse and lipid profile. Participants were asked to proceed with anthropometric measurements, buccal cell collection, lipid profile assessment, resting blood pressure measurement. Habitual coffee drinkers were required for the coffee testing.

3.7.1 Anthropometric measurements

Height and weight were obtained to calculate the body mass index (BMI). Their height was measured using stadiometer (Seca, German) and the reading was taken to the nearest 0.1 cm. Besides that, body weight was measured by the weighing scale (Hopkins, Singapore). Body composition such as BMI, body fat

and body water percentage, muscle mass, bone mass and BMR can be obtained by the same weighing scale while performing the body weight measurement as an additional information. The guideline of BMI classification was attached in Appendix D.

3.7.2 Buccal cell collection

Buccal cell collection via mouthwash was applied to collect the human genomic DNA from the participants. Donors were requested not to eat or drink any food and beverage (plain water was allowed) at least one hour before and rinse their mouth thoroughly upon the buccal cell collection. A paper cup containing 15 mL of the 0.9% saline solution (RinseCap, Malaysia) was given to the donor. Next, the donor was advised to perform the mouthwash as described: gargle 30 seconds for the left and right cheeks, respectively, and another 30 seconds for throat. After that, donor was requested to dispense the saline that containing buccal cells back into the paper cup. Finally, the mouthwash sample would be transferred to a sterile falcon tube and stored at -20°C in the chest freezer.

DNA isolation from buccal cells has been proved to yield high quality DNA for the genotyping of polymorphism (ArulJothi, et al., 2016). Cost-effective, non-invasive, and easy to perform are the most important reasons for the widely usage of buccal cells in DNA isolation. Besides that, not only the procedure of buccal cells is fast and simple, but Aidar and Line (2007) also claim that the

mouthwash sample can be stored under room temperature for up to 30 days prior to DNA isolation and PCR amplification.

3.7.3 Lipid profile assessment

Finger pricking was performed, and a cholesterol meter (Mission, USA) was utilized to assess the participant's non-fasting lipid profile, including the total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) and the ratio of total cholesterol to high-density lipoprotein cholesterol (TC/HDL-C). A push button lancet (Accu-Chek, Malaysia) was used to carry out the finger pricking. Prior to the finger pricking, light massage was applied on participant's hand to ensure enough blood could be drawn (18 µL). The first drop of blood was wiped off, followed by the collection of blood using capillary tube. Immediately, the blood was dispensed onto the lipid test strips (Mission, USA) that had been inserted into the cholesterol meter beforehand. Finally, the readings of the lipid profile would be shown on the cholesterol meter and recorded. The classification of lipid profile reading was attached in Appendix D.

3.7.4 Blood pressure measurement

Brachial blood pressure was measured on the left arm using the blood pressure monitor (Omron, Japan). Participants were asked to rest for 10 minutes before the measurement was carried out. Systolic blood pressure (SBP), diastolic blood pressure (DBP) and pulse rate were taken. Each measurement has been

triplicated and recorded to calculate for the average. The guidelines for blood pressure categories and classifications were attached in Appendix D.

3.7.5 Coffee testing

Participants (habitual coffee drinkers) were requested not to take in any food or beverage at least one hour before the coffee testing. After the pre- non-fasting lipid profile test and resting blood pressure measurement had been done, a cup of coffee (3g of instant black coffee dissolved in 150mL hot water) was given to the participants. The composition of the instant black coffee powder was being illustrated in Appendix E. This coffee powder was not being established in the market, not belong to any brand, and was solely extracted from Robusta (90%) and Arabica (10%) coffee bean. The caffeine on dry basis is 3.52%. Immediately after the participants had finished the coffee, their blood pressure would be measured at 0 minutes, 30 minutes, and 60 minutes after the coffee test. Similarly, each measurement would be triplicated and recorded to calculate for the average. Additionally, at 60 minutes of the coffee consumption, post-lipid profile test was conducted, and the readings were recorded.

3.8 Chemical materials

Chemicals involved in the genomic DNA extraction, ARMS-PCR, gel electrophoresis of the genomic DNA as well as the ARMS-PCR product were shown in Appendix F while the materials, instruments, and equipment used were shown in Appendix G.

3.9 Genomic DNA extraction

3.9.1 DNA extraction from buccal cell

DNA isolation was done by extracting the DNA from the buccal cell collected via mouthwash, followed the protocol provided by the manufacturer (Gene Direx). The commercial DNA extraction kit manufactured by Gene Direx was utilized to perform the extraction, namely the BR (cell harvesting), BC (lysis buffer) and BP (protein precipitation) solution. First, the saline solution consisting buccal cells was centrifuged at 10,000 g for 5 minutes. Next, the supernatant was discarded, and the pellet was resuspended in 50 µL of the BR solution and being pipetted to mix the mixture well. The purpose of adding BR solution was to prevent DNA degradation. After that, 300 µL of the BC solution was added to lyse the cells and the mixture was vortexed prior to be incubated in the water bath at 60°C for 10 minutes until the sample lysate became clear. During the incubation, the bottom of the falcon tube was tapped every 3 minutes interval. After the incubation, 100 µL of the BP solution was added into the falcon tube and immediately vortexed for 10 seconds before the mixture could be transferred into a 1.5 ml microcentrifuge tube. This step was to remove the protein content in the cells. Next, the mixture was incubated on ice for 5 minutes. Upon taking out from the ice, centrifugation at 16,000 g was carried out for 3 minutes for the microcentrifuge tube. After being centrifuged, the supernatant was transferred to another new 1.5 ml microcentrifuge tube before added with 300 µL of the isopropanol. Next, centrifugation at 16,000 g for 5 minutes was performed for the mixture and the supernatant was discarded. 300 µL of the 70% ethanol was then added to wash the pellet before another round of centrifugation at 16,000 g for 3 minutes was carried out. Supernatant was discarded and the

pellet was air-dried for 5 minutes before added with 100 μL sterile distilled water. Next, the microcentrifuge tube containing DNA sample added with the sterile distilled water was incubated at 60°C for 10 minutes to dissolve the DNA sample. The microcentrifuge tube was tapped every 3 minutes interval during the incubation. After that, the DNA sample was stored at -20°C for future use.

3.9.2 Quantification of the genomic DNA

NanoDrop® ND-1000 UV-Vis spectrophotometer was utilized in the quantification of the genomic DNA. By measuring the absorbance of the genomic DNA sample at 260 nm and 280nm wavelength, the concentration and purity of the genomic DNA can be determined. Concentration higher than 50 ng/μL was desired whereas the ratio of the 260nm/280nm which fell within 1.60 to 1.80 was considered as "pure". Before the concentration of the DNA sample can be measured, the nanodrop was calibrated prior to the DNA concentration measurement. This step required the arms of the machine to be put down and the system would automatically carry out the routine. After that, Kimwipe paper was used to clean the sample stage before 1 μL saline solution (0.9% NaCl) was used to blank the machine. This was done by pipetting the saline solution onto the bead of the sample stage. After that, the sample stage needed to be clean by Kimwipe paper, and each DNA sample was analyzed one by one. The process was similar to that of blanking with saline solution, which means that 1 μL of the DNA sample was pipetted onto the bead of the sample stage and the arms was lifted down carefully. After clicking on the "measure" button, the concentration as well as the purity of the DNA sample (260nm/280nm) was

shown. The bead of the sample stage required cleaning by using Kimwipe paper before the next sample was analyzed.

3.9.3 Qualification of the genomic DNA

Qualification of the genomic DNA was done by carrying out gel electrophoresis. The chemicals, materials, equipment and instruments needed included the followings: 1X TBE buffer, agarose powder, *HindIII* ladder, Novel Juice (DNA staining), gel casting tray, gel comb, gel tank, power supply and UV-Transilluminator. The 1X TBE buffer was prepared from 10X TBE buffer by dilution method, in which 100 mL of the 10X TBE buffer was diluted with 900 mL sterile distilled water in order to prepare 1L of 1X TBE buffer. After that, the 1X TBE buffer was store in a Schott bottle, for the usage of preparing agarose gel and used as running buffer. Besides that, a 1% agarose gel was required for the quality checking of genomic DNA. To prepare it, 0.33g of the agarose gel was mixed with 33mL of the 1X TBE buffer in a conical flask. Next, the conical flask containing the mixture was heated using a microwave to dissolve the agarose powder until it became clear. After that, the mixture was allowed to cool down by manually swirling the conical flask to prevent it from being solidified in the conical flask. Once the temperature of the mixture had been cooled down to approximately 50-60°C, it could be poured into the gel casting tray that had been fixed with gel comb. The agarose gel was being stood for 30 minutes to let it solidify before putting it into the gel tank.

After 30 minutes, the comb was removed, the gel was transferred into the gel tank and buffered with the 1X TBE running buffer until the level of the buffer was 5mm above the gel. While loading the well, the *HindIII* ladder was always loaded onto the first well whereas for the remaining well, each of them would then be loaded with DNA sample. Both the ladder and the DNA sample were required to be mixed with a DNA staining reagent (Novel Juice, supplied in 6X loading dye) and the mixing process was done on a parafilm, in which 1 µL of the Novel Juice was mixed with 3 µL DNA ladder or 5 µL DNA sample that being loaded into the well.

After all DNA samples had been loaded, the gel was run at 90V for 30 minutes. The gel was taken out from the tank and viewed under the UV light using the UV-Transilluminator to check for the genomic DNA quality after the running process was done. The gel image was captured, and the brightness was adjusted to a desired version.

3.9.4 Standardization of the genomic DNA

Prior to the ARMS-PCR amplification, all the DNA sample were required to be standardized to 50 ng/µL by diluting the DNA sample with sterile distilled water.

3.10 Genotyping

3.10.1 ARMS-PCR amplification

ARMS-PCR refers to the polymerase chain reaction that applies the allele-specific primers to replicate and amplify the targeted fragment of the DNA (ArulJothi, et al., 2016). According to Yang, et al. (2017), it is one of the simplest method to detect the SNPs. In ARMS-PCR, each DNA sample would be added with wildtype and mutant reverse primer, separately in two tubes, and the amplification of the DNA sample can only be achieved with the presence with that specific allele (wildtype or mutant allele). Unlike PCR-RFLP which requires the action of restriction enzyme, only PCR amplification and gel electrophoresis is needed in ARMS-PCR (Yang, et al., 2017).

Table 3.1 indicates the three *MTHFR* C677T primers used in the amplification of the PCR product of the C allele (226 bp) and T allele (226 bp), including one forward primer and two reverse primers (reverse wildtype and reverse mutant). The two different reverse primers were used to replicate the wild type and mutant allele, respectively. Each PCR tube represented one reaction with forward primer, reverse primer (wildtype or mutant), 1 unit of PCR Master Mix, DNA template and sterile distilled water. The volume and concentration of the components mentioned were shown in Table 3.2.

ARMS-PCR was applied to amplify the specific segment of the extracted DNA. Bio-Rad T100 PCR machine was used to perform the ARMS-PCR and it was

carried out on 18μL volume. Several steps involved in the PCR condition are shown in Table 3.3. After the pre-denaturation at 96°C for 2 minutes, 10 cycles of the denaturation (96°C for 15 seconds) and annealing (65°C for 60 seconds) was followed by another 20 cycles of final denaturation (96°C for 10 seconds), final annealing (61°C for 50 seconds) and final extension (72°C for 30 seconds). Lastly, the PCR products would be hold infinitely at 10°C until it was taken out to proceed with the gel electrophoresis. A 4% gel was used to run the PCR product at 80 V for 40 minutes. For DNA ladder, 50bp DNA ladder was used (Gene Direx, USA; PKT, Korea). Both the 50bp DNA ladder and PCR product were stained with Novel Juice before loaded into the well. To assess the presence of targeted amplicon size (226bp), the gel was viewed under UV-Transilluminator, and the gel image was captured.

Table 3.1: Primers for ARMS-PCR amplification.

Allele	Pairwise primers	Primers (5' to 3')	PCR amplicon
C allele (wild-type allele)	Forward	5'-GCG TGA TGA TGA AAT CGA-3'	226
	Reverse wildtype	5'GCG TGA TGA TGA AAT CGG-3'	
T allele (minor allele)	Forward	5'-GCG TGA TGA TGA AAT CGA-3'	226
	Reverse mutant	5'GCG TGA TGA TGA AAT CGA-3'	

(Zonouzi, et al., 2012)

Table 3.2: Volume and concentration of the components involved in ARMS-PCR amplification

PCR components	Stock Concentration	Final concentration	Volume (µL)
2x PCR Master Mix	5x	2x	9.0
Forward primer	10 µM	0.4 µM	0.72
Reverse primer	10 µM	0.4 µM	0.72
DNA template (50 ng/ µL)	-		1.00
Sterile distilled water	-	-	6.56
Total			18.00

Table 3.3: ARMS-PCR conditions

Stage	Temperature (°C)	Duration	Cycle
Pre-denaturation	96	2 min	1
Denaturation	96	15 sec	10
Annealing	65	60 sec	
Final denaturation	96	10 sec	20
Final annealing	61	50 sec	
Final extension	72	30 sec	
Hold	10	∞	

3.11 Statistical analysis

Socio-demographic data, personal and family's health status and disease history, medication used, dietary and supplement intake, etc. were studied. SPSS software (version 27) was used to carried out the statistical analysis. The association of different variables was presented using cross-tables in each analysis while Chi-square test was applied to test the association between the variables. Besides that, Chi-square test for Hardy-Weinberg was applied to compare the genotype distribution between habitual and non-habitual coffee drinkers.

Kolmogorov–Smirnov test was used to test the normality of the data. As there was a mixture of normally distributed and not-normally distributed data in the present study, parametric test, together with their non-parametric counterparts were utilized to test the hypothesis. The statistical tests used in the present study were as the following and stated in the way of parametric (non-parametric counterpart): one-way ANOVA (Kruskal-Wallis H test), paired T-test (Wilcoxon test), independent T-test (Mann-Whitney U test). The result was statistically significant when p-value less than 0.05.

One-way ANOVA and Kruskal-Wallis H test were used to determine if there was significant difference in the means of blood pressure, pulse and lipid profile between three or more independent group of participants. Tukey test and Dunn'a test were further utilized after the application of these two tests. Besides that, paired T-test and Wilcoxon test were carried out to determine the significant differences in the means of blood pressure, pulse and lipid profile before and after the participant drank the coffee. Furthermore, independent T-test and Mann-Whitney U test were used to compare the means of blood pressure, pulse and lipid profile between two independent group of participants.

CHAPTER 4

RESULTS

4.1 Demographic characteristics of the study population in UTAR, Kampar

The demographic data, including gender, age, ethnicity and educational level are presented in Table 4.1. A total of 298 respondent was recruited for the Part I, including 110 male and 188 female. Majority of the participants aged between 21 to 23, with the mean age of 21.16 ± 1.64 years old. Besides that, most of the respondents were Chinese (97%), while Malay and Indian accounted for 3%. Most of them were pursuing undergraduate degree (66.4%), followed by foundation studies (31.9%), one Master student and four with unknown education status (1.3%).

Table 4.1: Demographic characteristics of the recruited sample of Part I

Variable (Mean ± SD)	Frequency (n, %)
Gender	
Male	110 (36.9)
Female	188 (63.1)
Age (21.16 ± 1.64)	
18-20	105 (35.2)
21-23	172 (57.7)
24-26	20 (6.7)
27-29	1 (0.3)
Ethnicity	
Chinese	289 (97)
Malay	1 (0.3)
Indian	8 (2.7)
Educational level	
Foundation	95 (31.9)
Undergraduate degree	198 (66.4)
Master's degree	1 (0.3)
Unknown	4 (1.3)
Total	**298 (100%)**

4.1.1 BMI, physical activity and supplement intake

Table 4.2 illustrates respondents' BMI status, physical activity level and supplement intake. BMI was classified into underweight (<18.5 kg/m^2), normal (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2) and obese (>30 kg/m^2), according to the WHO guideline. Besides that, physical activity level was also recorded for both vigorous and moderate activity, ranges from no physical activity to seven times of workout a week. Majority of the respondents' BMI fell under normal range (60.1%) while 28.9% of the respondents were underweight, followed by overweight (7.7%) and obese (3.4%). In the study population, 54.4% of the respondents did not do vigorously activity whereas 49.7% of them did not practise moderate physical activity regularly. Only 1.3% and 2.3% of them carried out vigorous and moderate physical activity every day. In terms of the supplement used, 66.4% of the respondents took supplement, however, only 28.5% consumed food supplement.

Table 4.2: BMI, physical activity and supplement intake

BMI (Mean ± SD: 20.77 ± 3.58)	**Frequency (n, %)**
Underweight (<18.5 kg/m^2)	86 (28.9)
Normal (18.5 - 24.9 kg/m^2)	179 (60.1)
Overweight (25 - 29.9 kg/m^2)	23 (7.7)
Obese (>30 kg/m^2)	10 (3.4)
Physical activity	
Vigorous activity	
No vigorous activity in leisure time	162 (54.4)
1-2 times per week	55 (18.5)
3-4 times per week	52 (17.4)
5-6 times per week	25 (8.4)
7 times per week	4 (1.3)
Moderate activity	
No moderate activity in leisure time	148 (49.7)
1-2 times per week	79 (26.5)
3-4 times per week	46 (15.4)
5-6 times per week	18 (6)
7 times per week	7 (2.3)

Table 4.2 (Continued)

Supplement intake	198 (66.4)
Yes	100 (33.6)
No	
Food supplement intake	
Yes	85 (28.5)
No	213 (71.5)

4.1.2 Prevalence of coffee consumption among students at UTAR Kampar Campus

Figure 4.1 shows the prevalence of habitual coffee consumption among students at UTAR Kampar Campus while coffee consumption pattern is summarised in Table 4.3. Approximately one third (34.9%) of the participants were habitual coffee drinker (consume three or more than three times of coffee in a week), while the others (65.1%) were non-habitual drinker. In terms of the frequency, majority of the participants (23.1%) drank less than once per 6 months, followed by the intake of three or more than three times per week (19.8%). Only two of them drank until the extent of "more than three times per day".

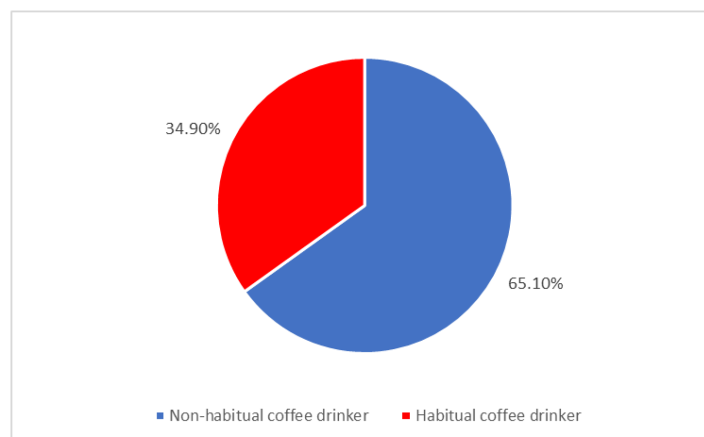

Figure 4.1: Prevalence of habitual coffee consumption among students at UTAR Kampar Campus

Table 4.3: Coffee consumption status and frequency

Variable	Frequency (n, %)
Coffee consumption status	
Habitual coffee consumption (3 times or above per week)	104 (34.9)
Non-habitual coffee consumption	194 (65.1)
Coffee consumption frequency	
Less than once per 6 months	69 (23.1)
Less than once per month	42 (14.1)
Once per month	22 (7.4)
2-3 times per month	24 (8.1)
1-2 times per week	37 (12.4)
3 or more than 3 times per week	59 (19.8)
Once per day	27 (9.1)
1-3 times per day	16 (5.3)
More than 3 times per day	2 (0.7)
Total	**298 (100)**

4.1.3 Knowledge, perception, attitude, and practice towards coffee consumption among the students at UTAR Kampar Campus

Knowledge, attitude, perceptions, and practice towards coffee consumptions is summarised in Appendix J. All participants (n=298) were required to answer the questions regarding the knowledge and perceptions in coffee consumption. However, only the participants (n=229) who claimed that they have consumed coffee for at least once in the past 6 months were required to answer the "coffee consumption attitude and practices" session. The knowledge and perception access the respondents' insights of the health impact and effect related to coffee intake. The attitude towards coffee intake investigates their measurement when purchasing coffee. Furthermore, coffee consumption practice shows their preferred coffee types as well as the event that they drank coffee the most, for example, they preferred to drink coffee with certain meal; did they also drank more frequently during final examination preparation period.

135 of the participants (45%) stood neutral for the statement that the ingestion of coffee may lead to increased disease risk (cancer, heart disease). 35% of them claimed that they drink coffee because of the taste, and they agreed that it helps in boosting their energy and making them stay awake. Meanwhile, they never thought that drinking coffee will lead to difficulty in sleep and they disagreed that friends and fashion were the reason of coffee consumption. In addition, majority (51.7%) were not concerned about the brand and origin of the coffee consumed.

Regarding the consideration when purchasing or ordering coffee, popular factors to be considered including price (45.4%), taste (50.2%) and strength (45.0%) whereby a coffee with smooth taste, strong aroma and inexpensive price would be preferred. Generally, most of the participants (31.4%) drank coffee during their breakfast, while drinking coffee right after waking up or during any event after breakfast (during lunch, afternoon tea, dinner, supper or before bed) were not a common practice among the study population. 26.2 % of the students would consume coffee during examination week, however, they did not do so when they were stressed. Instant coffee was the most preferred type (17.5% agreed) among the study population while majority claimed that it was because it tastes best (30/6%), followed by its convenience (16.6%).

4.1.4 Association between gender, BMI status and habitual coffee consumption

Among the participants, significant association was found between gender and coffee consumption status ($p < 0.001$) (Table 4.4). Males was more likely to be habitual coffee drinker than female. Furthermore, Table 4.5 shows that there was no significant association between coffee consumption and BMI status ($p > 0.05$).

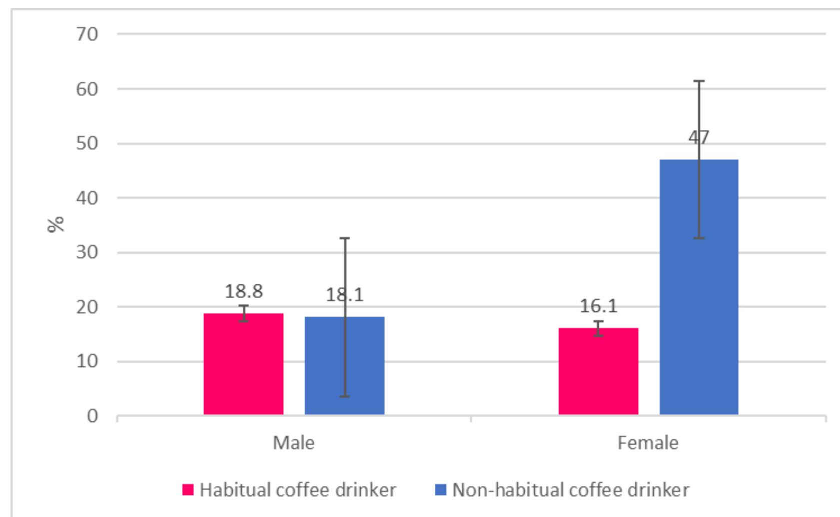

Figure 4.2: Prevalence of habitual coffee consumption among different genders

Table 4.4: Association between gender and coffee intake (habitual and non-habitual coffee drinker)

Gender	Coffee consumption status		χ^2	df	p
	Habitual coffee drinker (%)	Non-habitual coffee drinker (%)			
Male	56 (18.8)	54 (18.1)	19.671	1	< 0.001*
Female	48 (16.1)	140 (47.0)			

Table 4.5: Association between coffee intake (habitual or non-habitual coffee drinker) and BMI

BMI	Coffee consumption status		χ^2	df	p
	Habitual coffee drinker (%)	**Habitual coffee drinker (%)**			
Underweight	21 (7)	65 (21.8)			
Normal	71 (23.8)	108 (36.2)			
Overweight	8 (2.7)	15 (5)	6.062	3	0.109
Obese	4 (1.3)	6 (2)			
Total	104 (34.9)	194 (65.1)			

4.2 Prevalence of *MTHFR* (rs1801133) among the study population

4.2.1 Genomic DNA evaluation

DNA samples extracted from buccal cells were qualified and quantified prior to PCR amplification. DNA samples were standardized to 50 ng/µL before loaded on the 1% agarose gel. After that, gel electrophoresis was conducted in which the DNA samples were electrophoresed at 90V for 30 minutes. Figures 4.3 and 4.4 show the gel image of the genomic DNA before and after standardization. The genomic DNA's concentration and purity was shown in Appendix H.

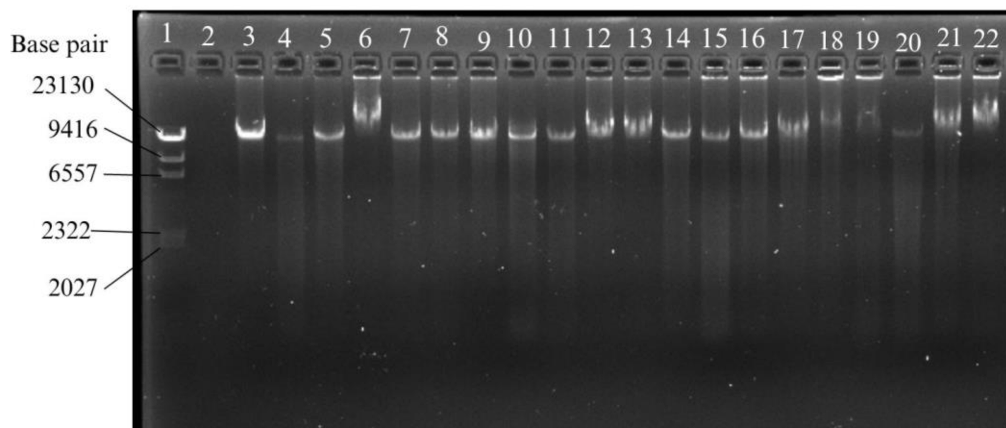

Figure 4.3: Gel image of DNA extraction using 1 % agarose gel. Lane 1: Lamda *HindIII* ladder; Lane 3 to Lane 22: genomic DNAs of different donors.

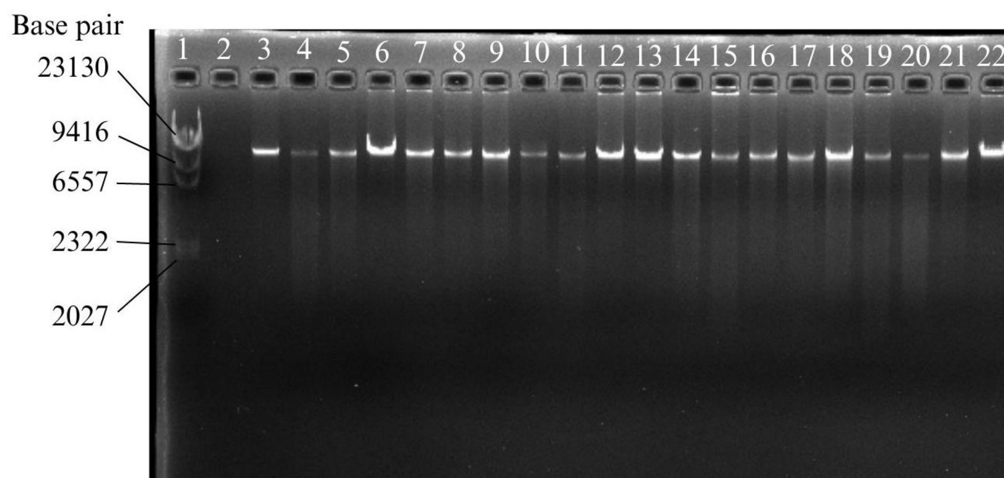

Figure 4.4: DNA standardization using 1 % agarose gel. Lane 1: Lamda *HindIII* ladder; Lane 3 to Lane 22: extracted DNA samples from different donors.

4.2.2 Genotyping of *MTHFR* C677T polymorphism

ARMS-PCR and gel electrophoresis were performed for the *MTHFR* (rs1801133) genotype identification. The PCR amplicon was 226 bp in size, regardless of the wildtype or mutant amplicon. After the PCR amplification, each PCR amplicon was loaded in two lanes (first lane for the DNA sample amplified with wildtype primer while second lane for the amplification using mutant primer). The forward primer was the same for both amplifications. 4% agarose gel was used, and the loaded sample would be electrophoresed at 80V for 40 minutes.

ARMS-PCR products on 4% agarose gel are demonstrated in Figure 4.5 and 4.6. The single band produced by wildtype primer was identified as homozygous wildtype (CC). The band amplified by mutant primer was detected as

homozygous mutant (TT) whereas individual was classified as heterozygous (CT) when band was generated by wildtype and mutant primers.

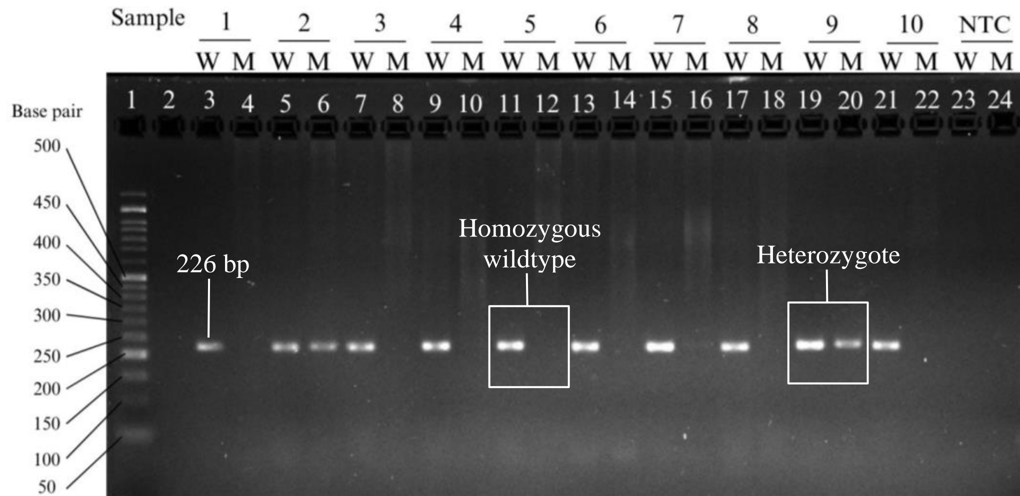

Figure 4.5: ARMS-PCR products on 4 % agarose gel. Lane 1: 50 bp ladder; Lanes 23 and 24: Controls; Lanes 3-4, 7-8, 9-10, 11-12, 13-14, 15-16, 17-18 and 21,22 show homozygous wildtype whereas lanes 5-6 and 19-20 show heterozygote.

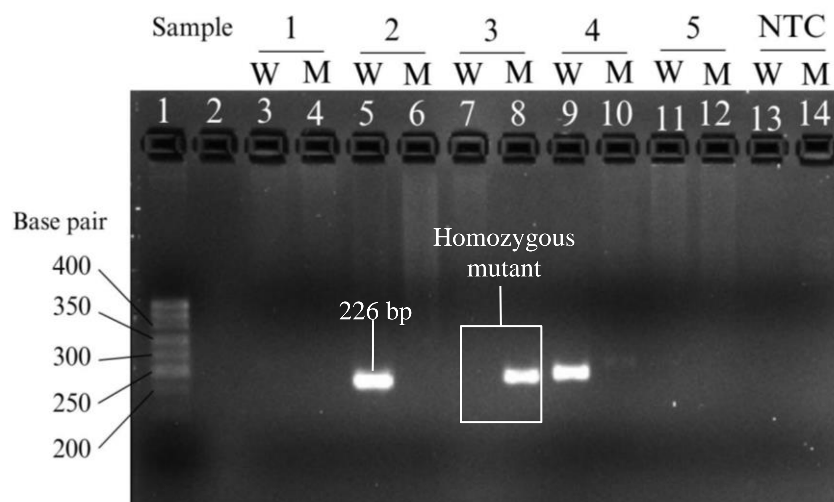

Figure 4.6: ARMS-PCR products on 4 % agarose gel. Lane 1: 50 bp ladder; Lanes 13 and 14: Controls; Lanes 5-6 and 9-10 show homozygous wildtype whereas lanes 7-8 show homozygous mutant.

4.2.3 Genotypic distribution of *MTHFT* C677T polymorphism among the recruited samples

Overall, a total of 106 participants who fulfilled the exclusion and inclusion criteria was recruited for the Part II of this study. Among the participants, 52 participants (49.1%) were homozygous wildtype (CC), 53 participants (50%) were heterozygote (CT) while one (0.9%) was homozygous mutant (TT) (Figure 4.7). 59 participants were considered as habitual coffee drinker while 47 of them were non-habitual coffee drinker. In habitual and non-habitual coffee drinker, the genotypic distribution was as the following: 27 (45.76%) and 25 (53.19%) participants were CC genotype, 32 (54.24%) and 20 (44.68%) of them were CT genotype while the only one (2.13) TT genotype was in the non-habitual coffee drinker group. Figure 4.8 shows the Hardy-Weinberg equilibrium calculation, the allelic frequency for C allele was 0.73 whereas T allele was 0.27.

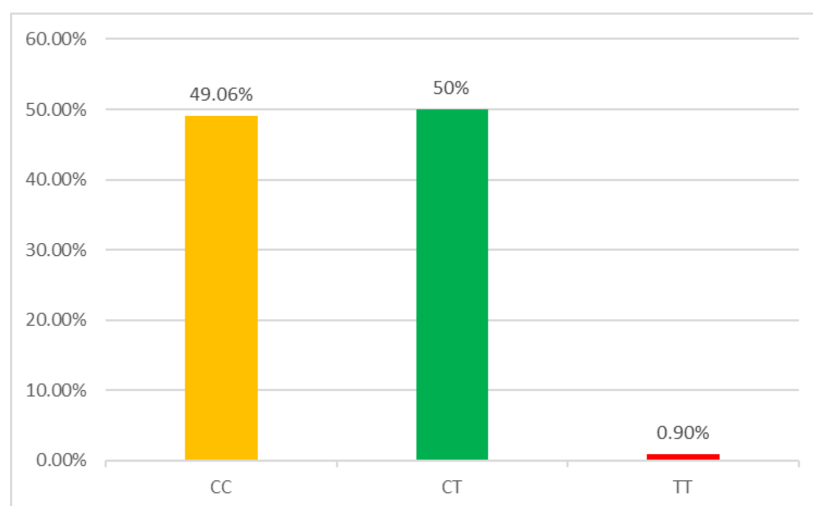

Figure 4.7: Genotypic distribution of *MTHFR* C677T genotype among the recruited samples

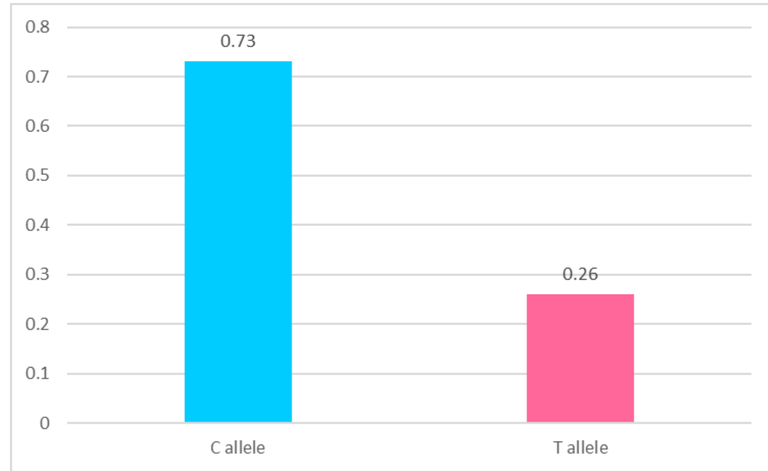

Figure 4.8: Allelic frequency of *MTHFR* C677T genotype among the recruited samples

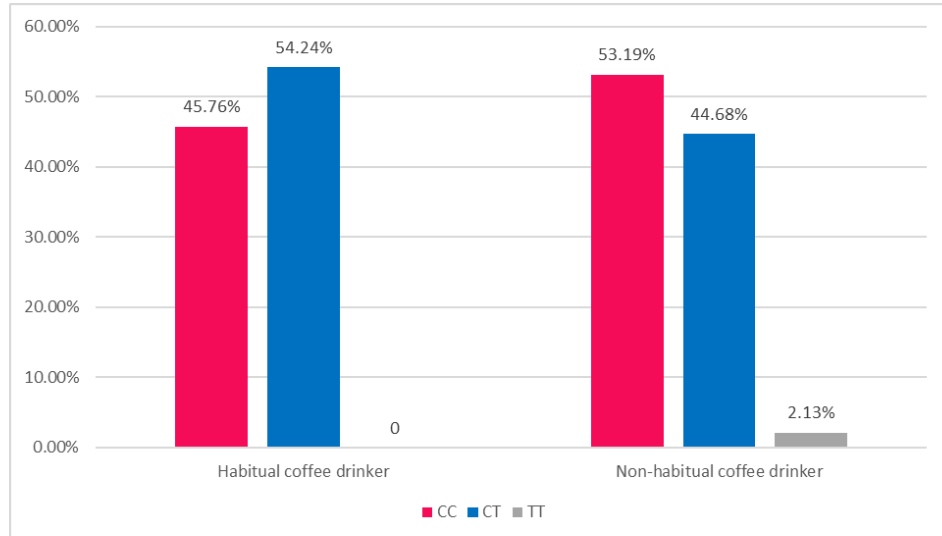

Figure 4.9: Genotypic distribution of *MTHFR* C677T genotype among habitual and non-habitual coffee drinkers

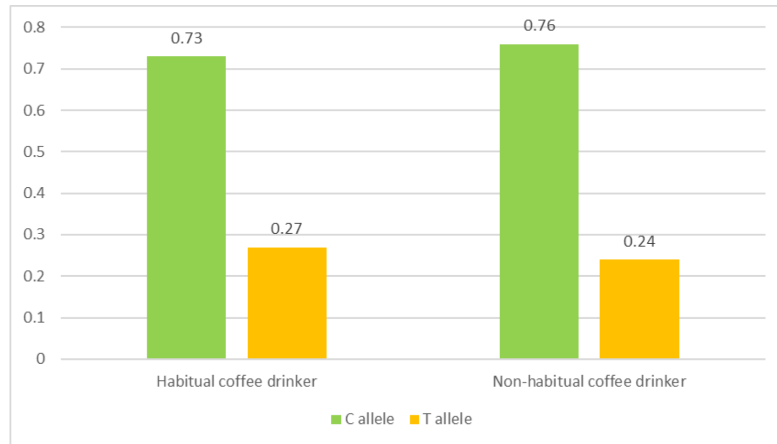

Figure 4.10: Allelic frequency of *MTHFR* C677T genotype among habitual and non-habitual coffee drinkers

Table 4.6: Distribution of *MTHFR* C677T genotype among the recruited samples

	Frequency (n, %)		
	Habitual coffee drinker (n=59)	**Non-habitual coffee drinker (n=47)**	**Overall (part II) (n=106)**
CC genotype	27 (45.76)	25 (53.19)	**52 (49.1)**
CT genotype	32 (54.24)	21 (44.68)	**53 (50.0)**
TT genotype	0 (0)	1 (2.13)	**1 (0.94)**
Total	**59 (100)**	**47 (100)**	**106**

4.2.4 Mean and standard deviation of BMI, blood pressure and non-fasting lipid profile

In Part II (n=106), resting blood pressure, pulse and non-fasting lipid profile of the total participants were further assessed prior to the commencement of the test (Table 4.7). The mean resting blood pressure was as following: resting SBP: 102.02 ± 14.215 mmHg; resting DBP: 65.10 ± 6.517 mmHg; pulse: 80.13 ± 10.349 beat/min. Over 90% of the respondents had normal SBP, DBP and pulse but seven of them were considered in prehypertension stage. Besides that, the mean of non-fasting lipid profile was recorded as TC: 4.33 ± 0.947 mmol/L;

TG: 1.11 ± 0.503 mmol/L; HDL-C (male): 1.10 ± 0.281 mmol/L; HDL-C (female): 1.63 ± 0.465 mmol/L; LDL-C: 2.42 ± 0.851 mmol/L; TC/HDL-C: 3.26 ± 0.999. Over 80% of the participants' TC, TG and LDL-C indicated low risk of developing chronic diseases. However, 32 female and male participants, respectively, had either intermediate risk or high risk in terms of the HDL-C level.

Among the 59 habitual coffee drinkers, their mean BMI was 20.65 ± 2.70 kg/m^2. The mean resting SBP was 103.65 ± 10.997 mmHg, DBP was 64.13 ± 6.332 mmHg while the mean pulse rate was 78.81 ± 9.839 beat/min. However, four of them was in prehypertension stage (based on SBP) whereas three participants had abnormal pulse rate. Table 4.7 shows that habitual coffee drinkers' mean TC was 4.43 ± 1.033 mmol/L, TG was 1.16 ± 0.580 mmol/L, HDL-C (male) was 1.09 ± 0.309 mmol/L, HDL-C (female) was 1.6 ± 0.397 mmol/L, LDL-C was 2.55 ± 0.924 mmol/L and the TC/HDL-C was 3.49 ± 1.096 mmol/L. Majority of the participants had low risk on their TC (81.4%), TG (83.1), LDL-C (84.7%). However, 53.1% female participants and 81.4% male participants showed undesirable HDL-C level. Only 55.9% of the habitual coffee drinkers had low risk TC/HDL-C ratio, perhaps due to low HDL-C level.

Table 4.7 demonstrates the resting blood pressure and serum non-fasting lipid profile among the 47 non-habitual coffee drinkers. The mean BMI was 19.74 ± 2.695 kg/m^2. The mean resting SBP was 99.98 ± 17.350 mmHg, DBP was 66.32

± 6.607 mmHg while the mean pulse rate was 81.78 ± 10.834 beat/min. Three readings from the SBP and one reading from the DBP was considered pre-hypertensive whereas one abnormal pulse rate was found. In addition, habitual coffee drinkers' mean TC was 4.21 ± 0.821 mmol/L, TG was 1.04 ± 0.382 mmol/L, HDL-C (male) was 1.15 ± 0.193 mmol/L, HDL-C (female) was 1.67 ± 0.519 mmol/L, LDL-C was 2.27 ± 0.729 mmol/L and the TC/HDL-C was 2.96 ± 0.771 mmol/L. Most of the participants' (over 80%) TC, TG, LDL-C, TC/HDL-C ratio were at low risk. Approximately 59.5% female's HDL-C level was at low risk. However, 80% male were having high risk and 20% of them were having undesirable HDL-C level.

Table 4.7: Mean and standard deviation of BMI, blood pressure, pulse and non-fasting lipid profile (n=106)

Variable	**Mean ± SD**		
	Habitual coffee drinker (n=59)	**Non-habitual coffee drinker (n=47)**	**Overall (part II) (n=106)**
Body mass index (BMI)	20.65 ± 2.700	19.74 ± 2.695	20.25 ± 2.730
Resting blood pressure			
Systolic blood pressure	103.65 ± 10.997	99.98 ± 17.350	102.02 ± 14.215
Diastolic blood pressure	64.13 ± 6.332	66.32 ± 6.607	65.10 ± 6.517
Pulse	78.81 ± 9.839	81.78 ± 10.834	80.13 ± 10.349
Non-fasting lipid profile			
TC	4.43 ± 1.033	4.21 ± 0.821	4.33 ± 0.947
TG	1.16 ± 0.580	1.04 ± 0.382	1.11 ± 0.503
HDL-C (male)	1.09 ± 0.309	1.15 ± 0.193	1.10 ± 0.281
HDL-C (female)	1.6 ± 0.397	1.67 ± 0.519	1.63 ± 0.465
LDL-C	2.55 ± 0.924	2.27 ± 0.729	2.42 ± 0.851
TC/HDL-C	3.49 ± 1.096	2.96 ± 0.771	3.26 ± 0.999

4.3 Hardy-Weinberg equilibrium for *MTHFR* (rs1801133) polymorphism

4.3.1 Hardy-Weinberg equilibrium for *MTHFR* (rs1801133) polymorphism

MTHFR C677T polymorphism was found associated with coffee intake in the study population, in which it did not in accordance with Hardy-Weinberg equilibrium. The H_0 and H_1 are as followed:

H_0: The observed values conform with the Hardy-Weinberg equilibrium.

H_1: The observed values do not conform with the Hardy-Weinberg equilibrium.

Table 4.8 shows a chi-square test. As the calculated χ^2 value (9.292) was larger than the tabulated χ^2 value (3.841), as a result, H_0 was rejected, hence, there was changes in the number of homozygous and heterozygous variant carriers based on its allele frequency.

Table 4.8: Genotypic distribution of *MTHFR* C677T polymorphism in the experimental study (n=106)

Genotype	Observed (O)	Expected (E)	$(O-E)^2$	$(O-E)^2/E$
CC	52	56	16	0.286
CT	53	42	121	2.881
TT	1	8	49	6.125
Σ	106	106		9.292

(Tabulated χ^2 = 3.841, df = 1, p = 0.05)

4.3.2 *MTHFR* (rs1801133) T allele was associated with coffee intake among habitual and non-habitual coffee drinker

Chi-square test was carried out to test the hypothesis set as below:

H_0: *MTHFR* C677T polymorphism is not associated with habitual coffee drinking

H_1: *MTHFR* C677T polymorphism is associated with habitual coffee drinking

The association between the *MTHFR* C677T polymorphism and habitual coffee drinker was found. As the calculated χ^2 value (8.038) was larger than the tabulated χ^2 value (3.841), hence, H_0 was rejected and H_1 was accepted. For non-habitual coffee drinker, the calculated χ^2 value (2.423) was smaller than the tabulated χ^2 value (3.841), therefore, H_0 was accepted. In conclusion, this result indicates that *MTHFR* C677T polymorphism is associated with habitual coffee drinking.

Table 4.9: Genotypic distribution of *MTHFR* C677T polymorphism in case group (habitual coffee drinker)

Genotype	Observed (O)	Expected (E)	$(O-E)^2$	$(O-E)^2/E$
CC	27	31	16	0.5161
CT	32	23	81	3.522
TT	0	4	16	4.000
Σ	59	59		8.0381

(Tabulated χ^2 = 3.841, df = 1, p = 0.05)

Table 4.10: Genotypic distribution of *MTHFR* C677T polymorphism in control group (non-habitual coffee drinker)

Genotype	Observed (O)	Expected (E)	$(O-E)^2$	$(O-E)^2/E$
CC	25	27	4	0.1481
CT	21	17	16	0.9412
TT	1	3	4	1.3333
Σ	47	47		2.4226

(Tabulated χ^2 = 3.841, df = 1, p = 0.05)

4.4 Blood pressure, pulse and non-fasting lipid profile of each genotype in coffee drinkers and non-coffee drinkers

4.4.1 Comparison of resting blood pressure, pulse and non-fasting lipid profile between habitual and non-habitual coffee drinker

Independent T-test (for normally distributed data) and Mann-Whitney U-test (for not normally distributed data) were conducted to study the differences in resting blood pressure, pulse and non-fasting lipid profile among the habitual and non-habitual coffee drinker, regardless of the genotype. As shown in Table 4.11, there was significant difference in HDL-C level and TC/HDL-C. The mean HDL-C of habitual coffee drinker (1.363 ± 0.438 mmol/L) was significantly lower than the non-habitual coffee drinker (1.557 ± 0.515 mmol/L) ($p < 0.05$). Besides that, habitual coffee drinker had significant higher TC/HDL-C than non-habitual drinker. In terms of the resting blood pressure, no significant result was observed ($p < 0.05$).

Table 4.11: Differences in resting blood pressure, pulse and non-fasting lipid profile between habitual and non-habitual coffee drinkers

	Habitual coffee drinkers (Mean ± SD)	Non-habitual coffee drinkers (Mean ± SD)	Mean difference ± SD	p
Resting blood pressure (mmHg)				
Systolic	103.654 ± 10.998	99.977 ± 17.350	3.677 ± 2.768	0.295^{b}
Diastolic	64.128 ± 6.332	66.321 ± 6.607	-2.193 ± 1.262	0.085^{a}
Pulse	78.812 ± 9.839	81.780 ± 10.834	-2.968 ± 2.012	0.143^{a}
Lipid profile (mmol/L)				
TC	4.432 ± 1.033	4.211 ± 0.821	0.221 ± 0.1185	0.234^{a}
TG	1.159 ± 0.580	1.042 ± 0.382	0.117 ± 0.098	0.370^{b}
HDL	1.363 ± 0.438	1.557 ± 0.515	-0.194 ± 0.093	0.034^{b*}
LDL	2.548 ± 0.924	2.268 ± 0.730	0.280 ± 0.165	0.093^{a}
TC/HDL-C	3.495 ± 1.096	2.957 ± 0.771	0.538 ± 0.182	0.010^{b*}

Remarks:
a: Independent T-test was used
b: Mann-Whitney U-test

4.4.2 Comparison of resting blood pressure, pulse and non-fasting lipid profile among habitual and non-habitual coffee drinker of different genotypes

One-way ANOVA (for normally distributed data) and Kruskal-Wallis H test (for not normally distributed data) were carried out to investigate the interaction of coffee consumption and genotypic difference on resting blood pressure, pulse and non-fasting lipid profile (Table 4.12). Significant difference in the TC/HDL-C was found among the habitual and non-habitual coffee drinker with different genotype. According to the results of post-hoc test (Tukey's test), among the CT genotype, the mean TC/HDL-C level of habitual coffee drinker (3.547 ± 1.177 mmol/L) was significantly higher than non-habitual coffee drinker (2.798 ± 0.776 mmol/L) ($p < 0.05$).

Table 4.12: Differences in resting blood pressure, pulse and non-fasting lipid profile between habitual and non-habitual coffee drinkers with different genotypes

Variable	Coffee drinkers (Mean ± SD)		Non-coffee drinkers (Mean ± SD)		p
	CC	CT	CC	CT+TT	
Resting blood pressure (mmHg)					
Systolic	103.016 ± 11.454	104.192 ± 10.752	98.985 ± 21.249	101.105 ± 11.870	0.752[b]
Diastolic	63.955 ± 6.678	64.273 ± 6.130	67.259 ± 6.340	65.255 ± 6.889	0.252[a]
Pulse	78.894 ± 9.848	78.742 ± 9.989	84.736 ± 10.702	78.421 ± 10.199	0.087[a]
Non-fasting lipid profile (mmol/L)					
TC	4.407 ± 1.046	4.453 ± 1.038	4.338 ± 0.836	4.067 ± 0.799	0.495[a]
TG	1.139 ± 0.513	1.175 ± 0.638	1.070 ± 0.421	1.009 ± 0.339	0.812[b]
HDL	1.369 ± 0.470	1.359 ± 0.416	1.601 ± 0.634	1.508 ± 0.341	0.214[b]
LDL	2.531 ± 0.855	2.562 ± 0.992	2.407 ± 0.751	2.110 ± 0.686	0.234[a]
TC/HDL	3.433 ± 1.010	3.547 ± 1.177	3.096 ± 0.754	2.798 ± 0.776	0.031[a*]

Remarks:
a: One-way ANOVA was used
b: Kruskal-Wallis H test was used

4.4.3 Comparison of resting blood pressure, pulse and non-fasting lipid profile between homozygous wildtype (CC) and T allele carriers (CT+TT)

Table 4.13 illustrates the differences in resting blood pressure, pulse and non-fasting lipid profile between CC genotype and T allele carriers, regardless of their coffee consumption status. As shown in Table 4.13, although not significant, CC genotype had higher TC, LDL and TC/HDL-C levels than T allele carriers. However, the HDL-C level was higher in CC genotype. Besides that, the mean resting SBP, DBP and pulse rate were all higher in CC genotype.

Table 4.13: Differences in resting blood pressure, pulse and non-fasting lipid profile between homozygous wildtype (CC) and T allele carriers (CT+TT)

	Wildtype (CC) (Mean ± SD)	T allele carrier (CT+TT) (Mean ± SD)	Mean difference ± SD	p
Resting blood pressure (mmHg)				
Systolic	101.078 ± 16.837	102.934 ± 11.216	-1.856 ± 2.769	0.980[b]
Diastolic	65.544 ± 6.665	64.673 ± 6.405	0.870 ± 1.269	0.494[a]
Pulse	81.703 ± 10.584	78.611 ± 9.980	3.092 ± 2.00	0.125[a]
Lipid profile (mmol/L)				
TC	4.374 ± 0.942	4.296 ± 0.959	0.078 ± 0.185	0.673[a]
TG	1.106 ± 0.468	1.107 ± 0.539	-0.001 ± 0.098	0.972[b]
HDL	1.480 ± 0.561	1.419 ± 0.391	0.061 ± 0.094	0.902[b]
LDL	2.472 ± .801	2.377 ± 0.901	0.094 ± 0.166	0.571[a]
TC/HDL-C	3.271 ± .903	3.2542 ± 1.090	0.029 ± 0.195	0.450[b]

Remarks:
a: Independent T-test was used
b: Mann-Whitney U-test was used

4.4.4 Comparison of resting blood pressure, pulse and non-fasting lipid profile between wildtype (CC) and heterozygote (CT) among habitual coffee drinkers

Table 4.14 presents the comparison of resting blood pressure, pulse and resting lipid profile between different genotype among the habitual coffee drinkers.

Similarly, there was no significant difference in the resting blood pressure, pulse and non-fasting lipid profile among the CC and CT genotype. Although not significant, CC genotype had lower TC, TG, LDL, TC/HDL-C, and higher HDL-C level than CT genotype. Regarding the resting blood pressure, CC genotype had lower reading for both SBP and DBP than CT genotype.

Table 4.14: Differences in resting blood pressure, pulse and non-fasting lipid profile between wildtype (CC) and heterozygote (CT) among habitual coffee drinkers

	Wildtype (CC) (Mean ± SD)	Heterozygote (CT) (Mean ± SD)	Mean difference ± SD	p
Resting blood pressure (mmHg)				
Systolic	103.016 ± 11.454	104.192 ± 10.752	-1.176 ± 2.895	0.686[a]
Diastolic	63.955 ± 6.678	64.273 ± 6.130	-0.319 ± 1.669	0.849[a]
Pulse	78.894 ± 9.848	78.742 ± 9.989	0.152 ± 2.594	0.953[a]
Lipid profile (mmol/L)				
TC	4.407 ± 1.046	4.453 ± 1.038	-0.046 ± 0.272	0.866[a]
TG	1.139 ± 0.513	1.175 ± 0.638	-0.035 ± 0.153	0.654[b]
HDL	1.369 ± 0.470	1.359 ± 0.416	0.010 ± 0.115	0.930[a]
LDL	2.531 ± 0.855	2.562 ± 0.992	-0.030 ± 0.243	0.901[a]
TC/HDL-C	3.433 ± 1.009	3.547 ± 1.177	-0.114 ± 0.288	0.695[a]

Remarks:
a: Independent T-test was used
b: Mann-Whitney U-test was used

4.4.5 Comparison of resting blood pressure, pulse and non-fasting lipid profile between homozygous wildtype (CC) and T allele carriers (CT+TT) among non-habitual coffee drinkers

For non-habitual coffee drinker, significant difference in resting pulse rate was found between CC genotype and T allele carriers (CT+TT), in which the mean pulse rate of CC genotype individual (84.736 ± 10.702 beat/min) was significantly higher than the T allele carriers (78.421 ± 10.199 beat/min) (p <

0.05) (Table 4.15). Although not significant, non-habitual coffee drinker had higher TC, TG, LDL-C level, TC/HDL-C, but higher HDL-C level if compared to the T allele carriers.

Table 4.15: Differences in resting blood pressure, pulse and non-fasting lipid profile between homozygous wildtype (CC) and T allele carriers (CT+TT) among non-habitual coffee drinkers

	Wildtype (CC) (Mean ± SD)	T allele carrier (CT+TT) (Mean ± SD)	Mean difference ± SD	p
Resting blood pressure (mmHg)				
Systolic	98.985 ± 21.249	101.105 ± 11.870	-2.120 ± 5.119	0.903[b]
Diastolic	67.259 ± 6.340	65.255 ± 6.889	2.005 ± 1.930	0.304[a]
Pulse	84.736 ± 10.702	78.421 ± 10.199	6.315 ± 3.061	0.045*
Lipid profile (mmol/L)				
TC	4.338 ± 0.836	4.067 ± 0.799	0.271 ± 0.239	0.263[a]
TG	1.070 ± 0.421	1.009 ± 0.339	0.062 ± 0.113	0.703[b]
HDL	1.601 ± 0.634	1.508 ± 0.341	0.093 ± 0.151	1.000[b]
LDL	2.407 ± 0.751	2.110 ± 0.686	0.298 ± 0.211	0.165[a]
TC/HDL-C	3.096 ± 0.754	2.798 ± 0.776	0.298 ± 0.224	0.813[b]

Remarks:
a: Independent T-test was used
b: Mann-Whitney U-test was used

4.4.6 Comparison of resting blood pressure, pulse and non-fasting lipid profile between habitual and non-habitual coffee drinkers of wildtype (CC) genotype

Differences in resting blood pressure and non-fasting lipid profile between habitual and non-habitual coffee drinker of homozygous CC genotype was also analyzed. From Table 4.16, it was observed that although not significant, the level of TC, TG, LDL-C level and TC/HDL-C was higher in habitual coffee drinker. Meanwhile, habitual coffee drinker had lower HDL-C level. In addition, the mean resting pulse rate of habitual coffee drinker (78.894 ± 9.848 beat/min)

was significantly lower than the non-habitual coffee drinker (84.736 ± 10.702 beat/min) ($p < 0.05$).

Table 4.16: Differences in resting blood pressure, pulse and non-fasting lipid profile between habitual and non-habitual coffee drinkers of wildtype (CC) genotype

	Habitual coffee drinkers (Mean ± SD)	Non-habitual coffee drinkers (Mean ± SD)	Mean difference ± SD	p
Resting blood pressure (mmHg)				
Systolic	103.016 ± 11.454	98.985 ± 21.249	4.032 ± 4.685	0.558[b]
Diastolic	63.955 ± 6.678	67.259 ± 6.340	-3.305 ± 1.809	0.074[a]
Pulse	78.894 ± 9.848	84.736 ± 10.702	-5.842 ± 2.850	0.046[a*]
Lipid profile (mmol/L)				
TC	4.407 ± 1.046	4.338 ± 0.836	0.069 ± 0.264	0.794[a]
TG	1.139 ± 0.513	1.070 ± 0.421	0.069 ± 0.131	0.608[b]
HDL	1.369 ± 0.470	1.601 ± 0.634	-0.232 ± 0.154	0.115[b]
LDL	2.531 ± 0.855	2.407 ± 0.751	0.124 ± 0.224	0.582[a]
TC/HDL-C	3.433 ± 1.009	3.096 ± 0.754	0.337 ± 0.249	0.181[a]

Remarks:
a: Independent T-test was used
b: Mann-Whitney U-test was used

4.4.7 Differences of resting blood pressure, pulse and non-fasting lipid profile between habitual and non-habitual coffee drinkers of T allele carrier (CT+TT) genotype

Among the T allele carriers, comparison of resting blood pressure and non-fasting lipid profile was investigated between habitual and non-habitual coffee drinkers. Table 4.17 indicates that the TC/HDL-C was significantly higher in habitual coffee drinkers as compared to those who did not drink coffee regularly ($p < 0.05$). Moreover, habitual coffee drinker had higher SBP and pulse rate, despite the result was not statistically significant.

Table 4.17: Differences in resting blood pressure, pulse and non-fasting lipid profile between habitual and non-habitual coffee drinkers of T allele carrier (CT+TT) genotype

	Habitual coffee drinkers (Mean ± SD)	Non-habitual coffee drinkers (Mean ± SD)	Mean difference ± SD	p
Resting blood pressure (mmHg)				
Systolic	104.192± 10.752	101.105 ± 11.870	3.087 ± 3.107	0.325[a]
Diastolic	64.273 ± 6.130	65.255 ± 6.889	-0.981 ± 1.786	0.585[a]
Pulse	78.742 ± 9.989	78.421 ± 10.199	0.321 ± 2.790	0.909[a]
Lipid profile (mmol/L)				
TC	4.453 ± 1.038	4.067 ± 0.799	0.386 ± 0.263	0.147[a]
TG	1.175 ± 0.638	1.009 ± 0.339	0.166 ± 0.149	0.418[b]
HDL	1.359 ± 0.416	1.508 ± 0.341	-0.149 ± 0.107	0.171[a]
LDL	2.562 ± 0.992	2.110 ± 0.686	0.452 ± 0.244	0.070[a]
TC/HDL-C	3.547 ± 1.177	2.798 ± 0.776	0.749 ± 0.266	0.0014[b*]

Remarks:
a: Independent T-test was used
b: Mann-Whitney U-test was used

4.5 Changes of blood pressure, pulse and lipid profile among habitual coffee drinkers after coffee consumption

Paired T-test (for normally distributed data) and Wilcoxon Test (for not normally distributed data) were utilized to study the acute effect of coffee consumption on blood pressure, pulse and lipid profile. The changes of resting blood pressure and lipid profile after coffee treatment was compared among all the coffee drinkers (Table 4.18), CC genotype coffee drinker (Table 4.19) and CT genotype coffee drinker (Table 4.20). Based on Table 4.18 and Table 4.19, no significant difference was observed for the changes in lipid profile, in both habitual coffee drinkers and coffee drinkers of CC genotype. However, in these two populations, significant changes in the resting blood pressure (SBP and DBP) and pulse rate were observed ($p < 0.05$), in which after the ingestion of coffee, SBP and DBP increased while pulse rate decreased, both significantly.

Among the habitual coffee drinker with CC genotype, although no significant result was found, there was an increment in the level of TC, TG, HDL-C and TC/HDL-C but a decrement in LDL-C level after coffee consumption (Table 4.19). Based on Table 4.20, the result indicates that among the CT genotype coffee drinker, their DBP increased while pulse rate decreased significantly ($p < 0.05$). In terms of their non-fasting lipid profile, only TC/HDL-C showed significant decrease after the intake of coffee ($p < 0.05$).

Table 4.18: Changes of blood pressure, pulse and lipid profile after coffee consumption for overall habitual coffee drinkers

	Habitual coffee drinkers		**Mean difference ± SD**	**p**
	Before (Mean ± SD)	**After (Mean ± SD)**		
Resting blood pressure (mmHg)				
Systolic	103.654 ± 10.998	105.904 ± 10.388	-2.250 ± 6.680	0.011[a*]
Diastolic	64.128 ± 6.332	66.778 ± 6.768	-2.650 ± 4.954	< 0.001[b]
Pulse	78.812 ± 9.839	71.634 ± 8.250	7.178 ± 6.966	< 0.001[a*]
Lipid profile (mmol/L)				
TC	4.432 ± 1.033	4.390 ± 1.045	0.042 ± 0.374	0.392[a]
TG	1.159 ± 0.580	1.092 ± 0.483	0.067 ± 0.445	0.527[b]
HDL	1.363 ± 0.438	1.380 ± 0.427	-0.017 ± 0.152	0.405[a]
LDL	2.548 ± 0.924	2.529 ± 0.961	0.019 ± 0.386	0.712[a]
TC/HDL-C	3.495 ± 1.096	3.401 ± 1.067	0.094 ± 0.424	0.094[a]

Remarks:
a: Paired T-test was used
b: Wilcoxon Test was used

Table 4.19: Changes of blood pressure, pulse and lipid profile after coffee consumption among habitual coffee drinkers with homozygous wildtype (CC) genotype

	Wildtype (CC)		Mean difference ± SD	p
	Before (Mean ± SD)	After (Mean ± SD)		
Resting blood pressure (mmHg)				
Systolic	103.016 ± 11.454	106.716 ± 10.781	-3.700 ± 5.932	0.003[a*]
Diastolic	63.955 ± 6.678	67.244 ± 6.674	-3.289 ± 4.567	< 0.001[a*]
Pulse	78.894 ± 9.848	72.144 ± 8.332	6.750 ± 6.560	< 0.001[a*]
Lipid profile (mmol/L)				
TC	4.407 ± 1.046	4.388 ± 1.045	0.020 ± 0.349	0.772[a]
TG	1.139 ± 0.513	1.098 ± 0.524	0.041 ± 0.243	0.383[b]
HDL	1.369 ± 0.470	1.366 ± 0.441	0.003 ± 0.172	0.920[a]
LDL	2.531 ± 0.855	2.535 ± 0.897	-0.004 ± 0.299	0.949[a]
TC/HDL-C	3.433 ± 1.009	3.398 ± 0.896	0.035 ± 0.501	0.718[a]

Remarks:
a: Paired T-test was used
b: Wilcoxon Test was used

Table 4.20: Changes of blood pressure, pulse and lipid profile after coffee consumption among habitual coffee drinkers with heterozygote (CT) genotype

	Heterozygous (CT)		Mean difference ± SD	p
	Before (Mean ± SD)	After (Mean ± SD)		
Resting blood pressure (mmHg)				
Systolic	104.192 ± 10.752	105.218 ± 10.166	-1.026 ± 7.113	0.421[a]
Diastolic	64.273 ± 6.130	66.384 ± 6.927	-2.111 ± 5.270	0.001[b*]
Pulse	78.742 ± 9.989	71.202 ± 8.287	7.540 ± 7.375	< 0.001[a*]
Lipid profile (mmol/L)				
TC	4.453 ± 1.038	4.393 ± 1.063	0.061 ± 0.399	0.394[a]
TG	1.175 ± 0.638	1.086 ± 0.454	0.088 ± 0.566	0.948[b]
HDL	1.359 ± 0.416	1.392 ± 0.421	-0.033 ± 0.134	0.168[a]
LDL	2.562 ± 0.992	2.524 ± 1.027	0.038 ± 0.451	0.641[a]
TC/HDL-C	3.547 ± 1.177	3.403 ± 1.207	0.144 ± 0.348	0.014[b*]

Remarks:
a: Paired T-test was used
b: Wilcoxon Test was used

4.6 Differences in the changes of blood pressure, pulse and lipid profile after coffee consumption between different genotypes

Independent T-test (for normally distributed data) and Mann-Whitney U test (for not normally distributed data) was conducted to study the differences in the

changes of blood pressure (with different intervals), pulse and lipid profile among different genotypes. No statistically significant was found in the differences of the lipid profile changes. For the changes in blood pressure and pulse rate, only changes in pulse rate of 2 different intervals (0 to 30 mins and 0 to 60 mins) was statistically significant between different genotypes ($p < 0.05$) (Table 4.21).

Table 4.21: Differences in the changes of blood pressure, pulse and lipid profile between homozygous wildtype (CC) genotype and heterozygous (CT) genotype

	Wildtype (CC) (Mean ± SD)	Heterozygote (CT) (Mean ± SD)	Mean difference ± SD	p
Changes of blood pressure (mmHg)				
Systolic				
R to 0 min	0.986 ± 4.498	-0.620 ± 5.741	1.606 ± 1.362	0.243[a]
R to 30 min	2.523 ± 5.543	-0.002 ± 6.435	2.525 ± 1.580	0.115[a]
R to 60 min	3.700 ± 5.932	1.026 ± 7.113	2.674 ± 1.725	0.127[a]
0 to 30 min	1.537 ± 5.805	0.619 ± 5.530	0.919 ± 1.478	0.537[a]
0 to 60 min	2.715 ± 5.811	1.646 ± 6.503	1.068 ± 1.619	0.512[a]
30 to 60 min	1.178 ± 5.254	1.028 ± 5.076	0.150 ± 1.348	0.912[a]
Diastolic				
R to 0 min	0.807 ± 3.558	0.422 ± 4.368	0.385 ± 1.05	0.716[a]
R to 30 min	2.636 ± 5.875	1.824 ± 4.882	0.812 ± 1.40	0.564[a]
R to 60 min	3.289 ± 4.567	2.111 ± 5.269	1.178 ± 1.296	0.594[b]
0 to 30 min	1.829 ± 5.072	1.401 ± 3.982	0.428 ±1.179	0.718[a]
0 to 60 min	2.482 ± 3.290	1.688 ± 4.140	0.794 ± 0.987	0.424[a]
30 to 60 min	0.653 ± 3.257	0.287 ± 3.669	0.366 ± 0.911	0.689[a]
Pulse				
R to 0min	1.737 ± 7.499	-1.309 ± 7.429	3.046 ± 1.950	0.150[b]
R to 30 min	-5.21 ± 7.842	-5.685 ± 6.575	0.471 ± 1.877	0.803[a]
R to 60 min	-6.750 ± 6.560	-7.540 ± 7.375	0.790 ± 1.833	0.668[a]
0 to 30 min	-6.952 ± 3.927	-4.377 ± 3.532	-2.575 ± 0.971	0.010[a*]
0 to 60 min	-8.487 ± 3.992	-6.231 ± 4.325	-2.257 ± 1.091	0.043[a*]
30 to 60 min	-1.536 ± 4.222	-1.854 ± 3.686	0.318 ± 1.030	0.758[a]
Changes of lipid profile (mmol/L)				
TC	-0.020 ± 0.349	-0.061 ± 0.399	0.041 ± 0.099	0.677[a]
TG	-0.042 ± 0.243	-0.088 ± 0.566	0.047 ± 0.117	0.503[b]
HDL	-0.003 ± 0.172	0.033 ± 0.134	-0.037 ± 0.04	0.360[a]
LDL	0.004 ± 0.299	-0.038 ± 0.451	0.041 ± 0.102	0.687[a]
TC/HDL-C	-0.035 ± 0.501	-0.144 ± 0.348	0.109 ± 0.111	0.332[a]

Remarks:
a: Independent T-test was used
b: Mann-Whitney U-test

CHAPTER 5

DISCUSSION

5.1 BMI, resting blood pressure, pulse rate and lipid profile

In this study, 298 participants were recruited for the cross-sectional study (Part I) and 106 eligible candidates were selected for the experimental study (Part II). These selected participants were recruited to examine the effect of *MTHFR* C677T variant and coffee intake on their blood pressure, pulse and lipid profile. Majority of the participants' BMI (60%) were within the normal range, followed by underweight (28.9%). Only 11.1% were overweight or obese. According to the NHMS 2022, 33.3% of the adolescents in Malaysia are either overweight or obese. The prevalence of overweight and obese in this study was slightly lower than the nation, perhaps due to the huge difference in ethnic distribution. According to Statista Research Department (2023), over 60% of the nation was made up of Malay and indigenous peoples while Chinese only contributed to 20%. However, 97% of the participants in this study were Chinese. It is important to note that the prevalence of obesity is different among the ethnicity, in which Malay and Indian recorded as highest (13.6% and 13.5%), therefore result in dissimilar findings (Rampal, et al., 2007).

Of the 106 selected candidates, resting blood pressure (SBP and DBP), pulse rate, and non-fasting lipid profile (TC, TG, HDL-C, LDL-C, TC/HDL-C) were further assessed. 71 (67%) of them had normal BMI, 99 (93.4%) of them have

normal SBP, 105 (99.1%) had normal DBP, 100 (94.3%) had normal pulse rate, 89 (84%), 93 (87.7%), 95 (89.6%) and 71 (67%) had normal value for TC, TG, LDL-C and TC/HDL-C, respectively. However, 32 (86.4%) males and 32 (46.4%) females had either low or borderline low HDL-C level. It could be due to high prevalence of sedentary lifestyle since majority of them do neither vigorous nor moderate physical activity regularly. Besides that, males were more likely to have undesirable HDL-C level than females. This is because estrogen does play a role in regulating the plasma HDL-C level. As claimed by Lamon-Fava and Micherone (2004), the administration of estrogen is associated with increased hepatic production of the Apolipoprotein A-I (apoA-I), which is the main component of HDL-C. Although testosterone was reported to be associated with higher HDL-C level in Thirumalai, Rubinow and Page (2015). The relationship between testosterone and HDL-C levels can be vary among individuals due to genetic variations, lifestyle factors, dietary pattern and health condition. However, these factors cannot be adjusted in the present study.

5.2 Prevalence of coffee consumption among students at UTAR Kampar Campus

The prevalence of habitual coffee consumption among students at UTAR Kampar Campus was 34.9%. Several possible reasons of coffee consumption as reported by the students including coffee aids in boosting the energy, helping them to stay awake and they liked the taste of coffee. Besides that, low price, smooth taste and the strength of coffee appeared to be the most important features that considered by the students at UTAR Kampar Campus when they

purchase the coffee while less concern was put on the brand and origin of the coffee. Regarding the types of coffee, majority (22.7%) preferred instant coffee due to the taste and conveniency. In terms of practice, students in the present populations preferred to drink coffee during breakfast.

Based on a cross-sectional study conducted by Annuar, et al. (2023), among the undergraduate pharmacy students in UiTM Puncak Alam, KPJ Healthcare University College, and the University of Brunei Darussalam, it showed that the prevalence of coffee consumption was 49.6% among the study population. Besides that, another cross-sectional study conducted in Perak, Malaysia indicated the prevalence of coffee consumption was 58% among the first-year medical students (Chan and Teoh, 2021). Different results may be due to different criteria were set for the individuals to be considered as habitual coffee drinkers. Besides that, different targeted population also contributed to the different results, in which students who studied in health-science related programmes had higher tendency to consume coffee to make them feel more awake so that they can cope with higher workload of study (AtikaRamli, et al., 2019; Chan and Teoh, 2021; Annuar, et al., 2023; Jaferi, et al., 2023). Despite another study reported higher coffee consumption prevalence (41.6%) in University of Lahore, Pakistan, it can be explained by different cultural background, ethnicity, and smaller sample size (n=167) (Nasir, et al., 2018).

5.3 Gender difference in coffee intake

Among the students in UTAR Kampar, the prevalence of coffee consumption was significantly higher in males (18.8%) if compared to females (16.1%). Similar result has been reported in a study conducted among young adults in Japan, of which significantly higher prevalence of coffee intake was found among males (50.8%) than females (32.8%) (Demura, 2013). Nevertheless, Kharaba, et al. (2022) and Riera-Sampol, et al. (2022) claimed that among university students in United Arab Emirates (UAE) and U.S, significantly, women drank more coffee since men were more preferred energy drinks. These contradicting findings may be due to cultural differences. Different culture may have distinct dietary pattern, lifestyle habits and social norms (Kharaba, et al., 2022). In addition, to explain in regards to the physiological make up, a study done by Doty and Cameron (2009) indicated that the perception of bitterness was stronger among female as compared to male. It could be one of the possible reasons why higher number of males is drinking coffee.

5.4 Association between BMI and coffee intake

In the present study, no significant association was found between BMI and coffee consumption. Meta-analysis showed no evidence in the association between high coffee intake with BMI among males, however, women with higher coffee consumption have significant higher risk of obesity or having higher BMI (Lee, et al., 2019). While Kim and Shin (2019) suggested that the BMI of coffee drinkers were significantly higher than the others among the Korean adults aged 40–69 years. Negative association between the intake of

coffee and body weight, BMI or obesity risk were found by Nordestgaard, Thomsen and Nordestgaard (2015) and Muhammad, et al. (2019) among the adults in Indonesia and Danish white populations. Wang, et al. (2017) proposed that among the US population, regular intake of coffee can even help in attenuating the risk of obesity and higher BMI. Nevertheless, the incidence of obesity and BMI are highly influenced by the differences in individual genetic background, ethnicity, age, dietary pattern and physical activity.

5.5 C677T gene polymorphism of *MTHFR* in the study

Geographic location, genetics and dietary intake serve as important factors in the differences in the genotypic and allelic frequency of *MTHFR* C677T polymorphism. In this study, the distribution of CC, CT and TT genotype was 49.1%, 50% and 0.94%. Besides that, the allelic frequency for C allele and T allele was 0.73 and 0.27, respectively.

Different genotype distribution of C677T polymorphism of *MTHFR* was detected in different studies. Two studies conducted among Malaysian showed that the genotypic frequency was 59.9%, 33.8%, 6.3% (Mejia Mohamed, 2011) and 57%, 30%, 13% (Choo, et al., 2011) for CC, CT and TT genotype, respectively. The differences may be due to genetic factor, food culture and race. The study of Mejia Mohamed (2011) and Choo, et al. (2011) focused on Malay population, whose genetics and dietary pattern differ from Chinese population. On the other hand, geographic location is another factor that may cause

genotype distribution to vary. For example, the distributions of the three genotypes: CC (19.5%,), CT (49.95%,) and TT (30.46%) among the Chinese population in northern China (Wang, et al., 2016) differs from the genotype distribution in southern China. Another study showed that the genotypic frequency of TT genotype increased along the southern-central-northern direction across the mainland of China (Song, et al., 2020).

Apart from that, the allelic frequency can be influenced by the geographical and ethnic variation. Chinese population in the northern and southern China have different allelic frequency (Lin, Liao and Zhang, 2023). In the mainland China, the 677T allelic frequency was 0.55 in the northern population and 0.25 in the southern population (Zhao, et al., 2018; Song, et al., 2020). Besides that, 677T allelic frequency among north Indian and Caucasian population were 0.11 and 0.32 (Yadav, et al., 2017; Nefic, Mackic-Djurovic and Eminovic, 2018).

5.6 Hardy-Weinberg equilibrium

Genotypic distribution and allelic frequency among the study cohort and coffee testing experimental group was tested for deviation from the Hardy-Weinberg equilibrium. Genotypic and allelic frequency in this study population were not in the Hardy-Weinberg equilibrium due to small sample size. Furthermore, selection that occurred as a result of the exclusion criteria for Part II may be one of the possible reasons. Hardy-Weinberg equilibrium believes that among a specific population, the genetic variation stays consistent from one generation

to the next without the presence of disturbing factors. Hence, this equilibrium assumes the five principles, which are “no mutations, no natural selection, no genetic drift, random mating, and a very large population” (Stark, 2005).

Genotypic and allelic frequency among habitual coffee drinkers did not conform with Hardy-Weinberg equilibrium. This result suggested that *MTHFR* C677T gene variant is associated to coffee drinking. T allele carriers will increase their susceptibility to coffee drinking. Although the sample size was small, but this result provided meaningful insights as the selected participants were screened based on the targeted criteria and focused on the Chinese students at UTAR Kampar Campus. In future, replication of findings with larger sample size will be targeted in order to validate and strengthen the conclusions.

Non-habitual coffee drinkers (control group) passed the Hardy–Weinberg equilibrium test, showing that the frequency of each genotype and allele were normal and the absence of any factors that influencing the genetic balance. In summary, this study cohort suggested evolution process happened among the study population while coffee was a factor that plays a role in the changes of genotypic and allelic frequency. Nevertheless, larger sample size with TT genotype is required in the future study to confirm the findings.

5.7 Epigenetics effect of caffeine

Over 1000 phytochemicals can be found inside a coffee bean. Caffeine, caffeic acid, phenolic compounds (chlorogenic acids), diterpenes (cafestol and kahweol) are some of the well-known compounds (de Melo Pereira, et al., 2020). Particularly caffeine, with the IUPAC name of 1,3,7-trimethylpurine-2,6-dione, is a kind of trimethyl xanthine that having three methyl groups attached at the positions 1, 3, and 7 of its chemical structure (National Center for Biotechnology Information, 2023). The impact of coffee on the development of various pathological conditions in human is still under studied while individual with different response to coffee has been proposed (Ding, Xu, and Lau, 2023). Ding, Xu, and Lau (2023) believed that coffee, particularly the caffeine content can exert epigenetics effect via several mechanisms, including DNA methylation, histone modification and the ncRNA expression. At molecular level, it regulates the gene activity by modifying the chromatin (Ding, Xu, and Lau, 2023).

5.8 Genetic interaction of *MTHFR* C677T variant with habitual coffee drinking to blood pressure, pulse and lipid profile

5.8.1 Effects of regular consumption of coffee on cardiometabolic risk factors

No significant difference in baseline resting SBP, DBP and pulse rate was found between the habitual coffee drinkers and non-habitual coffee drinkers. Alternation in blood pressure and pulse was caused by caffeine content in the coffee, however, tolerance may develop among regular coffee drinkers due to

the lack of pressor effect (Godos, et al., 2014). Besides that, although not significant, habitual coffee drinkers had higher mean SBP than non-habitual coffee drinkers. This result was supported by the study of Cicero, et al. (2023), who reported moderate coffee consumers had higher SBP than the non-coffee drinkers. Generally, the ingestion of coffee can have an acute effect on blood pressure as it stimulates the blood vessels, thus, increasing blood pressure. The methylation of caffeine may alter the blood pressure. It is important to understand that the impact of coffee on blood pressure can vary among individuals due to personalised nutrition.

At baseline, habitual coffee drinkers had significant lower mean HDL-C level and higher mean TC/HDL-C ratio than non-habitual coffee drinkers. Study on the long-term effect of coffee intake believed that regular coffee consumption led to elevated TC, potentially result in higher TC/HDL-C ratio (Cai, et al., 2012). Although to our knowledge, coffee either showed positive or no significant effect on HDL-C level, dietary fat intake, physical activity, body fat percentage, alcohol consumption, smoking status and genetic factors are the important elements that influence the HDL-C level (Jee, et al., 2001; Ghavami, et al., 2021). Despite majority of the participants were having normal body fat percentage, non-smoker, non-alcohol consumer, and physically inactive, the effect of dietary fat intake and genetic factors were unable to be adjusted in the present study. Therefore, important cofounding factors need to be considered in future studies to confirm the results.

5.8.2 The effects of *MTHFR* C677T polymorphism on cardiometabolic risk factors

C667T polymorphism is one of the most common polymorphisms of the *MTHFR* gene. It is a missense mutation in which the cytosine (C) at 677th is substituted by thymine (T), subsequently, encoding a thermolabile type of enzyme. *MTHFR* C677T variant may have reduced *MTHFR* enzyme activity. It will alter the homocysteine level whereby elevated homocysteine level is associated with cardiometabolic risk factors. In this study, no significant difference in non-fasting lipid profile were found between CC genotype and T allele carriers (CT+TT). Similar results were observed for these two genotypes among the habitual coffee drinkers and non-habitual coffee drinkers, respectively. According to the study conducted in Shaanxi, China and United States, the study population showed no significant difference in any parameters of lipid profile (TC, TG, HDL-C and LDL-C) (Mahesh, Cheng and Khalighi, 2019; Guan, et al., 2023). However, significant higher TC and LDL-C were observed among the T allele carriers (Zhang, et al., 2010; Guan, et al., 2023). These differences in findings can be caused by various factors, such as ethnicity, geographic location and health condition of the participants. Besides that, it may also relate to inadequate sample of TT genotype as C allele exerts protective effect on lipid profile (Chen, et al., 2021). The protective effect of C allele may also explain the insignificant difference in lipid profile between habitual and non-habitual coffee drinkers among the CC genotypes.

Among the habitual coffee drinkers, no significant difference in lipid profile can be observed. Up to date, the exact influence of coffee on health is still controversial. Karabudak, Türközü and Köksal (2015) also observed no influence of coffee on lipid profile. Despite chlorogenic acid and phenolic compound, as part of the complex structure of coffee, showed positive effect on cardiovascular health, however, coffee might increase TC, TG and LDL-C level (Cai, et al., 2012; Ghavami, et al., 2021). A *in vivo* study in rats indicated that treatment with chlorogenic acid after six weeks significantly lowered TC, TG, LDL-C while increased HDL-C (Wang, et al., 2019). The effect of coffee on cardiovascular health is highly variable due to various factors, such as cooking preparation, chemical additives, types of coffee and individual health condition.

No significant difference in blood pressure and pulse were found among the CC genotype and T allele carriers (CT+TT) as well as when comparison was done among the habitual coffee drinkers. However, study conducted among Ireland explained that healthy adults with TT genotypes had significant higher blood pressure if compared to the C allele carriers (Rooney, et al., 2022). Similar results had been found in a group of adults aged between 18-102 years old in Ireland (Ward, et al., 2020). The discrepancy of the result may be related to differences in age group, ethnicity, dietary pattern, geographical location, and the inadequate sample of TT genotype.

5.8.3 The influence of *MTHFR* C677T polymorphism on non-habitual coffee drinkers

Among the non-habitual coffee drinkers, CC genotype had significant higher mean resting pulse rate than T allele carriers (CT+TT). To date, there is limited information on the relationship between the *MTHFR* C677T gene variant and pulse rate. However, it is unlikely that a single nucleotide polymorphism will be the sole determinant of pulse rate. Multiple factors such as age, gender, increased body weight, physical activity and fitness level, smoking, alcohol consumption and the gene-gene interaction must be considered as part of the complex mechanisms in the regulation of pulse rate (Howden, et al., 2008; Summit and Böckelmann, 2016). Pulse rate was proven to be inherited while the interaction of several candidate genes was found to play a role in affecting the pulse rate (Howden, et al., 2008; Eijgelsheim, et al., 2010; Eppinga, et al., 2016).

5.8.4 Effect of regular coffee consumption on the cardiometabolic risk factors among the homozygous CC of *MTHFR* C677T polymorphism

Among the homozygote CC, significant difference was found in the pulse rate between habitual and non-habitual coffee drinkers. Although both of their readings were considered as normal, regular coffee consumers had significantly lower mean pulse rate than the non-frequent coffee drinkers. Despite it is widely known that increment in pulse rate right after coffee consumption is a normal physiological response to the caffeine content, however, long term effect of coffee on pulse rate shall be evaluated in future study. Gonzaga, et al. (2017)

suggested chronic caffeine intake did not significantly affect the pulse rate. Hence, not necessary the significant difference in their pulse rate were only due to the intake of coffee. As mentioned before, factors that thought to affect the pulse rate should be considered when examining the variation in pulse rate between the two groups. Future study should be done to confirm the causality between coffee and pulse rate so that it provides fundamental insight to the gene-coffee interaction on pulse rate.

5.8.5 Comparison of the resting blood pressure, pulse and non-fasting lipid profile between habitual and non-habitual coffee drinkers with different genotype

There was no significant difference in mean resting blood pressure (SBP and DBP) and mean pulse rate among habitual and non-habitual coffee drinkers in this study. Similar results were observed between habitual and non-habitual coffee drinkers with different genotypes.

The results were partially in line with the study conducted by Julius, Selamat and Ismail (2020), who claimed that significant difference in blood pressure was reported between frequent and non-frequent coffee consumers in males but not in females. In this study, dissimilar results were observed because there were more female participants in the study population (male: female = 1:2). Fatima, et al. (2020) indicated contrasting results as compared to the present study, whereby she claimed that SBP and DBP, as well as the pulse rate of regular

coffee drinkers, as measured on the left arm were significantly differed from the non-regular coffee drinkers. The findings of the study depend on the design of the experiments, types of study, ethnicity, etc. Godos, et al. (2014) also found that the absence of pressor effect on blood pressure was resulted from regular caffeine intake overtime. Fatima, et al. (2020) showed that individuals who consumed other caffeinated beverages (tea and energy drinks) would cause bias in the study. Therefore, the results may vary due to the consumption of other caffeinated beverage among the non-habitual coffee drinkers.

In the present study, significant difference was observed in the mean TC/HDL-C ratio between habitual coffee drinkers and non-habitual coffee drinkers. The result of multiple comparison indicated the mean TC/HDL-C ratio among habitual coffee drinkers of CT genotype was significantly higher than the non-habitual coffee drinkers of CT+TT genotype. Over the years, studies were carried out to investigate the effect of coffee consumption on serum lipid profile. However, dissimilar findings were remained (Naidoo, et al., 2011; Corrêa, et al., 2013; Sarriá, et al., 2018). Elevated homocysteine level is highly associated with *MTHFR* C677T polymorphism, in which the homozygosity of mutant T allele is at higher risk of developing hyperhomocysteinemia (Raghubeer and Matsha, 2021). In the epigenetics study, elevated homocysteine level induced by coffee was seen particularly obvious in TT genotypes (Strandhagen, et al., 2004). Liao, et al. (2006) demonstrated that decrement in HDL-C could be resulted from elevated homocysteine level since homocysteine inhibits the synthesis of major HDL apolipoprotein, apoA-I by the liver as well as enhancing

the clearance of the HDL-C. Together, decreased HDL-C and increased TC led to higher TC/HDL-C ratio among the habitual coffee drinkers of CT genotype, suspecting the undesirable effect shown by the polymorphic T allele. Therefore, it was predicted that regular intake of coffee could increase the risk of getting cardiovascular risk particularly in T allele carrier.

5.9 Changes of blood pressure, pulse and lipid profile after instant black coffee consumption

The mean blood pressure and pulse rate before and after coffee testing were normal among all subjects. Increment in blood pressure is considered a physiological response to the coffee, particularly the caffeine, happened right after the ingestion (Kujawska, et al., 2021). Significant increase in the mean blood pressure (SBP and DBP) after coffee ingestion were observed among the habitual coffee drinkers while there was decrement in the pulse rate. It may be due to a compensatory response to the increased blood pressure

The results indicated that drinking coffee could increase the risk of getting CVD. To date, there are still very limited studies on the direct impact of methylation on caffeine's effect on blood pressure. This result may significant important to enhance the understanding of the interaction between methylation, coffee and blood pressure. Habitual coffee drinkers showed significant increase in their mean SBP and DBP, but their mean pulse rate decreased significantly after the ingestion of coffee. Similar changes were observed among the homozygous C

allele habitual coffee drinkers. Although mean pulse rate decreased significantly among heterozygous (CT) habitual coffee drinkers, only DBP increased significantly. The ingestion of coffee was believed to increase the SBP and DBP by an average of 2.4 mmHg and 1.2 mmHg, respectively (Jee, et al., 1999). Meanwhile, the blood pressure-raising effect of coffee was obviously seen among the young population, which is similar to our study population. Caffeine as a psychoactive stimulant to the central nervous system, its ingestion supposed to give a rise in pulse rate. However, when discussing the effect, consideration of other factors needs to be included. For instance, the secondary response, which is also referred to as the vagal response after the initial stimulation of caffeine, is responsible in the decrement of pulse rate after coffee consumption. The increased heart rate right after coffee ingestion is detected by a group of specialised sensors at the arteries wall, which is known as the baroreceptors, as a consequence, parasympathetic nervous system will be activated and compensate with the increasing blood pressure, as a result, lead to a decrease in the pulse rate after certain period of time (Sudano, et al., 2005).

In terms of the lipid profile, overall readings of each component appeared to be normal among the subjects. When regardless of the genotype, no significant differences in the mean of TC, TG, HDL-C, LDL-C level and TC/HDL-C ratio were found. Similar results were observed in habitual coffee drinkers with CC genotype. It was conformed to many other studies that concluded the intake of coffee did not contribute to any changes in TC, TG, and LDL-C (Naidoo, et al., 2011; Karabudak, Türközü and Köksal, 2015). Only unfiltered coffee was

known to be associated with an increase in the TC, TG and LDL-C, as attributed to the cafestol and kahweol contents (Rodrigues and Klein, 2006; Cai, et al., 2012; Jee, et al., 2015). Nevertheless, the coffee used in this study, and the coffee consumed by the participants as reported in the questionnaire were the filtered type.

The mean TC/HDL-C ratio decreased significantly after the consumption of coffee among the habitual coffee drinkers with CT genotype. This result was dissimilar with the study of Hao, et al. (2006), of which coffee was thought to cause an increase in the homocysteine level particularly in the T allele whereby hyperhomocysteinemia is a contributing factor for higher TC and lower HDL-C level as mentioned by (Liao, et al., 2006). Meanwhile, originally, T allele carrier tends to have higher TC and lower HDL-C level, especially among the female population (Chen, et al., 2014). However, the influence of coffee on TC/HDL-C ratio, as well as TC and HDL-C (although the changes was not significant in this study) cannot be concluded solely by the acute effect of a session of coffee ingestion. The dissimilar findings from both studies are caused by differences in dietary factor and physical activity level. The daily food choices, food supplement intake (fish oil), and physical activity are the widely known factors that influence the lipid profile.

To ensure participants of well-balanced diet were recruited, dietary information was screened using food checklist by examining the frequency and portion of

specific food consumed over a month. For example, the intake of fruits and vegetables, high fat and/or high salt food, such as the processed food, fast food and fried food (Ching, et al., 1998; Ghavami, et al., 2023). Besides that, physical activity level was assessed to obtain the frequency, duration and intensity of the physical activity. However, under- or over-report, discrepancy in the understanding of food portion estimation, intensity of physical activity, and the inability to recall led to inaccuracy.

As comparing the post-coffee testing lipid profile between the CC and CT genotype, individuals with the CC genotype may metabolize caffeine more rapidly compared to CT genotype. It is possible that they may only experience a shorter duration of caffeine's effect and lower blood pressure, and not significant change to their lipid profile. However, coffee may exert beneficial effects on the TC/HDL-C ratio for the T allele carrier.

5.10 The TC to HDL-C ratio of the heterozygote (CT) of *MTHFR* C677T polymorphism

The ratio of TC to HDL-C is a measure used to assess the risk of CVDs. A higher TC/HDL-C ratio generally has increased risk of getting the CVDs. Among the heterozygote (CT), despite habitual coffee drinkers had significant higher baseline TC to HDL-C ratio than the non-habitual coffee drinkers, yet the experimental study (coffee testing) showed a significant decrease in the TC to HDL-C ratio after coffee consumption. Such contracting result can be

explained by other factors that impact on the lipid profile, including the diet and physical activity level as mentioned before. Important cofounding variables should be adjusted in future studies.

Besides that, not necessary an acute effect predicts the long-term effect of coffee on the lipid profile among the CT genotypes. Similar to blood pressure, significant increase in mean SBP and DBP had been found among habitual coffee drinkers yet in long term, tolerance may develop overtime. Future study should include the homozygote T allele genotype (TT), having a superior study design, such as randomized control trial, in which the researcher has control over more factors to have more accurate results.

5.11 Investigation of the changes of pulse rate after coffee consumption between *MTHFR* C677C and C677T variants

To the best of our knowledge, research on the relationship between coffee consumption and the *MTHFR* C677T polymorphism and how these two factors affect the elements of cardiometabolic risk factor is lacking. The trend of the mean pulse rate was consistent for CC and CT genotype, in which both of their mean pulse rate decreased after 30 minutes of coffee ingestion, and the decreasing trend persisted to 60 minutes. Possible reason for the decreasing pulse rate was stated previously, which is caused by the activation of parasympathetic nervous system that mediated through vagal nerves as a secondary response to the initial activation of sympathetic nervous system.

Significant differences were found between the CC and CT genotype in the changes of mean pulse rate at of two different intervals. At 30 minutes and 60 minutes after coffee ingestion, the mean decrement in pulse rate of CC genotype was significantly greater than CT genotype. In other word, the mean pulse rate of CC genotype tended to drop more so that it could back to normal after the initial activation of sympathetic nervous system. Study on the changes of pulse rate that relates to the *MTHFR* C677T variant is lacking, however, it is predicted that the 677T allele carrier tends to have higher pulse rate as elevated homocysteine level increases pulse rate (Moshal, et al., 2007). Therefore, possible reason to explain the smaller decrement of mean pulse rate in CT genotype could be genetically, they tend to have higher mean pulse rate at baseline.

No significant difference in the acute changes of blood pressure (SBP and DBP) and lipid profile were observed between CC and CT genotype after the intake of coffee. Although not statistically significant, the trends of these two parameters following coffee consumption provided insights to implicate the long-term effect on these parameters. Individuals' variation in the changes of blood pressure and lipid profile after coffee intake serve as a fundamental for future study (with longer duration of intervention) to prove the gene-diet interaction, meanwhile, determine the effect of certain bioactive compound, either present independently or in a bundle with others, such as coffee on different genotype of the *MTHFR* C677T polymorphism. Future study with the involving of TT genotype is necessary as C allele may act as protective allele

against the detrimental effect brought, hence, significant differences in the parameters could not be found between CC and CT genotype in this study.

5.12 Strengths

The *MTHFR* C677T gene variant has been widely used to study effects on the methylation process in the body. To the extent of our awareness, present study is the first of its kind to examine the joint effect of *MTHFR* C677T variant and coffee on the cardiometabolic risk factors. ARMS-PCR was triplicated for each sample to increase the validity of the genotype identification.

5.13 Limitations

The relationship between *MTHFR* C677T polymorphism, coffee consumption, blood pressure, pulse and lipid profile can be varied from individual to individual due to genetic factor, dietary pattern, physical activity level, health condition and etc. Certain collected data might not follow a specific distribution. Therefore, non-parametric methods were used in the analysis.

Findings of the present study were of low statistical power due to small sample size. In addition, the important cofounding variables, such as physical activity level, intake of supplement and other caffeinated beverages were not adjusted. Despite food checklist was applied to exclude participants with unbalanced diet, self-reported kind of data lack of accuracy. Other than that, long-term effects of

coffee could not be identified due to short experimental period. Furthermore, absence of TT genotype in the habitual coffee drinkers' group due to small sample size made the present study unable to investigate the effect of homozygosity of T allele on the cardiometabolic risk factors. Furthermore, there were indeed gender differences in lipid profile and blood pressure. Generally, male tends to have higher levels of TC and LDL-C. While female tends to have higher HDL-C. These are due to hormonal variations between genders.

5.14 Future recommendations

Future studies should be carried out using larger sample size and with more superior study design, such as randomized control trial to have greater control over dietary intake. Important cofounding factors, such as gender, diet and lifestyle habits need to be adjusted to ensure the internal validity of the study. In addition, homocysteine level should be assessed since the *MTHFR* C677T gene variant is strongly associated with serum homocysteine level. Coffee with various doses, together with longer duration of the intervention is encouraged to accomplish the study of dose-dependent chronic effects of coffee consumption on blood pressure and lipid profile. Moreover, as hormonal variation between genders do give an impact on the components of cardiometabolic risk factors, comparison between males and females in terms of their blood pressure, pulse and lipid profile should be carried out.

CHAPTER 6

CONCLUSION

In conclusion, present study suggested that there was association between *MTHFR* C677T polymorphism and habitual coffee consumption in the general study population and among the habitual coffee drinkers' group. The prevalence of habitual coffee drinking was 34.9% among the recruited 298 students in UTAR Kampar Campus. However, it was not representative of the prevalence of regular coffee drinking among the university students in Malaysia. This is because the ethnicity was mainly Chinese (97%) in present study and the prevalence might also differ in urban and rural area. 110 (36.9%) males and 188 (63.1%) females were included in this study. Significant association was found between gender and regular coffee consumption, in which the number of males was significantly higher than females among the frequent coffee drinkers.

There are three genotypes, namely CC (wildtype), CT (heterozygote) and TT (homozygous mutant) resulted from the *MTHFR* C677T polymorphism. The genotypic distribution for the wildtype, heterozygote and homozygous mutant were 49.1%, 50% and 1% in the study population, respectively. Regular consumption of coffee potentially increased the TC to HDL-C ratio among the T allele carriers. In addition, in the present study, regular coffee intake might exert negative effect on HDL-C parameters, regardless of the genotype. At baseline, no significant differences in the cardiometabolic risk factors between

the wildtype and T allele carrier among the young adult. However, wildtype non-frequent coffee drinkers tended to have a higher pulse rate as compared to their counterpart.

Among the overall and wildtype genotype habitual coffee drinkers, significant increment in their mean SBP and DBP but decrement in pulse rate were observed 1 hour after the coffee ingestion. No significant differences in lipid profile were recorded. On the other hand, only increment in DBP and decrement in pulse rate were found among the heterozygous habitual coffee drinkers. Meanwhile, the TC to HDL-C ratio decreased significantly after coffee intake.

Future studies with larger sample size, gender differences, study design using randomized control trial, adjusted cofounding variables and longer duration for intervention were required to confirm the findings of present study. Furthermore, epigenetics modifications play important role in mediating the nutrigenomics of coffee on human health. More research on gene-diet interaction on coffee and metabolic risk factors, gender, environmental factors should be established.

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APPENDICES

APPENDIX A

Ethical approval sheet

UNIVERSITI TUNKU ABDUL RAHMAN DU012(A)

Wholly owned by UTAR Education Foundation Co. No. 578227-M

Re: U/SERC/251/2022

22 November 2022

Dr Phoon Lee Quen
Department of Allied Health Sciences
Faculty of Science
Universiti Tunku Abdul Rahman
Jalan Universiti
Bandar Baru Barat
31900 Kampar, Perak

Dear Dr Phoon,

Ethical Approval For Research Project/Protocol

We refer to your application which was circulated for consideration of the UTAR Scientific and Ethical Review Committee (SERC). We are pleased to inform that your application for ethical approval of your research project (Undergraduate students' project) involving human subjects has been approved by SERC.

The details of the project are as follows:

Research Title	Epigenetic Effects of Instant Coffee in Lipid Metabolism
Investigator(s)	Dr Phoon Lee Quen Chin Karyn (UTAR Undergraduate Student) Tan Si Ni (UTAR Undergraduate Student) Wong Xiao Xian (UTAR Undergraduate Student)
Research Area	Science
Research Location	UTAR (Kampar Campus)
No of Participants	Minimum 200 participants (Age: 18 - 45)
Research Costs	Self-funded
Approval Validity	22 November 2022 - 21 November 2023

The conduct of this research is subject to the following:

(1) The participants' informed consent be obtained prior to the commencement of the research.

(2) Confidentiality of participants' personal data must be maintained; and

(3) Compliance with procedures set out in related policies of UTAR such as the UTAR Research Ethics and Code of Conduct, Code of Practice for Research Involving Humans and other related policies/guidelines.

(4) Written consent be obtained from the institution(s)/company(ies) in which the physical or/and online survey will be carried out, prior to the commencement of the research.

Kampar Campus : Jalan Universiti, Bandar Barat, 31900 Kampar, Perak Darul Ridzuan, Malaysia
Tel: (605) 468 8888 Fax: (605) 466 1313
Sungai Long Campus : Jalan Sungai Long, Bandar Sungai Long, Cheras, 43000 Kajang, Selangor Darul Ehsan, Malaysia
Tel: (603) 9086 0288 Fax: (603) 9019 8868
Website: www.utar.edu.my

Should you collect personal data of participants in your study, please have the participants in the research signed the attached Personal Data Protection Statement for your records.

The University wishes you all the best in your research.

Thank you.

Yours sincerely,

Professor Ts Dr Faidz bin Abd Rahman
Chairman
UTAR Scientific and Ethical Review Committee

c.c Dean, Faculty of Science
Director, Institute of Postgraduate Studies and Research

Kampar Campus : Jalan Universiti, Bandar Barat, 31900 Kampar, Perak Darul Ridzuan, Malaysia
Tel: (605) 468 8888 Fax: (605) 466 1313
Sungai Long Campus : Jalan Sungai Long, Bandar Sungai Long, Cheras, 43000 Kajang, Selangor Darul Ehsan, Malaysia
Tel: (603) 9086 0288 Fax: (603) 9019 8868
Website: www.utar.edu.my

APPENDIX B

Consent form in the questionnaire

Epigenetic Effects of Instant Coffee in Lipid Metabolism

Informed Consent

Aim / Purpose of the Research:

The objectives are:

1. To study the status, quality, and habitual coffee consumption.
2. To determine the association of targeted gene variants and instant coffee consumption.
3. To study the association of cardiometabolic risk factors and instant black coffee.
4. To determine the association of targeted gene variants, blood pressure and cardiometabolic risk factors.

Procedure, Risk and Discomfort:

Part I:

Volunteers, age 18 – 45 years will be recruited in the study. Firstly, you are invited to complete a survey and questionnaire on coffee consumption and food intake. Secondly, you are required to **rinse your mouth with water** to cleanse the mouth from food particles. Then, you are required to **rinse your mouth with saline solution**. The mouth rinse will be **collected in a disposable paper cup.** The mouth wash sample will be used for **genomic DNA extraction.** Buccal cell collection poses minimal risk and discomfort to donor as it is a non-invasive method. Thirdly, BMI and blood pressure reading will be taken down by using an automated blood pressure monitor. The **blood pressure** will be measured on the left arm after the participant has **rested for 5 minutes in a seated position**. Blood pressure measurement poses minimal risk and discomfort to participant as it is a non-invasive method involved in the measurement.

Part II:

Finger prick blood of the eligible participants will be collected to determine lipid levels (TC, TG, HDL-C, and LDL-C). Lastly, coffee testing will be provided. **Blood pressure will be measured before the instant coffee testing, 0, 30, 60 minutes after the oral administration of coffee.**

Confidentially

All information, samples and specimens you have supplied will be kept confidential by the principal investigator and the research team and will not be made available to the public unless disclosure is required by law.

Who to Contact

If you have any questions, you may contact : Dr Phoon Lee Quen ; Email : phoonlq@utar.edu.my. This proposal has been reviewed and approved by UTAR Scientific & Ethical Review Committee, which is a committee to confirm that research participants are protect from harm.

Disclosure

Data, samples and specimens obtained from this study will not identify you individually. The data, samples and specimens may be given to the sponsor and/or regulatory authorities and may be published or be reused for research purposes not detailed within this consent form. However, your identity will not be disclosed. The original records will be reviewed by the principal investigator and the research team, the UTAR Scientific and Ethical Review Committee, the sponsor and regulatory authorities for the purpose of verifying research procedures and/or data.

* Indicates required question

Consent form in the question (continued)

The flow of this project you need to know:

1. Part 1 - Questionnaire answering, BMI data and buccal cells collections will be conducted at the set booth (Block D, D014B or D215, exact location to be notified through WhatsApp).
2. The eligible participants who fulfill our research requirements will be invited to participate in Part II. A blood pressure monitoring period preceded by coffee intake which will be provided (to be informed).

Voluntary Participation

If you are eligible, your participation in this research is entirely voluntary. You understand that participation in this study is voluntary and that if you decide not to participate, you will experience no penalty or loss of benefits to which you would otherwise be entitled. If you decide to participate, you may subsequently change your mind about being in the study, and may stop participating at any time. You understand that you must inform the principal investigator of your decision immediately.

1. **Consent** *

Check all that apply.

☐ I have read and understood the condition stated above and I agree to voluntarily participate in these research studies.

Skip to question 2

2. Will you conduct your industrial training in the May/June 2023 trimester? *

Mark only one oval.

◯ Yes
◯ No

SECTION A: DEMOGRAPHICS

3. Name *

4. UTAR Email *

5. Current address *

6. Contact Number (e.g. 0123456789) *

7. Gender *

Mark only one oval.

◯ Male
◯ Female

APPENDIX C

Questionnaire

I) Section A: Demographics

SECTION A: DEMOGRAPHICS

3. Name *

4. UTAR Email *

5. Current address *

6. Contact Number (e.g. 0123456789) *

7. Gender *

Mark only one oval.

- Male
- Female

I) Section A: Demographics (continued)

8. Age *

9. Ethnicity *

Mark only one oval.

- Malay
- Chinese
- Indian
- Other:

10. Education Qualification *

Mark only one oval.

- Foundation
- Diploma
- Undergraduate degree
- Master degree
- Doctor of Philosophy

11. Place of Origin (Which state?) *

12. Residential Area (hometown) *

Mark only one oval.

- Urban
- Rural

13. Current height (cm) *

14. Current weight (kg) *

15. Amount of sleeping hours *

16. Usual bedtime *

Example: 8:30 AM

II) Section B: Personal health and family history

17. Usual wakeup time *

Example: 8:30 AM

Skip to question 18

Section B: Health History

B-1: Personal Health

18. 1. Please tick (√) at the appropriate column for each item. *

Mark only one oval per row.

	Yes	No
Do you have diabetes?	⬭	⬭
Do you have hypertension?	⬭	⬭
Do you have coronary heart disease?	⬭	⬭
Do you have cerebrovascular disease?	⬭	⬭
Do you have hypothyroidism?	⬭	⬭
Have you had any form of neurological disease? (seizures, epilepsy, etc)	⬭	⬭
Do you have any form of psychiatric disorder? (depression, anxiety, etc)	⬭	⬭
Are you pregnant?	⬭	⬭
Are you lactating?	⬭	⬭

19. 2. Do you have other disease? *
(If "Yes", please state at the "other" option)

Mark only one oval.

⬭ No

⬭ Other: ____________

II) Section B: Personal health and family history (continued)

20. 3. Have you been prescribed any medication? (If "Yes", please state at the "other" option) *

Mark only one oval.

- ⬭ No
- ⬭ Other: ____________________

21. 4. Do you smoke? (If "Yes", please state the amount (pack/week) at the "other" option) *

Mark only one oval.

- ⬭ No
- ⬭ Other: ____________________

22. 5. Have you been tested positive for COVID-19? *

Mark only one oval.

- ⬭ Yes
- ⬭ No

Skip to question 23

B.2: Family Health History

Please answer the questions as Yes or No. If "Yes", please state who and/or the disease at the "other" option.

23. 1. Do any of your parents (father/mother) have diabetes? *

If "Yes", Who? (state at the "other")

Mark only one oval.

- ⬭ No
- ⬭ Other: ____________________

24. 2. Do any of your parents (father/mother) have hypertension? *

If "Yes", Who? (state at the "other")

Mark only one oval.

- ⬭ No
- ⬭ Other: ____________________

25. 3. Do any of your parents (father/mother) have coronary heart disease? *

If "Yes", Who? (state at the "other")

Mark only one oval.

- ⬭ No
- ⬭ Other: ____________________

II) Section B: Personal health and family history (continued)

26. 4. Do any of your parents (father/mother) have cerebrovascular disease? *

If "Yes", Who? (state at the "other")

Mark only one oval.

- No
- Other: ____________

27. 5. Do any of your parents (father/mother) have hypothyroidism? *

If "Yes", Who? (state at the "other")

Mark only one oval.

- No
- Other: ____________

28. 6. Do any of your parents (father/mother) have other major diseases? *

If "Yes", Who and please state the disease at the "other" option.

Mark only one oval.

- No
- Other: ____________

29. 7. Do any of your parents (father/mother) have any form of neurological disease? (seizures, epilepsy, etc) *

If "Yes", Who? (state at the "other")

Mark only one oval.

- No
- Other: ____________

30. 8. Do any of your parents (father/mother) have any form of psychiatric disorder? (depression, anxiety, etc). *

If "Yes", Who? (state at the "other")

Mark only one oval.

- No
- Other: ____________

III) Section C: Habits and preferences to the consumption of coffee

Section C: Habits and Preferences to the Consumption of Coffee

31. Have you drunk at least one cup of coffee in the past 6 months? *

Mark only one oval.

- Yes *Skip to question 32*
- No *Skip to question 41*

Skip to question 32

32. 1. Coffee consumption frequencies: *

Mark only one oval.

- Less than once per month
- Once per month
- 2 – 3 times per month
- 1 -2 times per week
- 3 or more than 3 times per week
- Once per day
- 1 - 3 times per day
- More than 3 times per day

33. 2. In a typical day, how many cups of coffee do you drink? *

Mark only one oval.

- 2 cups or less
- 3 – 4 cups
- 7 – 8 cups
- 9 cups or more
- 5 – 6 cups

III) Section C: Habits and preferences to the consumption of coffee (continued)

34. 3. How often do you drink coffee on each of the following occasions? *

Mark only one oval per row.

	Never	Rarely	Often	Always
First thing after waking up in the morning?	◯	◯	◯	◯
With breakfast?	◯	◯	◯	◯
During your morning break?	◯	◯	◯	◯
With lunch?	◯	◯	◯	◯
During your afternoon break?	◯	◯	◯	◯
With dinner?	◯	◯	◯	◯
In the evening after dinner?	◯	◯	◯	◯
At bedtime?	◯	◯	◯	◯
During examination week?	◯	◯	◯	◯
When visiting friends or family?	◯	◯	◯	◯
When you are stressed?	◯	◯	◯	◯

35. 4. Please indicate which one of the following types of coffee you **use most**. *

Check all that apply.

- ☐ Instant
- ☐ Ground/ filter coffee (e.g. house of coffee)
- ☐ Instant decaffeinated
- ☐ Ground decaffeinated
- ☐ Cappuccino
- ☐ Espresso
- ☐ Latte
- ☐ Other: ____________________

III) Section C: Habits and preferences to the consumption of coffee (continued)

36. 5. Please indicate which one of the following types of coffee you **prefer most**. *

Mark only one oval.

- Instant
- Ground/ filter coffee (e.g. house of coffee)
- Instant decaffeinated
- Ground decaffeinated
- Cappuccino
- Espresso
- Latte
- Other: __________

37. 6. Which of the following is the **most important reason** for **using** the type of coffee specified in **Question 4**? Please tick (√).

Mark only one oval.

- It is the type I prefer.
- It tastes best.
- It is the most affordable.
- It is the most readily available.
- The place where I work/ study provides the most used type.
- Other members in my household prefer most used brand.
- Most convenient
- Other: __________

38. 7. If the type of coffee you **use most often differs from the type you prefer most**, which is the **most important reason** for this discrepancy? Please tick (√).

Mark only one oval.

- I cannot afford my preferred type.
- The place where I work/ study does not use the preferred type.
- Other members in my household prefer another type of coffee.
- Not applicable. The type of coffee I use most often is same as the type I prefer most.
- Other: __________

III) Section C: Habits and preferences to the consumption of coffee (continued)

39. 8. How important is each of the following to you when purchasing/ ordering coffee? *

Mark only one oval per row.

	Totally unimportant	Unimportant	Important	Very important
Low price	⬭	⬭	⬭	⬭
Smooth taste	⬭	⬭	⬭	⬭
Purchasing/ ordering a specific brand	⬭	⬭	⬭	⬭
The strength of the coffee	⬭	⬭	⬭	⬭
Purchasing/ ordering a local brand	⬭	⬭	⬭	⬭
Purchasing/ ordering an imported brand	⬭	⬭	⬭	⬭

40. 9. Do you consider yourself to be a coffee addict? *

Mark only one oval.

⬭ Yes

⬭ No

Skip to question 41

Untitled Section

III) Section C: Habits and preferences to the consumption of coffee (continued)

41. Please rank, in order of importance, each of the following reasons for not consuming coffee in the last 6 months.

Allocate a rank of "**1**" to the **most important reason** for not consuming coffee in the last 6 months, a rank of "2" to the second most important reason, etc. allocate a rank of "**6**" to the **least important reason** for not consuming coffee in the last 6 months. (**All the numbers from 1 to 6 should be chosen for this question, without equal rank.**)

Mark only one oval per row.

	1	2	3	4	5	6
I do not like the taste of coffee.	⬭	⬭	⬭	⬭	⬭	⬭
I do not like the smell of coffee.	⬭	⬭	⬭	⬭	⬭	⬭
Coffee is expensive.	⬭	⬭	⬭	⬭	⬭	⬭
I do not drink coffee for health reason.	⬭	⬭	⬭	⬭	⬭	⬭
I cannot sleep when drinking coffee	⬭	⬭	⬭	⬭	⬭	⬭
I prefer drinking tea.	⬭	⬭	⬭	⬭	⬭	⬭

Skip to question 42

Section D: Your Attitude and Perceptions Regarding Coffee Consumption

IV) Section D: Attitude and perceptions regarding coffee consumption

42. 1. To what extent do you agree with each of the following statements? *

Mark only one oval per row.

	Strongly disagree	Disagree	Neutral	Agree	Strongly agree
Drinking coffee increases my risk of getting cancer.	⬭	⬭	⬭	⬭	⬭
Drinking coffee increases my risk of heart disease.	⬭	⬭	⬭	⬭	⬭
I cannot fall asleep when I drink coffee during the day.	⬭	⬭	⬭	⬭	⬭
I prefer drinking tea to drinking decaffeinated coffee.	⬭	⬭	⬭	⬭	⬭
Drinking coffee gives me an energy boost.	⬭	⬭	⬭	⬭	⬭
I drink coffee because my friends do.	⬭	⬭	⬭	⬭	⬭
I drink coffee for the taste.	⬭	⬭	⬭	⬭	⬭
I drink coffee because it is fashionable.	⬭	⬭	⬭	⬭	⬭
Drinking coffee relaxes me.	⬭	⬭	⬭	⬭	⬭
I prefer drinking tea to drinking any coffee.	⬭	⬭	⬭	⬭	⬭
I cannot fall asleep when drinking coffee in the evening.	⬭	⬭	⬭	⬭	⬭
I drink coffee to help me stay awake.	⬭	⬭	⬭	⬭	⬭
I prefer local brands of coffee to	⬭	⬭	⬭	⬭	⬭

V) Section E: Food intake

imported ones.					
Drinking coffee makes me feel important.	⬭	⬭	⬭	⬭	⬭

Skip to question 43

Please list the foods and drinks you consume normally.

43. 1. Are you a vegetarian? *

Mark only one oval.

⬭ Yes

⬭ No

44. Breakfast (1st meal) and snacks: *

45. Lunch (2nd meal) and snacks: *

46. Dinner (3rd meal) and snacks: *

47. Supper: *

Skip to question 48

Section E: Food Intake

48. 1. How many **days in a week** do you eat/drink: *

Mark only one oval per row.

	0	1	2	3	4	5	6	7
Confectionery (local cakes, cakes, ice-cream, ABC, jelly, snacks, etc.)?	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Fruits (exclude canned fruits)	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Vegetables/ salads	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Plain water	⬭	⬭	⬭	⬭	⬭	⬭	⬭	⬭

VI) Section F: Food habit

F-1: Processed foods

51. Please tick at the appropriate columns of the amount of servings **per day.** *

Mark only one oval per row.

	0	1-2	3-4	5-6	More than 6
Any kind of milks	◯	◯	◯	◯	◯
Cereals	◯	◯	◯	◯	◯
Deli ham (chicken/beef)	◯	◯	◯	◯	◯
Sausage	◯	◯	◯	◯	◯
Bacon	◯	◯	◯	◯	◯
Burgers	◯	◯	◯	◯	◯
French fries	◯	◯	◯	◯	◯
Salad dressing	◯	◯	◯	◯	◯
Mayonnaise	◯	◯	◯	◯	◯
Frozen pizza	◯	◯	◯	◯	◯
Pickles	◯	◯	◯	◯	◯
Canned fruits (cocktails, pineapple, peach, etc)	◯	◯	◯	◯	◯
Canned vegetables (mushrooms, green peas, etc)	◯	◯	◯	◯	◯
Canned soups	◯	◯	◯	◯	◯
Canned tuna	◯	◯	◯	◯	◯
Canned spaghetti sauce	◯	◯	◯	◯	◯
Cheese	◯	◯	◯	◯	◯
Fast foods (Mc Donalds, KFC, pizza, Wingzone, etc)	◯	◯	◯	◯	◯
Instant noodles	◯	◯	◯	◯	◯
Instant porridges	◯	◯	◯	◯	◯
Butter	◯	◯	◯	◯	◯
Salted nuts, pumpkin/ sunflower	◯	◯	◯	◯	◯

VI) Section F: Food habit (continued)

F-2: Malaysian/Asian dishes

Section F: Food Habit

F-2: Malaysian/Asian Dishes:

54. Please tick at the appropriate columns of the amount of servings **per day**. *

Mark only one oval per row.

	0	1-2	3-4	5-6	More than 6
Roti Canai	⬭	⬭	⬭	⬭	⬭
Nasi Lemak	⬭	⬭	⬭	⬭	⬭
Char Kuey Teow	⬭	⬭	⬭	⬭	⬭
Rendang	⬭	⬭	⬭	⬭	⬭
Boba/Bubble Tea	⬭	⬭	⬭	⬭	⬭
Teh Tarik	⬭	⬭	⬭	⬭	⬭
Curry Mee	⬭	⬭	⬭	⬭	⬭
Mee Goreng/Maggie Goreng	⬭	⬭	⬭	⬭	⬭
Satay	⬭	⬭	⬭	⬭	⬭
Fried Chicken	⬭	⬭	⬭	⬭	⬭
Karipap	⬭	⬭	⬭	⬭	⬭
Mee Jawa	⬭	⬭	⬭	⬭	⬭
Naan Cheese	⬭	⬭	⬭	⬭	⬭
Hokkien Mee	⬭	⬭	⬭	⬭	⬭
Prawn Mee	⬭	⬭	⬭	⬭	⬭

VII) Section G: Vitamin/mineral supplement intake

Section G: Vitamin/Mineral Supplement Intake

Example: Multivitamin/ Multimineral, Vitamin A/ Carotenoids, B complex vitamin, Vitamin B12, Vitamin C, Folic acid/ B6, Iron, Calcium, Vitamin E, Zinc, etc.

57. 1. Did you take any vitamin/ mineral supplement within the last 12 months? *

Mark only one oval.

- ⬭ Yes *Skip to question 58*
- ⬭ No *Skip to question 61*

Skip to question 58

Untitled Section

58. 1. What type of vitamin/ mineral supplements do you take? List all. *

____

59. 2. What was you reason for taking vitamin/ mineral supplement? *

____

60. 3. How often did you take vitamin/ mineral supplement (**1** as **first type** that you have listed in Q1, **2** as the **second** type that you have listed in Q1, etc.). Please range it and give only one response in the 1 row if only one type of supplement is listed in Q1.

Mark only one oval per row.

	Occasionally	1-3 times per month	Once a week	More than once a week	Everyday
1	⬭	⬭	⬭	⬭	⬭
2	⬭	⬭	⬭	⬭	⬭
3	⬭	⬭	⬭	⬭	⬭
4	⬭	⬭	⬭	⬭	⬭
5	⬭	⬭	⬭	⬭	⬭

Skip to question 61

VIII) Section H: Food supplement intake

Section H: Food Supplement Intake

Example: Fish oil, Evening primrose oil, bird's nest, Essence of chicken, Haruan fish stock, Royal jelly, Collagen, Spirulina, Gingko biloba, Mangosteen extract, Sea cucumber products, Slimming products, Prune essence, Berry essence, Health powder (exclude slimming product), etc.

61. In the last 12 months, have you ever taken any food supplement? *

Mark only one oval.

- Yes *Skip to question 62*
- No *Skip to question 65*

Skip to question 62

Untitled Section

62. 1. What type of food supplement do you take? List all. *

____

63. 2. What was your reason for taking food supplement? *

____

64. 3. How often did you take food supplement (**1** as **first type** of supplement(s) that you have listed in Q1, **2** as the **second** type that you have listed in Q1, etc.). Please range it and give only one response in the 1 row if only one type of supplement is listed in Q1.

Mark only one oval per row.

	Occasionally	1-3 times per month	Once a week	More than once a week	Everyday
1	◯	◯	◯	◯	◯
2	◯	◯	◯	◯	◯
3	◯	◯	◯	◯	◯
4	◯	◯	◯	◯	◯
5	◯	◯	◯	◯	◯

Skip to question 65

IX) Section I: Physical activity

Section I: Physical Activity

I-1: Recreation, Sport, and Leisure-time Physical Activity

65. 1. Not counting any walking you have already mentioned, during the **last 7 days**, did you **walk for at least 10 minutes** at a time in your leisure time? *

Mark only one oval per row.

	No walking in leisure time	1-2	3-4	5-6	7
[] days per week	⬭	⬭	⬭	⬭	⬭

66. 2. How many **hours per day** did you usually spend on one of those days **walking** in your leisure time? State "0" as no walking in leisure time. *

67. 3. How many **minutes per day** did you usually spend on one of those days **walking** in your leisure time? State "0" as no walking in leisure time. *

IX) Section I: Physical activity (continued)

68. 4. During the **last 7 days**, did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming * in your leisure time?

Mark only one oval per row.

	No vigorous activity in leisure time	1-2	3-4	5-6	7
[ ] days per week	⬭	⬭	⬭	⬭	⬭

69. 5. How many **hours per day** did you usually spend on one of those days doing **vigorous** physical activities in your leisure time? State "0" as no vigorous physical activities in leisure time. *

70. 6. How many **minutes per day** did you usually spend on one of those days doing **vigorous** physical activities in your leisure time? State "0" as no vigorous physical activities in leisure time. *

71. 7. During the **last 7 days**, did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular * pace, and doubles tennis in your leisure time?

Mark only one oval per row.

	No	1-2	3-4	5-6	7
[ ] days per week	⬭	⬭	⬭	⬭	⬭

72. 8. How many **hours per day** did you usually spend on one of those days doing **moderate vigorous** physical activities in * your leisure time? State "0" as no moderate vigorous physical activities in leisure time.

73. 9. How many **minutes per day** did you usually spend on one of those days doing **moderate vigorous** physical activities in your leisure time? State "0" as no moderate vigorous physical activities in leisure time. *

Skip to question 74

Section I: Physical Activity I-2: Time Spent Sitting

I-2: Time Spent Sitting

IX) Section I: Physical activity (continued)

74. 1. During the **last 7 days**, how many **hours per day** did you usually spend **sitting** while at work, at home, while doing course work and during leisure time. on a **weekday/weekend days?** (exclude sitting in a motor vehicle) *

Mark only one oval per row.

	1-3	4-6	7-9	9-11	12 or more
Weekdays (Monday-Friday)	⬭	⬭	⬭	⬭	⬭
Weekend days (Saturday, Sunday)	⬭	⬭	⬭	⬭	⬭

75. 5. During the **last 7 days**, how many **minutes per day** did you usually spend **sitting** while at work, at home, while doing course work and during leisure time. on a **weekday/weekend day?** (exclude sitting in a motor vehicle) *

Mark only one oval per row.

	1-30	31-60	61-90	91-120	121-150	151-180	More than 180
Weekdays (Monday-Friday)	⬭	⬭	⬭	⬭	⬭	⬭	⬭
Weekend day (Saturday, Sunday)	⬭	⬭	⬭	⬭	⬭	⬭	⬭

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APPENDIX D

Guidelines on the classification of BMI, blood pressure and lipid profile

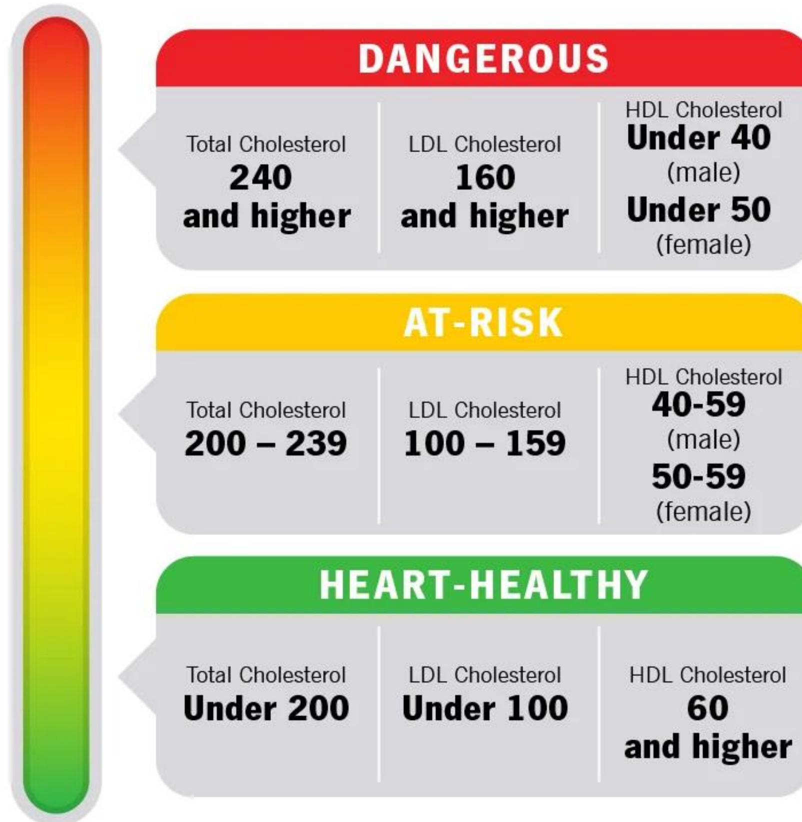
I) Classification of BMI (National Library of Medicine, n.d.)

Classification	BMI (kg/m^2)	Risk of comorbidities
Underweight	<18.5	Low (but risk of other clinical problems increased)
Normal range	18.5–24.9	Average
Overweight (preobese)	25.0–29.9	Mildly increased
Obese	≥30.0	
Class 1	30.0–34.9	Moderate
Class 11	35.0–39.9	Severe
Class 111	≥40.0	Very severe

II) Classification of clinic blood pressure levels in adults (Malaysian Society of Hypertension, 2018)

Classification*	Systolic (mmHg)		Diastolic (mmHg)	Prevalence in Malaysia[3]
Optimal	<120	and	<80	30.7
Normal	120-129	and/or	80-84	25.3
At Risk	130-139	and/or	85-89	18.6
Hypertension				
Stage 1 (Mild)	140-159	and/or	90-99	17.3
Stage 2 (Moderate)	160-179	and/or	100-109	5.7
Stage 3 (Severe)	≥180	and/or	≥110	2.4
Isolated Systolic Hypertension	≥140	and	<90	11.2

III) Classification of lipid profile (Cleveland Clinic, 2022)



APPENDIX E

Composition of instant black coffee powder

MATERIAL COMPOSITION DECLARATION

PRODUCT NAME	Instant Coffee Spray Dried Powder SS008-P		
PACKAGING	25 kg Carton Box		
NO.	**INGREDIENTS BREAKDOWN**	**PERCENTAGES (%)**	**ALLERGEN ? (YES / NO)**
1	Indian Robusta Bean	90%	No
2	Indian Arabica Bean	10%	No
3	-	-	-
4	-	-	-
	TOTAL	100%	NO

*** The Indian Coffee is from 100% coffee beans, there are no other materials being used during the extraction process.*

APPENDIX F

Chemicals that involved in the genomic DNA extraction, ARMS-PCR, gel electrophoresis of the genomic DNA as well as the PCR product

Chemicals	Manufacturer, Country
Saline solution (0.9%NaCl)	RinseCap, Malaysia
DNA extraction kit	Gene Direx, USA
BR solution	
BC solution	
BP solution	
Ethanol	Chemsol, Malaysia
Acetic acid	
Tris	1 st BASE, Singapore
Ethylenediaminetetraacetic acid (EDTA)	R&M Chemistry, Malaysia
Agarose powder	Vivantis, Malaysia
Novel juice	Gene Direx, USA
Hind III ladder/ Lambda DNA	Vivantis, Malaysia
50 bp DNA Ladder	Gene Direx, USA
	PKT, Korea
2x Power Taq PCR master mix	Takara Bio, Japan
	Simply Biologics, USA

APPENDIX G

Materials, instruments, and equipment used in DNA extraction and genotyping

Consumables/ Instruments	Manufacturer, Country
Conical flask (50 mL)	Favorit, Malaysia
Measuring cylinder (50 mL, 100 mL)	Favorit, Malaysia
Schott bottle (100 mL, 500 mL, 1000 mL)	Duran, Germany
Falcon tube	Nestle, China
1.5 mL microcentrifuge tube	Greiner Bio-one, Germany
PCR tube	Axygen Scientific, USA
Weighing machine	Hopkins, Singapore
Stadiometer	Seca, Germany
Micropipettes	Eppendorf, Germany Gilson, Germany
Pipette tips	Gilson, Germany
Water bath	Memmert, Germany
Analytical balance	A&D Corporation
Electrophoresis sets	Major Science, Taiwan
Microwave	Panasonic, Japan
-20° freezer	Panasonic, Japan
NanoDrop 2000 Spectrophotometer	Thermo Scientific, USA
Vortex	Gemmy Industrial Corporation, Taiwan
Centrifuge machine (15 mL)	Dynamica, India
Centrifuge machine (1.5 mL)	Thermo Scientific, USA
Nanophotometer	Thermo Scientific, USA
PCR machine	Biorad, USA

APPENDIX H

The concentration and purity of the DNA samples

Sample ID	Concentration of DNA (ng/µL)	Purity (260/280)
A2a (A2b)	10.7	1.52
A3a (A3b)	84.2	1.68
A4a (A4b)	48.9	1.59
A7	83.8	1.68
A8	115.9	1.86
A10	167.7	1.85
A11	28.3	1.71
A12a (A12b)	33.2	1.74
A13	68.6	1.74
A14	119.7	1.65
A15	72.1	1.77
A16a (A16b)	292.6	1.68
A18	194.6	1.64
A20a (A20b)	389.4	1.71
A21a (A21b)	123.0	1.61
A22	21.8	1.57
A23a (A23b)	252.5	1.67
A26 (A26 2R)	208.3	1.79
A27 (A27 2R)	7.8	1.58
A28	93.6	1.82
A32	751.5	1.54
A33	54.6	1.66
A35	8.9	1.17
A36	4061.9	1.51
A39a (A39b)	119.8	1.68
A40	104.4	1.61
A41	148.9	1.61
A42	628.0	1.76
A43a (A43b)	533.2	1.56
A44	1015.3	1.55
A45	174.2	1.58
A46	48.5	1.74
A47	36.4	1.62
A48 2R	364.6	1.56
A49/C74	46.9	2.06
A50/C3	11.0	1.38
YW	273.7	1.58
XJ	659.7	1.69
Evon	206.7	1.60
XY	143.5	1.64
SE	10.5	1.64
YY	62.8	1.62
VC	592.4	1.63
JK	387.2	1.75

E1	2304.6	1.59
E2	197.5	1.66
E3	119.0	1.58
B1	76.7	1.74
B2	10.2	1.64
B3b (B3a)	92.4	1.76
B5b (B5a)	157.2	1.66
B6a (B6b)	250.6	1.78
B8a (B8b)	361.6	1.71
B9	94.6	1.82
B11a (B11b)	211.4	1.65
B12	391.8	1.64
B13	97.6	1.70
B14	196.2	1.74
B15	64.3	1.75
B24a (B24)	173.3	1.66
B27	931.1	1.61
B28	148.3	1.58
B29a (B29b)	360.8	1.70
B31 2R (B31)	108.2	1.53
B32 (B32 2R)	38.0	1.61
B33	127.0	1.70
B35 2R (B35)	59.9	1.62
B36b (B36a)	793.4	1.72
B38	135.4	1.58
B39	173.5	1.67
B40	94.9	1.65
B41	156.6	1.63
B42	199.1	1.56
B44b (B44a)	322.1	1.68
B46	67.1	1.66
B47	177.0	1.67
B49	553.4	1.52
C70/B45	11.0	1.24
WC	21.7	1.74
TL	19.7	1.68
Jovy	9.8	1.69
CX	41.6	1.63
CY	54.6	1.66
D1	1766.0	1.68
D2	65.7	1.62
D4	216.1	1.63
D5(yoni)	166.9	1.63
D6	205.9	1.67
D7	257.9	1.70
D8	224.6	1.60
D9	63.1	1.62
D10	356.9	1.63
D11	914.3	1.68
D12	160.2	1.58

D13	337.1	1.65
D15	746.9	1.76
D16	25.7	1.75
D17	993.2	1.69
D18	198.8	1.65
D19	311.8	1.66
D20	456.8	1.65
D21	148.4	1.67
D22	296.3	1.66
D23	326.0	1.66
D24	375.0	1.67
D25	227.8	1.64

APPENDIX I

Normality test result

A) Variables: SBP, DBP, pulse, TC, TG, HDL-C, LDL-C, TC/HDL-C

I) All participant in Part II (n=106)

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
RSBP	.121	106	<.001	.804	106	<.001
RDBP	.064	106	.200*	.991	106	.741
pulse	.068	106	.200*	.983	106	.181
TC	.063	106	.200*	.965	106	.007
TG	.171	106	<.001	.841	106	<.001
HDL	.090	106	.033	.884	106	<.001
LDL	.082	106	.074	.966	106	.008
CHOL/HDL	.121	106	<.001	.925	106	<.001

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

II) Habitual coffee drinkers (n=59)

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
RSBP	.083	59	.200*	.979	59	.386
RDBP	.093	59	.200*	.980	59	.444
RBP	.091	59	.200*	.953	59	.022
TC	.102	59	.199	.955	59	.030
TG	.228	59	<.001	.820	59	<.001
HDL	.103	59	.189	.963	59	.069
LDL	.084	59	.200*	.970	59	.161
CHOL/HDL	.093	59	.200*	.950	59	.017

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

III) Non-habitual coffee drinkers (n=47)

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
RSBP	.196	47	<.001	.705	47	<.001
RDBP	.105	47	.200*	.972	47	.327
pulse	.070	47	.200*	.985	47	.806
TC	.050	47	.200*	.983	47	.737
TG	.176	47	<.001	.908	47	.001
HDL	.149	47	.010	.753	47	<.001
LDL	.083	47	.200*	.969	47	.240
CHOL/HDL	.168	47	.002	.888	47	<.001

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

IV) CC genotype (n=52)

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
RSBP	.172	52	<.001	.701	52	<.001
RDBP	.106	52	.200*	.983	52	.676
pulse	.075	52	.200*	.988	52	.878
TC	.096	52	.200*	.961	52	.087
TG	.196	52	<.001	.893	52	<.001
HDL	.140	52	.013	.825	52	<.001
LDL	.067	52	.200*	.977	52	.419
CHOL/HDL	.102	52	.200*	.957	52	.056

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

V) T allele carriers (CT+TT genotype) (n=54)

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
RSBP	.111	54	.094	.972	54	.233
RDBP	.100	54	.200*	.977	54	.393
pulse	.099	54	.200*	.949	54	.022
TC	.067	54	.200*	.961	54	.073
TG	.176	54	<.001	.778	54	<.001
HDL	.074	54	.200*	.984	54	.695
LDL	.118	54	.059	.941	54	.010
CHOL/HDL	.163	54	<.001	.886	54	<.001

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

VI) CC genotype among habitual coffee drinkers (n=27)

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
RSBP	.134	27	.200*	.927	27	.059
RDBP	.088	27	.200*	.973	27	.680
RBP	.109	27	.200*	.986	27	.971
TC	.147	27	.138	.942	27	.135
TG	.236	27	<.001	.872	27	.003
HDL	.143	27	.167	.949	27	.207
LDL	.071	27	.200*	.984	27	.942
CHOL/HDL	.121	27	.200*	.949	27	.202

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

VII) T allele carriers among habitual coffee drinkers (n=32)

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
RSBP	.103	32	.200*	.962	32	.314
RDBP	.121	32	.200*	.977	32	.718
RBP	.140	32	.113	.887	32	.003
TC	.088	32	.200*	.952	32	.164
TG	.234	32	<.001	.771	32	<.001
HDL	.090	32	.200*	.970	32	.512
LDL	.138	32	.128	.949	32	.134
CHOL/HDL	.116	32	.200*	.929	32	.036

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

B) Variables: changes of SBP, DBP, pulse, TC, TG, HDL-C, LDL-C, TC/HDL-C

II) All habitual coffee drinkers (n=59)

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
SBP	d R to 0	.078	59	.200*	.984	59	.610
	d R to 30	.063	59	.200*	.984	59	.623
	d R to 60	.075	59	.200*	.982	59	.524
	d 0 to 30	.070	59	.200*	.979	59	.406
	d 0 to 60	.069	59	.200*	.988	59	.838
	d 30 to 60	.095	59	.200*	.976	59	.287
DBP	d R to 0	.073	59	.200*	.963	59	.072
	d R to 30	.070	59	.200*	.974	59	.242
	d R to 60	.121	59	.031	.901	59	<.001
	d 0 to 30	.111	59	.065	.965	59	.087
	d 0 to 60	.101	59	.200*	.971	59	.173
	d 30 to 60	.109	59	.080	.973	59	.207
Pulse	d R to 0	.139	59	.006	.960	59	.053
	d R to 30	.074	59	.200*	.980	59	.436
	d R to 60	.066	59	.200*	.984	59	.618
	d 0 to 30	.060	59	.200*	.990	59	.903
	d 0 to 60	.073	59	.200*	.988	59	.826
	d 30 to 60	.097	59	.200*	.953	59	.024
DTC		.081	59	.200*	.985	59	.686
DTG		.163	59	<.001	.722	59	<.001
DHDL		.084	59	.200*	.984	59	.626
DLDL		.071	59	.200*	.985	59	.703
DCHOL/HDL		.113	59	.057	.955	59	.030

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

VI) CC genotype among habitual coffee drinkers (n=27)

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
SBP	d R to 0	.075	27	.200*	.975	27	.725
	d R to 30	.082	27	.200*	.977	27	.780
	d R to 60	.157	27	.087	.960	27	.370
	d 0 to 30	.113	27	.200*	.922	27	.044
	d 0 to 60	.095	27	.200*	.973	27	.693
	d 30 to 60	.156	27	.092	.907	27	.020
DBP	d R to 0	.127	27	.200*	.961	27	.396
	d R to 30	.144	27	.162	.939	27	.112
	d R to 60	.142	27	.174	.965	27	.475
	d 0 to 30	.126	27	.200*	.956	27	.298
	d 0 to 60	.119	27	.200*	.944	27	.150
	d 30 to 60	.115	27	.200*	.965	27	.480
Pulse	d R to 0	.132	27	.200*	.959	27	.359
	d R to 30	.085	27	.200*	.964	27	.458
	d R to 60	.094	27	.200*	.969	27	.563
	d 0 to 30	.115	27	.200*	.971	27	.624
	d 0 to 60	.128	27	.200*	.972	27	.644
	d 30 to 60	.131	27	.200*	.935	27	.090
DTC		.147	27	.139	.969	27	.572
DTG		.113	27	.200*	.963	27	.432
DHDL		.131	27	.200*	.974	27	.720
DLDL		.106	27	.200*	.976	27	.759
DCHOL/HDL		.113	27	.200*	.931	27	.072

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

VII) T allele carriers among habitual coffee drinkers (n=32)

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
SBP	d R to 0	.133	32	.161	.967	32	.429
	d R to 30	.092	32	.200*	.974	32	.621
	d R to 60	.102	32	.200*	.986	32	.936
	d 0 to 30	.097	32	.200*	.967	32	.433
	d 0 to 60	.096	32	.200*	.989	32	.978
	d 30 to 60	.077	32	.200*	.993	32	.998
DBP	d R to 0	.107	32	.200*	.936	32	.058
	d R to 30	.125	32	.200*	.925	32	.029
	d R to 60	.176	32	.013	.823	32	<.001
	d 0 to 30	.145	32	.083	.951	32	.150
	d 0 to 60	.136	32	.141	.964	32	.357
	d 30 to 60	.119	32	.200*	.975	32	.651
Pulse	d R to 0	.173	32	.016	.924	32	.027
	d R to 30	.130	32	.181	.911	32	.012
	d R to 60	.080	32	.200*	.980	32	.806
	d 0 to 30	.084	32	.200*	.957	32	.224
	d 0 to 60	.069	32	.200*	.985	32	.920
	d 30 to 60	.092	32	.200*	.949	32	.134
DTC		.139	32	.118	.958	32	.240
DTG		.229	32	<.001	.709	32	<.001
DHDL		.122	32	.200*	.934	32	.051
DLDL		.074	32	.200*	.981	32	.840
DCHOL/HDL		.123	32	.200*	.957	32	.231

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

APPENDIX J

Knowledge, attitude, perceptions, and practice towards coffee consumptions among students at UTAR Kampar Campus

Questions	Frequency (n, %)
Drinking coffee increases my risk of getting cancer	
Agree	25 (8.4)
Disagree	99 (33.2)
Neutral	**134 (45.0)**
Strongly agree	4 (1.3)
Strongly disagree	36 (12.1)
Drinking coffee increases my risk of heart disease	
Agree	79 (26.5)
Disagree	77 (25.8)
Neutral	**105 (35.2)**
Strongly agree	10 (3.4)
Strongly disagree	27 (9.1)
I cannot fall asleep when I drink coffee during the day	
Agree	59 (19.8)
Disagree	**75 (25.2)**
Neutral	66 (22.1)
Strongly agree	39 (13.1)
Strongly disagree	59 (19.8)
I prefer drinking tea to drinking decaffeinated coffee	
Agree	66 (22.1)
Disagree	48 (16.1)
Neutral	**97 (32.6)**
Strongly agree	51 (17.1)
Strongly disagree	36 (12.1)
Drinking coffee gives me an energy boost	
Agree	**102 (34.2)**
Disagree	45 (15.1)
Neutral	87 (29.2)
Strongly agree	38 (12.8)
Strongly disagree	26 (8.7)
I drink coffee because my friends do	
Agree	16 (5.4)
Disagree	95 (31.9)
Neutral	52 (17.4)
Strongly agree	6 (2.0)
Strongly disagree	**129 (43.3)**
I drink coffee for the taste	
Agree	**106 (35.6)**
Disagree	35 (11.7)
Neutral	75 (25.2)
Strongly agree	52 (17.4)
Strongly disagree	30 (10.1)

(Continued)

I drink coffee because it is fashionable	
Agree	18 (6.0)
Disagree	86 (28.9)
Neutral	63 (21.1)
Strongly agree	3 (1.0)
Strongly disagree	**128 (43.0)**
Drinking coffee relaxes me	
Agree	74 (24.8)
Disagree	56 (18.8)
Neutral	**97 (32.6)**
Strongly agree	26 (8.7)
Strongly disagree	45 (15.1)
I prefer drinking tea to drinking any coffee	
Agree	50 (16.8)
Disagree	50 (16.8)
Neutral	**109 (36.6)**
Strongly agree	50 (16.8)
Strongly disagree	39 (13.1)
I cannot fall asleep when drinking coffee in the evening	
Agree	
Disagree	59 (19.8)
Neutral	**70 (23.5)**
Strongly agree	65 (21.8)
Strongly disagree	50 (16.8)
	54 (18.1)
I drink coffee to help me stay awake	
Agree	**95 (31.9)**
Disagree	40 (13.4)
Neutral	65 (21.8)
Strongly agree	59 (19.8)
Strongly disagree	39 (13.1)
I prefer local brands of coffee to imported ones	
Agree	39 (13.1)
Disagree	49 (16.4)
Neutral	**154 (51.7)**
Strongly agree	7 (2.3)
Strongly disagree	49 (16.4)
Drinking coffee makes me feel important	
Agree	32 (10.7)
Disagree	79 (26.5)
Neutral	**90 (30.2)**
Strongly agree	10 (3.4)
Strongly disagree	87 (29.2)

Coffee consumption attitudes	Frequency (n, %)
Important reason to consider when purchasing/ordering coffee	
Low price	
Important	**104 (45.4)**
Totally unimportant	11 (4.8)
Unimportant	63 (27.5)

(Continued)

Very important	51 (22.3)
Smooth taste	
Important	**115 (50.2)**
Totally unimportant	12 (5.2)
Unimportant	18 (7.9)
Very important	84 (36.7)
Specific brand	
Important	69 (30.1)
Totally unimportant	28 (12.2)
Unimportant	**116 (50.7)**
Very important	16 (7.0)
Strength of the coffee	
Important	**103 (45.0)**
Totally unimportant	26 (11.4)
Unimportant	63 (27.5)
Very important	37 (16.2)
Whether it is local coffee	
Important	38 (16.6)
Totally unimportant	65 (28.4)
Unimportant	**122 (53.3)**
Very important	4 (1.8)
Whether it is imported brand	
Important	26 (11.4)
Totally unimportant	69 (30.1)
Unimportant	**127 (55.5)**
Very important	7 (3.1)
Do you consider yourself to be a coffee addict?	
Yes	67 (29.2)
No	**162 (70.7)**

Coffee consumption practices	Frequency (n, %)
How often do you drink coffee after waking up in the morning?	
Always	22 (9.6)
Never	**102 (44.5)**
Often	36 (15.7)
Rarely	69 (30.1)
How often do you drink coffee with breakfast?	
Always	45 (19.7)
Never	46 (20.1)
Often	**72 (31.4)**
Rarely	66 (28.8)
How often do you drink coffee during your morning break?	
Always	16 (7.9)
Never	80 (34.9)
Often	48 (21.0)
Rarely	**85 (37.1)**

(Continued)

How often do you drink coffee with lunch?	
Always	6 (2.6)
Never	83 (36.2)
Often	49 (21.4)
Rarely	**91 (39.7)**
How often do you drink coffee during your afternoon break?	
Always	8 (3.5)
Never	78 (34.1)
Often	52 (22.7)
Rarely	**91 (39.7)**
How often do you drink coffee with dinner?	
Never	**148 (64.6)**
Often	16 (7.0)
Rarely	65 (28.4)
How often do you drink coffee in the evening after dinner?	
Always	1 (0.4)
Never	**137 (59.8)**
Often	23 (10.0)
Rarely	68 (29.7)
How often do you drink coffee at bedtime?	
Always	1 (0.4)
Never	**171 (74.7)**
Often	9 (3.9)
Rarely	48 (21.0)
How often do you drink coffee during examination week?	
Always	60 (26.2)
Never	43 (18.8)
Often	**67 (29.3)**
Rarely	59 (25.8)
How often do you drink coffee when visiting friends or family?	
Always	11 (4.8)
Never	**95 (41.5)**
Often	41 (17.9)
Rarely	82 (35.8)
How often do you drink coffee when you are stressed?	
Always	17 (7.4)
Never	**105 (45.9)**
Often	44 (19.2)
Rarely	63 (27.5)
Types of mostly consumed coffee	
Instant coffee	**123 (53.7)**
Ground/ filtered coffee	53 (23.1)
Instant decaffeinated	4 (1.7)
Ground decaffeinated	4 (1.7)

(Continued)

Cappuccino	35 (15.3)
Espresso	29 (12.7)
Latte	54 (23.6)
Other	30 (13.1)
Types of preferred coffee	
Instant coffee	**52 (22.7)**
Ground/ filtered coffee	45 (19.7)
Instant decaffeinated	2 (0.9)
Ground decaffeinated	1 (0.4)
Cappuccino	31 (13.5)
Espresso	22 (9.6)
Latte	49 (21.4)
Other	32 (14.0)
Reason for most used coffee types	
It is the type I prefer	57 (24.9)
It tastes best	**70 (30.6)**
It is the most affordable	16 (7.0)
It is the most readily available	30 (13.1)
The place where I work/ study provides the most used type	11 (4.8)
Other members in my household prefer most used brand	6 (2.6)
Most convenient	38 (16.6)
Other	1 (0.4)
Reasons for the discrepancy of preferred and most used coffee types	
I cannot afford my preferred type	40 (17.5)
The place where I work/ study does not use the preferred type	50 (21.8)
Other members in my household prefer another type of coffee	20 (8.7)
Not applicable. The type of coffee I use most often is same as the type I prefer most	**115 (50.2)**
Other	4 (1.7)

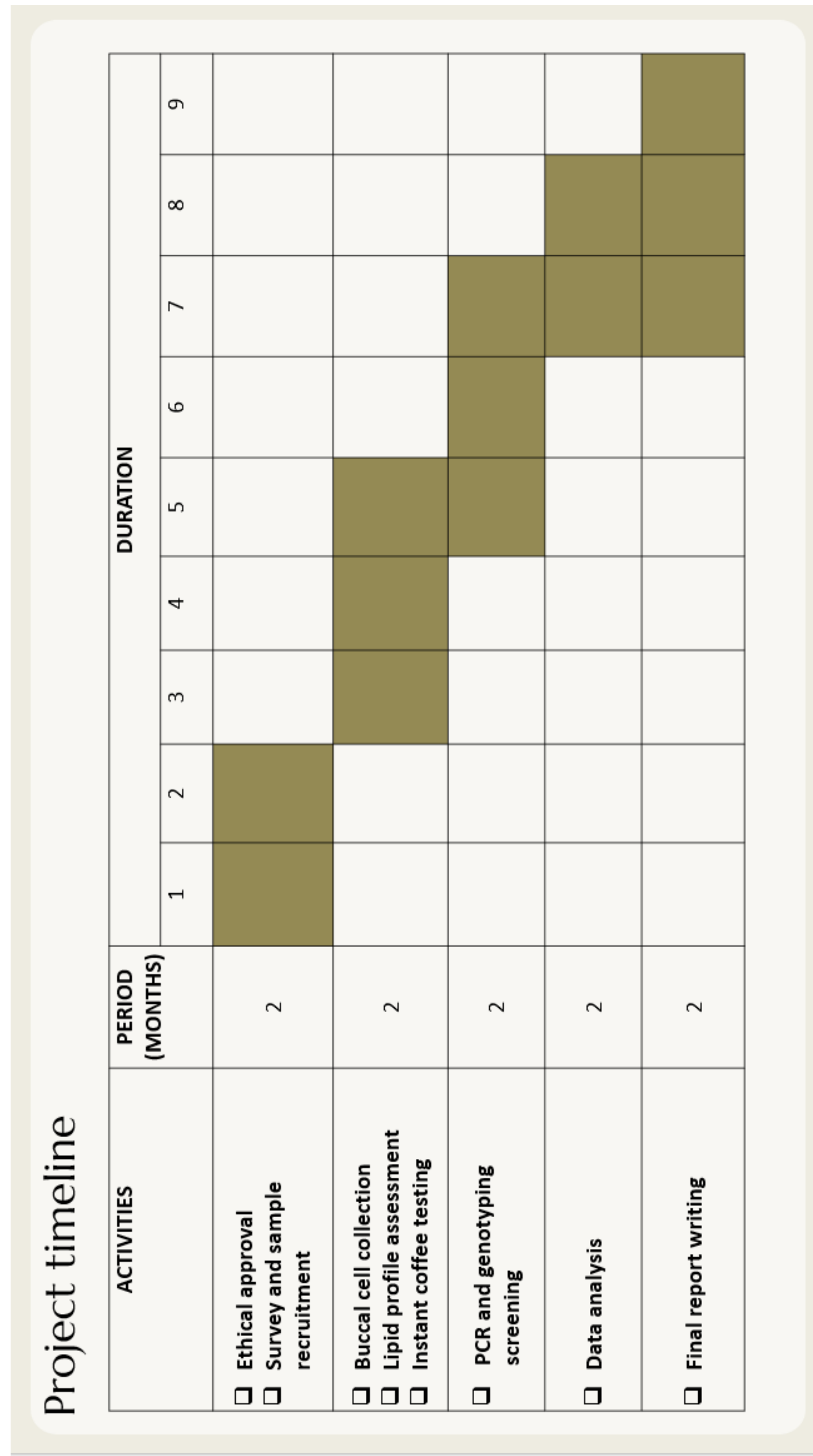
APPENDIX K

The frequency distributions according to participant's BMI, blood pressure and non-fasting lipid profile

Variable	Frequency (n, %)		
	Habitual coffee drinker (n=59)	**Non-habitual coffee drinker (n=47)**	**Overall (part II) (n=106)**
Body mass index (BMI)			
Underweight	13 (22.0)	15 (31.9)	28 (26.4)
Normal	42 (71.2)	29 (61.7)	71 (67.0)
Overweight	4 (6.8)	3 (6.4)	7 (6.6)
Resting blood pressure			
Systolic blood pressure			
Normal	55 (93.2)	44 (93.6)	99 (93.4)
Prehypertension	4 (6.8)	3 (6.4)	7 (6.6)
Diastolic blood pressure			
Normal	59 (100.0)	46 (97.9)	105 (99.1)
Prehypertension		1 (2.21)	1 (0.9)
Pulse			
Normal	56 (94.9)	44 (93.6)	100 (94.3)
Abnormal	3 (5.1)	3 (6.4)	6 (5.7)
Non-fasting lipid profile			
TC			
Desirable	48 (81.4)	41 (87.2)	89 (84.0)
Borderline high	7 (11.9)	5 (10.6)	12 (11.3)
High	4 (6.8)	1 (2.1)	5 (4.7)
TG			
Desirable	49 (83.1)	44 (93.6)	93 (87.7)
Borderline high	8 (13.6)	3 (6.4)	11 (10.4)
High	2 (3.4)		2 (1.9)
HDL-C (male)			
Desirable	5 (18.5)		5 (13.5)
Borderline high	10 (37.0)	8 (80.0)	18 (48.6)
High	12 (44.40)	2 (20.0)	14 (37.8)
HDL-C (female)			
Desirable	15 (46.9)	22 (59.5)	37 (53.6)
Borderline high	9 (28.1)	11 (29.7)	20 (29.0)
High	8 (25.0)	4 (10.8)	12 (17.4)
LDL-C			
Desirable	50 (84.7)	45 (95.7)	95 (89.6)
Borderline high	6 (10.2)	1 (2.1)	7 (6.6)
High	3 (5.1)	1 (2.1)	4 (3.8)
CHOL/HDL			
Desirable	33 (55.9)	38 (80.9)	71 (67.0)
Borderline high	21 (35.6)	8 (17.0)	29 (27.4)
High	5 (8.5)	1 (2.1)	6 (5.7)
Total	**59 (55.66%)**	**47 (44.33%)**	**106 (100%)**

APPENDIX L

Gann chart



APPENDIX M

Supervisor's comment

Universiti Tunku Abdul Rahman			
Form Title : Supervisor's Comments on Originality Report Generated by Turnitin for Submission of Final Year Project Report (for Undergraduate Programmes)			
Form Number: FM-IAD-005	Rev No.: 1	Effective Date: 3/10/2019	Page No.: 1of 1

FACULTY OF SCIENCE

Full Name(s) of Candidate(s)	ONG XUAN
ID Number(s)	20ADB05665
Programme / Course	Bachelor of Science (Honours) Dietetics
Title of Final Year Project	Heterozygous *MTHFR* C677T polymorphism (rs1801133) is associated with higher total cholesterol/high-density lipoprotein cholesterol ratio in habitual coffee drinkers

Similarity	**Supervisor's Comments (Compulsory if parameters of originality exceeds the limits approved by UTAR)**
Overall similarity index: 11 % **Similarity by source** Internet Sources: 9 % Publications: 6 % Student Papers: 2 %	
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Note Supervisor/Candidate(s) is/are required to provide softcopy of full set of the originality report to Faculty/Institute

Based on the above results, I hereby declare that I am satisfied with the originality of the Final Year Project Report submitted by my student(s) as named above.

Signature of Supervisor	Signature of Co-Supervisor
Name: Dr. Phoon Lee Quen	Name:
Date: 12-09-2023	Date:

APPENDIX N

Turnitin report

9/13/23, 10:03 PM   Turnitin - Originality Report - ONG XUAN FYP THESIS

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