**The Impact of Genetic Variability on the Relationship between Caffeine and Cardiometabolic Outcomes: A Systematic Review**

**Running title: Genetics of Caffeine and Cardiometabolic Health**

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**Abstract**

The relationship between caffeine consumption and cardiometabolic health has been reported, albeit with heterogenous results. Discrepancies in study results may be due to inter-individual variability between study participants. This systematic review aimed to identify the impact of genetics on the relationship between caffeine consumption and cardiometabolic outcomes. Electronic databases (PubMed and EMBASE) were searched for studies published until July 2021. Selected studies were of both intervention and observational design and included: 1) analysis of at least one of the selected cardiometabolic outcomes (type 2 diabetes, glucose/insulin levels, cardiovascular disease, blood pressure or hypertension, blood lipids and catecholamines levels), 2) adults aged 18-65 and 3) genetic analysis of individuals consuming caffeine. Seventeen studies were included: four randomised controlled trials and an interventional and quasi-experimental study, six population-based prospective cohort studies, three cross-sectional studies and three case-control studies. *CYP1A2* rs762551 and .*ADORA* rs5751876 were associated with glucose response when caffeine was consumed with carbohydrates. *CYP1A2* rs762551 moderated the association between coffee intake and hypertension. Moreover, *ADORA2A* rs5751876 and the *ADRA2B* I variants moderated the associations between caffeine and blood pressure. Studies that investigated the effects of genetic variations on cardiovascular disease and caffeine consumption reported equivocal findings (*CYP1A2*) or warrant replication (*COMT*, *ADORA*, and *TRIB1*). Elucidating the extent to which these genes moderate the association between caffeine and cardiometabolic outcomes will enable caffeine consumption advice to be tailored to specific individuals to optimise health.

**Keywords:** blood pressure; caffeine; cardiovascular disease; genetics; type 2 diabetes mellitus

**Introduction**

Caffeine, also known as 1,3,7- trimethylxanthine, is an alkaloid naturally found in over 60 plants, such as coffee beans, tea leaves, cola nuts, cocoa pods, and guarana berries and is one of the most widely consumed stimulants (Heckman et al., 2010; Mejia and Ramirez-Mares, 2014). Widespread consumption of caffeine is primarily due to its presence in coffee, the leading beverage worldwide, with an estimated daily consumption of approximately 1.4 billion cups (Cappelletti et al., 2015). The popularity of caffeine around the world has led to a growing interest in its effects on human health, with a particular attention on cardiometabolic health (Buscemi et al., 2016; Ramli et al., 2021).

When consumed as a beverage, caffeine is promptly and entirely absorbed within approximately one hour by the gastrointestinal tract, and it spreads throughout body fluid whereby it can enter cell membranes, including the brain (Cappelletti et al., 2015). Primarily, caffeine blocks adenosine receptors and inhibits phosphodiesterases (Ribeiro & Sebastião, 2010), leading to a build-up of cyclic adenosine monophosphate and enhancing the effect of catecholamines (De Caterina & El-Sohemy, 2016). Once absorbed, caffeine is metabolised in the liver by the 1A2 isozyme of the cytochrome P450 enzyme, which is encoded by the cytochrome P450 1A2 (*CYP1A2*) gene (Rasmussen et al., 2002). *CYP1A2* is responsible for nearly 95% of the caffeine metabolism, whereby it metabolises caffeine into paraxanthine (~ 80%), theobromine (~ 12%), and theophylline (~ 4%) (Guessous et al., 2014).

The role of caffeine in cardiometabolic health has been extensively studied (Chrysant, 2017). The acute consumption of caffeine or coffee may provoke a rise in systolic blood pressure (SBP), diastolic blood pressure (DBP) and catecholamine release (Eumann Mesas et al., 2011). However, these physiological effects may differ with chronic caffeine consumption and some studies have shown that habitual coffee consumption impacts blood pressure (BP) (Renda et al., 2012), whilst others have found no difference (Hou et al., 2021). Similarly, a systematic review and meta-analysis exploring the association between habitual coffee consumption and type 2 diabetes incidence suggested a 6% decreased risk of type 2 diabetes for each cup-per-day increase in coffee consumption (Carlström & Larsson, 2018). Contrary to this, acute caffeine ingestion was shown to reduce insulin sensitivity in healthy populations, suggesting that, in the short-term, caffeine might shift glycaemic homeostasis toward hyperglycaemia (Shi et al., 2016).

Besides the potential differences between acute and habitual caffeine and/or coffee consumption, there is heterogeneity in reported results (Renda et al., 2012; Carlström & Larsson, 2018; Hou et al.,2021). In part, this may be due to inter-individual differences, more specifically single-nucleotide polymorphisms (SNPs) affecting caffeine metabolism (Yang et al., 2010). *CYP1A2* rs762551, due to an A > C substitution at position -163, results in an impaired caffeine metabolism and has been consistently studied to categorise individuals into fast (AA genotype) and slow (AC/CC genotype) caffeine metabolisers (Nehlig, 2018). Some studies suggest that slow metabolisers have higher risk of cardiovascular disease (CVD) and hypertension with high coffee intake compared to fast metabolisers (Cornelis et al., 2006; Palatini et al., 2009).

Furthermore, caffeine metabolites (theophylline and paraxanthine) are adenosine receptor antagonists known to impact the effects of caffeine via a competitive binding to the A2A receptor (*ADORA2A*) (Nehlig, 2018). Adenosine has been shown to interact with the sympathetic nervous system by inhibiting the release of catecholamines (Rétey et al., 2007). The *ADORA2A* locus harbours several SNPs, the most studied being rs5751876 (Erblang et al., 2019). Several studies reported associations between caffeine consumption, *ADORA2A*, and glucose response (Banks et al., 2019), BP (Renda et al., 2012), and dyslipidaemia risk (Han et al., 2020) whereby the association between caffeine and the outcome of interest depended on the *ADORA2A* genotypes. Besides *CYP1A2* and *ADORA2A*, other genes have been studied as moderators of the association between caffeine and cardiometabolic health outcomes. These include the catechol-O-methyltransferase (*COMT*) and the tribbles pseudokinase 1 (*TRIB1)*, which catalyses the inactivation of adrenaline and noradrenaline and contributes to elevated triglycerides levels, respectively, both of which play a role in the cardiovascular effects of caffeine (De Caterina & El-Sohemy, 2016; Zhang et al., 2021). Likewise, the adrenoceptor Alpha 2B (*ADRA2B*) gene contributes to vasoconstriction and BP regulation and, unlike homozygotes for the D allele, those who are homozygotes for the I allele of the Ins+910Del variant are more likely to experience higher BP in response to caffeine, possibly mediated by adrenaline (Ranade et al., 2001; Renda et al., 2012). Finally, Max-like protein X interacting protein-like (*MLXIPL*) encodes the carbohydrate response element binding protein and the C allele of the rs7800944 contributes to decreased cholesterol levels, increased fasting glucose and increased coffee drinking behaviour compared to the T allele (Cornelis et al., 2021).

Taken together, genetic variations in genes regulating caffeine and catecholamine metabolism as well as sensitivity to caffeine may moderate the associations between caffeine/coffee and cardiometabolic outcomes. However, no previous systematic review has comprehensively synthesised this evidence. To this end, this review aimed to explore the associations between genetics, caffeine or coffee intake and cardiometabolic outcomes in the existing literature.

**Materials and methods**

Search strategy

This systematic review was registered in PROSPERO (<https://www.crd.york.ac.uk/prospero/>, identifier CRD42021268342). The reporting of this systematic review was guided by the standards of the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) Statement (PRISMA, 2020). Literature published until July 2021 was systematically searched by three investigators (G.J., P.M., and J.V.) using the electronic databases PubMed and EMBASE, with restriction to English written publications. This was accomplished using MeSH terms and keywords (Supplementary Table 1). Moreover, the reference lists of reviewed articles were checked to identify additional eligible publications. G.J., P.M., and J.V. independently screened titles, abstracts, and full texts, with disagreement resolved through consensus.

Study selection criteria

The studies included in this review were restricted to human participants and met all of the following criteria: a) RCTs or non-RCTs; b) analysis of at least one of the selected cardiometabolic outcomes: type 2 diabetes, blood glucose levels (fasting, postprandial, HbA1C), insulin levels and secretion/sensitivity, cardiovascular disease, BP or hypertension, blood lipids (triglycerides, total cholesterol, very low density lipoprotein (VLDL), low density lipoprotein (LDL), high density lipoprotein (HDL)), catecholamines levels (please refer to supplementary table for search strategy); c) adults aged 18-65; genetic analysis of individuals who consumed caffeine or coffee and underwent measurements of any of the specified outcomes. Studies using Mendelian Randomisation were excluded because health outcomes were compared by genetically predicted caffeine consumption (Lawlor et al., 2008). Non-English published literature and studies on populations with diseases that were not relevant to the foci of this review were excluded. Animal studies, reviews, conference proceedings, and editorials were also excluded. The manuscript selection process is summarised in Figure 1; whilst the list of included studies is presented in the study summary table (Table 1).

Risk of bias and quality assessment

Three reviewers (G.J., P.M., and J.V.) independently assessed the risk of bias of non-RCTs via the 13-item question tool developed by the Research Triangle Institute (RTI), Evidence-based Practice Centre (Viswanathan et al., 2013). This tool was used in a similar systematic review (Fulton et al., 2018). The Risk of Bias in Non-randomised Studies - of Interventions (ROBINS-I) tool (Sterne et al., 2016) was used to help reach the final judgment on risk of bias in each domain as well as the final risk of bias score for each study. The Cochrane Collaboration’s tool was applied to assess risk of bias for RCTs (Higgins et al., 2011). Conflicts in the risk of bias assessment were resolved by three investigators (G.J., P.M., and J.V.) through a consensus-based discussion.

Data extraction and analysis

The results of the data extraction procedure are presented in Table 1. Data extraction was conducted independently by three investigators (G.J., P.M., and J.V.) and conflicts were resolved through consensus. For all included studies, the following information was extracted: first author’s name, year of publication, study design, participants’ characteristics (i.e., number, gender, age, and ethnicity), caffeine and/or coffee consumption measurement, SNP(s) and gene(s) observed, and the main outcome(s), including the assumed risk estimates.

A narrative synthesis of study results was conducted. The studies were categorised based on the outcomes of the studies (Type 2 diabetes and glucose, blood pressure/hypertension, and cardiovascular disease) and summarised as such in tables and described in the text. Outcomes (e.g. type 2 diabetes, hypertension, CVD or observed changes in blood glucose, blood lipids and blood pressure) were reported with metrics used by the study authors. We prioritised results that included all three key variables of interest (caffeine/coffee dose/intake, genotype and cardiometabolic outcome).

Additionally, the studies were summarised in the text according to study type (i.e. acute, long-term coffee/caffeine supplementation/ingestion and habitual coffee/caffeine intake). This was to explore heterogeneity in studies and decided on due to the discrepancies in results between studies exploring acute and habitual intakes.

**Results**

Searching process and results

The complete search generated 2,469 records. Of these, 613 were duplicates; therefore, 1,856 were initially screened and assessed for eligibility, with 14 studies meeting the inclusion criteria. An additional 3 records were added manually, thus leading to 17 studies included in this systematic review.

Characteristics of the included studies

The main characteristics and outcomes of the included studies are shown in Table 1. Of the 17 included studies, four were randomised controlled trials (RCT), three were case-control studies (CCS), three were cross-sectional studies (CSS), one was an interventional and quasi-experimental study, and the remaining six were population-based prospective cohort studies.

Risk of bias assessment

The results for risk of bias assessment are presented in Tables 2 and 3. For the RCTs, two studies were judged as overall low risk of bias (Renda et al., 2012; Yoshihara et al., 2019) and two as high risk of bias (Robertson et al., 208; Banks et al., 2019). For individual domains, only one study showed an unclear risk of bias for random sequence generation (Robertson et al., 2018), and three showed a low risk of bias. For allocation concealment, one study showed an unclear risk of bias (Banks et al., 2019), whilst three studies showed a low risk of bias. Only two RCTs demonstrated a high risk of bias for blinding of participants and researchers (Robertson et al., 2018; Banks et al., 2019), which was due to the nature of the administered nutritional interventions. Regarding the blinding of outcome assessment, one study showed an unclear risk of bias (Robertson et al., 2018) and three studies demonstrated a low risk of bias. Finally, all RCTs were categorised as having a low risk of bias for incomplete outcome data, selective reporting, and other bias.

Out of the 13 non-RCTs, two studies were judged as having overall low risk of bias (Cornelis et al., 2006; Kohno et al., 2013) and the remaining 11 as high risk of bias (Happonen et al., 2006; Palatini et al., 2009; Brathwaite et al., 2011; Palatini et al., 2015; Casiglia et al., 2017; Casiglia et al., 2018; Soares et al., 2018; Zhou et al., 2019; Han et al., 2020; Liu et al., 2020; Cornelis, 2021). Regarding individual domains, four studies showed a high risk of bias (Happonen et al., 2006; Zhou et al., 2019; Liu et al., 2020; Cornelis, 2021), and eight showed a low risk for selection bias. For performance bias, six studies showed a high risk of bias (Palatini et al., 2009; Palatini et al., 2015; Casiglia et al., 2017; Casiglia et al., 2018; Zhou et al., 2019; Cornelis, 2021), whilst seven showed a low risk. For measurement of bias, three showed a high risk (Brathwaite et al., 2011; Casiglia et al., 2018; Liu et al., 2020), two showed an unclear risk (Casiglia et al., 2017; Han et al., 2020), and the remaining eight studies showed a low risk. For missing data bias, three studies showed an unclear risk (Palatini et al., 2009; Brathwaite et al., 2011; Casiglia et al., 2017), three studies showed a high risk (Happonen et al., 2006; Han et al., 2020; Liu et al., 2020), and the remaining seven studies showed a low risk. For selection of the reported results bias, only two studies showed a high risk of bias (Casiglia et al., 2017; Casiglia et al., 2018), whilst the remaining studies showed a low risk of bias. For the confounding category, six of the 12 studies displayed a high risk of bias (Palatini et al., 2015; Casiglia et al., 2018; Soares et al., 2018; Han et al., 2020; Liu et al., 2020; Cornelis, 2021), and the remaining seven displayed a low risk of bias.

Reporting of the outcomes

Glucose control and type 2 diabetes mellitus

Five of the included studies have reported several concordant interactions between genetics, caffeine consumption and glucose control. Banks et al., (2019) showed that short-term glucose response was influenced by SNPs in the *CYP1A2* and *ADORA2A* genes, with such response differing based on whether caffeine was consumed with carbohydrate (0.75g/kg carbohydrate in the form of sucrose and dextrose mixed with 7.5ml/kg of water with 4mg/kg of pharmaceutical grade caffeine anhydrous powder) versus the same carbohydrate meal consumed on its own. Glucose levels were higher in the CC genotype (mean ± 95% CI difference = 15.71 ± 11.71 mg/dL), but not in the CT and TT genotype (mean ± 95% CI difference = 3.15 ± 10.46 mg/dL) of the *ADORA2A* rs5751876 SNP, when caffeine was consumed with carbohydrate. Glucose levels increased 30 min post-caffeine ingestion (mean ± 95% CI difference = 45.36 ± 8.26 mg/dL) and decreased back to baseline levels from 30 to 60 min (mean ± 95% CI difference = − 48.67 ± 10.61 mg/dL). Similarly, a higher glucose AUC was observed in the CC genotype in the carbohydrate + caffeine condition versus the carbohydrate condition (mean ± 95% CI difference = 1142.14 ± 664.42 mg/dL·min).; however, no differences in glucose AUC were observed for the CT and TT genotype (mean ± 95% CI difference = 248.18 ± 711.0). A similar outcome was shown for the slow caffeine metabolisers of the *CYP1A2* rs762551 during the carbohydrate + caffeine condition compared to the carbohydrate (mean ± 95% CI difference = 24.00 ± 17.33 mg/dL) condition at 60-min, whereas there was no difference in the fast caffeine metabolisers (mean ± 95% CI difference = −3.55 ± 21.06 mg/dL). Whilst in the short-term caffeine seems to increase the acute postprandial glucose response, especially in slow caffeine metabolisers, the longer-term effects of coffee (4 cups of coffee/day for 12 weeks) also appeared to be somewhat associated with a greater postprandial glucose response in the slow caffeine metabolisers compared to fast slow metabolisers of the *CYP1A2* rs762551 SNP (Robertson et al., 2018). Likewise, caffeine (from coffee and other caffeine sources) was associated with higher glucose levels in individuals who reported drinking caffeine within ~1h before blood draw compared to those who did not drink caffeine within the hour. When stratified by genotype, higher glucose levels were observed in female participants who were slow caffeine metabolisers of *CYP1A2* rs2472297 and with the CC genotype for *MLXIPL* rs7800944, but not males with the same genotype (Cornelis et al., 2021).

Similar to the acute caffeine feeding trial and the 12-week chronic coffee supplementation, Palatini et al., (2015) observed an increased incidence of impaired fasting glucose (IFG) in hypertensive patients, especially among those who were slow caffeine metabolisers of the *CYP1A2* rs762551 SNP after a follow up of 6.1 years. Among the participants stratified by *CYP1A2* genotype, heavy coffee drinkers (> 3 cups of coffee/day) who were slow caffeine metabolisers had a higher adjusted risk of IFG glucose (HR 2.8, 95 % CI 1.3–5.9) compared to abstainers, whereas this association was not statistically significant among fast caffeine metabolisers (HR 1.7, 95 % CI 0.8–3.8). A different cross-sectional study aimed to explore how interactions between *CYP1A2* and coffee intake, assessed as self-reported long-term coffee consumption, influenced glucose levels (Kohno et al., 2013). Unlike other reported associations with glucose response post-caffeine ingestion, *CYP1A2* SNPs were not strongly associated with IFG/IGT or type 2 diabetes. However, when participants were stratified into smokers and non-smokers, there was a significant association in smokers between coffee intake and type 2 diabetes in slow metabolisers for the *CYP1A2* rs762551 variant (p = 0.008) and fast metabolisers for the *CYP1A2* rs2969514 variant (p < 0.0001).

Blood pressure response and hypertension

Three studies assessing acute caffeine and coffee intake on BP outcomes stratified by *CYP1A2*, *ADORA2A* and *ADRA2B* genotypes reported several concordant changes in BP following caffeine and caffeinated coffee consumption. For example, Renda et al., (2012) found that acute caffeinated vs decaffeinated coffee consumption (40-ml dose of decaffeinated preparation with 3mg/kg of caffeine added vs decaffeinated preparation with no added caffeine) increased BP, and this association was influenced by genetics. BP was measured three times at baseline and then at 6-min intervals after coffee intake for the following 2h. It found a change in peak SBP of 4 mmHg following caffeinated coffee ingestion (147 ± 12 mmHg after caffeinated and 143 ± 12 mmHg after decaffeinated coffee ingestion, p < 0.001), which was modulated by the *ADORA2A* rs5751876 (TT genotype) and the *ADRA2B I* variant; however, in this study, no significant association between *CYP1A2* genotypes and BP following coffee consumption was found. However, a higher dose of acute caffeine (6mg/kg) consumption affected BP response from baseline to 1h post consumption in another intervention study (Soares et al., 2018). It was observed that the slow caffeine metabolisers of the *CYP1A2* rs762551 had increased basal-DBP when compared to fast caffeine metabolisers; however, DBP increased in both fast and slow caffeine metabolisers groups (67.4 ± 7.0 vs 71.5 ± 8.6mmHg and 74.7 ± 7.0 vs 78.6 ± 5.6mmHg; p < 0.05), respectively. Notably, the post-caffeine DBP of the slow caffeine metabolisers was higher than the post-caffeine DBP of the fast caffeine metabolisers. Importantly, this increase was modified by physical activity status depending on whether fast and slow caffeine metabolisers were sedentary or physically active individuals. As such, the SBP of sedentary fast caffeine metabolisers increased from 116.4 ± 10.9 to 121.7 ± 8.7 mmHg (p < 0.05) after the caffeine challenge. Similarly, acute caffeine consumption increased the DBP from 72.4 ± 6.1 mmHg to 75.7 ± 5.7 mmHg (p < 0.05) in the sedentary fast caffeine metabolisers, from 75.9 ± 10.3 mmHg to 80.0 ± 7.6mmHg (p < 0.05) in the sedentary slow caffeine metabolisers, and from 70.4 ± 4.2 mmHg to 74.5 ± 8.6 mmHg in the physically active slow caffeine metabolisers (Soares et al., 2018). Another intervention study (Yoshihara et al., 2019), investigating the contribution of habitual coffee consumption as well as an acute coffee ingestion on BP changes found that the *ADORA2A* rs5751876 SNP was not associated with BP increase, regardless of daily caffeine consumption (low-daily caffeine consumption: ≤ 90 mg per day, equivalent to one cup of coffee; high-daily caffeine consumption: > 90 mg per day, equivalent to more than one cup of coffee). There was, however, a significant contribution of caffeine to the elevation of SBP and DBP in slow caffeine metabolisers (only individuals who were homozygous for the C allele) of the *CYP1A2* rs762551 SNP among the low-daily caffeine consumption group. Stratified analysis with daily caffeine consumption showed that the SBP change in the slow metabolisers was 11.8 ± 5.9 compared to fast metabolisers and slow metabolisers with one C allele (4.1 ± 5.5) (p < 0.001), while the change in DBP was 9.5 ± 5.5 compared to fast metabolisers and slow metabolisers with one C allele (5.5 ± 3.8) (p < 0.001). In the same study, acute caffeine consumption (2g of Nescafe Gold containing ~ 1.8 mg), following a series of four calculation tests, increased SBP and DBP regardless of *ADORA2A* and *CYP1A2* SNPs.

Similar to the acute effects of caffeine, caffeine consumption over the long-term (e.g., during a median follow-up of 8.2 years in individuals screened for stage 1 hypertension) was associated with an increased probability of physician-diagnosed hypertension in slow caffeine metabolisers of the *CYP1A2* genotype, but not in fast caffeine metabolisers (Palatini et al., 2009). Hypertension hazard was observed in both moderate (1-3 cups/day) and heavy (≥ 4 cups/day) coffee drinkers who were slow caffeine metabolisers. Specifically, for slow caffeine metabolisers the hazard ratios of hypertension were 1.00 in abstainers (reference), 1.72 (95%CI, 1.21–2.44) in moderate coffee drinkers (p = 0.03), and 3.00 (1.53–5.90) in heavy drinkers (p = 0.001). In contrast, hazard ratios for fast caffeine metabolisers were 0.80 (0.52–1.23, p = 0.29) for moderate drinkers and 0.36 (0.14–0.89, p = 0.026) for heavy drinkers.

Short- and long-term impact on cardiovascular disease

The studies on caffeine consumption and risk of CVD yielded inconsistent results. Two population-based cohort studies have looked at the association between *CYP1A2* genotypes and heart failure (Casiglia et al., 2017) and atrial fibrillation incidence (Casiglia et al., 2018); however, they did not find increased risk of either heart failure or atrial fibrillation incidence depending on genotype. In fact, Casiglia et al., (2017) showed a protective effect of caffeine on HF in participants who were slow caffeine metabolisers, while Casiglia et al., (2018) initially observed a greater protective effect of caffeine on AF in slow caffeine metabolisers followed up for 12 years; however, this was no longer significant following multivariate analysis. Unlike *CYP1A2* genotypes, several SNPs of the *ADORA* loci, however, showed an association with dyslipidaemia incidence in a different population-based cohort study, albeit the incidence associated with SNPs was dependent on sex (Han et al., 2020). For example, it was shown that higher coffee consumption (≥ 1 cup/day) influenced dyslipidaemia incidence, but this was only applicable to female participants with the G minor allele of *ADORA1* rs10800901 (OR: 0.727, 95% CI: 0.560–0.944, p = 0.0168), and C minor allele of *ADORA2B* rs2779212 (OR: 0.645, 95% CI: 0.506–0.823, *p* = 0.0004) as well as the G major allele of *ADORA3* rs2786967 (OR: 0.818, 95% CI: 0.676–0.989, *p* = 0.0384). Male participants carrying the T minor allele of *ADORA2A* rs5760423 were shown to have a higher dyslipidaemia risk, when consuming < 1 cup/day of coffee.

*COMT* genotypes and CVD incidence were observed in a cross-sectional study (Brathwaite et al., 2011) and a population-based cohort study (Happonen et al., 2006). Brathwaite et al., 2011 observed a significant association (*p* = 0.001) between habitual caffeine intake (> 2 cups/day) and an increased heart rate in participants with the Val/Met and Met/Met genotypes, with an adjusted OR (95% CI) of 1.43 (0.64 - 3.20) and 2.98 (1.04 - 8.51), respectively. While Happonen et al., 2006 showed that CHD incidence was more likely among heavy coffee drinkers (≥ 4 cups/day) with the Met/Met genotype (OR: 3.2; 90% CI: 1.2 – 8.4) compared to the Val/Met and Val/Val genotypes. Other associations between genetics, caffeine consumption and CVD incidence were reported by three case-control studies. For example, Liu et al., (2020) showed a protective effect of the *TRIB1* rs17321515 and CHD incidence with coffee consumption in the GG genotype (OR, 0.62; 95% CI, 0.45–0.85, *p* = 0.0330), but no association was observed between CHD incidence and *CYP1A2* genotypes. MI incidence associated with coffee consumption, however, appeared to be influenced by *CYP1A2* genotypes and amount of cups of coffee consumed daily, since slow caffeine metabolisers had an OR (95% CI) of MI of 1.64 (1.14-2.34) for ≥ 4 cups/d, as compared to ≤ 1 cup/d (Cornelis et al., 2006). By contrast, Zhou et al., (2019) reported no significant gene-coffee interactions with CVD that might be influenced by the *CYP1A2* gene and/or a caffeine genetic risk score (GRS), constructed based on 8 genome-wide significant variants, including the most prominent loci near the aryl hydrocarbon receptor gene (*AHR)* and *CYP1A2* (rs4410790, rs6968554, rs10275488, rs2892838, rs12909047, rs35107470, rs2470893, and rs2472297) which are known to code for biological candidate proteins involved in caffeine metabolism.

**Discussion**

Our systematic review assessed the evidence for associations between genetics, coffee/caffeine intake and cardiometabolic outcomes. Overall, seventeen studies were available, and some consistent findings emerged.

Glucose control and type 2 diabetes mellitus

Caffeine can increase glucose levels by different mechanisms, such as increasing plasma adrenaline and augmenting its action as a general adenosine receptor antagonist, which together lead to a reduction in glucose disposal rates (Battram et al., 2005). The studies included in this systematic review highlighted some plausible associations between SNPs in the *CYP1A2*, *ADORA2A* and *MLXIPL* genes and glucose metabolism. For example, Banks et al., (2019) and Palatini et al., (2015) suggested that slow caffeine metabolisers of the *CYP1A2* rs762551 SNP might be at increased risk for higher glucose levels and IFG post-caffeine consumption, with such risk being proportionate to the amount of caffeine consumed (Banks et al., 2019) and, in other cases, even mediated by the interaction of smoking habit on caffeine metabolism (Kohno et al., 2013). Likewise, Cornelis et al., (2021) showed similar glucose responses post-caffeine consumption in slow caffeine metabolisers with the variant in the *CYP1A2* gene, although this effect was exclusively observed in female participants. Other genetic variants in the *MLXIPL* and *ADORA2A* genes (rs7800944 and rs5751876, respectively) also seemed to worsen glucose response. Specifically, short-term studies showed that consumption of caffeinated coffee may increase the AUC for glucose response, especially when consumed together with simple carbohydrates, including sucrose and dextrose (Banks et al., 2019), whilst long-term studies suggested an elevated glucose response with habitual caffeinated coffee consumption in individuals with the CT and TT genotypes of the *MLXIPL* gene (Cornelis et al., 2021).

BP response and hypertension

The relationship between BP, hypertension and caffeine is of major interest because caffeine is widely recognised as a potent inducer of an overall acute hypertensive response (James, 2004). As anticipated, most studies focused on candidate genes and relevant SNPs, with *CYP1A2* rs762551 being the most extensively studied. Among the included studies, consistent associations were reported between fast and slow caffeine metabolisers of the *CYP1A2* rs762551 SNP, SBP and DBP responses (Soares et al., 2018; Yoshihara et al., 2019) and risk of hypertension following caffeine consumption (Palatini et al., 2009). Other studies such as Renda et al., (2012) noted a relationship between the TT genotype of *ADORA2A* (rs5751876) as well as the *ADRA2B* insertion II variant, and the increase in peak SBP 2h post-caffeine consumption. Taken together, studies were consistent in revealing that caffeine exposure has the potential to interact with genetic predisposition in relation to BP. Previous data suggested that a reduction of 5 mmHg in SBP may decrease the CVD burden by 9% (Chobanian et al., 2003). Therefore, this is of paramount importance to healthcare since antihypertensive treatment has been shown to be effective in only 50% of cases in England, and 60 and 70% in USA and Canada, respectively (Joffres et al., 2013).

Short- and long-term impact on cardiovascular disease

Four of the studies included in this section reported interactions between *CYP1A2* rs762551 and caffeine, although with conflicting findings. For example, Casiglia et al., (2018) observed a significant trend for AF decrease in all *CYP1A2* genotypes, suggesting that caffeine activity on AF is not influenced by genetics. However, caffeine intake was only measured during the first screening and, therefore, changes in caffeine consumption during the follow-up period were not considered which poses a considerable bias on the overall results. In contrast to these findings, Cornelis et al., (2006) found that coffee consumption was associated with an increased MI risk in slow caffeine metabolisers of the *CYP1A2* rs762551 SNP, while fast caffeine metabolisers consuming up to 3 cups of coffee per day had a decreased risk. These findings agree with previous literature describing a J- or U-shaped association between MI risk and coffee consumption (Kleemola et al., 2000; Panagiotakos et al., 2003). Thus, from a clinical perspective slow caffeine metabolisers of the *CYP1A2* rs762551 SNP might benefit from reducing their daily coffee intake. The reported associations between caffeine intake, genetics and HF and CVD risks were more ambiguous. The authors found no established health risks from caffeine consumption associated with genetic variants of the *CYP1A2* and *AHR* genes neither on incident HF events (Casiglia et al., 2017) nor on CVD factors, including CAD, stroke and peripheral artery disease (Zhou et al., 2019). This, however, does not imply that genetics, and in particular SNPs of the *CYP1A2* do not moderate the effect of coffee consumption on CVD risks. In addition, the possible additive effects of other components present in coffee (e.g., bioactive compounds including polyphenol) rather than caffeine itself cannot be ruled out (Guessous et al., 2014). The associations between SNPs of the *ADORA2A* (rs57604223) gene and dyslipidaemia risk observed by Han et al., (2020) suggested an increased dyslipidaemia occurrence in participants who were habitual coffee drinkers (≥ 1 cup/day) with the TG or TT genotype; however, the opposite effect was observed with other genetic variants. Other SNPs of the *TRIB1* and *COMT* genes were also associated with CVD risk factors. For example, Liu et al., (2020) reported an association between habitual coffee consumption and lower CHD prevalence, albeit only in the GG genotype, and Happonen et al., (2006) observed that those with a Met/Met genotype of the *COMT* gene were more susceptible to CHD with higher coffee consumption compared to the Val/Val genotype. Brathwaite et al., (2011) also observed that participants with the *COMT* Met/Met genotype reported increased heart rate following more than 200mg/day of caffeine (more than two cups of coffee) regardless of *CYP1A2* genotype, thus suggesting that this effect is independent of the rate of caffeine metabolism. The authors mentioned this effect might be due to the hemodynamic effects of caffeine in stimulating unfavourable cardiovascular outcomes via catecholamine signalling (Brathwaite et al., 2011).

Quality assessment of evidence, limitations and strengths

The quality assessment of the included studies found that the strength of evidence reviewed for the interactions between caffeine consumption, cardiometabolic outcomes and selected SNPs appears to be moderate for RCTs and low for non-RCTs. The included RCTs presented low and unclear risk in the selection and detection bias, with only two exhibiting a high risk in performance bias due to the participants’ awareness of the intervention. All RCTs displayed a low risk for attrition, reporting, and other bias. Similarly, the non-RCTs exhibited mostly low risk of bias, with only a minority describing a high risk, for selection, performance, reporting and attrition. However, the majority of non-RCTs displayed a high risk of bias for the confounding category, a limitation often encountered in observational studies (Metelli & Chaimani, 2020). For example, some studies included a few confounders introduced by self-reported caffeine intake (Johnson & Fendrich, 2005; Addicott et al., 2009) and a lack of control over dietary factors that are known to influence glucose response, regardless of caffeine intake (e.g., saturated fat and dietary fibre consumption) (Lattimer & Haub, 2010; Imamura et al., 2016). The implementation of self-reported dietary data collection techniques adopted in most studies also poses some issues, such as participant bias (Ravelli et al., 2020). Another limitation is related to the high heterogeneity among studies in terms of type of coffee and dose of caffeine consumed and way of administration (coffee versus caffeine capsules). For example, caffeine content in coffee differs based on how it is prepared. Espresso coffee contains about 60 mg of caffeine per cup, soluble coffee caffeine content ranges between 30 and 90 mg and American coffee provides around 180-300 mg of caffeine per cup (Borghi, 2022), making these differences in caffeine content responsible for some of the uncertainties about the role of coffee consumption on cardiometabolic health. Furthermore, differences in terms of experimental protocol adopted (i.e., acute versus long-term, with and/or without physical activity), characteristics of the study population (i.e., healthy and/or with established risk factors), genotyping and genetic variants assessed and, ultimately, measurements (i.e., gold standard technique such as high-performance liquid chromatography versus surrogate methodologies) make the comparison among the studies difficult to perform.

Potential practical applications

The consumption of coffee in appropriate amounts should not be discouraged in individuals with pre-diabetes, hypertension and CVD, and The Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) have considered a safe limit for caffeine consumption to be 400 mg per day (EFSA, 2015; FDA, 2018). Nevertheless, one-size-fits-all recommendations may not be safe enough for individuals who are slow caffeine metabolisers, whose risks for certain conditions including hypertension, pre-diabetes and heart attack may increase by consuming the same amount of caffeine that is currently considered safe. Identifying if an individual is a slow or fast caffeine metaboliser can only be achieved via the use of genetic testing, which, in the future, may be recognised as a tool to guide caffeine recommendations according to genotype.

5. Conclusion and recommendations for future research

When taken together, these findings partly support the associations between caffeine consumption, genetics and cardiometabolic outcomes. From our review, we can infer that both *CYP1A2* and *ADORA2A* genes influence glucose response following caffeine consumption, at least in the short-term; however, further work is warranted to elucidate their effects on glucose control in the long-term. Similarly, studies included in the review suggest an increase in BP following caffeine consumption that is mostly influenced by the *CYP1A2* and *ADORA2A* genes. However considering the extent to which genetic variability and caffeine consumption impact CVD, more caution is needed since our findings were ambiguous. Based on our review, future research in this area must, at least, consider the *CYP1A2*, *ADORA2A*, and *COMT* genes as potential genetic modifiers of the above-mentioned associations. We recommend future studies to implement an integrative approach to assess caffeine effects on cardiometabolic outcomes since these may derive from complex interactions including genetics, physiological and behavioural factors. In this regard, considering the influence of physical activity on the reported associations may be a good starting point. Finally, dose and time-dependent studies are needed to better identify the effects of caffeine on cardiometabolic health. The results of these studies could be implemented into developing caffeine consumption advice to be tailored according to genotype to maintain and/or improve cardiometabolic health and reduce the incidence of cardiometabolic disorders across different populations.

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**Conflict of Interest**

YM serves as a scientific consultant for MyHealthChecked PLC. Leta Pilic is the founder of Optimyse Nutrition LTD.

**CRediT authorship contribution statement**

JV: conceptualisation, methodology, systematic review process (search, title & abstract screening, report screening, data extraction, quality assessment), validation, formal analysis, investigation, resources, data curation, writing – original draft, writing – review & editing, project administration. PM: conceptualisation, methodology, systematic review process (search, title & abstract screening, report screening, data extraction, quality assessment), validation, formal analysis, investigation, resources, data curation, writing – review & editing, project administration. GJ: conceptualisation, methodology, systematic review process (search, title & abstract screening, report screening, data extraction, quality assessment), validation, formal analysis, investigation, resources, data curation, writing – review & editing, project administration. YM: writing – review & editing, supervision, project administration. LP: conceptualisation, methodology, writing – review & editing, supervision, project administration.

**Availability of data and materials statement**

Information not provided in the manuscript or supplementary material is available upon request.

**Tables**

**Table 1.** Characteristics of the studies included in this systematic review (reported in alphabetical order by health outcomes)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **First Author** | **Study design**  | **Participants**  | **Caffeine consumption measurement and intervention**  | **SNP(s) and gene(s) observed**  | **Outcome(s)** |
| **Glucose control and Type 2 Diabetes Mellitus** |
| Banks et al., (2019) | Randomised, single-blind, cross-over design | Male = 18Age (y) ± SD = 23.1 ± 1.3Ethnicity = Caucasians | Caffeine consumption questionnaire.Intervention: 4mg/kg of caffeine powder mixed with carbohydrate in a liquid drink vs 0.75 g carbohydrate/Kg mixed with 75 ml/kg of water | *ADORA2A* rs5751876*CYP1A2* rs762551 | When examining *ADORA2A* 1976T > C genotype, there was a significant genotype × condition interaction (F1,16 = 7.24, *p* = 0.02, np2 = 0.31). Participants with the CC genotype for *ADORA2A* rs5751876 had elevated glucose levels and greater glucoseAUC following a carbohydrate + caffeine vs carbohydrate only meal. Similarly, when examining *CYP1A2* −163C > A genotype, there was a significant genotype × condition × time interaction (F1,16 = 4.534, *p* = 0.018, np2 = 0.221) for glucose responses. Participants with the AC/CC genotype for *CYP1A2* rs762551 had greater glucose levels 60-min post meal during carbohydrate + caffeine vs carbohydrate (*p* = 0.022). |
| Cornelis (2021) | Population-based cross-sectional study  | Total = 370,193 (included for genetic analysis)Age (y) = 37-73 Ethnicity = Caucasians | FFQ(coffee, and tea consumption). Coffee consumption was self-reported prior to blood sampling | *CYP1A2* rs2472297*MLXIPL* rs7800944 | Women with CC or CT genotypes for *CYP1A2* rs2472297 exhibited over two-fold higher glucose levels when drinking caffeine within ~ 1 hour of blood sampling (*p* = 0.004). Similarly, women with CC genotype for *MLXIPL* rs7800944 exhibited higher glucose levels with habitual coffee consumption (*p* = 0.004).  |
| Kohno et al., (2013) | Cross-sectional study | Male = 2263Age (y) ± (SD) = 52.4 ± 0.9 Ethnicity = Japanese | FFQ for weekly and daily coffee consumption  | *CYP1A2* (rs2069514, rs762551) | IFG/IGT decreased with increasing consumption of coffee regardless of CYP1A2 genotypes. The effect modifications of CYP1A2 genotypes on the association between coffee and type 2 diabetes was statistically significant among current smokers with the CYP1A2 rs762551 variant (*p* = 0.008) and rs2969514 variant (*p* < 0.0001). |

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Palatini et al., (2015) | Prospective longitudinal study | Total = 1,180 (639 were included for genetic analysis)Age (y) ± (SD) = 33.0 (8.5)Follow-up = 6.1 yearsEthnicity = Caucasians, Italians  | FFQ (coffee consumption, PA, alcohol, cigarette smoking). | *CYP1A2* rs762551 | Following adjustments for several confounders, the OR for IFG associated with coffee consumption among carriers of the slow \*1F allele was 1.42 (CI 0.91–2.22, *p* = 0.13) for moderate drinkers and 2.78 (1.32–5.88, *p* = 0.0076) for heavy drinkers. The corresponding HRs for participants who were homozygous for the rapid \*1A allele were 1.33 (CI 0.67–2.67, *p* = 0.42) and 1.71 (0.76–3.84, *p* = 0.20), respectively. |
| Robertson et al., (2018) | Open parallel arm- randomised control trial | Coffee group = 19 Control group = 8Age (y) = 18 – 42Ethnicity = Caucasians  | Caffeine concentration was measured by HPLC. Intervention: 4 cups x 2 g/day instant coffee for 12 weeks vs no caffeine for 12 weeks  | *CYP1A2* rs762551 | Prior to coffee consumption, participants who were slow metabolisers had a higher baseline glucose level compared to fast metabolisers (*p* < 0.05). However, post-intervention reduced postprandial glycaemia was only observed in the slow metabolisers, whilst the opposite effect was observed in fast metabolisers (*p* < 0.05).  |
| Blood pressure response and hypertension  |
| Palatini et al., (2009) | Population-based cohort study | Total = 553(72.5% were men and 27.5% women)Age (y) ± (SD) = 33.2 (8.6)Ethnicity = Caucasians, Italian  | FFQ (coffee consumption, PA,alcohol, cigarette smoking). | *CYP1A2* rs762551 | Carriers of the slow \*1F allele (59%) had a HR for hypertension of 1.00 for abstainers (reference), 1.72 (95 % CI, 1.21–2.44) for moderate coffee drinkers (*p* = 0.03), and 3.00 (1.53 – 5.90) for heavy drinkers (*p* = 0.001). Conversely, HRs for coffee drinkers with the \*1A/\*1A genotype were 0.80 (0.52–1.23, *p* = 0.29) for moderate drinkers and 0.36 (0.14–0.89, *p* = 0.026) for heavy drinkers. Participants with the slow \*1F allele had a higher urinary epinephrine with coffee consumption (*p* = 0.001). |

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| Renda et al., (2012) | Prospective, double-blind, randomised study with crossover design  | Male = 110Age (y) ± (SD) = 26 ± 42 Ethnicity = Caucasians, Italian  | Caffeine and catecholamines were measured by blood sampling at 30-min for caffeine measurement and at 2-h following caffeine consumptionIntervention: 40 mL decaffeinated preparation without any added caffeine vs 40ml decaffeinated preparation + 3 mg/kg caffeine | *CYP1A2*-734A/C; *ADORA2A*-1976C/T; *AMPD1*-C34T; *ADRA1A*-Arg347Cys; *ADRA2B*-Ins+910Del; *ADRB1*-Arg389Gly; *ADRB1*-Ser49Gly; *ADRB2*-Arg16Gly; *ADRB2*-Glu27Gln; *ADRB2* -Thr16Ile; *ADRB3*-Trp64Arg  | Unlike decaffeinated coffee, caffeinated coffee was associated with a mean (± SD) significant increase in SBP of 4 ± 12 mmHg and in DBP of 3 ± 10 mmHg (*p* < 0.001 for both). Similarly, plasma caffeine and adrenaline raised following caffeinated coffee, but not decaffeinated. Out of the 11 SNPs analysed, significant relations were observed between the *ADORA2A* TT variant and the increase in SBP peak (r = 0.214, *p* = 0.024) and between the *ADRA2B* I variant and the increase in both SBP mean (r = 0.226, *p* = 0.017) and peak (r = 0.225, *p* = 0.018).  |
| Soares et al., (2018) | Interventional and quasi-experimental | Total = 37 (29 men and 8 women)Age (y) = 19 – 50 Ethnicity = Caucasians  | FFQ | *CYP1A2* rs762551 | Participants with the AC genotype had increased baseline DBP and post-caffeine SBP compared to those with the AA genotype (p < 0.05). Similarly,PAonly influenced the BP responses to acute caffeine ingestion in participants with the AC genotype (p < 0.05). |

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| Yoshihara et al., (2019) | Single-centre, prospective, double-blind, randomised trial.  | Caffeine group = 130 (105 men and 25 women)Decaffeinated group = 71 (53 men and 18 women)Age (y) = 20 – 37Ethnicity = Japanese  | FFQIntervention: 1 cup of 150 ml of coffee with 2 g of instant coffee + 200 mg of caffeine was added in the caffeine group | *ADORA2A* rs5751876 *CYP1A2* rs762551  | Caffeine intake increased BP and calculation speed (*p* < 0.05); however, this was not dependent on *ADORA2A* and *CYP1A2* SNPs. In stratified analysis, a statistically significant change inBP was observed in the caffeine group (90 mg/d) for the *CYP1A2* gene. Change in SBP in the *CYP1A2* rs762551 CC group (mean ± SD 1⁄4 11.8 ± 5.9) was higher than SBP in the AA/AC group (4.1 ± 5.5) (*p* < 0.001). |
| **Cardiovascular disease**  |
| Brathwaite et al., (2011) | Cross-sectional  | Female = 801Male = 344Age (y) = 20-29Ethnicity = Caucasians, East Asian, South Asian  | Validated caffeine consumption questionnaire | *COMT* rs4680 *CYP1A2* rs762551 | The Met/Met *COMT* genotype was significantly associated with an increased heart rate among participants who habitually consumed more than 200 mg/day of caffeine (*p* = 0.001). *CYP1A2* genotypes were not associated with any health outcome and did not modify the effect of *COMT* genotypes on heart rate.  |
| Casiglia et al., (2017) | Population-based cohort study | Total = 1,475 Age (y) ± (SD) = 60.0 ± 16.7Follow-up = 12 years Ethnicity = Caucasian, Italian | 7-day food diary  | *CYP1A2* rs762551 | Following a 12-year follow-up, 125 new HF cases were recorded. When participants were stratified according to gender and genotype, a protective effect of caffeine against HF was only identified in C-carrier men (*p* < 0.0001), but not in C-carrier women and in AA homozygous independent of sex. |
| Casiglia et al., (2018) | Population-based cohort study  | Total= 1,475 Age (y) ± (SD) = 60.0 ± 16.7 Follow-up = 12 yearsEthnicity = Caucasian, Italian  | Caffeine intake was calculated from a food diary by using a prespecified formula | *CYP1A2* rs762551 | A higher caffeine intake (> 165 mmol/day or > 320 mg/day) was associated with a lower AF incidence. The risk of an ischaemic stroke decreased with increasing caffeine intake (*p* for trend < 0.001). A significant trend towards AF reduction was detected in all *CYP1A2* genotypes (*p* < 0.01), regardless of genetic control.  |

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| Cornelis et al., (2006) | Case-control study  | Cases = 2014 Controls = 2014 Age (y), mean (SD) = 58.4 (11.0) for cases; 58.1 (11.3) for controls Ethnicity = Hispanic Americans  | FFQ (caffeinated coffee, tea, cola beverages, and chocolate) | *CYP1A2* rs762551 | Participants who were C-carriers had an increased risk of MI associated with coffee consumption (*p* = 0.04 for gene x coffee interaction). The OR (95% CI) of MI was 1.64 (1.14-2.34) for ≥ 4 cups/d, as compared to **≤** 1 cup/d. The corresponding OR (95% CI) among those who were homozygous for the ‘rapid’ A allele was 0.99 (0.66-1.48).  |
| Han et al., (2020) | Population-based cohort study | Male = 2527Female = 2371Age (y)= 43 – 74Follow-up every 2 years from 2005 to 2018Ethnicity = East Asian, Korean | FFQ | *ADORA1* (rs10800899, rs6701725, rs10800901); *ADORA2A* (rs5760423); *ADORA2B* (rs17715109; rs2779212); *ADORA3* (rs3393; rs2786967) | Female participants with the minor allele of *ADORA1* rs10800901 (OR: 0.727, 95% CI: 0.560–0.944, *p* = 0.0168), and *ADORA2B* rs2779212 (OR: 0.645, 95% CI: 0.506–0.823, *p* = 0.0004), and the major alleles of *ADORA3* rs2786967 (OR: 0.818, 95% CI: 0.676–0.989, *p* = 0.0384) had a decreased dyslipidaemia risk (defined as elevated levels of LDL cholesterol and total cholesterol and low levels of HDL cholesterol) with coffee consumption. Male participants with the minor alleles of *ADORA2A* rs5760423 had an increased dyslipidaemia risk when consuming more coffee (≥ 1 cup/day of coffee) (OR: 1.352, 95% CI: 1.014–1.802, *p* = 0.0402). |

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| Happonen et al., (2006) | Population-based prospective cohort study | Male = 773Age (y) ± (SD) = 51. ± 6.6 Follow-up = 13 yearsEthnicity = Finnish  | 4-day food diary (household measures for cups of coffee and tea) | *COMT* rs4680  | Heavy coffee drinkers (> 814 ml) with the Met/Met genotype had an OR (90% CI) of 3.2 (1.2 – 8.4) for CHD, compared to those with a high *COMT* activity. Urinary adrenaline excretion was also 2-fold higher in heavy coffee drinkers with a low *COMT* activity compared to non-drinkers (*p* = 0.008 for trend).  |
| Liu et al., (2020) | Case-control study | Total cases and controls = 1116 and 7853, respectivelyAge (y) = 30 – 70Ethnicity = Taiwanese  | FFQ | *TRIB1* rs17321515 *CYP1A2* rs762551  | There was an association between *TRIB1* rs17321515 and coffee consumption on CHD risk (*p* = 0.0330 for interaction). Following stratification by rs17321515 genotypes, coffee consumption remained significantly associated with a lower risk of CHD only among participants with the GG genotype (OR, 0.62; 95% CI, 0.45–0.85). No association of *CYP1A2* rs762551 with coffee consumption on CHD was observed.  |
| Zhou et al., (2019) | Case-control study  | Total = 347,077 (including 8,368 incident CVDscases)Age (y) = 37 – 73Ethnicity = White British, Caucasians | Participants were asked to report how many cups of coffee (including decaffeinated) they had drunk per day  | *CYP1A2* rs762551 Secondary analyses = caffeine-GRSincluding *AHR* and *CYP1A2* (rs4410790, rs6968554, rs10275488, rs2892838, rs12909047, rs35107470, rs2470893, and rs2472297) | *CYP1A2* genotype and caffeine-GS were not associated with CVD (including CAD, stroke, and peripheral artery disease), (*p* ≥ 0.22 for all comparisons). No interaction was found to be significant between the *CYP1A2* genotype or caffeine-GRS and coffee consumption regarding CVD (*P* ≥ 0.53).  |

Abbreviations: glucose AUC, glucose area under the curve; AF, atrial fibrillation; FFQ, food-frequency questionnaire; MI, myocardial infarction; OR, odds ratio; HF, heart failure; CHD, coronary heart disease; CAD, coronary artery disease; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; HR, hazard ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; PA, physical activity; HPLC, high-performance liquid chromatography; BP, blood pressure; CVD, cardiovascular disease; GRS, genetic risk score

**Table 2.** Risk of bias assessment using the Cochrane Collaboration’s Tool for RCTs

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| **First Author** | **Random Sequence Generation** | **Allocation Concealment** | **Blinding of Participants and Researchers** | **Blinding of Outcome Assessment** | **Incomplete Outcome Data** | **Selective Reporting** | **Other Bias** | **Overall judgment** |
| Banks et al., (2019) |  |  |  |  |  |  |  |  |
| Renda et al., (2012) |  |  |  |  |  |  |  |  |
| Robertson et al., (2018) |  |  |  |  |  |  |  |  |
| Yoshihara et al., (2019) |  |  |  |  |  |  |  |  |

Key: Low risk of bias (green); Unclear risk of bias (yellow); High risk of bias (red)

**Table 3.** Risk of bias assessment using the Research Triangle Institute (RTI) Item Bank and Risk of Bias in Non-randomised Studies of Interventions (ROBINS-I) for observational studies

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| **First Author** | **Selection Bias** | **Performance Bias** | **Measurement bias** | **Missing data bias** | **Selection of the reported results bias** | **Confounding** | **Overall judgment** |
| Brathwaite et al., (2011) |  |  |  |  |  |  |  |
| Casiglia et al., (2018) |  |  |  |  |  |  |  |
| Casiglia et al., (2017) |  |  |  |  |  |  |  |
| Cornelis (2021) |  |  |  |  |  |  |  |
| Cornelis et al., (2006) |  |  |  |  |  |  |  |
| Han et al., (2020) |  |  |  |  |  |  |  |
| Happonen et al., (2006) |  |  |  |  |  |  |  |
| Liu et al., (2020) |  |  |  |  |  |  |  |
| Kohno et al. (2013) |  |  |  |  |  |  |  |
| Palatini et al., (2009) |  |  |  |  |  |  |  |
| Palatini et al., (2015) |  |  |  |  |  |  |  |
| Soares et al., (2018) |  |  |  |  |  |  |  |
| Zhou et al., (2019) |  |  |  |  |  |  |  |

Key: Low risk of bias (green); Moderate risk of bias (yellow); High risk of bias (red)

**Figure legends**

**Figure 1.** PRISMA flowchart of the study selection process