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Combined Effect Of Coffee Consumption And Cigarette Smoking On Serum Levels Of Vitamin B12, Folic Acid, And Lipid Profile In Young Male: A Cross-Sectional Study

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Objective: To investigate the associations of coffee consumption and/or smoking on certain clinical outcomes including total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), vitamin B12, and folic acid in a population of young healthy men.

Method: This cross-sectional study was conducted in Amman, Jordan, over 4 months. Participants were approached for study participation and asked to fill a questionnaire about their anthropometric information, habitual smoking, and coffee consumption during the last 3 months. Their fasting blood samples were taken to measure TC and LDL-C.

Results: Healthy male participants (n=117) in the age range of 18 to 26 years were recruited. Mean serum TC was higher in heavy coffee consumers (C++) group (≥3 cups/day) with or without smoking (M= 179.9±34.59 mg/dL and 195.94±23.69 mg/dL) in comparison with moderate coffee consumers (C+) group (1–2 cups/day) (M= 158.1±24.82 mg/dL and 177.23 ±34.17 mg/dL), and the mean level was higher in subjects who were coffee consumers only than smokers who were coffee consumers. LDL-C levels were higher in participants who were coffee consumers (M= 103.06±34.82mg/dL and 118.06±19.31 mg/dL) than smokers who were coffee consumers (M= 88.6±22.40 mg/dL and 108.26±37.57 mg/dL). No significant difference was noted regarding HDL-C, vitamin B12, and folic acid.

Conclusion: Our findings showed that heavy coffee consumption was more associated with hyperlipidemia than cigarette smoking. Accordingly, we conclude that moderate coffee consumption may reduce the risk of cardiovascular diseases or their consequences in male.

Keywords: coffee, cigarette smoking, cholesterol, lipid profile, CVD

Introduction

Coffee consumption has been associated with several risk factors leading to cardiovascular diseases (CVDs), including hyperhomocysteinemia and hyperlipidemia.[1–4] Frequent coffee consumption has beneficial effects in reducing the risk of diabetes mellitus (DM) type 2, obesity, liver disease, CVDs, some types of cancer, Parkinson's disease, and Alzheimer's disease.[5,6] Controversies concerning coffee consumption benefits and risks in regards for CVDs were mentioned in many studies.[6–9]

Lack of vitamin B12 and/or folate, which are necessary vitamins in homocysteine (Hcy) metabolism,[10,11] leads to elevation in Hcy levels. Hyperhomocysteinemia is a

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risk factor for atherosclerosis, due to numerous mechanisms including oxidative stress and endothelial dysfunction that promotes thrombosis.[10,12]

Therefore, and because of their close relationship, vitamin B12 and folate deficiencies can be an indirect indication of hyperhomocysteinemia. Both folate and vitamin B12 are Hcy determinants, hence positively associated with CVDs.[10,13]

It was shown that caffeine, as well as polyphenols which are found in coffee can raise total Hcy,[14,15] and to have an association with CVDs.[16] Nevertheless, moderate to no effect of coffee consumption on plasma folate and vitamin B12 were noted in previous studies.[3]

Thus, the mechanism behind total Hcy increase remains uncertain. Similarly, elevated total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels were found to be largely attributable to the boiled coffee, rather than filtered coffee.[13] Hyperlipidemia is well known to be one of the most contributing factors for the risk of CVDs including thrombosis.[17]

Correspondingly, the association of cigarette smoking with CVDs can be attributed to increased oxidative stress due to excessive free radicals resulted from burning tobacco and other chemicals of the cigarettes.[18,19]

Nicotine, which is the main component of cigarette smoke, causes coronary death by provoking ventricular arrhythmias. Cardiac effects of nicotine happen due to the stimulation of nicotinic receptor, which enhances the release of catecholamine.[20]

Generally, 50% of smokers drink coffee and report drinking almost twice as much coffee per day as nonsmokers.[21] Many reports have pointed that caffeine and cigarette smoking has a negative effect on human blood pressure and considered to be a risk for CVD.[22]

Both caffeine consumption and cigarette smoking are well known to yield considerable changes in the cardiovascular hemodynamics.[22] Caffeine acutely raises both systolic and diastolic blood pressure, but has no significant effect on the heart rate. However, cigarette smoking leads to acute increase in blood pressure and heart rate. In addition, high comorbidity of smoking and caffeine in CVD patients support the synergistic association between coffee consumption and cigarette smoking on the hemodynamic status.[23] Accordingly, it is essential to better understand the biological factors that may be associated with these co-occurrences.[21]

Therefore, this research aims to investigate the associations of habitual coffee consumption and smoking on certain clinical outcomes including body mass index (BMI), fasting blood glucose (FBG), TC, high-density lipoprotein cholesterol (HDL-C), LDL-C, serum triglyceride (TG), folic acid and vitamin B12 level, in a population of young healthy men.

Materials And Methods

Study Design And Participants

This cross-sectional study was conducted in Amman, Jordan, over 4 months, January to April 2015. Young male students studying at Applied Science Private University (ASU) in Amman were approached for study participation. Inclusion criteria included male, ASU, student of the Faculty of Pharmacy, in the age range from 18 to 26 years, and do not have any acute or chronic medical conditions. The numbers of female smokers are low in comparison with male smokers in study sample society; moreover, the female smokers do not like to state that they are smokers because of cultural issues. Exclusion criteria included students who did not speak Arabic or who used any medications for either an acute and/or a chronic condition during the past 2 months prior to the study entry.

The protocol of this study was approved by the ASU ethics committee under reference DRGS-2013/2014-8-2 for the protection of human subjects and was conducted in accordance with the Declaration of Helsinki. The purpose of this research, details of the experimental protocol, and the risks involved in the research were clarified to the participants verbally and provided in an information sheet. All participants signed a written consent and were asked to fill a health screening questionnaire prior to the study.

Participants were then asked to fill a questionnaire that included two parts: Part A involved the anthropometric information and clinical characteristics. Part B involved questions regarding smoking and coffee consumption during the last 3 months prior to study entry.

Following to questionnaire completion, subjects were asked to have their blood samples taken for analysis. Following to analysis, the participants were divided into four groups according to daily coffee intake and whether they are smoker or not: group 1 (NS/C+) were nonsmokers and moderate coffee consumers (1–2 cups/day); group 2 (NS/C++) were nonsmokers and heavy coffee consumers (≥3 cups/day); group 3 (S/C+) were smokers and moderate coffee consumers (1–2 cups/day); group 4 (S/C++) were smokers and heavy coffee consumers (≥3 cups/day). Smoker participants consume about 20±5 cigarettes/day.

Coffee Preparation

Participants were drinking boiled unfiltered Turkish coffee (Alameed Coffee, Amman, Jordan). The coffee was prepared by mixing 6.5–13.0 g of Alameed Turkish coffee with 150–200 mL of boiling water (this was equivalent to 1–2 teaspoons of coffee powder) resulting in 150 mg of caffeine per cup.[24]

Blood Glucose And Lipid Profile

Fasting blood samples were collected in 5-mL serum tubes with a clot activator (VACUETTE® Z Serum [Sep] Clot Activator; GBO) from the participants at 8 a.m.. Then, the samples were stored at room temperature for around 45–60 mins, after which they were centrifuged at 4000 rpm for 10 mins. Aliquots of at least 1 mL of serum were measured into Eppendorf tubes[25] and stored at −20°C until the time of their assay. One Touch® test strips (LifeScan; Johnson & Johnson, Palmitas, CA, USA) were used to measure the levels of FBG, TG, TC, and HDL-C were determined using enzymatic colorimetric kits (Linear Chemicals, Barcelona, Spain). As for LDL-C, it was calculated from the equation ($C_{LDL} = C_{plasma} - C_{HDL} - TG/5$) that was recorded in a previous study.[26]

Serum Vitamin B12 And Folic Acid

The serum was analyzed using the electrochemiluminescence immunoassay. Cobas immunoassay analyzer (Roche Diagnostics) was used to measure vitamin B12 in serum and electrochemiluminescence immunoassay (ECLIA) kit (Roche Diagnostics) was used to measure the folic acid in serum.

Body Mass Index

On the day of evaluation, BMI (kg/m^2) was calculated for each participant according to their height (m) and weight (kg) recorded on the day.

Hematology Parameters

Total leukocyte count, differential leukocyte count, platelet count, hemoglobin, hematocrit, and red blood cells (RBC) indices and mean platelet count were measured for all participants. Complete blood count was performed on the COBAS MICROS OT 18 (Roche, France).

Statistical Analysis

Statistical analyses were performed using the statistical software package SPSS version 19.0 for Windows (Chicago, IL, USA). The *t*-test statistical analysis was used to evaluate the significant differences in independent variables (IDVs) between the groups. Significance of the differences between the various groups was evaluated by two-way ANOVA following Student's *t*-test. It was conducted to determine whether the interaction effect of coffee consumption and cigarette smoking was more or less significant than the main effect for each term alone on the studied parameters. The results were considered statistically significant when $p<0.05$.

The stepwise multiple regressions and univariate analysis were used to evaluate the effects of IDVs, serum lipid profile, BMI, body weight (BW), FBG, vitamin B12, folic acid, RBCs, hemoglobin, packed cell volume (PCV), mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) on lipid profile parameters, B12 and folic acid levels as a dependent variable (DV) in the four study groups.

Results

A total of 117 healthy male pharmacy students in the age range of 18 to 26 years were invited to participate in this study. Samples were taken from all eligible participants who agreed to follow the study protocol as shown in Figure 1. Participants were categorized into four groups, NS/C+ group (n=39, 33.3%), NS/C++ group (n=21, 18.0%), S/C+ group (n=22, 18.8%), and S/C++ group (n=5, 29.9%).

Descriptive Analysis

There were no significant differences in clinical parameters and characteristics of the study participants subdivided by their coffee consumption into two groups: moderate coffee consumers (C+), n=61 and heavy coffee consumers (C++), n=56, as shown in Table 1. Regardless of their smoking behavior, there was a trend of higher vitamin B12 (364.41±124.76 pg/mL) and folic acid (9.98 ±3.46 ng/mL) in C++ group compared to vitamin B12 (350.89±135.56 pg/mL) and folic acid (9.64±3.15 ng/mL) in C+ group, with no significant difference as shown in Table 1. This trend was clearer, when the study participants were further subdivided by their combined smoking and coffee consumption behavior into four groups. In S/C++ group, vitamin B12 (377.01±138.44 pg/mL) and folic acid (10.42±3.79 ng/mL) were the highest among others as presented in Table 2. Vitamin B12 (375.63±153.33 pg/mL) was also high in S/C+ group in comparison to others, Table 2. The TC (185.581±31.85

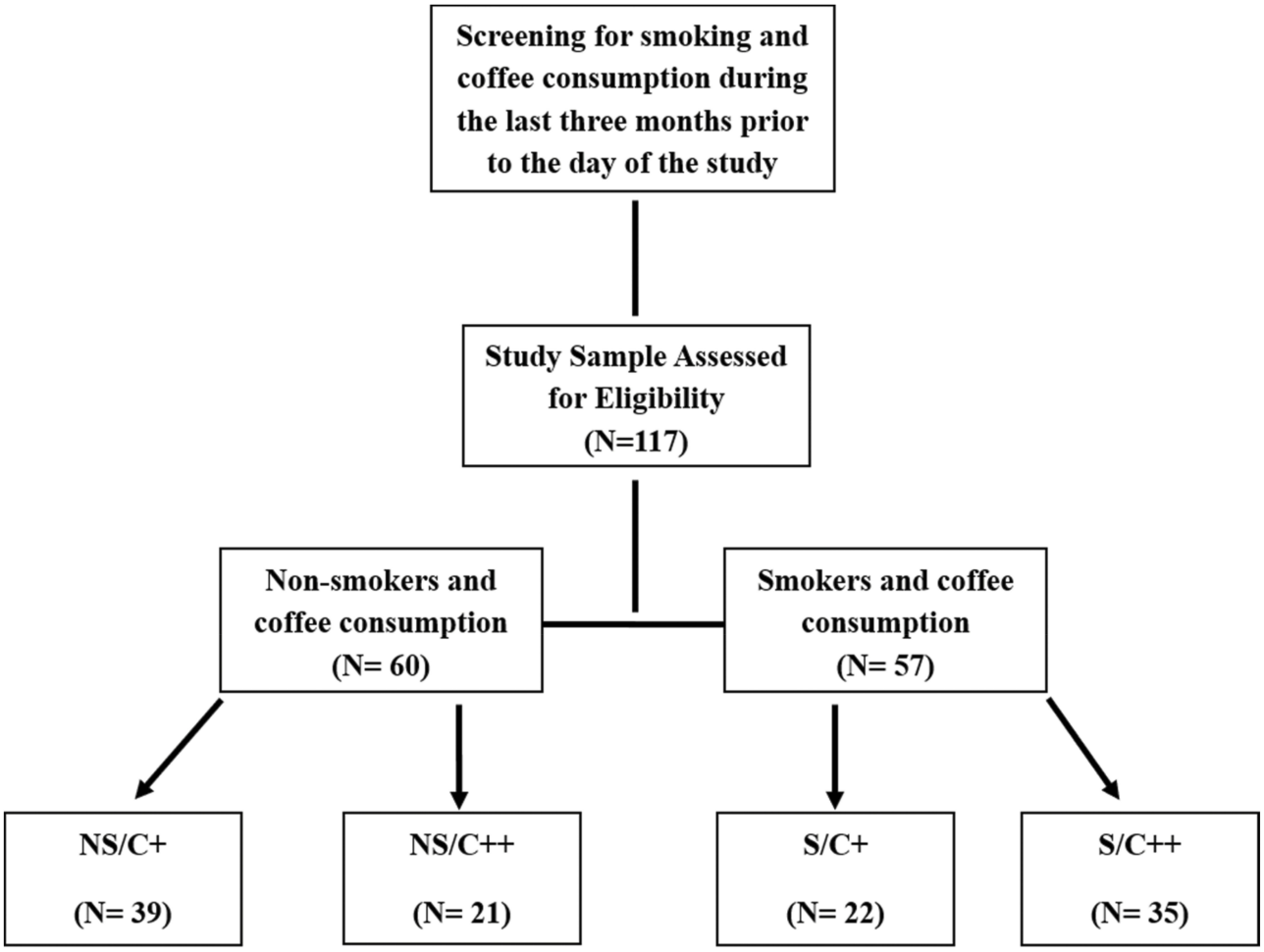

Figure 1 Schematic diagram of study design.
Abbreviations: N, sample size; NS/C+, nonsmokers and moderate coffee consumers; NS/C++, nonsmokers and heavy coffee consumers; S/C+, smokers and moderate coffee consumers; S/C++, smokers and heavy coffee consumers.

mg/dL) and LDL-C (111.73±32.41 mg/dL) in C++ group compared to TC (169.73±31.99 mg/dL) and LDL-C (97.39 ±31.13 mg/dL) in C+ group were higher with no significance difference as shown in Table 3. The mean serum TC and LDL-C were higher in NS/C++ group (195.94±23.69 and 118.06±19.31 mg/dL, respectively) and S/C++ group (179.90±34.59 and 108.26±37.57 mg/dL, respectively) in comparison to other groups, Table 4. The combination of smoking and coffee drinking behavior showed a lowering effect on TG serum levels of the two smokers' groups (S/C +: 110.95±58.43 and S/C++: 124.13±51.15 mg/dL) compared to the nonsmokers (NS/C+: 139.58±61.57 and NS/C ++: 139.12±46.92 mg/dL) as shown in Table 4.

No significant differences were noted between the different groups for the FBG, HDL-C, BMI, BW, vitamin B12, folic acid, RBCs, Hgb, PCV, MCV, MCH, and MCHC clinical parameters (Tables 1, 2, and 4).

Results Of Two-Way ANOVA Comparing The Four Study Groups

Combined versus single effect of cigarette smoking and coffee consumption on clinical parameters of the participants (FBG, BMI, BW, vitamin B12, folic acid, RBC, Hgb, PCV, MCV, MCH, and MCHC) using two-way ANOVA test showed no significant difference as presented in Table 5. Regarding the lipid profile, no significant differences were noted in the serum HDL-C and TG levels among all studied groups, while in TC and LDL-C levels there was significant differences between the groups regarding TC in smokers (p=0.008) and in coffee consumers (p= 0.002; Table 6); results were not significant for participants who smoked and drink coffee (p=0.812). In addition, LDL-C was significant in coffee consumers (p=0.01), and not significant in smokers and smokers who consumed coffee (p=0.067 and p=0.723, respectively;

Table 1 Clinical Parameters And Characteristics Of The Study Participants Subdivided By Their Coffee Consumption Into Two Groups; Moderate (C+) And Heavy (C++) Coffee Consumers (Mean±SD)

Clinical Parameters	Participants (n=117)		
	C+ (n=61)	C++ (n=56)	Total (n=117)
FBG (mg/dL)	87.05±8.38	86.84±8.32	86.95±8.32
BMI (kg/m^2)	25.49±4.05	25.91±4.86	25.70±4.45
BW (kg)	79.10±14.04	80.10±15.87	79.58±14.89
B12 (pg/mL)	350.89±135.56	364.41124.76	357.41130.06
Folic acid (ng/mL)	9.64±3.15	9.98±3.46	9.80±3.29
RBCs (× 10^{12}/L)	5.23 0.65	5.43±0.79	5.3 ±0.72
Hgb (g/dL)	15.92±1.16	15.98±1.49	15.94±1.32
PCV (%)	46.05±4.59	46.68±5.61	46.34±5.07
MCV (fL)	88.10±4.44	86.45±5.12	87.34±4.81
MCH (pg)	30.61±2.72	29.59±3.48	30.13±3.12
MCHC (g/dL)	34.72±2.40	34.33±2.55	34.54±2.46

Abbreviations: C+, moderate coffee consumers (1–2 cups/day); C++, heavy coffee consumers (>3 cups/day); FBG, fasting blood glucose; BMI, body mass index; BW, body weight; B12, vitamin B12, RBCs, red blood cells; Hgb, hemoglobin (g/dL); PCV, packed cell volume; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; N, sample size.

Table 6). There were no significant differences in the other parameters between the four study groups (p>0.05).

Results indicated that serum levels of TC and LDL-C of C + were lower than those of C++ as presented in Table 3. Also, there was a significant increase in the levels of TC and LDL-C in smokers and coffee consumers, but not in the participants who used a combination of both as seen in Table 6.

Table 3 Lipid Profile Parameters Of The Study Participants Subdivided By Their Coffee Consumption Into Two Groups; Moderate (C+) And Heavy (C++) Coffee Consumers (Mean±SD)

Lipid Profile Parameters	Participants (n=117)		
	C+ (n=61)	C++ (n=56)	Total (n=117)
TG (mg/dL)	128.35±61.41	129.44±49.72	128.88±55.77
TC (mg/dL)	169.73±31.99	185.58±31.85	177.41±32.75
HDL-C (mg/dL)	51.65±7.79	49.15±9.49	50.39±8.72
LDL-C (mg/dL)	97.39±31.13	111.73±32.41	104.34±32.41

Abbreviations: C+, moderate coffee consumers (1–2 cups/day); C++, heavy coffee consumers (>3 cups/day); TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; N, sample size.

Two-way ANOVA analysis indicated a strong effect of smoking and coffee consumption on TC and LDL-C ($p < 0.05$). Although there were no statistically significant differences in terms of TG levels between the groups, smoking effect on TG approached significance ($p = 0.062$).

Results Of Multiple Linear Regression (Stepwise Method) Comparing The Four Study Groups

Nonsmoker And Moderate Coffee Consumers (NS/C+) Group

The multivariate association between hematological parameters, age and lipid profile parameters as IDVs and lipid profile parameters, vitamin B12 and folic acid as DVs showed an influential relationship of the IDVs (MCHC,

Table 2 Clinical Parameters And Characteristics Of The Study Participants Subdivided By Their Combined Smoking (Nonsmoker (NS)/Smoker (S)) And Coffee Consumption (Moderate (C+)/Heavy (C++)) Behavior Into Four Groups (Mean±SD)

Clinical Parameters	Participants (n=117)			
	NS/C+ (n=39)	NS/C++ (n=21)	S/C+ (n=22)	S/C++ (n=35)
FBG (mg/dL)	87.31±8.97	86.71±6.97	86.59±7.39	86.91±9.14
BMI (kg/m^2)	26.01±3.39	25.52±3.84	24.68±4.92	26.14±5.41
BW (kg)	80.78±12.17	79.33±15.59	76.40±16.59	80.55±16.27
B12 (pg/mL)	337.23±124.73	344.00±98.46	375.63±153.33	377.01±138.44
Folic acid (ng/mL)	9.85±3.05	9.23±2.72	9.27±3.35	10.42±3.79
RBCs (× 10^{12}/L)	5.15±0.61	5.54±1.01	5.38±0.69	5.38±0.68
Hgb (g/dL)	15.95±1.33	15.97±1.44	15.87±0.78	15.98±1.54
PCV (%)	45.74±4.54	47.12±6.04	46.62±4.75	46.47±5.49
MCV (fL)	88.36±4.01	86.36± 3.15	87.61±5.21	86.50±5.86
MCH (pg)	30.91±2.52	29.76±2.43	30.06±3.06	29.51±3.90
MCHC (g/dL)	34.96±2.39	34.46±2.20	34.27±2.40	34.27±2.73

Abbreviations: NS/C+, nonsmoker and moderate coffee consumers (1–2 cups/day); NS/C++, nonsmoker and heavy coffee consumers (>3 cups/day); S/C+, smoker and moderate coffee consumers (1–2 cups/day); S/C++, smoker and heavy coffee consumers (>3 cups/day); FBG, fasting blood glucose; BMI, body mass index; BW, body weight; B12, vitamin B12, RBCs, red blood cells; Hgb, hemoglobin (g/dL); PCV, packed cell volume; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; N, sample size.

Table 4 Lipid Profile Parameters Of The Study Participants Subdivided By Their Combined Smoking (Nonsmoker (NS)/Smoker (S)) And Coffee Consumption (Moderate (C+)/Heavy (C++)) Behavior Into Four Groups (Mean±SD)

Lipid Profile Parameters	Participants (n=117)			
	NS/C+ (n=39)	NS/C++ (n=21)	S/C+ (n=22)	S/C++ (n=35)
TG (mg/dL)	139.58±61.57	139.12±46.92	110.95±58.43	124.13±51.15
TC (mg/dL)	177.23±34.17	195.94±23.69	158.1±24.82	179.9±34.59
HDL-C (mg/dL)	51.64±7.31	50.53±9.69	51.65±8.61	48.39±9.45
LDL-C (mg/dL)	103.06±34.82	118.0 ±19.31	88.6±22.40	108.26±37.57

Abbreviations: NS/C+, nonsmoker and moderate coffee consumers (1–2 cups/day); NS/C++, nonsmoker and heavy coffee consumers (>3 cups/day); S/C+, smoker and moderate coffee consumers (1–2 cups/day); S/C++, smoker and heavy coffee consumers (>3 cups/day); TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; N, sample size.

Table 5 Combined Versus Single Effect Of Cigarette Smoking And Coffee Consumption On Clinical Parameters Of The Participants Using 2-Way ANOVA Test

Clinical Parameters	Cigarette Smoking (CS, n=57)		Coffee Consumption (CC, n=117)		Combined Effects Of CS And CC (n=57)	
	F-Test	P-Value	*F*-Test	*P*-Value	*F*-Test	P-Value
FBG (mg/dL)	0.026	0.873	0.007	0.934	0.080	0.777
BMI (Kg/m^2)	0.152	0.698	0.281	0.597	1.122	0.292
BW (kg)	0.263	0.609	0.191	0.663	0.821	0.367
B12 (pg/mL)	1.984	0.162	0.026	0.872	0.011	0.915
Folic acid (ng/mL)	0.208	0.650	0.161	0.689	1.808	0.182
RBC (× 10^{12}/L)	0.049	0.826	1.521	0.221	1.498	0.224
Hgb (g/dL)	0.016	0.900	0.054	0.816	0.021	0.886
PCV (%)	0.011	0.917	0.303	0.583	0.471	0.494
MCV (fL)	0.084	0.772	2.205	0.141	0.182	0.671
MCH (pg)	0.672	0.415	1.554	0.216	0.195	0.660
MCHC (g/dL)	0.661	0.418	0.213	0.646	0.225	0.637

Abbreviations: FBG, fasting blood glucose; BMI, body mass index; B12, vitamin B12, RBCs, red blood cells; Hgb, hemoglobin; PCV, packed cell volume; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration.

Table 6 Combined Versus Single Effect Of Cigarette Smoking And Coffee Consumption On Lipid Profile Parameters Of The Participants Using 2-Way ANOVA Test

Lipid Profile Parameter	Cigarette Smoking (CS, n=57)		Coffee consumption (CC, n=117)		Combined Effects Of CS And CC (n=57)	
	F-Test	P-Value	*F*-Test	P-Value	*F*-Test	P-Value
TG (mg/dL)	3.567	0.062	0.303	0.583	0.349	0.556
TC (mg/dL)	7.399	0.008	9.823	0.002	0.057	0.812
HDL-C (mg/dL)	0.337	0.563	1.418	0.237	0.342	0.560
LDL-C (mg/dL)	3.424	0.067	6.984	0.010	0.127	0.723

Abbreviations: TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; N, sample size.

MCH, MCV, Age and HDL-C) respectively on TG serum concentration according to the fifth multiple linear regression model indicators (R: 0.894, R^2: 0.800, F-test: 12.808, B1: −27.332, B2: −24.463, B3: 7.220, B4: 23.303, B5:-2.374, p-value: <0.05), n=39, Table 7. The fifth multiple linear regression model by its predictors explained approximately 80.0% of the variance in TG levels in this group. On the other hand, LDL-C, TG, HDL-C and FBG respectively influenced TC serum concentrations according to the fourth multiple linear regression model indicators (R: 0.995, R^2: 0.990, F-test: 421.098, B1: 0.989, B2: 0.204, B3: 0.925, B4: −0.308, p-value: <0.05), which

Table 7 The Multivariate Association Between Hematological Parameters, Age And Lipid Profile Parameters As Independent Variables And Lipid Profile Parameters, Vitamin B12 And Folic Acid As Dependent Variables In Nonsmoker And Moderate Coffee Consumers Group (NS/C+, n=39) By Using Multiple Linear Regression (Stepwise Method)

Dependent Clinical Parameters	Univariate Effects Estimates (Independent Parameters)	Coefficients			
		R	R^2	*F*-Test	P-Value
TG (mg/dL)	MCHC (g/dL)	0.476	0.227	5.875	0.025
	MCH (pg)	0.642	0.412	6.645	0.006
	MCV (fL)	0.814	0.663	11.78	0.000
	Age (years)	0.859	0.738	11.98	0.000
	HDL-C (mg/dL)	0.894	0.800	12.81	0.000
TC (mg/dL)	LDL-C (mg/dL)	0.947	0.896	172.975	0.000
	TG (mg/dL)	0.977	0.954	195.519	0.000
	HDL-C (mg/dL)	0.992	0.985	388.666	0.000
	FBG (mg/dL)	0.995	0.990	421.098	0.000
LDL-C (mg/dL)	TC (mg/dL)	0.947	0.896	172.975	0.000
	TG (mg/dL)	0.980	0.960	227.223	0.000
	HDL-C (mg/dL)	0.993	0.986	434.076	0.000
	FBG (mg/dL)	0.995	0.991	458.942	0.000
B12 (pg/mL)	Folic acid (ng/mL)	0.451	0.204	5.112	0.035
Folic acid (ng/mL)	B12 (pg/mL)	0.451	0.204	5.112	0.035

Abbreviations: TG, triglycerides; MCHC, mean cell hemoglobin concentration; MCH, mean cell hemoglobin; MCV, mean cell volume; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; FBG, fasting blood glucose; B12, vitamin B12; *R*, Pearson linear correlation coefficient; R^2, determinant coefficient.

explained approximately 98.9% of the variance in TC levels among participants in the same mentioned group. LDL-C level was influenced by TC, TG, HDL-C and FBG respectively according to the fourth multiple linear regression model indicators (*R*: 0.995, R^2: 0.991, *F*-test: 458.942, B1: 1.000, B2: −0.206, B3: −0.923, B4: 0.302, *p*-value: <0.05). And this explained approximately 99.1% of the variance in LDL-C levels. Folic acid and vitamin B12 influenced each other interchangeably in a significant way according to the multiple linear regression model indicators (*R*: 0.451, R^2: 0.204, *F*-test: 5.112, B: 17.720, *p*-value: <0.05, which explained approximately 20.4% of the variance in vitamin B12 levels in NS/C+ group.

Nonsmoker And Heavy Coffee Consumers (NS/C++) Group

The multivariate association between hematological parameters, BMI, and LDL-C as IDVs and lipid profile parameters, TG, TC, and LDL-C as DVs showed an influential relationship of the IDV BMI on TG serum concentration according to the multiple linear regression model indicators (*R*: 0.910, R^2: 0.828, *F*-test: 19.197, B: 13.111, *p*-value: <0.05) and that explained approximately 82.8% of the variance in TG levels, n=21, Table 8. TC was influenced by LDL-C and MCH respectively according to the second multiple linear regression model indicators (*R*: 0.992, R^2: 0.983, *F*-test: 87.603, B1: 2.398, B2: 14.400, *p*-value: <0.05), which explained approximately 98.3% of the variance in TC levels. Interestingly, both MCH and Folic acid respectively influenced LDL-C according to the second multiple linear regression model indicators (*R*=0.996, R^2=0.993, *F*-test=209.279, B1= −8.551, B2=1.341, P-value= 0.001), which almost explained the variance in LDL-C levels by 99.3% in NS/C++ group as shown in Table 8.

Smoker And Moderate Coffee Consumers (S/C+) Group

The multivariate association between hematological parameters, BW, TC and TG as IDVs and lipid profile parameters; TG, TC and LDL-C and vitamin B12 as DVs showed an interchangeable influential relationship of the TC and TG serum concentrations on each other, according to the multiple linear regression model indicators (*R*: 0.647, R^2: 0.418, *F*-test9.344, B: 1.738, *p*-value: <0.05) and that explained approximately 41.8% of the variance in TG and TC levels, n=22, Table 9. Besides 31.4% MCV influential effect on LDL-C serum concentrations

Table 8 The Multivariate Association Between Hematological Parameters, BMI And LDL-C As Independent Variables And Lipid Profile Parameters; TG, TC And LDL-C As Dependent Variables In Nonsmoker And Heavy Coffee Consumers Group (NS/C++, n=21) By Using Multiple Linear Regression (Stepwise Method)

Dependent Clinical Parameters	Univariate Effects Estimates (Independent Parameters)	Coefficients			
		R	R^2	*F*-Test	P-Value
TG (mg/dL)	BMI (kg/m^2)	0.910	0.828	19.197	0.012
TC (mg/dL)	LDL-C (mg/dL) MCH (pg)	0.912 0.992	0.831 0.983	19.661 87.603	0.011 0.002
LDL-C (mg/dL)	MCHC (g/dL) Folic acid (ng/mL)	0.970 0.996	0.941 0.993	63.997 209.279	0.001 0.001

Abbreviations: TG, triglycerides; BMI, body mass index; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; R, Pearson linear correlation coefficient; R^2, determinant coefficient.

Table 9 The Multivariate Association Between Hematological Parameters, Body Weight TC, And TG As Independent Variables And Lipid Profile Parameters; TG, TC And LDL-C And Vitamin B12 As Dependent Variables In Smoker And Moderate Coffee Consumers Group (S/C+, n=22) By Using Multiple Linear Regression (Stepwise Method)

Dependent Clinical Parameters	Univariate Effects Estimates (Independent Parameters)	Coefficients			
		R	R^2	*F*-Test	P-Value
TG (mg/dL)	TC (mg/dL)	0.647	0.418	9.344	0.009
TC (mg/dL)	TG (mg/dL)	0.647	0.418	9.344	0.009
LDL-C (mg/dL)	MCV (fL)	0.560	0.341	5.949	0.030
B12 (pg/mL)	PCV (%) Body weight (kg)	0.658 0.803	0.433 0.645	9.918 10.878	0.008 0.002

Abbreviations: TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; MCV, mean vell Volume; PCV, packed cell volume; *R*, Pearson linear correlation coefficient; R^2, determinant coefficient.

according to the multiple linear regression model indicators (*R*: 0.560, R^2: 0.314, *F*-test: 5.949, B: 2.538, *p*-value: <0.05). Then, vitamin B12 was influenced by PCV and BW respectively according to the second linear regression parameters (*R*: 0.803, R^2: 0.645, *F*-test: 10.878, B1: 32.014, B2: 4.676, *p*-value: <0.05), and this explained 64.5% of the variance in vitamin B12 levels in NS/C+ group.

Smoker And Heavy Coffee Consumers (S/C++) Group

The multivariate association between hematological parameters and lipid profile parameters as IDVs and lipid profile parameters and vitamin B12 as DVs showed an influential relationship of HDL-C and MCV respectively on TG according to the second linear regression model indicators (*R*: 0.703, R^2: 0.495, *F*-test: 8.818, B1: −3.103, B2: 4.906, *p*-value: <0.05) and that explained approximately 64.5% of the variance in TG levels, n=35, Table 10. Besides 93.5% approximate explanation of the variance in TC levels was due to the influential effect of both LDL-C and TG based on the second linear regression model indicators (*R*: 0.967, R^2: 0.935, *F*-test: 129.863, B1: 0.784, B2: 0.169, *p*-value: <0.05). Then, TC and RBCs influenced HDL-C based on the second linear regression model indicators (*R*: 0.647, R^2: 0.419, *F*-test: 6.497, B1: −0.153, B2: −5.144, *p*-value: <0.05), and this explained 41.9% of the variances in HDL-C levels in the mentioned group. On the other hand, LDL-C was influenced by TC, TG, and HDL-C respectively according to the third linear regression analysis indicators (*R*: 0.970, R^2: 0.941, *F*-test: 91.044, B1: 1.106, B2: −0.229, *p*-value: >0.05) with no significant difference, and this explained 94.1% of the variances in LDL-C serum concentrations. Last, vitamin B12 and folic acid was interchangeably influenced by each other according to the multiple linear regression parameters (*R*: 0.635, R^2: 0.403, *F*-test: 12.815, B: 21.353, *p*-value: <0.05), and this explained 40.3% of the variance in vitamin B12 levels in NS/C+ group.

Table 10 The Multivariate Association Between Hematological And Lipid Profile Parameters As Independent Variables And Lipid Profile Parameters And Vitamin B12 As Dependent Variables In Smoker And Heavy Coffee Consumers Group (S/C++, n=35) By Using Multiple Linear Regression (Stepwise Method)

Dependent Clinical Parameters	Univariate Effects Estimates (Independent Parameters)	Coefficients			
		R	R^2	*F*-Test	P-Value
TG (mg/dL)	HDL-C (mg/dL) MCV (fL)	0.472 0.703	0.222 0.495	5.433 8.818	0.031 0.002
TC (mg/dL)	LDL-C (mg/dL) TG (mg/dL)	0.930 0.967	0.865 0.935	122.115 129.863	0.000 0.000
HDL-C (mg/dL)	TC (mg/dL) RBCs ($\times 10^{12}$/L)	0.524 0.647	0.275 0.419	7.201 6.497	0.015 0.008
LDL-C (mg/dL)	TC (mg/dL) TG (mg/dL) HDL-C (mg/dL)	0.930 0.962 0.970	0.865 0.926 0.941	122.115 112.348 91.044	0.000 0.000 0.000
B12 (pg/mL)	Folic acid (ng/mL)	0.635	0.403	12.815	0.002
Folic acid (ng/mL)	B12 (pg/mL)	0.635	0.403	12.815	0.002

Abbreviations: TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; MCV, mean cell volume; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; RBCs, red blood cells; B12, vitamin B12; *R*, Pearson linear correlation coefficient; R^2, determinant coefficient.

Discussion

In this study, it was demonstrated that LDL-C levels were more positively associated with coffee consumption than cigarette smoking. Also, lower LDL-C and TC levels were noted in **S/C+** group compared to all other groups of the study. Accordingly, our findings may refer to a potential absorptive effect of cigarette smoking and/or moderate coffee consumption (MCC) on the negative consequences of excessive consumption of coffee as shown in the combined effect of both factors by the results of two-way ANOVA test. However, hyperlipidemic levels of LDL-C and TC have been correlated with the separate effect of HCC and cigarette smoking in humans[18,27,28] and animal models.[29–32] Nevertheless, some studies showed that caffeine has significant effects[11,23] or no effect on the lipid profile.[22] It can be concluded that feedback from studies linking coffee consumption to blood lipid levels and its relationship to CVD risk is still vague and needs more research depth. Despite the importance of factors such as coffee type and method of preparation,[33–36] the rate of consumption is now gaining more attention in the relation to lipid profile alterations.[33,37]

Accordingly, monitoring of lipid profile alterations is considered an essential public health tool for the prevention or reduction of the risk of CVD[38,39] despite a causal relationship and practical consequences for treatment are less clear.[40]

In consistent with our findings, Panagiotakos et al indicated that MCC showed a weak correlation with hypercholesterolemia compared to HCC or very HCC.[33] Further and based on the evidences from recent studies, MCC is safe and beneficial in both healthy persons as well as patients with CVD or DM.[41] Results of a systematic review for four prospective studies (196,256 participants, 41,184 diagnosis of hypertension) indicated that habitual MCC is not associated with higher risk of hypertension.[42] Many studies have positively addressed HCC and cigarette smoking as stress associated factors.[43,44] Accordingly, the variations of stress predictors such as LDL-C and TC were mostly coffee rate consumption dependent, as seen in prior associated studies.[44,45] In this context, our results confirmed the importance of the daily coffee consumption rate on an individual's health as noted in previous studies.

It is well known that catecholamines and cortisol mediate short and long terms of stress respectively[46] including the stress effects of caffeine and/or nicotine.[47]

Nicotine stimulates the secretion of catecholamines which enhances lipolysis.[28] Moreover, caffeine acts synergistically in the presence of lipolytic hormones, such as epinephrine. It was previously pointed that the effect of caffeine on adrenoceptors is dose dependent.[48] Blocking beta-adrenoceptors decreased caffeine effect, which indicates that some of the metabolic effects of caffeine are mediated by the catecholamines.[49]

The Adenosine receptor (A2b) in turn regulates hyperlipidemia and atherosclerosis.[50] Therefore, adenosine mediated mechanism is a possible alternative pathway that may explain how MCC reduced LDL-C and TC in smokers as shown in this study. Caffeine is most likely exerted an antagonism mechanism by blocking adenosine receptors (mainly A_{2A} receptor), thereby competitively inhibiting its action.[51,52] Many recent reports displayed predictions for cholesterol interaction sites on the A_{2A} adenosine receptor.[53,54]

Furthermore, it has been hypothesized that caffeine concentration inversely interfered with internalization of LDL-C.[55] However, coadministration of both caffeine and nicotine showed a synergistic inhibitory effect on adenosine receptors' activity when compared to either nicotine or caffeine treated mice.[56] Thus, we suggest that moderate doses of caffeine produce metabolic effects that are different from those of large doses via up-down regulatory mechanism. This may also clarify in part the acceleration of drive lipid oxidation associated with HCC.

Another important finding of this study was that serum vitamin B12 levels were more positively associated with cigarette smoking factor than coffee consumption factor. Similar findings were reported that the coffee consumption has neither short- nor long-term detectable effects on lipid peroxidation or on plasma Hcy concentrations in healthy nonsmokers.[57] Moreover, no longer association has been observed between coffee consumption and plasma Hcy after adjustment for plasma folic acid concentration.[34,58] Conversely, MCC was associated with lower risk of hyperhomocysteinemia and could exert a protective effect against some cardiovascular risk factors.[59]

Although the results of studies on vitamin B12 and folic acid as well as for Hcy levels in smokers are still unclear,[60,61] higher levels of vitamin B12 in smokers as shown in our study may represent a protective role for smokers who are C+.[61] Ulvik et al showed that coffee consumption was associated with reduced plasma concentration of folate.[13] However, inconsistencies among the prospective or cross-sectional studies require more attention of the controlled clinical trials to clarify the effect of coffee on vitamin B12 and folic acid levels.

Consequently, stepwise regression test observed that TG levels were the most crucial factor responsible for the significant variation in TC and LDL-C associated with the rate of coffee consumption. Prior studies have shown that increased consumption of unfiltered coffee results in a dose-dependent increase in serum levels of TG, TC, and LDL-C.[7,62] Based on a review of 54 published studies, TC, TG, and LDL-C levels in smokers were 3%, 9.1%, and 1.7% respectively higher than nonsmokers.[3] These findings were inconsistent with multivariate associations between TG and LDL-C with TC seen in smoker groups of this study. Regarding TC level alterations, approximately 40% was explained by the effect of TG in S/C+ group, whereas in S/C++ group, LDL-C explained about 85% of those alterations. Therefore, the combination of HCC with cigarette smoking may increase TC and LDL-C levels via nicotine-mediated stress mechanism results in increased hepatic synthesis of TG and very-low-density lipoprotein cholesterol (VLDL-C) as pointed previously.[6] Finally, smoking-induced erythropoiesis associated factors such as MCV and PCV were significantly positively correlated with serum levels of LDL-C and vitamin B12 in S/C+ group. In the same group, TC levels were significantly correlated with TG levels. These notable findings indicated that significant alterations in LDL-C and vitamin B12 levels were more associated with cigarette smoking than coffee consumption, whereas elevated LDL-C levels seen in S/C++ were strongly associated with TC levels (R= 0.930, R^2= 0.865). Overall, our findings confirmed that HCC associated with elevated TC and LDL-C levels in male smokers.

Study Limitations

The major limitation of this study was that coffee consumption information was collected by a self-administered questionnaire. In addition, we have no information about the amount of coffee consumed per cup, particularly the cup size is varied with ranges of 150–200 mL. Also, we did not measure stress markers such as cortisol to evaluate coffee consumption induced stress on the lipid profile parameters.

Conclusion

Our findings showed that HCC was more associated with hyperlipidemia than cigarette smoking. Accordingly, we conclude that MCC may reduce CVDs risk or their consequences in male.

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Disclosure

The authors report no conflicts of interest in this work.

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