Effect of Coffee Consumption on Blood Glucose and Lipid Profile Levels in Male Students at Nnamdi Azikiwe University, Nnewi Campus, Anambra State.

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ABSTRACT

Introduction: Coffee is one of the most consumed beverages worldwide and it contains several biologically active components which may have an impact on human health. **Aim**: This is an experimental study designed to evaluate the effect of coffee intake on plasma glucose and serum lipid profile levels in male students of College of Health Science, Anambra State, Nigeria. **Methods:** A total of 30 male participants aged between eighteen (18) and thirty (30) years were randomly recruited for the study. Five milliliters (5mls) of baseline samples (after an overnight fast) were collected from participants at day 0 as baseline samples and levels of glucose and lipid profile were evaluated. Subsequently, in addition to their normal diet, each of the participants received a cup of caffeinated coffee prior to their breakfast daily for a period of 21 days. After overnight fasting, post research (test 1st & 2nd) samples (fasting blood sample) were collected on days 11 and 22 respectively and the levels of glucose and lipid profile were re-evaluated. Plasma glucose and lipid concentrations were determined using enzymatic methods respectively. Also, the body mass index (BMI) and blood pressure readings of participants were determined before and after coffee consumption. **Results:** The results showed that there were significant increases in the mean plasma glucose and serum HDL-C concentrations 11 days (intermediate consumption) and 22 days (post consumption) following coffee intake when compared with baseline levels respectively ($p<0.05$). However, the mean BMI, SBP, DBP and serum levels of TC, TG and LDL-C remained unaltered after coffee consumption ($p>0.05$) respectively. **Conclusion:** The present study has shown that short term consumption of coffee may cause significant alterations in plasma concentration of glucose and serum HDL-C levels with no significant effects on BMI and serum concentrations of TC, TG and LDL-C. However, further studies using larger population size may be necessary in validating these findings.

KEY WORDS: Coffee, Plasma glucose, lipid profile, diabetes, cardiovascular diseases.

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INTRODUCTION

Non-communicable diseases (NCDs) are diseases that are not transmissible directly from one person to another. NCDs also known as chronic diseases tend to be of long duration and are the result of a combination of genetic, physiological, environmental and behavioral factors (1). There are four main classes of NCDs including cardiovascular diseases (CVDs), cancers, chronic respiratory diseases and diabetes. Together, these four groups of diseases account for over 80% of all premature non-communicable disease (NCD) deaths (1). Globally, NCDs result in the death of 41 million people each year, accounting for 71% of all deaths worldwide and each year, 15 million people die from NCD between the ages of 30 and 69 years; with over 85% of these premature deaths occurring in low and middle income countries with cardiovascular diseases accounting for most NCD deaths (17.9 million deaths) and 1.6 million deaths resulting from diabetes each year (1).

Diabetes is a global health problem, with the World Health Organisation (WHO) estimating the prevalence to be 9% of the adult population in 2014 (2). It affects almost 350 million people worldwide, the majority of which (90%) have T2DM. If poorly controlled, it can result in serious complications, including cardiovascular disease, nephropathy and retinopathy, and it is estimated to have caused 1.5 million deaths in 2012 (2). On the other hand, cardiovascular diseases (CVDs) are a group of disorders of the heart and blood vessels. CVDs are the number 1 cause of death globally: more people die annually from CVDs than from any other cause. In 2016, an estimated 17.9 million people died from CVDs, representing 31% of all global deaths. Of these deaths, 85% are due to heart attack and stroke (3). Also, In Nigeria, CVDs accounted for 11% of all deaths in 2018 (1). Metabolic disorders, such as obesity, dysregulated glucose homeostasis, dyslipidemia, and abnormal elevation of systolic and diastolic blood pressure are important risk factors for cardiovascular disease (CVD) and are among the major contributors for overall mortality (4). Overweight and obese population have rapidly increased worldwide leading to a concomitant rise of type 2 diabetes incidence, especially in the highest income regions. Hypertension and dyslipidemia affect 20%–40% of the population, showing a significant association with elevated BMI, waist circumference, and fasting blood glucose (6). Altogether, these conditions represent a major public health issue that could potentially be reduced by the adoption of a healthier lifestyle. Importantly, both CVDs and diabetes are caused by modifiable factors such as behavioural risk factors such as tobacco use; unhealthy diet and obesity, physical inactivity and harmful use of alcohol (1).

Coffee is a complex mixture of more than a thousand different chemicals, many of which are reported to be biologically active (7). There are two main species of coffee which are traditionally used for making Arabica and Robusta coffees, with the former representing the most diffused species worldwide (8). It has been reported that arabica coffee contains more lipids, while robusta contains more caffeine and polyphenols (9). Coffee contains large amounts of bioactive compounds including caffeine, phenolic compounds, trigonelline, diterpenes and soluble fiber (10).

Among phenolic compounds, chlorogenic acids (CGAs) are the most abundant in coffee, representing more than 98% of its total phenolic content, while the remaining 2% is composed of alkylmethoxyphenols, alkylphenols, methoxyphenols, and other phenolics such as pyrogallol, catechol, and phenol (11). Coffee has been extensively studied for antioxidant activity (12-14) and

has been described as probably the most relevant source of dietary antioxidant compounds (15), which are thought to counteract the action of reactive oxygen species (ROS), the main contributors to the development of oxidative stress.

Furthermore, a number of recent studies reported a substantial positive effect of coffee consumption on human health, especially in relation with cardio-metabolic risk factors (16-18). Coffee consumption seems to be reasonably associated with decreased risk of diabetes and cardiovascular disease (CVD) (19-21). Although, a number of authorities have evaluated the effect of coffee consumption on plasma glucose and serum lipid profile levels in other countries, researches in this regard seems to be scanty in Nigeria. Therefore, the present study seeks to evaluate the effect of coffee consumption on plasma glucose and serum lipid profile levels in male students of College of Health Sciences and Technology, Anambra State, Nigeria.

MATERIALS AND METHODS

Study design

This is an experimental study designed to evaluate the effect of coffee intake on plasma glucose and serum lipid profile levels in male students of College of Health Sciences, Anambra State, Nigeria. A total of 30 male participants within the age range of eighteen (18) to thirty (30) were randomly recruited for the present study. The protocols for the study were properly explained to the prospective participants and thereafter, written consents were obtained from participants prior to the commencement of the study. Each participant was advised to abstain from coffee and similar food consumption for a period of three weeks. Afterwards, 6mls each of baseline samples (after an overnight fast) was collected from the participants at day 0 as baseline samples, and levels of glucose and lipid profile (TC, TG, LDL-C, HDL-C) were evaluated. Subsequently, in addition to their normal diet, each of the participants received a cup of caffeinated coffee early in the morning before meal daily for a period of 21 days. After an overnight fast, 6mls of post research (1^{st} test ^{and} 2^{nd} test) samples was collected on day 11 and 22 respectively and the levels of glucose and lipid profile were re-evaluated. Blood glucose (fasting blood sugar) and lipid concentrations (Total cholesterol, Triglyceride, Low density lipoprotein cholesterol and High-density lipoprotein cholesterol) were determined using enzymatic methods. Also, a structured questionnaire was used to obtain relevant information such as age, height, sex, demographic factors, dietary patterns, physical activities, medical history, lifestyle and history of coffee intake, while participants' weight and blood pressure readings were obtained using weighing scale and sphygmomanometer respectively before and after coffee consumption.

Preparation of coffee

The coffee used for this study was commercially prepared and marketed by Tesco. Each of the participants was given the same quantity of coffee comprising of a mixture of 150milliliter of water and 1gram of coffee powder.150ml of hot water was measured using a measuring cup and a gram of coffee measured using a weighing scale was added to it. Afterwards, 50mg of non-dairy creamer was added.

Inclusion criteria

Apparent healthy male participants aged between 18 and 30 years who consented to the study were included for this study.

Exclusion criteria
Individuals consuming coffee and similar foods, Diabetic and hypertensive individuals, and those diagnosed with ulcer and cardiovascular disease, alcoholics and smokers or those outside the age bracket of 18-30 years were excluded from the present study.

Ethical Consideration
The ethical approval for this study was sought and obtained from the Ethics Committee of Faculty of Health Sciences and Technology, Anambra State, Nigeria (reference number:ERC/FHST/NAU/2018/169 and dated: September 24, 2018).

Estimation of total cholesterol (TC)
Total Cholesterol level was estimated using enzymatic method as described by Roeschlau *et al*. (22).

Estimation of triglycerides
Triglyceride level was estimated with the enzymatic method as described by Tietz, (23).

Estimation of high-density lipoprotein cholesterol (HDL-C)
HDL level was estimated using the method described by Burstein *et al.* (24).

Estimation of low-density lipoprotein cholesterol (LDL-C)
LDL level was estimated using the enzymatic method described by Assman *et al*. (25).

Estimation of plasma glucose (FBS)
Plasma glucose level was determined using glucose oxidase method as described by Barham and Trinder, (26).

Statistical analysis
The data obtained was statistically analyzed using Statistical package for Social Sciences (SPSS) Version 23.0. Paired students' t-test were used to compare means. The results were expressed as mean±SD and confidence limit was chosen at 95% ($p<0.05$). $p<0.05$ was considered statistically significant.

RESULTS
The result of analysis of variance showed that the mean concentrations of plasma glucose and serum high density lipoprotein cholesterol (HDL-C) were significantly different amongst the groups ($F=11.246$ and 29.387) ($P<0.05$) respectively, whereas, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), total cholesterol (TC), triglyceride (TG) and low density lipoprotein cholesterol (LDL-C) levels did not differ significantly amongst the groups ($P>0.05$). See table 1.
There were no significant differences in the mean values of BMI, DBP, SBP when compared between baseline (day 0), intermediate consumption (day 11) and post (day 22) coffee consumption respectively ($p>0.05$). Also, the mean serum levels of TC, TG and LDL-C did not differ significantly when compared between the groups ($p>0.05$). See table 1.
However, a significant increase was observed in the mean level of plasma glucose when compared between baseline (0 day) and intermediate consumption (day 11) of coffee (3.93±0.39 Vs 4.37±0.32; $p=0.011$). Also, there was a significant increase in the mean plasma concentration of glucose when compared between baseline and post (day 22) coffee intake (3.93±0.39 Vs 4.71±0.69; $p=0.000$). Furthermore, the mean plasma glucose concentration differed significantly when compared between the intermediate (day 11) and post (day 22) coffee consumption (4.37±0.32 Vs 4.71±0.69; $p=0.042$). See table 1.

Interestingly, the mean serum HDL-C level was significantly increased in intermediate consumption of coffee than in baseline (3.21±0.98 Vs 1.11±0.98; p=0.000). Also, the mean serum HDL-C level was significantly increased in post consumption of coffee than in baseline (2.97±1.17 Vs 1.11±0.98; p=0.000), but the mean serum level of HDL-C did not differ significantly when compared between intermediate and post coffee consumption (p>0.05). See table 1.

BMI, Blood pressure, plasma glucose and serum lipid profile levels of male students before and after coffee consumption (Mean±SD; n=20).

Variables	BMI (Kg/m^2)	SBP (mmHg)	DBP (mmHg)	Glucose (mmol/L)	TC (mmol/L)	TG (mmol/L)	HDL-C(mmol/L)	LDL-C (mmol/L)
Baseline (1)	22.27 ±3.21	122.00 ±13.08	77.94 ±8.67	3.93 ±0.39	5.05 ±1.34	1.17 ±0.41	1.11 ±0.32	3.54 ±1.31
Intermediate consumption (2)	22.39 ±3.03	121.44 ±12.77	74.44 ±9.27	4.37 ±0.32	4.59 ±1.13	0.97 ±0.44	3.21 ±0.98	3.21 ±0.98
Post consumption (3)	21.94 ±3.02	122.17 ±9.98	78.39 ±7.76	4.71 ±0.69	4.47 ±1.12	1.07 ±0.26	2.97 ±1.17	2.97 ±1.17
1 Vs 2 (p-value)	0.915	0.890	0.227	0.011*	0.260	0.119	0.000*	0.388
1 Vs 3 (p-value)	0.748	0.967	0.877	0.000*	0.152	0.452	0.000*	0.142
2 Vs 3 (p-value)	0.668	0.858	0.174	0.042*	0.753	0.411	0.426	0.537

***Results are expressed as Mean±SD and are statistically significant at p<0.05(Confidence Interval (CI) is set at 95%).**

DISCUSSION

Coffee has been the focus of major attention due to its global consumption and impact on human health (27). It is among the most widely consumed pharmacologically active beverages, and its consumption has become a regular part of daily life worldwide (28). Coffee contains several biological active compounds which may affect human health (29).

In this study, the effect of coffee consumption on plasma glucose and lipid profile levels in male students was evaluated. The present finding indicates that there was no significant effect of coffee consumption on the body mass index (BMI) of the participants. A potential explanation to this result could be related to the quantity of coffee consumed by the participants. A study by Gavrieli *et al.* stated that by increasing the amount of coffee there is also a potential alteration of the equilibrium between the actions of the different coffee compounds (30). In line with this, Salinardi *et al.* hypothesized that when a small amount of coffee is consumed, the dominant effect comes from the compounds that promote energy intake, but when a higher coffee amount is consumed, then the balance is changed in favor of compounds such as caffeine, chlorogenic acids and mannooligosaccharides that prevent energy consumption thereby inducing a reduction in body weight (31). However, some authorities had earlier recorded differing result from our present finding (32-36).

Also, the present study observed no significant effects of coffee consumption on the mean values of DBP and SBP when compared between before and after coffee

consumption. The possible reason for this result is not clearly understood. This is in line with the finding of Mesas *et al*. who recorded no significant difference in the mean blood pressure after two weeks of coffee consumption (37). In contrast to the present study, Nurminen *et al*. suggested that acute coffee intake increases blood pressure (38).

Interestingly, there was a significant increase in the mean glucose value when compared before and after coffee consumption. This increase may have been potentiated by the caffeine component of the coffee which causes alterations in glucose homeostasis by decreasing glucose uptake into skeletal muscle, thereby causing elevations in blood glucose concentration. Previous studies have documented acute hyperglycemia and reduced insulin sensitivity or impaired glucose tolerance after short term consumption of coffee (39-41). The present finding is in consonance with some previous studies which reported that the ingestion of caffeinated coffee with either a high or low glycemic index (GI meal) significantly impairs acute blood glucose management and insulin sensitivity thereby resulting in increased mean glucose level (42-44). Additionally, Van-Dam *et al.* observed an increase in mean glucose value after coffee consumption in a population-based Hoorn Study, which included Dutch men and women (45). Furthermore, in a systematic review and meta-analysis, Shi *et al.* concluded that coffee intake might shift glycemic homeostasis toward hyperglycemia (46). However, some other studies have also documented results which are in contrast with the present results (47-49).

This present study observed no significant differences in the mean serum TC, TG and LDL-C levels in the studied subjects when compared before and after coffee consumption. This may be due to the type of coffee beans utilized in this study or it could be as a result of the quantity of coffee consumed in the present study. The coffee beans used in this research was derived from *Coffea robusta* which has been reported to have fewer amounts of cafesol and kahweol than other types of coffee (50). Cafestol, and to a lesser extent kahweol, have been shown to increase total cholesterol, low-density lipoprotein cholesterol (LDL-C) and triglyceride concentrations, without substantial effects on high-density lipoprotein cholesterol (HDL-C) concentrations (51, 52). Also, previous studies have indicated that increased consumption of coffee results in a dose-dependent increase in serum levels of TC, LDL-C and triglycerides (TG) (53-55). Relatively small quantities of coffee was consumed by the subject compared to the quantity consumed in other previous research works hence the result. Consistent with these findings, Zargar *et al*. reported no significant changes in the serum levels of TC and LDL-C with a significant reduction in TG level following coffee consumption in a study population of 49 adult participants (56). Furthermore, the reports of Karabudak *et al.* is in keeping with the present study (55). By contrast, a meta-analysis study by Cai *et al*. showed that coffee was directly related to increased total cholesterol, LDL-cholesterol and triglycerides when consumed boiled or non-filtered (57). Similarly, Rezq and Fathy, (58) revealed that administration of boiled and Turkish coffee induced a significant increase in atherogenic index represented as increase in total lipids, TG, TC, LDL-C and VLDL-C. Also, Abd El-Fatta, (59) showed a significant elevation of serum total cholesterol, TC, TG, LDL-C, with a significant decrease of HDL-C in rats fed on diet supplemented with low or high dose of coffee. Furthermore, Onuegbu *et al.* observed a significant increase in the mean

total serum cholesterol concentration and LDL-cholesterol concentration in healthy human subjects after regular administration of coffee (60).

Another key finding in this present study is a significant increase in the mean serum level of HDL-C when compared before and after coffee consumption. However, no significant difference was observed in the mean serum level of HDL-C when compared between intermediate and post coffee consumption. The reason for the alterations in the level of HDL-C is not clearly understood but could however be attributed to the additives used in this study. Cheung *et al.* which suggested that the addition of flavoring substances, such as sugar and cream, to coffee resulted in significant alterations in the serum level of HDL-C (61). However, the findings in this study is in contrast with work by Onuegbu *et al.* who reported non-significant difference in the mean HDL cholesterol concentration when healthy humans were administered coffee over a period of time (60). Driessen *et al.* observed no association of coffee consumption with HDL cholesterol (62). Karabudak *et al.* indicated that there was no significant association between coffee consumption and serum HDL-C level (55).

Conclusion

In conclusion, the present study have shown that short term consumption of coffee may cause significant alterations in plasma glucose and serum HDL-C concentrations without significant alterations in the mean BMI and serum concentrations of TC, TG and LDL-C. However, further studies using larger population size may be necessary in validating these findings.

REFERENCES

1. WHO (2018). Fact sheets: Non-communicable diseases (NCD). www.who.int/news_room/fact-sheet/detail/noncommunicable-diseases. Retrieved 20 April, 2019.

2. World Health Organisation (WHO) (2015). Fact Sheet 312 - Diabetes. Available from: http://www.who.int/mediacentre/factsheets/fs312/en/. Retrieved April 12, 2019.

3. WHO (2017). Fact sheets: Cardiovascular diseases (CVDs). www.who.int/news_room/fact-sheet/detail/cardiovasculardiseases-(cvds). Retrieved 20 April, 2019.

4. GBD. Mortality and Causes of death collaborators, Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet, 2015; 385 (9963): 117–171.

5. Danaei G, Finucane MM, Lu Y, Singh GM, Cowan MJ, Paciorek CJ, et al. Global Burden of Metabolic Risk Factors of Chronic Diseases Collaborating, National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. Lancet, 2011, 378:31–40.

6. Hedner T, Kjeldsen SE, Narkiewicz K. State of global health—hypertension burden and control. Blood Pressure, 2012; 21 (Suppl. 1):1–2.

7. Butt MS, Sultan MT. Coffee and its consumption: benefits and risks. Crit Rev Food Science and Nutrition, 2011; 51(4):363-373.

8. International Coffee Organization. Trade statistics Available online:

http://www.ico.org/show_news.asp?id=656. Retrieved on 30 April 2019.

9. Cagliani LR, Pellegrino G, Giugno G, Consonni R. Quantification of *Coffeaarabica* and *Coffeacanephora var. robusta* in roasted and ground coffee blends. Talanta, 2013; 106:169–173.

10. Farah A. Coffee Constituents. In Coffee: Emerging Health Effects and Disease Prevention; Chu, Y.F., Ed.; Wiley-Blackwell: Oxford, UK, 2012; pp. 21–58.

11. Tresserra-Rimbau A, Medina-RemónA, Estruch R, Lamuela-Raventós RM. Coffee Polyphenols and High Cardiovascular Risk Parameters. In Coffee and Health and Disease Prevention; Preedy, V.R., Ed.; Academic Press: San Diego, CA, USA, 2014; pp. 387–394.

12. CarvalhoDdo C, Brigagao MR, dos Santos MH, de Paula FB, Giusti-Paiva A, Azevedo L. Organic and conventional Coffeaarabica L.: a comparative study of the chemical composition and physiological, biochemical and toxicological effects in Wistar rats. Plant Foods and Human Nutrition, 2011; 66:114–121.

13. Correa TA, Monteiro MP, Mendes TM, Oliveira DM, Rogero MM, Benites CI, et al. Medium light and medium roast paper-filtered coffee increased antioxidant capacity in healthy volunteers: results of a randomized trial. Plant Foods Hum Nutrition, 2012; 67:277–282.

14. Viana AL, Fonseca M, Meireles EL, Duarte SM, Rodrigues MR, Paula FB. Effects of the consumption of caffeinated and decaffeinated instant coffee beverages on oxidative stress induced by strenuous exercise in rats.Plant Foods and Human Nutrition, 2012; 67:82–87.

15. Vinson JA. Polyphenols: Total amounts in foods and beverages and U.S. per capital consumption. Abstract number AGFD 10; Proceedings of the American Chemical Society 230th National Meeting; Washington, DC, USA, 2005.

16. Abrahao SA, Pereira RG, de Sousa RV, Lima AR, Crema GP, Barros BS. Influence of coffee brew in metabolic syndrome and type 2 diabetes. Plant Foods and Human Nutrition, 2013; 68:184–189.

17. Salomone F, Li Volti G, Vitaglione P, Morisco F, Fogliano V, Zappala A, et al. Coffee enhances the expression of chaperones and antioxidant proteins in rats with nonalcoholic fatty liver disease. Transl Res, 2013; 163:593–602.

18. Grosso G, Marventano S, Galvano F, Pajak A, Mistretta A. Factors associated with metabolic syndrome in a Mediterranean population: role of caffeinated beverages. J Epidemiol, 2014; 24:327–333.

19. O'Keefe JH, Bhatti SK, Patil HR, DiNicolantonio JJ, Lucan SC, Lavie CJ. Effects of habitual coffee consumption on cardiometabolic disease, cardiovascular health, and all-cause mortality. J Am College of Cardiology, 2013; 62:1043–1051.

20. Jiang X, Zhang D, Jiang W. Coffee and caffeine intake and incidence of type 2 diabetes mellitus: a meta-analysis of prospective studies. Eur J of Nutrition, 2014; 53:25–38.

21. Shokouh P, Jeppesen PB, Hermansen K, Laustsen C, Stødkilde-Jørgensen H, Hamilton-Dutoit SJ, et al. Effects of Unfiltered Coffee and Bioactive Coffee Compounds on the Development of Metabolic Syndrome Components in a

High-Fat-/High-Fructose-Fed Rat Model. Nutrients, 2018; 10(1547):2-15.

22. Roeschlau P, Bernt E, Gruber JW. Enzymatic procedure for cholesterol determination.*J Clinic Chemistry and Clinical Biochemistry, 1974;* 12: 403.

23. Tietz NW, Saunders WB. & Co. *Clinical Guide to Laboratory Tests;* 3rd Edition, Philadelphia, 1995; Pp 578-580.

24. Burstein M, Scholnick HR, Morfin R. Rapid method for the isolation of lipoproteins from serum by precipitation with polyanions. Scand J Clinical and Laboratory Investigation, *1980;* 40: 583-595.

25. Assman G, Jabs HU, Kohnert U, Nolte W, Schriewer H. LDL-C determination in blood serum following precipitation of LDL with polyvinyl sulphate. *AnalyticaChimica Acta,*1984; 140: 77-83.

26. Barham D, Trinder P. Determination of Blood Glucose using Glucose oxidase method. *Analyst*; 1972; 97:142.

27. Giuseppe G, Agnieszka M, Godos J, Andrzej P, Salvatore S, Bes-Rastrollo M, et al. Long-Term Coffee Consumption Is Associated with Decreased Incidence of New-Onset Hypertension: A Dose–Response Meta-Analysis. *Nutrients*, 2017; **9**(8): 890.

28. Jae-Hoon B, Jae-Hyung P, Seung-Soon IM, Dae-Kyu S. Coffee and health. Integrative medicine research, 2014; 3(4):189–191.

29. Godos J, Pluchinotta FR, Marventano S, Buscemi S, Li-Volti G, Galvano F, et al. Coffee components and cardiovascular risk: beneficial and detrimental effects. Int J Food Science and Nutrition, 2014; 65(8): 925-936.

30. Gavrieli A, Karfopoulou E, Kardatou E, Spyreli E, Fragopoulou E, Mantzoros CS, et al. Effect of Different Amounts of Coffee on Dietary Intake and Appetite of Normal Weight and Overweight/Obese Individuals.Obesity, 2013; 21**:** 1127-1132.

31. Salinardi TC, Rubin KH, Black RM. Coffee mannooligosaccharides, consumed as part of a free-living, weight-maintaining diet, increases the proportional reduction in body volume in over weight men.The Journal of Nutrition, 2010; 140:1943-1948.

32. Bouchard DB, Robert R, Ian J. Coffee, Tea and Their Additives: Association with BMI and Waist Circumference. Obesity Facts, 2010; 3**:**345–352.

33. Ohnaka K, Ikeda M, Maki T, Okada T, Shimazoe T, Adachi M. Effects of 16-week consumption of caffeinated and decaffeinated instant coffee on glucose metabolism in a randomized controlled trial. J Nutrition and Metabolism, 2012; 2012:207-426.

34. Lelyana R. Effect of Coffee Daily Consumption Uric Acid Level and Body Weight to Prevent Metabolic Syndrome.JNanomed Nanotechnology, 2016; 7**:** 400.

35. Jeonghee L, Hye YK, Jeongseon K. Coffee Consumption and the Risk of Obesity in Korean Women. Nutrients, 2017; 9(12): 1340.

36. Muhammad HFL, Sulistyoningrum DC, Huriyati E, Lee YY, Muda WAW. The Interaction between Coffee: Caffeine Consumption, UCP2 Gene Variation, and Adiposity in Adults—ACross-Sectional Study. Journal of Nutrition and Metabolism, 2019; 2019:2-7, Article ID 9606054, https://doi.org/10.1155/2019/9606054.

37. Mesas AE, Leon-Muñoz LM, Rodriguez-Artalejo F, Lopez-Garcia E. The effect of coffee on blood pressure and cardiovascular disease in hypertensive individuals: a systematic review and meta-analysis. Am J Clinical Nutrition, 2011; 94(4):1113-1126.

38. Nurminen ML, Niittynen L, Korpela R, Vapaatalo H. Coffee, caffeine and blood pressure: a critical review. Euro J Clinical Nutrition, 1999; 53(11):831-839.

39. Tunnicliffe JM, Shearer J. Coffee, glucose homeostasis, and insulin resistance: physiological mechanisms and mediators. Applied Physiology, Nutrition, and Metabolism, 2008; 33(6):1290-1300.

40. Greenberg JA, Owen DR, Geliebter A. Decaffeinated coffee and glucose metabolism in young men. Diabetes Care, 2010; 33(2):278-280.

41. Wedick NW, Brennan AM, Sun Q, Hu FB, Mantzorost CS, Van-Dam RM. Effect of caffeinated and decaffeinated coffee on biological risk factors for type 2 diabetes: a randomized controlled trial. Nutrition Journal, 2011; 10: 93.

42. Van-Dam RM, Wilrike JP, Verhoef P. Effects of Coffee Consumption on Fasting Blood Glucose and Insulin Concentrations Randomized controlled trials in healthy volunteers. diabetes care, 2005; 27 (12): 2990-2992.

43. Bidel S, Hu G, Sundvall J, Kaprio J, Tuomilehto J. Effects of coffee consumption on glucose tolerance, serum glucose and insulin levels--a cross-sectional analysis. Hormone and Metabolic Research, 2006; 38(1):38-43.

44. Moisey LL, Kacker S, Bickerton AC, Robinson LE, Graham TE. Caffeinated coffee consumption impairs blood glucose homeostasis in response to high and low glycemic index meals in healthy men. Am J Clinical Nutrition, 2008; 87(5):1254-1261.

45. Van-Dam RM, Dekker JM, Nijpels G, Stehouwer CD, Bouter LM, Heine RJ. Coffee consumption and incidence of impaired fasting glucose, impaired glucose tolerance and type 2 diabetes: the Hoorn Study. Diabetologia, 2004; 47:2152-2159.

46. Shi X, Xue W, Liang S, Zhao SJ, Zhang X. Acute caffeine ingestion reduces insulin sensitivity in healthy subjects: a systematic review and meta-analysis. Nutrition Journal, 2016; 15: 103.

47. Gustavo DP, Juliane CSZ, Joyce AT, João FM. Does long-term coffee intake reduce type 2 diabetes mellitus risk?.Diabetology and Metabolic Syndrome, 2009; 1: 6.

48. Rafael B, Hofit C, Yehuda K, Aviv S, Dror H. Triglycerides and HDL Cholesterol: Stars or second leads in diabetes?.Diabetes Care, 2009; 32(suppl 2): S373–S377.

49. Rehab RW, Rabea AA, Amal AZ, Farah M.E, Elham GB, Omar KS. The Relationship between Habitual Coffee and Tea Consumption and Type 2 Diabetes Mellitus among Libyan Adults. The International Journal of Pharma Research & Review, 2015; 4(5): 34-39.

50. Speer K, Kölling-Speer I. The lipid fraction of the coffee bean.Braz J Plant Physiology, 2006; 18 (1):201-216.

51. Heckers H, Gobel U, Kleppel U. End of the coffee mystery: diterpene alcohols raise serum low-density lipoprotein cholesterol

and triglyceride levels. J Internal Medicine, 1994; 235: 192-193.
52. Urgert R, Katan MB. The cholesterol-raising factor from coffee beans. J Royal Society of Medicine, 1996; 89:618–623.

53. Aro A, Teirilä J, Gref CG. Dose-dependent effect on serum cholesterol and apoprotein B concentrations by consumption of boiled, non-filtered coffee.Atherosclerosis, 1990; 83**:** 257–261.

54. Jee SH, He J, Appel LJ, Whelton PK, Suh I, Klag, MJ. Coffee consumption and serum lipids: a meta-analysis of randomized controlled clinical trials. Am J Epidemiology, 2001; 153: 353–362

55. Karabudak E, TürközüD, Köksal E. Association between coffee consumption and serum lipid profile. Exp Therapeutic Medicine, 2015; 9(5): 1841–1846.

56. Zargar A, Auttapibarn C, Hong SH, Larson TJ, Hayworth KH, Ito MK. The effect of acute café latte ingestion on fasting serum lipid levels in healthy individuals. J ClinLipidology, 2013;7:165–168.

57. Cai L, Ma D, Zhang Y, Liu Z, Wang P. The effect of coffee consumption on serum lipids: a meta-analysis of randomized controlled trials.Euro J Clinical Nutrition, 2012;66: 872–877.

58. Rezq AA, Fathy NM. Effect of regular drinking of boiled, filtered or Turkish coffee and its impact on some biochemical parameters relevant to atherogenicity and the functions of the kidney and liver in rat model.Euro J Biological Research, 2010; 2(3): 46-54.

59. Abd El-Fattah, H.M. Nutritional Interaction Effect of Zinc and Coffee on Serum Lipid Profile and Copper in Rats.The Egyptian Journal of Hospital Medicine, *2008;* 33**:** 492– 502.

60. Onuegbu AJ and AgbedanaEO.The effects of coffee consumption on serum lipids and lipoprotein in healthy individuals.Afr J Medicine and Medical Science, 2001; 30(1-2):43-45.

61. Cheung RJ, Gupta EK, Ito MK. Acute coffee ingestion does not affect LDL cholesterol level. Annals of Pharmacotherapy, *2005;* 39:1209–1213.

62. Driessen MT, Koppes LL, Veldhuis L, Samoocha D, Twisk JW. Coffee consumption is not related to the metabolic syndrome at the age of 36 years: the Amsterdam Growth and Health Longitudinal Study. Euro J Clinical Nutrition, 2009; 63:536–542