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Research Article

The effect of excessive coffee consumption, in relation to diterpenes levels of medium-roasted coffee, on non-high-density lipoprotein cholesterol level in healthy men

Shady Awwad[1], Ahmed Abu-Zaiton[2], Reem Issa[3], Rana Said[3], Ahmad Sundookah[4], Maha Habash[5], Beisan Mohammad[6], Mahmoud Abu-Samak[7]

1 *Department of Pharmaceutical Chemistry & Pharmacognosy, Faculty of Pharmacy, Applied Science Private University, Amman, Jordan*
2 *Department of Biological Sciences, Al Al-Bayt University, Mafraq, Jordan*
3 *Department of Pharmaceutical Sciences, Pharmacological and Diagnostic Research Center (PDRC), Faculty of Pharmacy, Al-Ahliyya Amman University, Amman, Jordan*
4 *Department of clinical Nutrition and Dietetics, Faculty of Pharmacy, Applied Science Private University, Amman, Jordan*
5 *Michael Sayegh Faculty of Pharmacy, Aqaba University of Technology, Aqaba, Jordan*
6 *Department of Pharmaceutical Sciences, Fakeeh College for Medical Sciences, Jeddah, Saudi Arabia*
7 *Department of Clinical Pharmacy and Therapeutics, Faculty of Pharmacy, Applied Science Private University, Amman, Jordan*

Corresponding author: Shady Awwad (sh_awwad@asu.edu.jo)

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Abstract

This study was designed to determine the levels of coffee oil and diterpenes and evaluate the correlation between the effect of excessive roasted coffee consumption on non-high-density lipoprotein cholesterol (non-HDL) and the roasting degree effect on the levels of coffee oil and diterpenes extracted from *Coffea arabica*. The coffee oil and diterpenes were extracted using soxhlet and liquid-liquid extraction. Sixty-six healthy normolipidemic male participants were assigned for this study which consisted of two stages. The first stage is the coffee abstaining stage where subjects were requested to abstain from drinking coffee for 2 weeks. The second stage is the coffee drinking stage which consisted of groups (the control group and coffee-drinking group). The levels of TC, TG, LDL-C, HDL-C, and non-HDL were determined in all participants before and after the experiment. The results indicated that the coffee roasting degree demonstrated a significant increase in the levels of coffee oil and diterpenes ranging from 9.31% (green coffee) to 15.64% (dark roast) and from 0.205% (green coffee) to 0.300% (dark roast) for diterpenes. In conclusion, the current study revealed that excessive consumption of medium roasted coffee was associated with elevated non-HDL levels in normotensive nonsmoker healthy men which might be attributed to the positive association between the degree of roasting and diterpenes levels.

Keywords

coffee, non-HDL, lipid profile, coffee oil, diterpenes, roasting degree

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PENSOFT.

Introduction

Coffee is considered one of the most popular beverages and most traded products around the world due to its physiochemical, health benefits, chemical composition, and physiological properties (Speer and Kolling-Speer 2006; Ciaramelli et al. 2019). This genus contains many different distinct species but the two major ones are *Coffea arabica* (Arabica) and *Coffea canephora* (Robusta) (Belandria et al. 2016). On the other hand, the world coffee production of *C. arabica* corresponds to 70–80% (de Oliveira et al. 2014), and because of this wide and increasing consumption around the world, there has been a notable interest and attention in investigating and evaluating the possible influences of coffee's bioactive compounds on human health (de Lucia et al. 2009).

Coffee has a very unique chemical composition since it contains several compounds. It is a complex substance and contains more than two thousand bioactive compounds (Yashin et al. 2017). Among these compounds; are lipids, diterpenes, caffeine, carbohydrates, phenolic compounds, minerals, vitamins, melanoidins, and volatile aroma compounds (Jeszka-Skowron et al. 2015). The contents of the lipids, which are also identified as coffee oil, in the coffee range from 7–17%. These coffee lipids consist of the following major components; triglycerides (TG) 75%, diterpenes 15–20%, and sterols 2–5% (Kurzrock and Speer 2001; Barbosa et al. 2014). However, these values vary between green and roasted coffee beans, which indicates that the roasting conditions affect directly the content of the later compounds and the quality of coffee (Budryn et al. 2012). This means that the coffee content of oil and diterpenes is not a function only of the chemical composition of coffee beans but also depends on some other factors such as the type of coffee, degree of roasting (temperature), brewing methods, and the extraction process (Speer and Kolling-Speer 2006). Isolation, quantification of the coffee lipids, and evaluation of their health effects have gained a notable amount of attention because of their enormous positive health effects which include; anticarcinogenic effects, antioxidant effects, anti-inflammatory activities, and induction of the degradation of toxic compounds (Dias et al. 2010).

On the other hand, although some reports have mentioned that the coffee's bioactive ingredients may have cardiovascular health benefits (Ranheim and Halvorsen 2005; Pandurang 2012), high levels of both low-density lipoprotein cholesterol (LDL-C) and total cholesterol (TC) have been linked to the consumption of a large amount of coffee (Barbosa et al. 2014; Moeenfard et al. 2015). Paradoxically, similar results regarding high-density lipoprotein cholesterol (HDL-C) have been reported (Uto-Kondo et al. 2010). To solve this dilemma, measurement of non-high-density lipoprotein cholesterol (non-HDL-C) was conducted by subtracting HDL from TC to provide a better assessment of cardiovascular disease (CVD) risk (Bergmann 2010). However, the effects of coffee consumption rate on some specific lipid profile parameters, particularly non-HDL-C, are still unclear (Cai et al. 2012; Karabudak et al. 2015). The results of many studies highlighted the coffee consumption rate for non-HDL-C was also mixed (Cheung et al. 2005; Zargar et al. 2013). Therefore, the effect of the degree of roasting on diterpenes content can be linked to clarify the effect of roasted coffee consumption on non-HDL (Pietinen et al. 1988). The unique structure of the coffee, the variability and the complex number of compounds, and their properties and health effects had brought our attention to investigate this rich and distinctive compound (Belandria et al. 2016). Few studies have investigated the effect of coffee consumption rate on non-HDL levels. Some studies showed a positive correlation (Pietinen et al. 1988) while others showed that coffee consumption rate did not affect the non-HDL levels (Cheung et al. 2005; Zargar et al. 2013).

Based on available data to date, no studies have correlated the association between coffee's degree of roasting and content of diterpenes with the effect of excessive medium-roasted coffee consumption on non-HDL levels in healthy Jordanian men. Therefore, the current study aims to evaluate the correlation between the effect of excessive medium-roasted coffee consumption on non-high-density lipoprotein cholesterol (non-HDL) and the effect of the degree of roasting on the levels of coffee oil and diterpenes extracted from *C. arabica*.

Materials and methods

Study area

This study was conducted from October 2018 to February 2019.

Reagents

The following chemicals and reagents were used in this investigation: Petroleum ether was purchased from Honeywell Research Chemicals (France). Methyl *t*-butyl ether (MTBE) and potassium hydroxide pellets were purchased from LOBA Chemie (India). Ethanol was purchased from Gainland Chemical Company (UK). Anhydrous sodium sulfate was purchased from Merck. The coffee beans (*C. arabica*, which originated from Ethiopia, Ghana, & India) were purchased from the local market (Al-Ameed Coffee Company).

Roasting and grinding of coffee beans

The coffee beans were roasted at 165 °C, 180 °C, and 195 °C (light, medium, and dark) using a Kn-8828-2 coffee roaster (Hottop USA, Cranston, RI, USA). Three different degrees of roasting were used in this experiment. Roasting conditions including the degree of roasting temperature, and roasting time, were described in a previous study (Choi et al. 2018). The coffee beans roasting conditions are summarized in Table 1. After roasting, the coffee beans were ground (grain size below 1 mm) using a coffee grinder (900 N, Yang-Chia Machine Works, Co. Ltd., Taiwan). Then, the coffee extracts were stored in the freezer until the day of extraction and analysis.

Table 1. Types of coffee, weight, roasting temperature, and roasting time.

Type of coffee	Abbreviation	Weight (gram)	Roosting temperature (°C)	Roosting time (min)
Green beans	G	100 g	–	–
Light roast	L	100 g	165	20
Medium roast	M	100 g	180	20
Dark roast	D	100 g	195	20

Extraction and quantification of oil and total diterpenes contents in coffee

Extraction and quantification of oil content in green and roasted coffee beans

The levels of (coffee lipids) coffee oil were extracted and determined according to the experimental procedures reported by Speer and Kolling-Speer (2006) and Guercia et al. (2016) with several modifications and developments were conducted (Speer and Kolling-Speer 2006; Guercia et al. 2016). First, five grams of the green or roasted coffee were placed in a Florence flask and the Soxhlet extraction was executed at 55–60 °C over sixteen hours of reflux with 250 mL MTBE (boiling point: 55 °C). Next, the sample was dried for 30–40 minutes at 105 °C till the solvent was evaporated by rotary evaporation and then the mass of the oil (lipid) extract was gravimetrically measured as g/100 g of ground coffee.

Extraction and quantification of total diterpenes in the extracted coffee oil

The levels of total diterpenes were gravimetrically assessed according to previous chemical procedures with several modifications and developments (Clarke and Macrae 1985; Speer and Kolling-Speer 2006). The extracted coffee oil from the previous part was saponified with (200 mL of 10% potassium hydroxide – ethanol solution) in a water bath under nitrogen. Then, the organic layer was evaporated using a rotary evaporator. A 350 mL of distilled water and 300 ml MTBE was added and the mixture was put on an agitator for 8–12 hours, then the contents were transferred to a separatory funnel. The aqueous phase was discarded and the ether layer was washed with distilled water several times to get rid of potassium hydroxide. Anhydrous sodium sulfate was added to absorb the remaining traces of water. Next, the contents were transferred into the Soxhlet apparatus, and the ether was evaporated until 100 mL was left. Then, a volume of 200 mL of petroleum ether (30–40 °C) was added. The contents were cooled in the refrigerator until yellow crystals were formed in the ether phase. Next, the mixture was filtered and the ether was discarded. The crystals were recrystallized several times (3 times) in the ether layer until pure crystals were obtained. The obtained crystals represent a mixture of cafestol and kahweol. Finally, the crystals were determined, weighed, and the percentage of diterpenes (cafestol and kahweol) in the coffee oil was calculated.

Preparation of coffee drinks

Participants were requested to drink boiled unfiltered Turkish coffee (Al-Ameed Coffee, Amman, Jordan). The coffee drinks were prepared by mixing 6.5–13.0 g of Al-Ameed Turkish coffee with 125 mL of boiling water and they were allowed to boil gently for about 1–2 min with continuous stirring (which is equivalent to 1–2 teaspoons of coffee powder) resulting in 150 mg of caffeine per cup (Jeon et al. 2013). Next, the drinks were allowed to stand for about 1 min to settle before decanting. The prepared coffee drinks were distributed to the volunteers every other day in tightly closed glass bottles. The volunteers were requested to store the coffee bottles refrigerated. However, they have the choice to drink the coffee either cold or heated slightly.

Study design and participants

This study was carried out at the Applied Science Private University (ASU), Amman, Jordan during the period from October 2018 to February 2019. Sixty-eight healthy Jordanian men, normolipidemic, normotensive, nonsmoker males with body mass index (BMI) ranging from 18 to 44 kg/m^2, and aged from 27 to 35 years from Amman (Jordan) participated in the study. Anthropometric data of the participants were measured by a group of research assistants who filled out a questionnaire that included questions on anthropometric and lifestyle habits in a face-to-face interview with each participant on the day of blood sample collection. To avoid confusing parameters that are known to impact the leptin and salivary testosterone (ST) levels, the participants that were diagnosed with chronic diseases such as CVDs, diabetes, hepatic, or endocrine disorders and those who had been taking any type of medication for the last two months prior to the study, were disqualified.

The study consisted of two main stages as shown in the consort flow diagram (Fig. 1). Stage one (abstaining-coffee): all subjects were requested to abstain from drinking any type of coffee or consume any food item that contains coffee for two weeks. At the end of this stage, an overnight fasting blood sample was taken from each subject. Stage two (drinking-coffee): At the beginning of this stage, the subjects were divided into two groups, the coffee-drinking group (CD) and the control group (C). Participants of the CD group (n=33) were requested to drink four (125 ml/ cup) cups a day as the sole source of coffee for four weeks. While the participants of the control group (n=33) were requested to continue abstaining from any type of coffee for four weeks. At the end of this stage, another overnight fasting blood sample was taken from each subject. It is worth noting that during the coffee-drinking stage, the researchers met each volunteer every other day to ensure good compliance with the protocol of the study.

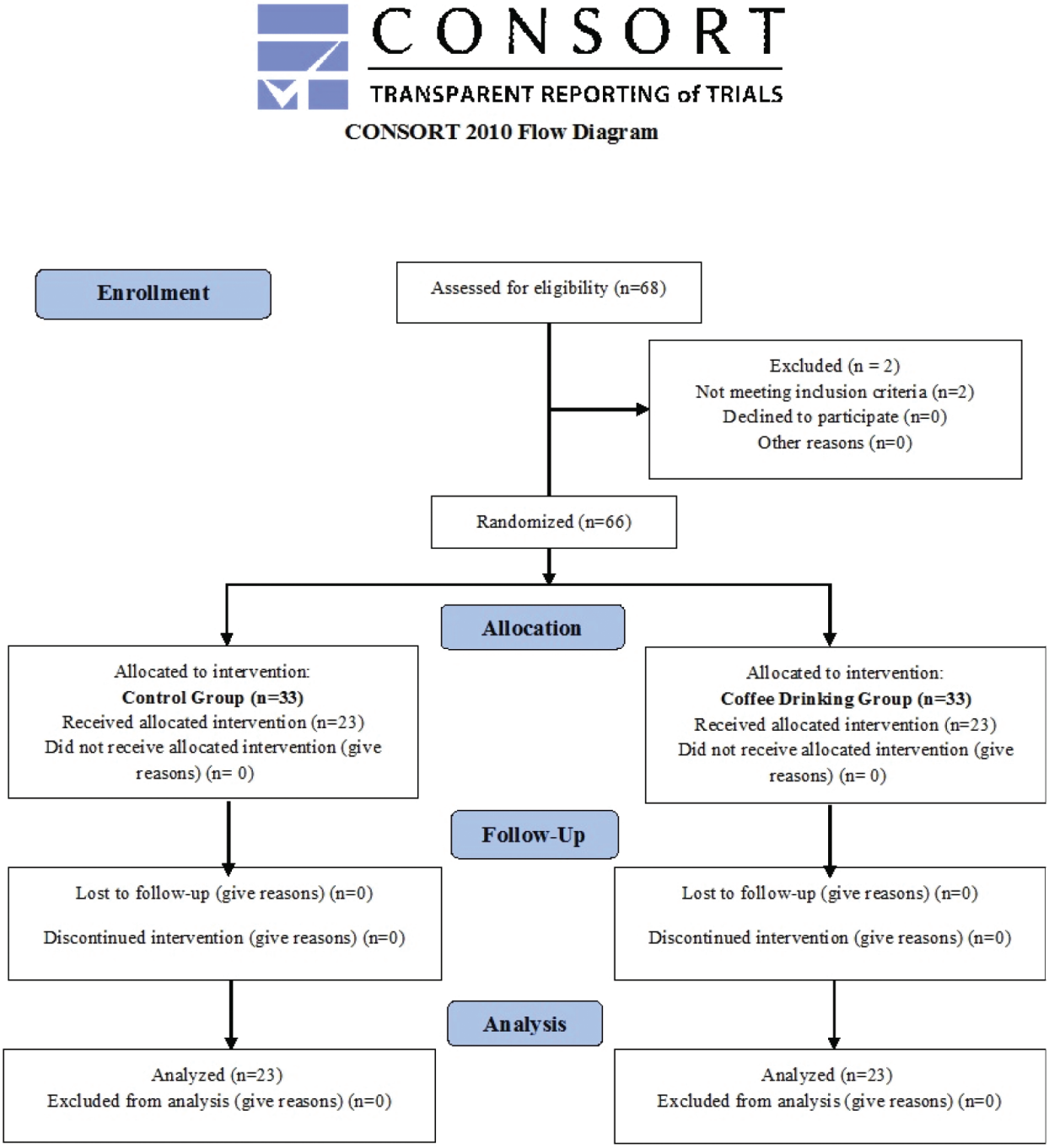

Figure 1. CONSORT flow diagram for the study indicating the number of subjects screened, recruited, randomly assigned to different groups.

Informed consent and ethical considerations

This study was approved by the ASU ethics committee for the protection of human subjects, (Ethical approval No. = 2018-PHA-2). This work was done in accordance with the Helsinki Declaration. All participants were provided with an informed consent, which contained details of the experimental protocol. The participants fully understood the main objective and the risks of this investigation. They were informed of being free to withdraw from the investigation at any stage. They were also provided written informed consent before the commencement of the study and were asked to complete a health screening questionnaire before they participated in this investigation.

Coffee drinks

The amount of coffee grounds used in the preparation of coffee drinks was 80 g per liter of boiling water. Each subject was requested to consume four (125 ml/cup) cups a day, consequently, the amount of coffee grounds consumed was equivalent to 40 g/day which might represent the consumption of heavy coffee drinkers (Abu-Taha et al. 2020). In previous studies, the amounts of coffee that were requested to be consumed were 58.8 and 56.0 g/

day, respectively (Aro et al. 1978; Urgert et al. 1996). Such amounts were used to induce the effect within a shorter period of time (on average 4 weeks) since it is perhaps not possible for human subjects to comply with studies restrictions for longer periods.

Data collection or measurement

Anthropometric data, which includes age, body weight (BW), height (Ht), and body mass index (BMI), were collected following standard procedures (Hamden et al. 2013; Jeon et al. 2013).

Lipid profile measurement

The baseline fasting-blood samples were collected at the end of the coffee-withdrawal stage and, the follow-up samples were collected at the end of the coffee-drinking stage. Blood samples were collected by nurses' research assistants using the method described previously. The overnight fasting venous blood samples were then obtained, centrifuged and stored at -20 °C until being assayed. TC, TG, and HDL-C were assessed using the enzymatic colorimetric kits (Linear Chemicals, Barcelona, Spain). LDL-C was routinely estimated by the Friedewald equation (Friedewald et al. 1972). The non-HDL-C levels were calculated by subtracting the HDL-C from TC. The lipid profile was measured at the laboratories of the department of clinical pharmacy and therapeutics – ASU-Pharmacy College.

Statistical analysis

The measured analytical parameters were analyzed and presented as means, SD, *P*-value, Pearsons' correlation odd ratio using the SPSS (Version 22, SPSS Inc., Chicago, IL) at the statistical significance of $p<0.05$, and $p<0.01$. The statistical analysis was performed using a statistical software package SPSS, version 19.0 for Windows (Chicago, IL, USA). The T-test statistical analysis was used to compare the differences in demographic and clinical findings between the means of the two studied groups. The Pearson analysis was used to find if there was any correlation between the participants' characteristics and serum leptin levels.

Results

Oil and total diterpenes content in coffee samples

The results indicated a positive correlation between the oil or diterpenes contents in the four different types of coffee with the degree of roasting. The oil (lipid) and diterpenes contents of the raw and the various studied roasted coffee are shown in Table 2. The oil content percentage in the green beans was the lowest (9.31%), while increasing the roasting temperature increased the oil contents in coffee. Specifically, the light roast was 10.02%, the medium roast was 14.43%, and the dark roast was 15.64%. While the percentages of the diterpenes in coffee showed a similar positive correlation but their contents showed no consistent correlation in coffee oil. The diterpenes percentage in the green beans was 0.205%, the light roast was 0.207%, the medium roast was 0.289%, and the dark roast was 0.300%.

Table 2. The percentage of oil and diterpenes in green beans and roasted coffee.

Type of Coffee	% of Oil	% of Diterpenes	
	(g/100 g coffee) (Mean ± SD)	(g/100 g oil) (Mean ± SD)	(g/100 g coffee) (Mean ± SD)
G	9.31 ± 0.28	2.21 ± 0.17	0.205 ± 0.007
L	10.02 ± 0.42	2.07 ± 0.12	0.207 ± 0.021
M	14.43 ± 0.08	2.00 ± 0.25	0.289 ± 0.035
D	15.64 ± 0.25	1.92 ± 0.27	0.300 ± 0.071

SD: Standard deviation; G: green beans, L: light roast; M: medium roast; D: dark roast.

The results indicated that the lowest content of coffee oil and diterpenes was found in the green coffee, 0.4655 g, and 10.25 mg, respectively. While the highest content of coffee oil and diterpenes was found in the dark roasted coffee, 0.782 g, and 15.00 mg, respectively. The contents and percentage of coffee oil appeared to increase with increasing the roasting temperature which was indicated by several studies (Ariga et al. 2018). However, the calculated percentage of diterpenes in the coffee ground seems to increase with increasing the roasting temperatures due to loss of moisture and some organic matter (Speer et al. 1992; Speer and Kolling-Speer 2006), while the percentage of diterpenes in the oil content stayed constant with a slight decrease. On the other hand, increasing the roasting temperature increases led to a significant decrease in the diterpenes content (cafestol and kahweol) (Sridevi et al. 2011). The following figure shows the correlation between the levels of coffee oil and diterpenes versus the degree of roasting (Fig. 2).

The results demonstrated a positive correlation between coffee oil content and degree of roasting. Furthermore, the levels of total diterpenes also demonstrated a positive correlation with the degree of roasting. The levels of total diterpenes are illustrated on a lipid basis for each type of coffee to analyze the behavior and stability of the total diterpenes during the roasting stages, dismissing the rise in the lipid levels (Fig. 3).

It was indicated previously that the roasting process can the water content in the coffee beans to decrease but it can help to release the lipid from coffee and increase the coffee oil (lipid) content (Araujo and Sandi 2006). The previous figure demonstrated a negative correlation between the (coffee diterpenes/coffee oil) ratio and the degree of roasting. The levels of diterpenes on a lipid basis were decreased by around 13% (when comparing dark-roasted coffee with green coffee).

Baseline values of the participants

The mean age of participants was 31.18 ± 3.74 years with a BMI of (28.52 ± 4.3) which means they were overweight. Descriptive analysis for anthropometric parameters is shown in Table 3.

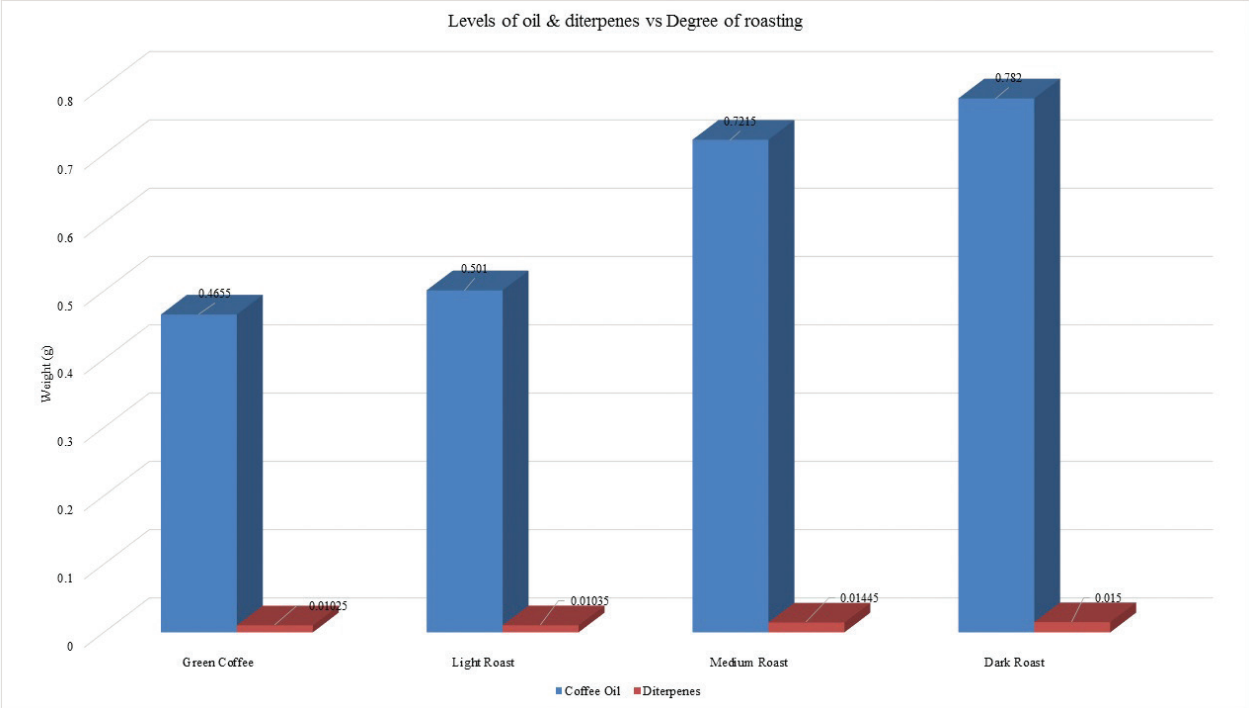

Figure 2. The levels of coffee oil and total diterpenes in green and roasted coffee.

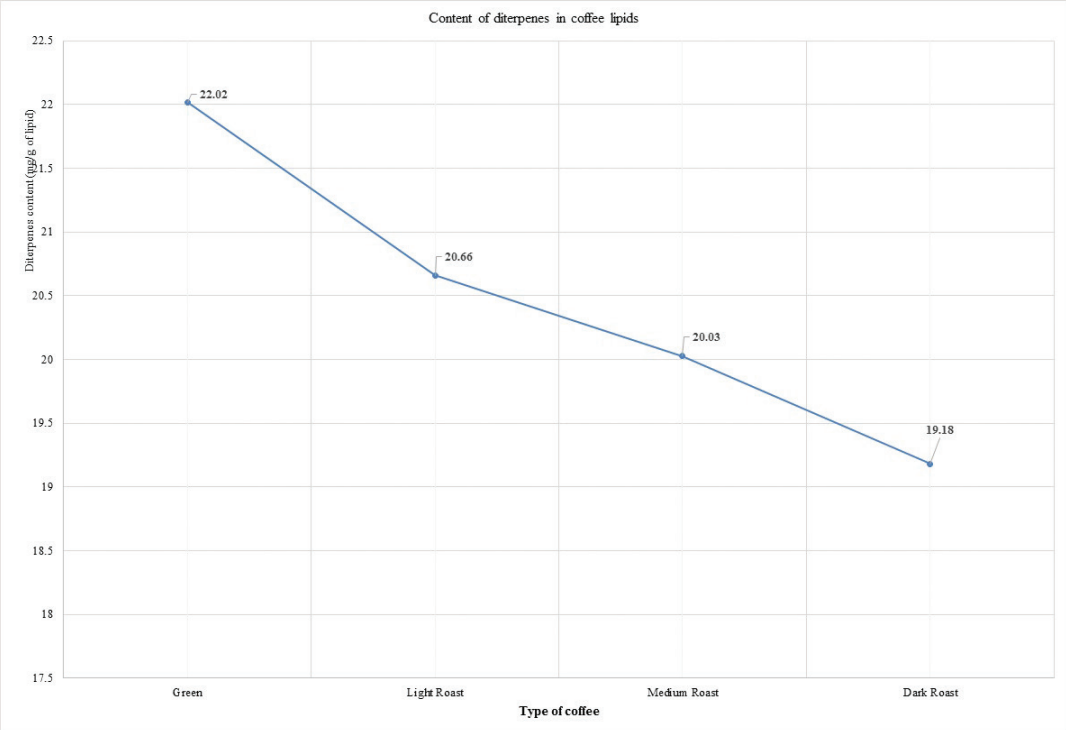

Figure 3. The levels of total diterpenes on coffee oil basis (lipid basis) versus the types of coffee with different degrees of roasting.

Table 3. Baseline descriptive statistics of the anthropometric parameters (n=48).

Parameter	Range	Mean ± SD
Age (year)	27 to 35	31.18 ± 3.74
Weight (kg)	51–116	79.7 ± 1.10
Height (cm)	156–188	166.33 ± 7.93
BMI (kg.m-2)	18.4–41.08	28.52 ± 4.3

SD: standard deviation; BMI: body mass index; kg: kilogram; cm: centimeter.

Changes in serum non-HDL and lipid profile levels within study groups at baseline and follow-up

The lipid profile variables, which include the serum TC, HDL-C, TG, and LDL-C, were determined for two groups (control and MCD) after two weeks of abstaining from all

types of coffee. The baseline results pointed out that lipid profile variables were very similar and no significant differences were observed between the control and medium coffee drinking group (MCD). This indicated that the initial values of all the studied lipid profile variables are homogenous. An independent sample *T*-test was conducted before and after coffee intake by MCD to present any expected significant differences between the two study groups (P^b-value). The independent *T*-test indicated that the MCD group was significantly different from the control group regarding the non-HDL, TC, and LDL-C at the end of the study (P^B=0.001). Except for HDL-C, four weeks of medium roasting coffee intake increased the levels of TC, TG, LDL-C, and non-HDL significantly from 179.5 to 205.3 ng/mL, 93.1 to 122.3 ng/mL, 118 to 134.7, and 136.9 to 165.7 ng/m, P<0.001, respectively (Table 4). A significant follow-up change in non-HDL levels was approximately 29 ng/ml (Fig. 4), which means the powerful hyperlipidemic effect of excessive medium roasted coffee consumption.

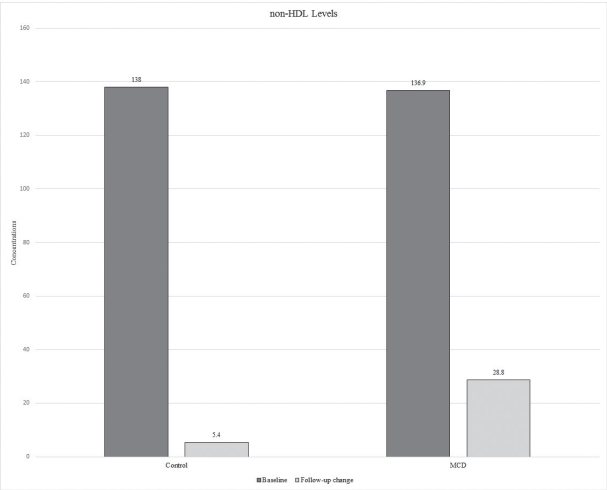

Figure 4. The non-HDL levels in the control and MCD groups.

Discussion

There have been numerous studies that were performed to examine the relationship between the content of coffee oil and diterpenes with the degree of roasting temperature. The majority of these studies demonstrated a positive correlation between the degree of roasting coffee and the contents of coffee oil and diterpenes. The coffee oil levels ranged from 0.4655 g to 0.782 g and the total diterpenes ranged from 10.25 mg to 15.00 mg. Thus, an increase in the contents of coffee oil and diterpenes was observed for *C. arabica* when the degree of roasting changed to higher limits, confirming the strong impact of the degree of roasting on their levels. These results proved the fact that coffee lipids and diterpenes are heat resistant and thermally stable compounds compared to other coffee bioactive compounds and it also indicated a higher heat susceptibility of the coffee sample at a higher degree of roasting (Dias et al. 2014). The findings of our study showed a good and similar agreement with several previous studies where whenever the degree of roasting

Table 4. Fasting serum levels of lipid profile and non-HDL at the baseline and 4 weeks follow-up of the study.

	Control	MCD	t-test	P^B -value
		TC		
Baseline	180.0 ± 3.1	179.5 ± 1.5	0.25147	0.404921
Follow-up change	5.7 ± 2.6	25.8 ± 1.6**	-5.94676	0.000505
dependent T-test	3.7972	13.1626		
P^A	0.03207	0.0009473		
		HDL-C		
Baseline	42.3 ± 3.6	42.9 ± 1.2	-0.41079	.695493
Follow-up change	0.3 ± 0.9	0.3 ± 0.4	-0.36322	0.728892
dependent T-test	0.5774	1.006		
P^A	0.6042	0.3885		
		TG		
Baseline	96.0 ± 2.5	93.1 ± 3.4	1.19022	0.278913
Follow-up change	21.2 ± 2.8	29.2 ± 3.0	-1.50065	0.184118
dependent T-test	14.5832	16.8586		
P^A	0.0006992	0.0004545		
		LDL-C		
Baseline	118.5 ± 4.4	118.0 ± 1.9	0.26301	0.801
Follow-up change	1.5 ± 1.5	16.7 ± 1.6	-1.74–4.9167	0.002666
dependent T-test	0.5348	18.0783		
P^A	0.6299	0.0003692		
		Non-HDL		
Baseline	138	136.9	3.39683	0.014553
Follow-up change	5.4 ± 1.9	28.8 ± 5.3	-7.94968	.000211
dependent T-test	5.5018	10.94		
P^A	0.01182	0.001635		

* MCD: medium coffee drinking group; TC: total cholesterol; HDL-C: high density lipoprotein-cholesterol; TG: triglecerdes; LDL-C: low density lipoprotein-cholesterol; non-HDL; non-high density lipoprotein-cholesterol; P^A: *P*-value for paired *t*-test between baseline and 4 weeks follow-up of the study for each group; P^B: *P*-value for independent T-test between baseline means of the two study groups.

coffee increases, the levels of coffee oil and total diterpenes increase as well. It has been previously indicated that increasing the degree of roasting can lower the content of water in the coffee beans and hence increase the release of coffee oil, diterpenes, and several coffee bioactive compounds (Dias et al. 2013; Ariga et al. 2018; Awwad et al. 2021).

This study was also designed to assess the effect of excessive medium roasted coffee consumption on non-HDL-C levels in normolipidemic adult males with regard to the diterpenes levels in medium roasted coffee. The main finding of this study was that at four weeks follow-up, excessive roasted coffee intake with drinking 4 (125 ml/cup) cups a day significantly increased non-HDL-C levels. The same rate of coffee intake also elevated serum TC and LDL-C. The previous studies confirmed that excessive coffee consumption increases serum levels of TC and LDL-C without affecting those of HDL-C or TG (Haffner et al. 1985; Kark et al. 1985; Pietinen et al. 1988; Merkhan et al. 2021). Though, the levels of TC and LDL-C in hyperlipidemic adults have been correlated with the distinct influence of hepatocellular carcinoma (HCC), certain plasma protein markers, and cigarette smoking in animal and humans model. However, several studies revealed that caffeine levels have a major influence or no influence on the lipid profile parameters (Ashakumary and Vijayammal 1997; Vlachopoulos et al. 2004; Nystad et al. 2010; Al Hariri et al. 2016; Abu-Taha et al. 2020).

Based on the present study, however, it cannot be ascertained that the plateau levels of lipid profile were reached

within four weeks because of the lack of measurements between the beginning and the end of the study, and since the present study was limited by four weeks, the possibility that serum levels of TC and LDL-C might rise further if coffee intake continues for longer periods cannot be excluded. It has been known that medium roasted coffee is the most common type of coffee among the other types of coffee in Jordan. This prompted the design of this study to evaluate the diverse effects of the high consumption rate of medium roasted coffee. The attention was drawn to the importance of verification to clarify whether higher intakes of roasted coffee (compared to the moderate consumption rate, 1–2 cups/day) influence the non-HDL level as a predictor of CVD risk (Abu-Taha et al. 2020).

The significant positive correlation observed between coffee consumption and lipid profile parameters did not include HDL-C, which was consistent with a previous study (Cai et al. 2012). It seems that the significant follow-up elevation of non-HDL-C that was observed in the MCD study group might have resulted from the significant increase in TC and LDL-C levels, which referred that non-HDL-C seems to be a potential predictor of CVD and strokes. While excessive coffee consumption has been associated with several risk factors leading to CVDs including also hyperlipidemia (Christensen et al. 2001), moderate coffee consumption was linked with a reduced risk of CVDs (Abu-Taha et al. 2020; Hasoun et al. 2021). Nevertheless, several studies reported that coffee consumption has an insignificant or beneficial impact on the lipid profile in healthy subjects (Robertson et al. 2018; Roshan et al. 2018). It was also reported that coffee reduced the levels of blood TG's in subjects with high cholesterol levels after eight weeks (Sarria et al. 2018). However, inconsistent results from previous studies delivered limited proof that coffee and its bioactive constituents can control lipid metabolism.

In this context, coffee has adverse or beneficial effects on blood lipids profile and its mechanisms are still being investigated (Robertson et al. 2018; Roshan et al. 2018). Therefore, the non-HDL-C results, which were indicated in the current study, may be clarified paradoxical observations of circulatory lipoproteins alteration linked to coffee consumption. However, the results of the few relevant studies are also inconclusive due to the inconsistency of their findings (Cheung et al. 2005; Karabudak et al. 2015). It has been revealed that elevated lipids levels in coffee drinkers were not related only the caffeine but also to other ingredients of coffee (Jee et al. 2001). Therefore, extraction and quantification of the selected coffee bioactive constituents became very essential to propose potential mechanisms of action of coffee and its bioactive compounds on lipid metabolism (Harpaz et al. 2017).

The extraction of the coffee oil and diterpenes have been a very difficult mission. The diterpenes constituents can be determined by extraction as total (esterified with different fatty acids) or free diterpenes and it is more economic to assess their levels as total diterpenes instead of the free diterpenes. As the free diterpenes exist only as minor components and their determination requires an effective separation technique from the major components. Several techniques were reported for the extraction of coffee oil and diterpenes. The soxhlet extraction method was proven to be a common and very efficient method for extracting coffee oil and diterpenes. Several organic solvents were investigated to evaluate the efficiency of the extraction such as n-Hexane, petroleum ether, tert-butyl methyl ether, and diethyl ether (Speer and Kolling-Speer 2006). Furthermore, it was demonstrated that the diterpenes yield that was obtained from *C. arabica* by soxhlet extraction increases with increasing the roasting temperature which was consistent with our findings (Kitzberger et al. 2013). In agreement with our results, a positive association between the coffee roasting temperature and its coffee oil and diterpenes content might be attributed to the heat effect that decreases the water content while breaking down the oil cells in the sample (Ariga et al. 2018). On the other hand, increasing the roasting temperature can lead to a major decrease in the yield of coffee oil. This is attributed to using high temperatures can cause the water and volatile compounds contents in coffee to evaporate (Purnamayanti et al. 2017). Furthermore, the levels of total diterpenes can be affected inversely by the degree of roasting (Rendon et al. 2017). An interesting correlation between the degree of roasting and the levels of coffee oils and diterpenes was demonstrated by Araujo and Sandi. They attempted to extract the coffee oils and diterpenes from green and roasted coffee beans using the supercritical carbon dioxide extraction technique (Araujo and Sandi 2006). Their findings indicated a positive correlation between the degree of roasting and the content of coffee oil but a negative correlation with the content of diterpenes. Since the soxhlet extraction procedure was proven to be effective in extracting coffee oil and diterpenes, this technique was chosen to determine the amount of coffee oil and the total diterpenes using MTBE as an extracting solvent and it showed a good and promising efficiency because the diterpenes' solubility is high when high temperatures are conducted in the soxhlet extraction procedure. Furthermore, it is believed that at high temperatures, the solvent molecules tend to infiltrate the coffee particles easily and solubilize the coffee constituents since at higher temperatures solvents are likely to have low viscosity and high kinetic energy compared to lower temperatures (Novaes et al. 2019). Moreover, the coffee's degree of roasting illustrated a positive correlation with the content of coffee oil in coffee or the total diterpenes in coffee and their amounts increase with raising the temperature of roasting as indicated previously by several studies (Ariga et al. 2018). On the other hand, the results illustrated that the content of total diterpenes in the lipid content was reduced as the roasting degree increased since these compounds were thermally unstable upon heating and they tend to undergo thermal degradation during the roasting process which showed a good agreement with the findings of previous investigations (Speer and Kolling-Speer 2006; Dias et al. 2010).

To the best of our knowledge, this is the first study that investigated the effect of coffee consumption on serum lipids, specifically non-HDL. The lack of studies around this topic has brought our attention to investigate the possible correlation between coffee consumption and non-HDL levels.

Conclusions

The degree of roasting of coffee is positively proportional to the percentages of the coffee oil and total diterpenes, which is believed to be linked to the hyperlipidemic effect that is resulted from excessive consumption of roasted coffee in healthy men. The present study, in relation to the effect of degree of roasting on the total diterpenes levels in coffee beans, concluded that the excessive consumption of medium roasted coffee significantly elevated the non-HDL levels in healthy men.

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Supplementary material 1

IRB Research Protocol

Authors: Shady Awwad, Mahmoud Abu-Samak

Data type: Protocol

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