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RESEARCH

Association between caffeine intake and liver biomarkers in non-alcoholic fatty liver disease

Non-alkolik yağlı karaciğer hastalığında kafein alımı ve karaciğer biyobelirteçleri arasındaki ilişki

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Abstract

Purpose: Non-alcoholic fatty liver disease (NAFLD) is the most common cause of elevated liver enzymes in developed countries. The aim of this study is to examine the effects of caffeine intake on some parameters of liver metabolism in individuals with NAFLD.
Materials and Methods: A total of 20 female and 20 male subjects between the ages of 19 to 64, who were diagnosed with NAFLD, were included in the study. To determine caffeine intake, a specially developed caffeine-food frequency questionnaire was administered by a trained dietitian. Individuals were categorized into 3 groups according to their caffeine intake; Group 1: ≤150 mg/day, Group 2: 150-250 mg/day, and Group 3: ≥250 mg/day. Anthropometric measurements and biochemical parameters were recorded. Protein quantities in serum samples were determined by ELISA method.
Results: The body fat composition of group 1 was higher than group 2. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels of group 3 were higher than group 1 and group 2, respectively. High-density lipoprotein cholesterol (HDL-C) level of group 3 was lower than group 1 and group 2. There was a positive correlation between total caffeine intake and ALT level. There was no significant difference between the caffeine intake groups in terms of serum protein levels. In addition, there was not found significant correlation between serum protein levels and total caffeine intake.
Conclusion: Caffeine intake of ≥250 mg/day may increase ALT and AST levels and decrease HDL-C level in individuals with NAFLD.
Keywords: Non-alcoholic fatty liver disease, caffeine, alanine aminotransferase, aspartate aminotransferase, high-density lipoprotein

Öz

Amaç: Non-alkolik yağlı karaciğer hastalığı (NAFLD), gelişmiş ülkelerde karaciğer enzimlerindeki yükselmenin en yaygın nedenidir. Bu çalışmanın amacı, NAFLD olan bireylerde kafein alımının karaciğer metabolizmasının bazı parametreleri üzerindeki etkilerini incelemektir.
Gereç ve Yöntem: Çalışmaya NAFLD tanısı almış 19-64 yaş arasında toplam 20 kadın ve 20 erkek birey dahil edilmiştir. Kafein alımını belirlemek için, deneyimli bir diyetisyen tarafından özel olarak geliştirilmiş kafein-besin alım sıklığı anketi uygulanmıştır. Bireyler kafein alımlarına göre 3 gruba ayrılmıştır; Grup 1: ≤150 mg/gün, Grup 2: 150-250 mg/gün ve Grup 3: ≥250 mg/gün. Antropometrik ölçümler ve biyokimyasal parametreler kaydedilmiştir. Serum örneklerindeki protein miktarları ELISA yöntemi ile belirlenmiştir.
Bulgular: Grup 1'in vücut yağ kompozisyonu grup 2'den daha yüksektir. Grup 3'ün alanin aminotransferaz (ALT) ve aspartat aminotransferaz (AST) düzeyleri sırasıyla grup 1'den ve grup 2'den daha yüksektir. Grup 3'ün yüksek yoğunluklu lipoprotein kolesterol (HDL-C) düzeyi grup 1 ve grup 2'den daha düşüktür. Toplam kafein alımı ile ALT düzeyi arasında pozitif bir ilişki vardır. Kafein alım grupları arasında serum protein düzeyleri açısından anlamlı bir farklılık yoktur. Ayrıca serum protein seviyeleri ile toplam kafein alımı arasında anlamlı bir korelasyon bulunmamıştır.
Sonuç: ≥250 mg/gün kafein alımı, NAYKH olan bireylerde ALT ve AST düzeylerini yükseltebilir ve HDL-C düzeyini düşürebilir.

Anahtar kelimeler: Non-alkolik yağlı karaciğer hastalığı, kafein, alanin aminotransferaz, aspartat aminotransferaz, yüksek yoğunluklu lipoprotein

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INTRODUCTION

Fat accumulation of more than 5% in hepatocytes without viral infection, any etiology of liver disease, and excessive alcohol use (>10g/day, >20g/day for women and men, respectively) are defined as non-alcoholic fatty liver disease (NAFLD)[1]. The pathogenesis of NAFLD is still unclear, however, mechanisms in disease development and progress are complex and multifactorial[2].

Today, drinking coffee is one of the popular lifestyle choices[3]. Caffeine, chlorogenic acid, cafestol, and kahweol are examples of bioactive components in coffee that have antioxidative and anti-fibrotic activities[4,5]. Caffeine might be the reason behind the hepato-protective effects of coffee[6]. Caffeine, structurally similar to adenosine, has an adenosinergic effect[7]. This effect inhibits hepatic stellate cells, which are the main mediators of liver fibrosis, and therefore prevents fibrosis. Although there are numerous other bioactive compounds in the coffee grounds, they are present at very low concentrations compared to caffeine concentration[7]. In addition, both coffee and caffeine intake were examined, and consistent effects were observed for both. However, the effect of decaffeinated coffee was not similar[7]. Recent studies demonstrated a decrease in liver enzymes and fibrosis with an increase in coffee intake[4,5,8-11]. Coffee, especially caffeine rather than its other components, is considered to have an anti-fibrotic effect of hepatocytes and hepatic stellate cells with various mechanisms[6,12].

Caffeine may be effective in the reduction of ALT, AST, and GGT levels, known as an indicator of liver injury by its role as antioxidative and adenosine receptor antagonist[13]. The liver has an important role in lipogenesis and cholesterol metabolism[6]. Studies suggest that coffee brewing methods may also cause changes in cholesterol levels[4,6,14]. The diterpenes (kahweol and cafestol) in coffee can be effective on serum lipid[6]. Boiled coffee increases serum cholesterol compared to filter coffee, suggesting that it has a higher concentration of diterpenes than filter coffee[6,14].

Hepatocyte apoptosis plays a critical role in the pathogenesis of NAFLD[15]. In the early stages of apoptosis, caspases cut epithelium-specific intermediate filament cytokeratin 18 (CK18) into 3 fragments[16,17]. Recent studies have shown that CK18 is a good biomarker for NASH[15,18,19]. Irisin has protective effects on the liver by reducing lipogenesis and lipid accumulation and stimulating glycogenesis[20]. Fibroblast growth factor (FGF) 21 directly affects lipid metabolism by reducing hepatic lipid accumulation independent of insulin[21]. The high serum FGF21 level is associated with NAFLD and its risk factors[22].

Despite the evidence supporting the effect of caffeine intake on NAFLD, studies in this field present inconclusive results on the threshold level at which caffeine can have beneficial effects. Even though caffeine intake was investigated in many studies, there is little or no evidence showing to extent of caffeine intake in NAFLD for improving the effectiveness of these biochemical markers and serum proteins. Therefore, our study attempts to present data on the threshold value of caffeine intake on health-promoting benefits in NAFLD. This study hypothesizes that liver and lipid biomarkers and related serum protein levels would improve with high dietary caffeine intake. The aim of this study is to examine the relationship between caffeine intake and ALT, AST, HDL levels, and CK18, irisin, FGF21 protein levels in individuals with NAFLD.

MATERIAL AND METHODS

Sample

Subjects were recruited from the Department of Gastroenterology, Kecioren Training and Research Hospital. A total of 120 volunteers were assessed for study eligibility. According to eligibility criteria, 40 subjects (20 females and 20 males) between the ages of 19 to 64, were deemed eligible and randomly recruited in the study to minimize selection bias. All 40 subjects completed the study.

Procedure

The diagnosis of NAFLD was based on exceeding the upper limits of indicators of liver function and determination of fatty liver by ultrasonography. Indicators of liver function include alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), and alkaline phosphatase (ALP). In addition, individuals who had any of the following conditions were excluded from the study; any other liver disease, viral hepatitis, chronic inflammatory disease, abdominal or bariatric surgery, alcohol intake (>20g/day, >30g/day for women and men, respectively), pregnancy, mental illness. Individuals were diagnosed

with NAFLD by a trained gastroenterologist and referred to the clinical dietitian. Qualified dietitian administrated the questionnaire face-to-face in the Hacettepe University Department of Nutrition and Dietetics. Voluntary participants signed an informed consent form. Due to the sensitive nature of the questions asked in this study, survey respondents were assured raw data would remain confidential and would not be shared. The application flow of the study is summarized in Figure 1. This research was approved by the Institutional Review Board, Non-Interventional Clinical Research Ethics Committee of Hacettepe University (protocol number: GO 17/309; date of approval: 04.04.2017) and conforms to the provisions of the Declaration of Helsinki.

Questionnaire

Questionnaire was designed to gather information on demographic characteristics, use of alcohol and tobacco products, physical activity level, and caffeine intake. The questionnaire was designed by collecting the list of questions from qualified health professionals and conducting a content validity testing. Firstly, the researchers conducted an extensive literature review to identify factors that may be associated with caffeine intake in NAFLD. Researchers compiled a list of queries based on the knowledge of clinical dietitians and public health experts in the topic. Subsequently, a content validation form was prepared. In this format, the relevance of each question was scored on a 1-point scale (0-not relevant, 1-relevant). The content validation was assessed by a review panel of eight clinical dietitians and public health specialists. Universal agreement would similarly be scored as 1 if all responses to a question were recoded as 1. After calculations, scale content validity index was 0.973 based on average and 0.913 based on universal agreement method. Furthermore, Cronbach's Alpha was calculated as 0.854 for internal consistency.

Assessment of caffeine intake

A caffeine-food frequency questionnaire (Caffeine-FFQ) was formed to evaluate caffeine intake of all participants. All of the caffeine-containing foods, beverages, athletic food supplements, and drugs were questioned retrospectively for the last six months. Caffeine-containing foods and beverages in the questionnaire include coffee, tea, cola, chocolate, energy drinks, and cacao. Quantity (grams, mL) and frequency of caffeine sources (never, every day, 1 to 2 times a week, 3 to 4 times a week, 5 to 6 times a week, once every 15 days, once a month) were recorded. The average daily caffeine intake amounts of the individuals were determined according to the recorded data. The data obtained from the Caffeine-FFQ (total coffee intake average) were validated according to the food frequency questionnaire and the 24-hour dietary recall data to minimize measurement bias. For the validation, the comparison of means approach was used.

Assessment of anthropometric measurements

Body weight, waist, hip, and neck circumference were measured for all participants. Body weight was measured with bare feet and minimal clothing to the nearest 0.1 kg using a standard scale. The body compositions of participants were measured using a bioelectrical impedance analysis (BIA) device (TANITA TBF-215, Tokyo, Japan). The principles followed before BIA measurement were as follows: Absence of a pacemaker or metal in individuals, not drinking alcohol and not doing heavy physical activity 24-48 hours before the measurement, not drinking tea or coffee and not eating 4 hours before the measurement, and not drinking a lot of water before measurement. Weight in kilograms divided by height in meters squared is how the body mass index (BMI) is computed. Subjects raised their arms while standing, and the waist circumference was measured at the midline between the lower rib and iliac bone. Hip circumference was measured from the widest part of the hip parallel to the floor using a non-stretch tape in a standing position. Waist/Hip ratio was calculated by dividing waist circumference with hip circumference. Using a non-stretch tape, the neck's circumference was measured below the laryngeal prominence and perpendicular to the long axis of the neck. All circumferences were recorded to the nearest 0.1 cm.

Biochemical parameters

Biochemical parameters including ALT, AST, GGT, ALP, creatinine, lipid profiles, total bilirubin, direct bilirubin, fasting glucose, and fasting insulin levels were tested routinely. Lipid profiles include high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol, triglyceride.

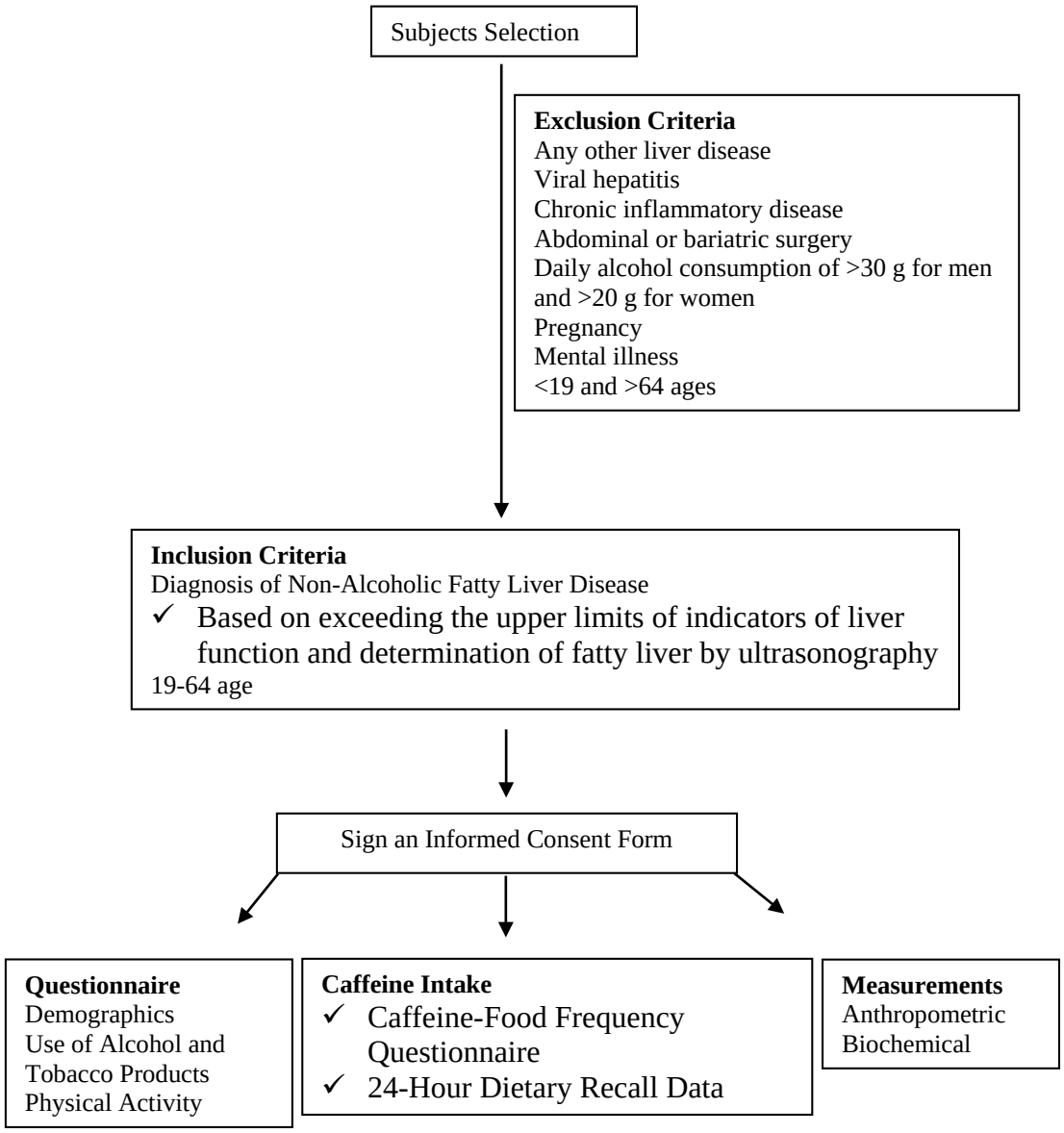

Figure 1. Study flow chart

This information was collected from patient files. The insulin resistance levels of individuals were calculated with homeostasis model of assessment insulin resistance (HOMA-IR) formula; [fasting blood glucose (mg/dl) * fasting insulin level (μU/ml)]/405[23].

Enzyme-linked immunosorbent assays (ELISA)

Blood samples were collected from all subjects after an overnight fast to analyze CK18, irisin, and FGF21. These samples were centrifuged at 5000 rpm and 4 ºC for 15 minutes. The serum samples (supernatant) were stored at -80 ºC until subsequent analysis. Serum

CK18 (Elabscience, USA, Catalog No: E-EL-H2072), tumor necrosis factor (TNF)-alpha (BOSTER, USA, Catalog No: EK0525), interleukin (IL)-6 (BOSTER, USA, Catalog No: EK0410), irisin (Elabscience, USA, Catalog No: E-EL-H2254) and FGF21 (BOSTER, USA, Catalog No: EK0994) proteins were determined by Enzyme-Linked Immunosorbent Assay (ELISA) analysis method. The serum samples were examined for duplicate in each of the five 96-well plates according to manufacturer's procedures, at Hacettepe University Department of Nutrition and Dietetics, Scientific Research Laboratory. The plates were read by a spectrophotometer with 450 nm wavelength within 30 minutes to determine the amounts of protein in the serum.

Statistical analysis

Sample size was determined via power analysis using anticipated means for biomarker levels in accordance to the previous research data [9,19] with 90% confidence interval and 0.05 type-I error (G*Power software, version 3.1.9.2, Franz Faul, Universität Kiel, Dusseldorf, Germany). Simple randomization method was used to recruit subjects. The statistical analyses were performed by Statistical Package for Social Sciences software (IBM SPSS Statistics version 21.0). Chi-square test was used to compare smoking and physical activity status between different treatment groups. Kolmogorov–Smirnov test of normality was used to test the distribution of variables. Independent sample t-test and Mann-Whitney u test were used to analyze whether the difference between the means was significant. One-way ANOVA was used for comparison of means among more than two groups. For post hoc analysis, least significant difference was used with equal variances, and Games-Howell test was used with unequal variances. Pearson correlation test was used to calculate the correlation between numerical data. There was no missing data. Data were presented as means ± standard deviation and percentage. Differences were considered significant at $p<0.05$.

RESULTS

Table 1 summarizes the characteristics of 20 male and 20 female participants with NAFLD. The average age of males is 39.7±10.66 years and the average age of females is 48.1±13.01 years. BMI is 32.4±4.69 kg/m^2 in males and 33.2±6.12 kg/m^2 in females. Waist-hip ratio is 1.0±0.06 cm in males while 0.9±0.07 cm in females. Neck circumference is 44.2±3.53 cm in males and 39.5±3.78 cm in females. Body fat composition is 31.2±6.78% in males while 40.4±6.42% in females. There was no significant difference in the groups in characteristics such as smoke (%), exercise (%), BMI (kg/m^2), waist-hip ratio (cm), neck circumference (cm) and body fat composition (%).

The caffeine intake of males and females is shown in Table 1. The total caffeine intake of males (256.8±198.15 mg) is slightly higher females (211.2±126.33 mg) (p=0.646). When the intake of caffeine sources was analyzed, the caffeine intake with coffee types in males higher than females (55.0±153.63 mg, 54.9±91.19 mg, respectively, p=0.998). The caffeine intake with tea types is 188.0±127.48 mg/day in males and 153.1±72.59 mg/day in females (p=0.295). The caffeine intake with carbonated drinks in males slightly higher females (5.4±11.39 mg, 0.4±1.28 mg, respectively, p=0.069). In addition, the caffeine intake with cocoa products in males (7.2±11.30 mg) was slightly higher females (2.8±5.17 mg) (p=0.126). There was no significant difference between the groups in caffeine intake with energy drinks.

By ensuring that the number of individuals in the groups was close, the cut-off values specific to this study were determined for the caffeine intake dose. Subjects were categorized into three groups according to their caffeine intake; Group 1: ≤150 mg/day, Group 2: 150-250 mg/day, and Group 3: ≥250 mg/day. Table 2 summarizes anthropometric measurements, liver parameters, lipid, and glucose profiles of individuals according to caffeine intake groups. Accordingly, the body fat composition of group 1 was higher than group 2 (40.2±6.63%, 32.3±8.50%, respectively, p=0.008). In addition, the difference between BMI and waist-hip ratio of the groups was not significant. ALT and AST levels of group 3 were higher than group 1 (p=0.029, p=0.050) and group 2 (p=0.011, p=0.007), respectively (Table 2). HDL-C level of group 3 was lower than group 1 (p=0.015) and group 2 (p<0.001) (Table 2). However, there was no significant difference between the groups in terms of other biochemical parameters and serum proteins.

Table 1. Some demographics and anthropometric measurement and caffeine intake amounts

Characteristics	Male (n=20)	Female (n=20)	*p*
Age (y)	39.7±10.66*	48.1±13.01*	0.031†
Smokers (%)	15.0	20.0	0.520‡
Exercisers (%)	15.0	25.0	0.429‡
BMI (kg/m²)	32.4±4.69*	33.2±6.12*	
Waist–hip ratio (cm)	1.0±0.06*	0.9±0.07*	
Neck circumference (cm)	44.2±3.53*	39.5±3.78*	
Body fat composition (%)	31.2±6.78*	40.4±6.42*	
Total Caffeine Intake (mg/day)	256.8±198.15*	211.2±126.33*	0.646†
Coffee drinks (mg/day)	55.0±153.63*	54.9±91.19*	0.998†
Tea drinks (mg/day)	188.0±127.48*	153.1±72.59*	0.295†
Carbonated drinks (mg/day)	5.4±11.39*	0.4±1.28*	0.069†
Cocoa products (mg/day)	7.2±11.30*	2.8±5.17*	0.126†

*Data are shown as mean±standard deviation. †t-test or mann-whitney u test were used for analysis. ‡Chi-square test was used for analysis. BMI, body mass index.

Table 2. Some anthropometric measurements, biochemical parameters and TNF-alpha, IL-6, CK18, irisin, and FGF21 according to caffeine intake

	Caffeine Intake (mg/day)			
	≤150 (n=13)	150-250 (n=16)	≥250 (n=11)	*p*
	mean±SD	mean±SD	mean±SD	
Body weight (kg)	91.2±14.20	82.7±18.18	89.5±22.01	0.415
BMI (kg/m²)	34.9±5.44	31.2±5.66	32.7±4.51	0.196
Waist–hip ratio (cm)	0.9±0.95	0.9±0.74	1.0±0.58	0.331
Body fat (%)	40.2±6.63	32.3±8.50	35.5±6.71	0.027*
ALT (U/L)	81.2±42.24	75.8±28.85	121.0±58.25	0.026*
AST (U/L)	49.4±20.44	43.2±17.86	66.9±25.75	0.022*
GGT (U/L)	53.1±24.44	73.4±33.90	97.0±113.83	0.264
ALP (U/L)	74.0±18.62	85.8±28.35	84.9±28.16	0.419
Creatinine (mg/dL)	0.8±0.18	0.8±0.12	0.7±0.18	0.378
HDL-C (mg/dL)	49.0±11.81	55.9±11.34	37.3±10.16	0.001*
LDL-C (mg/dL)	140.7±53.39	142.2±46.67	130.4±52.54	0.823
T-C (mg/dL)	209.9±58.15	212.2±38.76	243.4±61.06	0.229
Triglyceride (mg/dL)	192.0±106.10	181.5±137.52	369.7±351.80	0.062
Total bilirubin (mg/dL)	0.7±0.32	0.9±0.41	0.9±0.37	0.356
Direct bilirubin (mg/dL)	0.3±0.14	0.3±0.12	0.3±0.12	0.264
Fasting glucose (mg/dL)	123.6±34.77	105.7±30.45	94.1±17.81	0.055
Plasma insulin (µIU/ml)	23.0±12.40	20.8±25.81	19.1±12.11	0.883
HOMA-IR	7.0±4.76	5.7±6.96	5.0±2.61	0.656
TNF-alpha (pg/mL)	3.6±2.89	3.3±2.94	4.3±2.64	0.686
IL-6 (pg/mL)	5.4±2.64	4.0±2.23	4.7±1.73	0.252
CK-18 (mU/mL)	228.0±9.70	208.7±31.99	225.6±14.93	0.053
Irisin (pg/mL)	3.3±0.58	3.1±0.78	3.3±0.33	0.569
FGF21 (pg/mL)	112.3±22.85	149.1±87.32	118.2±28.23	0.209

Anova test was used for analysis. ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CK-18, cytokeratin-18; FGF21, fibroblast growth factor 21; GGT, gamma-glutamyl transferase; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model of assessment-insulin resistance; IL-6, interleukin-6; LDL-C, low-density lipoprotein cholesterol; SD, standard deviation; T-C, total cholesterol; TNF-alpha, tumor necrosis factor-alpha. *$p<0.05$.

Correlations between caffeine intake and biochemical parameters, age, and body fat composition (%) of subjects with NAFLD are shown in Table 3. There was a negative correlation between total caffeine

intake and age (r=-0.398, p=0.011), and a positive correlation between total caffeine intake and ALT level (r=0.346, p=0.029). However, correlations between caffeine intake and body fat composition and other biochemical parameters were not significant.

Table 3. Correlations between caffeine intake and some biochemical parameters, age and body fat composition

	Caffeine Intake (mg/day)	
	r	***p***
Age (y)	-0.398	0.011*
Body fat composition (%)	-0.178	0.271
ALT (U/L)	0.346	0.029*
AST (U/L)	0.249	0.122
Triglyceride (mg/dL)	0.177	0.274
HDL-C (mg/dL)	-0.283	0.077
LDL-C (mg/dL)	-0.069	0.674
T-C (mg/dL)	0.113	0.489
Fasting Glucose (mg/dL)	-0.288	0.072
HOMA-IR	-0.071	0.661

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model of assessment-insulin resistance; LDL-C, low-density lipoprotein cholesterol; r, pearson correlation coefficient; T-C, total cholesterol. *p<0.05.

Table 4. Correlations between serum proteins and biochemical parameters and anthropometric measurement

	TNF-alpha		**IL-6**		**CK-18**		**Irisin**		**FGF21**	
	r	***p***	**r**	***p***	**r**	***p***	**r**	***p***	**r**	***p***
Waist–hip ratio (cm)	0.146	0.367	0.048	0.771	-0.130	0.423	0.271	0.090	-0.106	0.515
BMI (kg/m^2)	-0.037	0.822	0.106	0.517	0.228	0.157	0.050	0.759	-0.011	0.945
Body fat (%)	-0.050	0.758	0.170	0.295	0.436	0.005*	-0.101	0.535	0.061	0.710
ALT (U/L)	-0.102	0.533	0.212	0.190	0.047	0.772	0.271	0.091	-0.191	0.237
AST (U/L)	-0,179	0,269	0.450	0.004*	0,165	0,308	0.216	0.180	-0.137	0.398
GGT (U/L)	-0,157	0,333	0.323	0.042*	-0,084	0,608	-0.027	0.869	-0.039	0.813
ALP (U/L)	0,037	0,819	0.461	0.003*	-0,019	0,906	0.029	0.858	0.042	0.796
Total Bilirubin (mg/dL)	-0,165	0,309	-0,025	0,879	-0.415	0.008*	0.167	0.303	-0.093	0.566
Direct Bilirubin (mg/dL)	-0,226	0,161	0,131	0,421	-0.400	0.011*	0.160	0.325	-0.053	0.747
Triglyceride (mg/dL)	-0.055	0.736	0.268	0.095	0.255	0.112	0.054	0.742	0.085	0.603
HDL-C (mg/dL)	0.131	0.420	0.099	0.545	-0.230	0.154	-0.001	0.997	0.159	0.328
LDL-C (mg/dL)	-0.346	0.029*	0.368	0.020*	-0,037	0,822	0.128	0.430	-0.065	0.691
T-C (mg/dL)	-0.305	0.055	0.111	0.494	0.119	0.466	0.029	0.857	0.071	0.663
Fasting glucose (mg/dL)	-0.180	0.265	0.289	0.070	0.157	0.333	0.050	0.757	0.095	0.561
HOMA-IR	0,208	0,199	0.317	0.046*	0,103	0,527	0.200	0.216	-0.153	0.346
Total caffeine intake (mg/day)	0.063	0.699	-0.099	0.545	-0.012	0.940	0.048	0.770	-0.041	0.802

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CK-18, cytokeratin-18; FGF21, fibroblast growth factor; GGT, gamma-glutamyl transferase; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model of assessment-insulin resistance; IL-6, interleukin-6; LDL-C, low-density lipoprotein cholesterol; r, pearson correlation coefficient; T-C, total cholesterol; TNF-alpha, tumor necrosis factor-alpha. *p<0.05.

Table 4 summarizes correlations between serum proteins and biochemical parameters, anthropometric measurement of subjects with NAFLD. There was a negative correlation between TNF-alpha and LDL-C (r=-0.346, p=0.029). In addition, there were positive correlations between IL-6 and AST (r=0.450, p=0.004), GGT (r=0.323, p=0.042), ALP (r=0.461, p=0.003), LDL-C (r=0.368, p=0.020) and HOMA-IR (r=0.317, p=0.046). In addition, there was a positive correlation between CK-18 and body fat composition (%) (r=0.436, p=0.005), and a negative correlation between CK-18 and total (r=-0.415, p=0.008) and direct bilirubin (r=-0.400, p=0.011).

DISCUSSION

There is an inverse relationship between coffee intake and the risk of liver diseases[5,11,24-28]. This beneficial effect of coffee is thought to originate from caffeine, one of the active compounds in its structure[7]. The amount and sources of caffeine intake differ among societies. In this study, total caffeine intake is similar both in males (256.8±198.15 mg) and females (211.2±126.33 mg). Caffeine intake up to 400 mg is reported to be safe for a healthy adult[29]. This value is equivalent to about 5 cups of espresso or 8 cups of tea. In this study, black tea was the first source of caffeine for both males (185.8±28.70 mg) and females (140.2±60.57 mg). The secondary source was espresso (21.5±84.60 mg) for males and instant coffee (17.4±29.43 mg) for females. Although coffee is the primary source of caffeine around the world, the primary caffeine source in Turkey is black tea. In addition, many factors (age, nutritional habits, climate condition etc.) can affect caffeine intake[30]. In this study, total caffeine intake was found to be lower than other countries[8,31]. It may be due to black tea being the primary source of caffeine in Turkey, whereas coffee is the primary source of caffeine in other countries. In this study, there was a negative correlation between age and caffeine intake. Older individuals with liver disease may think foods and beverages containing caffeine are unhealthy[10].

Studies have reported that lower liver enzymes are associated with coffee intake[11,31]. However, the dose required to exert these beneficial effects is unknown. Despite the evidence supporting the protective effect of coffee, the underlying mechanism is still unclear[31]. Caffeine in coffee is thought to have beneficial effects on the liver via adenosine receptor antagonism or antioxidant effects[7,13]. In a study by Ruhl and Everhart, there was a significantly greater reduction in serum ALT level in caffeine intake compared to coffee intake[24]. In another study, there was a positive correlation (r=0,173; p=0,02) between total caffeine intake and ALT level[9]. Graeter and colleagues reported no correlation between caffeine intake and serum ALT level[32]. In an experimental study, Turkish coffee (unfiltered) has increased aminotransferase level[33]. In this study, caffeine intake of ≥250 mg/day showed higher levels of ALT and AST in individuals with NAFLD. However, ALT and AST levels showed no significant difference between caffeine intake of < 150 mg/day and 150-250 mg/day (Table 2). According to these results, caffeine intake of <250 mg/day may benefit biochemical parameters. In addition, the effect of caffeine on reducing liver enzyme levels may be more pronounced in individuals with high liver damage [34].

Coffee may have some effects on inflammatory cytokines. Fukushima et al. examined the effect of coffee on inflammatory cytokine gene expression in high-fat diet fed mice. As a result, coffee may be a potent anti-inflammatory stimulant[35]. Recent animal studies have highlighted antioxidative and anti-inflammatory effects, particularly in steatohepatitis[36,37]. Furthermore, adipocyte cytokine secretions such as TNF-alpha, IL-6, and IL-8 may increase during the development of NAFLD[38,39]. Coffee can improve fatty liver by reducing inflammatory cytokine secretion, increasing insulin sensitivity, suppressing hyperglycemia, and antioxidant effect [5]. In this study, the effect of caffeine intake on inflammatory proteins was not observed. This situation may be explained by caffeine intake being lower in Turkey compared to the other countries. In addition, many factors are involved in the pathogenesis of NAFLD, and more studies are needed to clearly understand the effect mechanism of caffeine. There was a positive correlation between CK18, which is a liver fibrosis indicator, and body fat composition (%) in this study. Increased body fat composition (%) is an indication of increased visceral adiposity. Increased lipid accumulation in liver may exacerbate pathology and histological findings of the disease. High energy, saturated fat, animal protein, sugar, cholesterol, low fiber, and polyunsaturated fatty acid intake are closely associated with NAFLD. Nutrient intakes may affect NAFLD markers[40]. Therefore, the effect on ALT, AST, and HDL-C

levels seen with ≥250 mg caffeine intake may be affected by nutrient intake.

The strength of this study is that it evaluates total caffeine intake in the diet. Many studies examining the protective effect of caffeine in NAFLD have focused on coffee intake rather than total caffeine intake. Caffeine-FFQ is designed and developed for this study. Caffeine-FFQ included all food and beverages, medicines, and nutritional supplements containing caffeine.

The present study has some limitations. Firstly, NAFLD diagnosis was decided via ultrasonography criteria. Liver biopsy, which is accepted as the gold standard in the diagnosis of NAFLD, or fibroscan were not performed. This situation has created limitations in classifying individuals according to NAFLD severity. Moreover, caffeine intake was evaluated for only last 6 months. Finally, nutrient intakes have not been eliminated, which may slightly affect NAFLD markers.

In conclusion, although the mechanism of action is not known precisely, caffeine may have a protective effect on the development and progress of NAFLD. However, this protective role may appear up to a certain caffeine intake threshold. In this study, ALT, AST, and HDL-C levels were adversely affected for individuals with NAFLD intake ≥250 mg/day caffeine. However, there is a need for further studies to determine the action mechanism, and the intake suggested dose of caffeine in NAFLD.

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