

# Effects of Green Coffee Bean Extract Supplementation on Patients with Non-Alcoholic Fatty Liver Disease: A Randomized Clinical Trial

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## Abstract

**Background:** Non-alcoholic fatty liver disease (NAFLD) is a major worldwide public health problem with no effective treatment options. Green coffee bean extract (GCBE) is a rich source of bioactive phytochemicals with a variety of biochemical and physiological effects.

**Objectives:** The aim of this study was to investigate the effects of GCBE on the management of patients with NAFLD.

**Methods:** 44 patients with NAFLD were enrolled in a parallel, double-blind, placebo-controlled clinical trial. The participants were administered either GCBE or placebo (1 gram/day) for 8 weeks. They also were advised to follow a standard energy-balanced diet and physical activity. Liver ultrasonography, anthropometric variables, and biochemical parameters were compared at pre- and post-intervention.

**Results:** GCBE significantly improved the levels of aspartate aminotransferase (AST), triglyceride (TG), total cholesterol, free fatty acids (FFAs), fasting blood sugar (FBS), homeostasis model assessment insulin resistance (HOMA-IR) index, high-sensitivity C-reactive protein (hs-CRP), and total antioxidant capacity (TAC) compared to the placebo group. On the other hand, there were no significant differences between the two groups in body weight, HDL-cholesterol, LDL-cholesterol, LDL-C to HDL-C ratio, insulin, degree of steatosis, aspartate transaminase (AST), alkaline phosphatase (ALP), and tumor necrosis factor-alpha (TNF- $\alpha$ ).

**Conclusions:** GCBE supplementation may benefit patients with NAFLD. These beneficial effects may be due to the possible ability of GCBE to improve insulin sensitivity and its anti-inflammatory and antioxidant properties.

**Keywords:** Non-Alcoholic Fatty Liver Disease, Green Coffee Bean Extract, Chlorogenic Acid, Polyphenols, Hepatic Steatosis, Phytochemicals

## 1. Background

Non-alcoholic fatty liver disease (NAFLD) represents a spectrum of histological abnormalities ranging from hepatocellular steatosis to more severe non-alcoholic steatohepatitis (NASH), which may progress to hepatic fibrosis and cirrhosis (1). The increasing prevalence of NAFLD is tied to the rising epidemic of obesity (2), and now it is affecting approximately one third of the population in the developed countries and Asia (3). The prevalence of NAFLD in the obese population is estimated to be as high as 60%, and NASH is diagnosed in 19% of these individuals (4). Consequently, the prognosis of patients with steatosis is benign despite a 1% - 2% risk of progressing to cirrhosis over 15 - 20 years (5). Unfortunately, up to 5% - 11% of NASH patients develop end-stage liver disease (6); thus, it is estimated that NASH will become the major cause of liver transplantation by the year 2020 (7). In addition to the increased risk of

liver-related mortality, NAFLD is also independently associated with an increased risk for certain malignancies, type 2 diabetes, dyslipidemia, cardiovascular disease (CVD), and chronic kidney disease (8). Hence, there is an urgent need for an effective treatment for NAFLD.

Currently, there is no approved pharmacotherapy for NAFLD; and dietary modification and lifestyle changes focusing on weight reduction are recommended as the primary treatment in the management of NAFLD. However, unfortunately weight reduction is neither easy to achieve nor to sustain (9). Hence, as NAFLD therapy still remains an issue of concern, a wide variety of treatment approaches with several mechanisms are under evaluation. In the last few years, the effects of nutraceuticals on NAFLD have received much attention (10). Many clinical trials have also evaluated the effects of resveratrol, silymarin, omega-3 fatty acids, carnitine, vitamin E, and vitamin D on liver function (10).

Coffee, as a nutraceutical, is one of the most popular consumed beverages worldwide (11). Coffee is a source of bioactive phytochemicals including methylxanthines (e.g. caffeine), amino acids, phenolic acids, and polyphenols (11). Research has shown that consumption of coffee may play a protective role against various diseases of modern society, such as obesity, type 2 diabetes, cancer, and cardiovascular disorders (12) as well as neurodegenerative disorders (13, 14). More recently, a number of studies have suggested the protective effect of coffee intake and coffee constituents on liver function (15). A growing number of epidemiologic evidence has shown an inverse association between coffee consumption and the risk of NAFLD (16). Coffee consumption has also been associated with reduced levels of hepatic aminotransferases and progression of pre-existing liver disease (17, 18). Besides, experimental studies have demonstrated that coffee and coffee polyphenols improved insulin sensitivity, fatty liver, and abdominal fat (16). These beneficial effects of coffee consumption may be explained by its bioactive phytochemicals (11).

Bioactive phytochemicals of coffee have drawn attention due to their reported biological properties such as antioxidant and anti-inflammatory activities (19), increased fatty acid oxidation and insulin sensitivity, and modulation of glucose absorption and utilization (20). However, coffee roasting process destroys significant amounts of the bioactive phytochemicals that are believed to be responsible for the biological actions, such as chlorogenic acid (11). To avoid loss of some compounds with health beneficial effects, coffee can also be used as green coffee bean extract (GCBE) (11). GCBE is made up of unroasted coffee beans and contains higher amounts of bioactive phytochemicals than that for the usual roasted coffee that is currently used (11, 21).

## 2. Objectives

Therefore, the aim of the present study was to investigate the effects of GCBE supplementation on serum lipid profiles, insulin resistance, oxidative stress, inflammatory biomarkers, and serum liver enzymes in overweight and obese patients with NAFLD in a randomized-controlled trial.

## 3. Methods

### 3.1. Materials

The green coffee bean extract (GCBE) utilized for this study is a commercially prepared supplement (Nature's Way, USA). Each capsule of GCBE contained 500 mg of GCBE

and is claimed by the manufacturer to be an alcoholic extract of Arabic coffee standardized to 50% chlorogenic acid (250 mg). Placebo capsules were packaged at the Pharmacy Department of Ahvaz Jundishapur University of Medical Sciences. Placebo capsules and package were identical in appearance to the GCBE capsules and contained the same amount of edible starch.

### 3.2. Study Population

A total of 98 men or women aged 20 to 70 years who were diagnosed with NAFLD by ultrasonography and increased levels of alanine transaminase (ALT) were recruited from the gastroenterology outpatient clinic of Imam Khomeini hospital, Ahvaz, Iran.

### 3.3. Inclusion Criteria

Participants' eligibility included: age greater than 20; diagnosis of NAFLD determined by ultrasound (steatosis score equal or greater than 1) and serum levels of ALT higher than 19 U/l for women and 30 U/l for men (22), and body mass index (BMI = weight (kg)/height<sup>2</sup> (m)) greater than 24.9 and less than 35.

### 3.4. Exclusion Criteria

Patients with other causes of fatty liver (i.e. alcohol consumption; steatogenic drugs such as calcium channel blocker, methotrexate, tamoxifen, amiodaron, and corticosteroids; and parenteral nutrition); any other known forms of hepatic disease (such as viral hepatitis, autoimmune liver diseases, hereditary hemochromatosis, Wilson's disease, toxic hepatitis, etc.); any other known metabolic disease (diabetes, hypothyroidism, Cushing's syndrome, renal failure, cancer and etc.); women who were pregnant or lactating; hormone replacement therapy; taking other supplements in the past 6 months and during the study; history of bariatric surgery in the past or strict weight loss diets in the past 6 months; and compliance with supplements consumption less than 90% in any follow-up visit, were excluded.

### 3.5. Ethical Approval

The trial protocol was approved by the ethics committee of Ahvaz Jundishapur University of Medical Sciences (No. IR.AJUMS.REC.1394.710) and registered at the Iranian registry of clinical trial (No. IRCT2016030626941N1). Written informed consent was obtained from all participants.

### 3.6. Study Design

A total number of 44 NAFLD patients who fulfilled the selection criteria were enrolled in the study. This study was an 8-week double-blind, placebo-controlled, parallel-arm randomized clinical trial. Thus, the participants were randomly allocated to GCBE or placebo groups (1:1 ratio) using block randomization method with a block size of 6. The list of randomization was computer-generated and a person, who was not aware of the nature of the trial, packed the supplements and placebo capsules in numbered bottles based on the list. The other person who was not aware of random sequences allocated the patients to the numbered bottles.

### 3.7. Intervention

Participants in the GCBE group ( $n = 22$ ) assigned to receive two GCBE capsules daily for 8 weeks. They were advised to take one capsule 30 minutes before breakfast and the other 30 minutes before lunch. NAFLD patients in the placebo group ( $n = 22$ ) assigned to receive the same amount of placebo for the same time. Safety of the dose and duration of the study has been verified in previous studies (23, 24). The capsules were given to the patients at the time of randomization and at the 4<sup>th</sup>-week follow-up visit. The compliance was assessed by counting unused capsules, which were returned to the researchers at each followed-up visit. At the first visit, all participants were also advised to keep an energy balanced diet and physical activity, according to the clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults from the national institutes of health and the North American association for the study of obesity (25). Furthermore, they were asked to exercise at least for 30 minutes every day. Patients were followed-up by a call at weekly intervals for awareness of any adverse reactions and to be reminded of the supplements consumption and adherence to the trial protocol.

### 3.8. Clinical, Paraclinical, and Dietary Intake Assessments

Body weight (WT), height (HT), waist circumference (WC), and hip circumference (HC) were measured according to the World Health Organization's recommendation at baseline (week 0) and at the end of the study (8<sup>th</sup> week). The waist/hip ratio (WHR) and BMI were also calculated. To reduce measurement errors, all the anthropometric parameters were made by one person. Ten milliliters of fasting blood sample were obtained from each patient after 10–12 hours of overnight fasting at baseline and at the end of the study. The serum of the blood samples was stored at  $-80^{\circ}\text{C}$  until further assays.

Serum enzyme activities of alanine aminotransferase (ALT) and aspartate transaminase (AST) were measured by using the kinetic method (Pars Azmoon Co, Tehran, Iran); and for alkaline phosphatase (ALP), serum activity were determined by using p-nitrophenol phosphate as substrate (kinetic ALP/DGKC method, Pars Azmoon Co, Tehran, Iran). Total cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were measured by the enzymatic photometric method (Pars Azmoon Co, Tehran, Iran). Triglyceride (TG) was measured by the colorimetric enzymatic method (Pars Azmoon Co, Tehran, Iran). Fasting blood glucose levels were assayed using the glucose oxidase method (GODPAP, Pars Azmoon Co, Tehran, Iran).

Commercially available enzyme-linked immunosorbent assay (ELISA) kits following the manufacturers' protocols were used to measure serum levels of fasting insulin (Insulin-R monobind, Lake Forest, USA), high-sensitivity C-reactive protein (hs-CRP) (CRP Elisa Kit- LDN Labor Diagnostika Nord GmbH&Co KG, Nordhorn, Germany), free fatty acids (FFAs) (HANGZHOU EAST BIOPHARM CO., LTD, China), total antioxidant capacity (TAC) (ZellBio GmbH, Germany), and tumor necrosis factor-alpha (TNF- $\alpha$ ) (IBL Co., Ltd. Hamburg, Germany). All biochemical measurements were done in the same laboratory.

The homeostasis model assessment insulin resistance index (HOMA-IR) was used to calculate insulin resistance according to the formula:  $\text{HOMA-IR} = \text{fasting glucose (mg/dL)} \times \text{fasting insulin (IU/mL)} / 405$ .

To assess dietary intake, a 3-day 24-hour dietary recall (two weekdays and a weekend) was obtained from all the participants at baseline and at the end of the study. Nutritional analysis of dietary intakes was performed by using modified Nutritionist IV software (version 3.5.2, First Data-Bank; Hearst Corp, San Bruno, California). Furthermore, by using the short-form of international physical activity questionnaire (IPAQ), the physical activity of each patient was assessed at baseline and at the end of the study.

Both before and at the end of the study, the same trained ultrasound reader blinded to the groups performed a liver ultrasonography (General Electric LOGIQ 400 CL- Using probe 3.5/5 MHz, USA). To obtain a semi-quantitative evaluation of the severity of the fat deposition in the liver, a scoring system was adopted. Based on parameters, including liver echo-texture, the brightness of the liver, the contrast ratio of the liver-to-kidney, and blurred vessels, the degree of hepatic fatty infiltration was scored from I to III.

### 3.9. Statistical Analysis

Distribution of variables was assessed by using Shapiro-Wilk test. Paired t-test, Wilcoxon paired rank

test, and MacNemar test were respectively used for within-group comparisons of quantitative normally distributed, non-normally distributed, and categorical variables. Between-group differences were assessed by independent samples t-test, Mann-Whitney U test, and chi-square test for normally distributed, non-normally distributed, and categorical variables, respectively. All statistical analyses were performed using SPSS software version 16 (SPSS Inc, Chicago, IL, USA). A two-sided P Value less than 0.05 was considered significant in all analyses.

## 4. Results

### 4.1. Patient Inclusion, Study Completion, Safety and Compliance

All 44 NAFLD patients allocated to the trial groups (GCEB group, n = 22; placebo group, n = 22) completed the study. Thus, 44 patients entered into the final analysis. No subject showed hypersensitivity reaction or complained about abnormal events during the trial. Patient compliance with study treatment was 96% in the GCEB group versus 98% in the placebo groups.

### 4.2. Baseline Characteristics

Baseline characteristics of the study participants are shown in Table 1. As can be seen, there was an overall homogeneity in variables between the two groups at baseline. Thus, there were no statistically significant between-group differences in all variables at baseline ( $P > 0.05$  for all variables).

### 4.3. Anthropometric, Nutritional Variables and NAFLD Severity

Within- and between-group changes in terms of all the variables are summarized in Table 2. In within-group comparisons, body weight and BMI significantly decreased in both groups ( $P < 0.001$ ); however, these changes were not significant in between-group comparisons. After the intervention, GCEB group showed a significantly greater reduction in waist circumference than placebo group. However, similarly these changes were not significant between the two groups. There were no significant differences in hip circumference and waist/hip ratio (WHR) in within- and between-group comparisons. There was no significant difference in the improvement of physical activity between the arms ( $P = 0.336$ ); however, it significantly improved within the groups. No significant differences were also found between the groups in estimated energy intake and percentage of carbohydrate, protein, and fat in the diet. However, in within-group comparisons, estimated energy intake significantly decreased in both groups and the percentage of fat in diet significantly increased in the placebo

group. At the end of the study, there were no significant differences between the two groups in percentages of NAFLD grades.

### 4.4. Biochemical Parameters

Table 3 demonstrates the biochemical parameters in both groups before and after the intervention.

#### 4.4.1. Liver Enzymes

Serum ALT levels were significantly lower at the end of the study in the GCEB group than the placebo group ( $P < 0.001$ ). However, serum AST and ALP changes during the study were not statistically different between the two groups.

#### 4.4.2. Markers of Insulin Resistance

Reductions in FBS and HOMA-IR were significantly greater in the GCEB group than the placebo group ( $P = 0.01$ ;  $P = 0.04$ , respectively) although fasting insulin did not change significantly in any group.

#### 4.4.3. Serum Lipid Parameters

After intervention, TG, total cholesterol, and FFAs significantly reduced in the GCEB group compared to the placebo group ( $P = 0.037$ ;  $0.025$ ;  $0.02$ , respectively); in contrast, the mean changes in the improvement of LDL-C, HDL-C, and LDL-C to HDL-C ratio were not significant ( $P = 0.363$ ;  $0.593$ ;  $0.95$ , respectively) between the two groups.

#### 4.4.4. Inflammatory and Antioxidant Markers

Serum hs-CRP concentrations between the two groups showed a significant reduction; in contrast, concentrations of TNF- $\alpha$  were not significantly different between the groups. Finally, TAC levels significantly increased at the end of the study in the GCEB group compared to the placebo group.

## 5. Discussion

Scientific studies have shown that coffee consumption may be beneficial in NAFLD through a direct effect on the liver as well as beneficial systemic metabolic effects. Experimental and human studies have also demonstrated that green coffee bean extracts (GCBEs) enhance energy metabolism and expenditure, decrease blood lipid levels, improve glucose tolerance, and support weight management (26). GCBE contains chlorogenic acid (CGA) as the principal constituent, and most of the health benefits of decaffeinated coffee and its by-product have been attributed to chlorogenic acid (26). The present study is the first randomized trial that investigated the effect of

**Table 1.** Baseline Characteristics in Two Groups

Characteristics	GCBEGroup (N = 22)	Placebo Group (N = 22)	P Value
Gender (male/female), % <sup>a</sup>	50 / 50	50 / 50	1
Smoker, No. <sup>a</sup>	1	2	1
Age, y <sup>b</sup>	41.36 ± 7.69	44.50 ± 5.24	0.123
Height, cm <sup>b</sup>	168.87 ± 10.91	169.53 ± 8.48	0.823

<sup>a</sup>Data are tested by chi-square test.<sup>b</sup>Data are expressed as mean ± standard deviation and tested by independent samples t-test.**Table 2.** Within- and Between-Group Comparisons of the Changes from Baseline to the End of the Intervention for Fatty Liver Status, Anthropometric and Nutritional Variables in Both Groups

Variables	GCBEGroup (N = 22)			Placebo Group (N = 22)			Between Group P	
	Before	After	Within group P	Before	After	Within group P	Before	After
Grade of NAFLD, % <sup>a</sup>			0.31			0.31	1	1
I, (No.)	54.5 (12)	50 (11)		54.5 (12)	50 (11)			
II, (No.)	31.8 (7)	36.4 (8)		31.8 (7)	36.4 (8)			
III, (No.)	13.6 (3)	13.6 (3)		13.6 (3)	13.6 (3)			
Weight, kg <sup>b</sup>	88.81 ± 6.73	85.68 ± 5.73	< 0.001	90.25 ± 6.99	88.60 ± 6.69	< 0.001	0.492	0.128
BMI, kg/m <sup>2b</sup>	31.27 ± 2.58	30.24 ± 2.63	< 0.001	31.45 ± 2.18	30.87 ± 2.16	< 0.001	0.809	0.385
WC, cm <sup>b</sup>	103.18 ± 8.93	102.23 ± 9.01	0.012	105.26 ± 7.44	104.64 ± 8.27	0.130	0.406	0.360
HC, cm <sup>b</sup>	104.56 ± 6.27	103.88 ± 5.60	0.057	105.09 ± 7.19	104.85 ± 7.38	0.471	0.799	0.624
WHR <sup>c</sup>	0.98 (.88, 1.10)	0.97 (.86, 1.10)	0.615	0.99 (.91, 1.26)	0.99 (.90, 1.29)	0.425	0.390	0.663
PA (METmin/week) <sup>c</sup>	214.36 (135, 323)	235.63 (172, 372)	0.002	224.45 (175, 487)	247.40 (189, 401)	0.007	0.622	0.336
Energy, kcal/d <sup>b</sup>	2230.59 ± 156.06	2220.45 ± 152.95	0.005	2160.63 ± 146.61	2136.77 ± 134.37	0.030	0.133	0.061
CHO, % <sup>b</sup>	57.73 ± 3.50	56.18 ± 2.76	0.006	58.62 ± 2.80	55.89 ± 3.15	0.002	0.360	0.744
Fat, % <sup>b</sup>	29.17 ± 3.24	30.24 ± 2.89	0.149	28.78 ± 3.04	29.75 ± 2.95	0.033	0.681	0.584
protein, % <sup>b</sup>	13.08 ± 2.98	13.57 ± 3.92	0.534	12.59 ± 1.97	14.35 ± 4.43	0.086	0.520	0.540

Abbreviations: BMI, Body Mass Index; CHO, Carbohydrate; HC, Hip Circumference; MET, Metabolic Equivalent of Task; NAFLD, Non-Alcoholic Fatty Liver Disease; PA, Physical Activity; WC, Waist Circumference; WHR, Waist to Hip Ratio.

<sup>a</sup>Data are tested by McNemar test (within the groups) and chi-square test (between the groups).<sup>b</sup>Data are expressed as mean ± standard deviation and tested by Paired t-test (within the groups) and independent samples t-test (between the groups).<sup>c</sup>Data are expressed as mean (minimum, maximum) and tested by Wilcoxon paired rank test (within the groups) and Mann-Whitney U test (between the groups).

CGA-rich GCBEG supplementation in patients with NAFLD. Briefly, the results of this parallel-arm trial indicate that daily supplementation with one gram GCBEG for 8 weeks significantly and beneficially affected serum levels of ALT, TG, total cholesterol, FFAs, hs-CRP, TAC, FBS, and HOMA-IR index between the arms (Table 3).

GCBEG has been gaining popularity as a potential weight loss supplement (27). Several plausible mechanisms through which GCBEG and/or CGA may exert its effects on weight management have been suggested, such as improving lipolytic activity by modification of metabolic pathways (28), and decrease in lipid absorption by inhibi-

tion of the pancreatic lipase (29). In this study, although weight reduction in both groups compared to baseline state was significant, GCBEG supplementation was not associated with a significantly greater weight loss compared to the placebo group. A reasonable explanation for the weight loss achieved in the two groups may be the decrease in energy intake and improvement in physical activity that occurred during the study. In contrast to our trial, a systematic review and meta-analysis of three out of five eligible human trials by Onakpoya et al. (27) showed a significant mean difference in body weight in GCBEG-treated groups compared to the placebo group (mean difference:



**Table 3.** Within- and Between-Group Comparisons of the Changes from Baseline to the End of the Intervention for Biochemical Parameters

Variables	GCBE Group (N = 22)			Placebo Group (N = 22)			Between Group P	
	Before	After	Within group P	Before	After	Within group P	Before	After
<b>Liver enzymes markers</b>								
ALT, IU/L <sup>a</sup>	33.04 ± 4.28	26.04 ± 3.30	< 0.001	33.63 ± 4.42	34.04 ± 4.81	0.617	0.655	< 0.001
AST, IU/L <sup>a</sup>	36.77 ± 11.10	36.00 ± 11.13	0.385	37.13 ± 9.84	36.77 ± 9.96	0.730	0.909	0.810
ALP, IU/L <sup>b</sup>	187.04 (134, 317)	186.04 (127, 302)	0.614	186.04 (140, 321)	192.36 (132, 314)	0.721	0.751	0.851
<b>Markers of insulin resistance</b>								
FBS, mg/dL <sup>a</sup>	105.81 ± 8.79	98.90 ± 7.08	0.008	108.95 ± 14.28	106.59 ± 11.17	0.159	0.386	0.010
Insulin, $\mu$ U/mL <sup>a</sup>	14.52 ± 2.91	13.65 ± 1.69	0.070	13.80 ± 2.38	13.65 ± 2.37	0.611	0.377	0.622
HOMA-IR <sup>a</sup>	3.79 ± 0.85	3.24 ± 0.36	0.006	3.67 ± 0.57	3.57 ± 0.63	0.290	0.571	0.041
<b>Serum lipid parameters</b>								
TG, mg/dL <sup>a</sup>	226.36 ± 52.56	188.59 ± 39.70	< 0.001	223.04 ± 40.48	220.63 ± 57.36	0.750	0.792	0.037
T- chol, mg/dL <sup>a</sup>	211.77 ± 33.42	194.81 ± 25.32	< 0.001	214.40 ± 33.84	214.95 ± 31.91	0.841	0.796	0.025
LDL-C, mg/dL <sup>a</sup>	139.95 ± 17.12	137.54 ± 24.26	0.551	142.90 ± 19.81	137.18 ± 18.02	0.065	0.599	0.363
HDL-C, mg/dL <sup>a</sup>	46.36 ± 11.56	46.00 ± 12.18	0.653	44.09 ± 5.73	44.40 ± 6.63	0.747	0.415	0.593
LDL to HDL ratio <sup>a</sup>	3.16 ± 0.73	3.13 ± 0.76	0.780	3.29 ± 0.59	3.14 ± 0.60	0.222	0.530	0.95
FFAs, mMol <sup>a</sup>	0.89 ± 0.44	0.66 ± 0.35	< 0.001	0.94 ± 0.35	0.92 ± 0.34	0.626	0.716	0.020
<b>Inflammatory and antioxidant Markers</b>								
TNF- $\alpha$ , pg/mL <sup>a</sup>	9.64 ± 3.92	8.63 ± 5.00	0.161	8.27 ± 3.17	8.82 ± 4.09	0.279	0.211	0.896
hsCRP, mg/L <sup>b</sup>	1.42 (.36, 3.38)	1.08 (.47, 2.26)	< 0.001	1.49 (.44, 2.75)	1.50 (.40, 3.04)	0.846	0.324	0.012
TAC, mMol <sup>a</sup>	1.20 ± .22	1.58 ± 0.37	< 0.001	1.26 ± .22	1.28 ± .24	0.649	0.400	0.003

Abbreviations: ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; ALP, Alkaline Phosphatase; FBS, Fasting Blood Sugar; FFAs, Free Fatty Acids; HDL-C, High-Density Lipoprotein Cholesterol; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; hsCRP, High-Sensitivity C-Reactive Protein; LDL-C, Low-Density Lipoprotein Cholesterol; TAC, Total Antioxidant Capacity; TC, Total Cholesterol; TG, Triglyceride; TNF- $\alpha$ , Tumor Necrosis Factor-Alpha.

<sup>a</sup>Data are expressed as mean  $\pm$  standard deviation and tested by Paired t-test (within the groups) and independent samples t-test (between the groups).

<sup>b</sup>Data are expressed as mean (minimum, maximum) and tested by Wilcoxon paired rank test (within the groups) and Mann-Whitney U test (between the groups).

-2.47 kg; 95% CI: -4.23, -0.72). However, the author declared that all included studies had poor methodological quality and there was a significant heterogeneity amongst them. These inconsistent results may also be explained by different dosages, GCBE types, and duration of the studies.

NAFLD is associated with alterations in normal metabolic pathways, which have adverse consequences on health, such as perturbation in lipid and glucose metabolism and insulin sensitivity (30, 31). Hence, insulin sensitizers as well as lipid- and glucose-lowering agents have become important options for the management of NAFLD and its cardiovascular complications. Beneficial effects of GCBE and CGA on the improvement of insulin resistance (IR), lipid profile, and glucose metabolism have been shown in experimental and human studies (32-34).

IR is frequently found in NAFLD patients, and is as-

sociated with both steatosis emergence and disease progression to its more advanced forms (35). In the present study, the mean values of HOMA-IR in both groups were greater than those of healthy adults (36). Similar to our study that showed GCBE significantly reduced FBS and HOMA-IR index, Sarria et al. in a randomized, controlled, crossover trial on 52 healthy subjects, who consumed three servings/day of the green/roasted (35:65) coffee blend for eight weeks, showed that HOMA-IR and FBS significantly reduced (21). Epidemiological and cross-sectional studies also showed an inverse association between coffee consumption and HOMA-IR. In addition to differences in dosages, coffee types, and duration of studies, an explanation for the trials' findings (37-39) regarding no beneficial effect of coffee on HOMA-IR may be the differences in weight of the studied samples. This is because when the

results of Pham et al. (40) study were stratified by BMI, an inverse association between coffee and HOMA-IR was observed only in the overweight/obese subjects but not in the normal weight ones; thus, it is possible that in this study, the observed effect was strengthened as a result of overweight/obesity in all participants.

NAFLD is a consequence of elevated plasma FFAs resulting in steatosis and dyslipidemia (20). Plasma FFAs levels are usually elevated in obesity states due to: a) more FFAs release from enlarged adipose tissue, b) possible reduction of FFAs clearance, and c) inhibition of the anti-lipolytic action of insulin by FFAs. The liver is the main organ in which, FFAs are oxidized or esterified into TG (20). FFAs elevation is responsible for NASH, IR, decrease in skeletal muscle glucose uptake, and increased hepatic gluconeogenesis (20). Therefore, reducing plasma FFAs and promoting FFAs uptake and oxidation in the liver may be an interesting strategy in the management of NAFLD.

Another finding of this study is the antilipidemic effect of GCBE. Our results showed that GCBE supplementation significantly reduced serum levels of FFAs, TG, and TC; however, LDL-C, HDL-C, and LDL-C/HDL-C ratio did not change significantly. The results of human trials investigating the effects of coffee on serum lipid levels are conflicting. McAnlis et al. (41) reported coffee did not affect serum lipid levels, whereas Yukawa et al. (32) found that consumption of 24 g coffee per day, for one week, significantly reduced serum levels of TC and LDL-C. Some of our results also are consistent with the findings of some previous experimental studies. Sudeep et al. showed that CGA supplementation significantly reduced plasma and hepatic levels of TG and FFAs in hyperlipidemic obese Wistar rats (20). Further molecular analysis by authors also confirmed that CGA promotes FFA catabolism by modulating the regulatory enzymes of FFA catabolism in the liver. These molecular modulation effects of CGA have also been shown to regulate glucose and lipid metabolism in HepG2 human hepatoma cell line (34). Moreover, Rodriguez de Sotillo and Hadley reported that intravenous infusion of CGA significantly reduced TG and TC in the blood and liver of Zucker rats (33).

The progression from simple steatosis to more advanced forms of NAFLD is also affected by inflammation and oxidative stress (42). Several studies have shown that NAFLD patients have significantly higher serum levels of markers of oxidative stress and inflammation (43, 44). Thus, reducing oxidative stress and inflammation in NAFLD patients would be an important strategy to slow down NAFLD progression and decrease the risk of cardiovascular complications.

Our results showed that supplementation with 1g GCBE for 8 weeks significantly decreased the hs-CRP con-

centration and increased the level of TAC; however, the level of TNF- $\alpha$  was not significantly affected. Hs-CRP is synthesized predominantly in the liver and its serum levels may be more closely linked to the extent of hepatic inflammation (45). In a high-fat-diet (HFD)-induced NASH model in male Wistar rats, Vitaglione et al. showed that coffee in drinking water significantly improved levels of glutathione and malondialdehyde (46).

Finally, in this study, a significant improvement in ALT was also observed in the GCBE group. However, the degree of steatosis did not significantly change in either group. Given the overall positive outcome achieved by GCBE supplementation, a significant reduction in ALT is justifiable. Because, as mentioned above, an improvement in inflammation, oxidative stresses status, IR, and hyperlipidemia have been associated with a significant improvement in liver function.

The ultrasonography method used for detecting the degree of steatosis could not reveal changes as precisely as detected by more accurate methods such as Fibroscan. In addition to using this subjective and insensitive method, low sample size and short duration of the study can be considered as the main limitation of this study.

In summary, the results of this trial show that GCBE supplementation in patients with NAFLD has a beneficial effect on liver enzymes, insulin resistance, as well as glucose and lipid metabolism. These beneficial effects may be due to the possible ability of GCBE to improve insulin sensitivity and its anti-inflammatory and antioxidant properties. Therefore, GCBE supplementation in NAFLD patients provided some interesting outcomes that need to be investigated in future studies.

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## Footnotes

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