# Treatment of Non-alcoholic Fatty Liver Disease with Curcumin: A Randomized Placebo-controlled Trial

**Sepideh Rahmani,[1] Sedigheh Asgary,[2]* Gholamreza Askari,[1] Mahtab Keshvari,[2] Mahdi Hatamipour,[3] Awat Feizi[4] and Amirhossein Sahebkar[5,6]**

[1]Food Security Research Center, Isfahan University of Medical Sciences, Isfahan, Iran
[2]Isfahan Cardiovascular Research Center, Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan, Iran
[3]Neurogenic Inflammation Research Center, Nanotechnology Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
[4]Department of Epidemiology and Biostatistics, School of Health, Isfahan University of Medical Sciences, Isfahan, Iran
[5]Biotechnology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[6]Metabolic Research Centre, Royal Perth Hospital, School of Medicine and Pharmacology, University of Western Australia, Perth, Australia

Non-alcoholic fatty liver disease (NAFLD) is a global health problem. Although many aspects of NAFLD pathogenesis have been understood, there is a paucity of effective treatments to be used as the second line when lifestyle modification is insufficient. Curcumin, a natural polyphenol from turmeric, has been shown to be effective against development of hepatic steatosis and its progression to steatohepatitis, yet these beneficial effects have not been explored in clinical practice. The aim of this study is to investigate the effects of curcumin on hepatic fat content as well as biochemical and anthropometric features of patients with NAFLD. In this randomized double-blind placebo-controlled trial, patients with ultrasonographic evidence of NAFLD were randomly assigned to receive an amorphous dispersion curcumin formulation (500 mg/day equivalent to 70-mg curcumin) or matched placebo for a period of 8 weeks. Liver fat content (assessed through ultrasonography), glycemic and lipid profile, transaminase levels, and anthropometric indices were evaluated at baseline and at the end of follow-up period. The clinical trial protocol was registered under the Iranian Registry of Clinical Trials ID: IRCT2014110511763N18. Compared with placebo, curcumin was associated with a significant reduction in liver fat content (78.9% improvement in the curcumin vs 27.5% improvement in the placebo group). There were also significant reductions in body mass index and serum levels of total cholesterol, low-density lipoprotein cholesterol, triglycerides, aspartate aminotransferase, alanine aminotransferase, glucose, and glycated hemoglobin compared with the placebo group. Curcumin was safe and well tolerated during the course of trial. Findings of the present proof-of-concept trial suggested improvement of different features of NAFLD after a short-term supplementation with curcumin. Copyright © 2016 John Wiley & Sons, Ltd.

*Keywords:* fatty liver; insulin resistance; metabolic syndrome; curcuminoids; *Curcuma longa*.

## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most prevalent chronic liver disease that is closely associated with obesity, diabetes, dyslipidemia, metabolic syndrome, and cardiovascular disease (Angulo, 2002). NAFLD is characterized by the accumulation of neutral lipids, mainly, triglycerides (TGs), in liver cells, and can progress to non-alcoholic steatohepatitis (NASH), liver fibrosis, cirrhosis, and eventually hepatocellular carcinoma (Adams *et al.*, 2005). Owing to the rising epidemiology of obesity and type 2 diabetes, NAFLD has become a public health concern with a projected prevalence of 6–35% among adults worldwide (Vernon *et al.*, 2011). Adherence to dietary and lifestyle modifications is the first step to correct hepatic fat accumulation and prevent NAFLD progression to NASH. However,

most patients fail to achieve proper weight control, and the compliance with lifestyle modification is often low. In spite of the high prevalence, there is a paucity of effective treatments that could reverse the pathophysiology of NAFLD and blunt the progression of NAFLD to NASH. Therefore, there is a surge of interest to find effective and safe treatments for this important clinical problem. For this purpose, natural products and medicinal plants have been the subject of increasing attention owing to their wide availability, low cost, multi-target action, and potential safety (Foroughi *et al.*, 2014, Asgharian *et al.*, 2016, Pezeshki *et al.*, 2016). According to the findings of a recent Cochrane review, some herbal medicines possess beneficial effects on hepatic fat content (assessed through either ultrasonography or computed tomography) and reduction of hepatic transaminase levels (Liu *et al.*, 2013). Moreover, a recent systematic review and meta-analysis of 62 randomized controlled trials involving 5904 patients showed better effects of Traditional Chinese Medicine in normalization of alanine aminotransferase (ALT) and disappearance of radiological steatosis in

* Correspondence to: Sedigheh Asgary, Isfahan Cardiovascular Research Center, Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan.
E-mail: sedighehasgary@gmail.com

NAFLD (Shi *et al.*, 2012). These large analyses have prompted more attention to the potential utility of natural products in the treatment of NAFLD.

Curcumin, a naturally occurring polyphenol from turmeric, has been the subject of extensive research over recent decades owing to its capacity for interaction with hundreds of molecular targets and numerous pharmacological benefits (Sahebkar, 2013). Curcumin has been reported to possess antioxidant (Panahi *et al.*, 2016a; Panahi *et al.*, 2016b; Panahi *et al.*, 2015c; Panahi *et al.*, 2012a; Sahebkar *et al.*, 2013), anti-inflammatory (Panahi *et al.*, 2015c; Panahi *et al.*, 2014c; Panahi *et al.*, 2012b; Sahebkar, 2014a), anticancer (Panahi *et al.*, 2014c), antiarthritic (Panahi *et al.*, 2014b), antidepressant (Esmaily *et al.*, 2015; Panahi *et al.*, 2015a), anti-diabetic (Chuengsamarn *et al.*, 2012; Chuengsamarn *et al.*, 2014; Ramirez *et al.*, 2013), and lipid-lowering (Panahi *et al.*, 2014a; Sahebkar *et al.*, 2014) properties in clinical practice. The capacity of curcumin to improve insulin sensitivity, decrease lipogenesis, and attenuate inflammation and oxidative stress could be employed for the prevention of hepatic steatosis and its progression to steatohepatitis (Shapiro and Bruck, 2005). This hypothesis has been tested in several experimental studies, and the results have confirmed the efficacy of curcumin in reducing hepatic TG content and other histopathological and biochemical features in dietary-induced models of NAFLD/NASH (Um *et al.*, 2013; Wang *et al.*, 2014). In spite of the positive experimental findings, proof-of-concept studies in humans are lacking. The present study aimed to evaluate the efficacy of short-term curcumin supplementation in patients with NAFLD.

## MATERIALS AND METHODS

**Subjects.** Adult subjects referring to the Endocrinology and Metabolism Research Center with symptoms of metabolic syndrome including waist circumference $\geq 102$ cm (male) or $\geq 88$ cm (female), blood pressure $\geq 130/85$ mm Hg, triglycerides $\geq 1.7$ mmol/L, high-density lipoprotein cholesterol (HDL-C) $< 1.03$ mmol/L (males) or $< 1.29$ mmol/L (females), and fasting blood glucose $\geq 6.1$ mmol/L were screened for eligibility. Inclusion criteria were diagnosis of NAFLD (grades 1–3) according to liver ultrasonography. Exclusion criteria were pregnancy or breastfeeding, NAFLD secondary to alcohol consumption, smoking, consumption of hypoglycemic, hypolipidemic, and antiinflammatory medications as well as any drug known to affect hepatic function, and the presence of hepatitis, coronary, renal, pulmonary, and thyroid diseases.

Eligible subjects were randomly assigned to curcumin (500 mg/day of an amorphous dispersion preparation comprising 70-mg curcuminoids) ($n = 40$) or placebo ($n = 40$). Curcumin and placebo powder were filled into shape-matched, size-matched, and color-matched capsule and dispensed in identical blinded bottles.

The study protocol was given approval by the institutional Ethics Committee, and written informed consent was obtained from participants. The clinical trial protocol has been registered under the ID: IRCT2014110 511763N18.

**Anthropometric measurements.** Height and weight of subjects were measured with the accuracy of 0.1 cm and 0.1 kg, respectively. Measurements were performed in the standing position, with minimal clothing and no shoes at baseline and the 8th week. Body mass index was calculated as weight (kg) divided by height (m) squared.

**Blood sampling and biochemical measurements.** Fasted blood samples were collected from the drawn from a cubital vein at baseline and after 8 weeks of supplementation. Blood samples were centrifuged for 10 min at a speed of 2000–2500 g to separate the serum. Serum samples were kept at $-80\,^{\circ}$C until analyses.

Total cholesterol, low-density lipoprotein cholesterol (LDL-C), HDL-C, triglycerides, ALT, aspartate aminotransferase (AST), fasting blood sugar (FBS), and glycated hemoglobin (HbA1c) were measured at baseline and at the end of study using routine enzymatic assays with commercial kits (Pars Azmoon, Tehran, Iran).
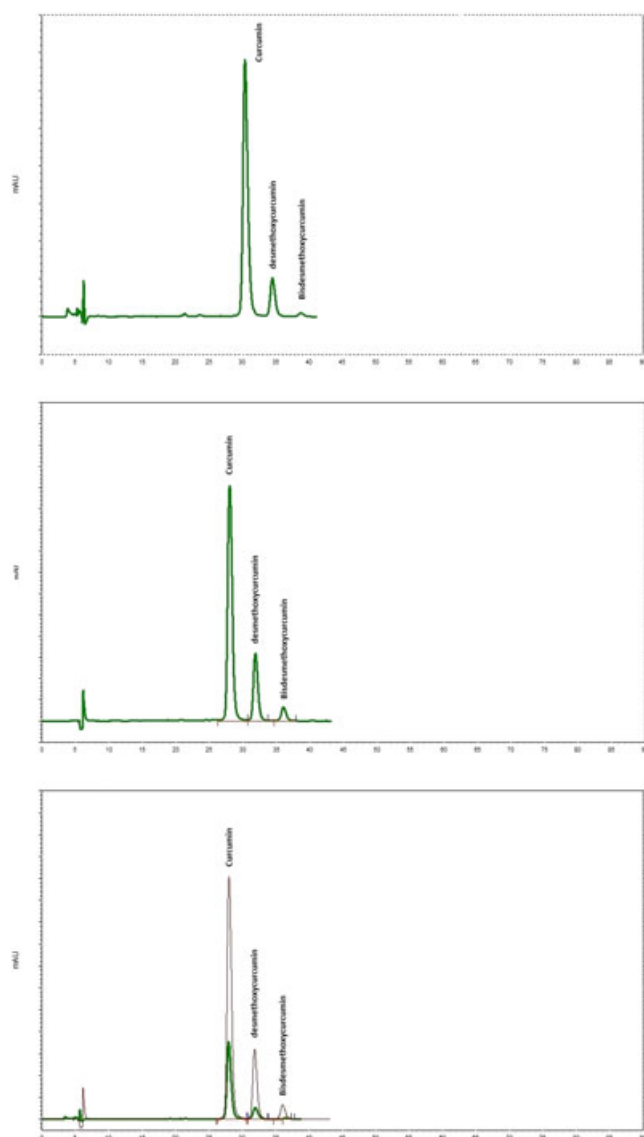
**Liver ultrasonography.** Liver fat content and the severity of hepatic steatosis were evaluated at baseline and after 8 weeks of supplementation using ultrasonography with Esaote Medical ultrasound machine (convex 3.5 MHz) at the beginning and the end of the study. Ultrasound assessments were performed by the same expert radiologist blinded to the type of allocation, and with the same instrument. Assessments were performed in the fasted state, with the subjects in the supine position.

Right and left lobes of the upper and lower surfaces of liver were studied. Echogenicity of the liver, the presence or absence of bulky tumors cystic or solid and calcification was assessed. Intrahepatic bile ducts, portal vein, and hepatic artery were evaluated. Hepatic steatosis was graded as 0 (lack of fat accumulation), 1 (mild increase in echogenicity with normal visualization of the diaphragm and intrahepatic vessel borders), 2 (moderate increase in echogenicity with slightly impaired visualization of the diaphragm and intrahepatic vessel borders), and 3 (severe increase in echogenicity with markedly impaired visualization of the diaphragm, intrahepatic vessel borders, and the posterior portion of the right hepatic lobe).

**Chromatographic quantification.** Identification and determination of curcuminoids in the amorphous dispersion preparation were carried out using an isocratic high-performance liquid chromatography (Shimadzu, Japan) method on a reverse phase C18 column (200 mm × 4.6 mm × 5 μm, Atlantis T3; Waters, Milford, MA, USA). The eluent system comprised tetrahydrofuran and water (containing citric acid at a concentration of 1 mg/mL) in a 4:6 ratio. The flow rate was set at 1 mL/min, and detection was performed at 420 nm. The injection volume was set to 20 μL. Identification and quantification of curcuminoids in the amorphous dispersion preparation were performed by comparing retention times of peaks with the corresponding retention times of United States Pharmacopeia (USP) reference standards. High-performance liquid chromatography

assays revealed the content of curcuminoids to be $140\,mg/g$ product (Fig. 1).

**Statistical analysis.** Statistical analyses were performed using the SPSS software version 11.5 (SPSS Inc., Chicago, IL, USA). The study population size was estimated at the significance level of 95%, with a power of 80% and an effect size of 0.7 for AST and ALT. Data were expressed as mean ± SD or number (%). Within-group comparisons were performed using paired samples $t$-test (for normally distributed data) or Wilcoxon signed-ranks test (for non-normally distributed data). Between-group comparisons were performed using independent samples $t$-test (for normally distributed data) or Mann–Whitney $U$-test (for non-normally distributed data). Categorical variables were compared using chi-squared test. Binary logistic regression analysis was used to adjust for the effect of potential confounders on the association between curcumin supplementation and changes the severity of NAFLD according to ultrasonographic findings. For this analysis, change in the ultrasonographic findings (categorized as either improvement or lack of improvement) was entered into the model as dependent variable. A $p$-value of $<0.05$ was considered as statistically significant in all analyses. Statistical power was calculated using the Power and Sample Size Calculation (PS) software version 3.0 (Dupont and Plummer, 1998).



**Figure 1.** Identification and determination of curcuminoids in the curcumin preparation used in this study. Upper and middle chromatograms refer to the study preparation and USP reference standard. Overlaid chromatogram is shown in the lower plot.

# RESULTS

## Baseline characteristics

Seventy-seven subjects completed the study. There were only three drop-outs in the curcumin group because of stomachache and nausea (Fig. 2). The number of drop-outs did not differ between the study groups ($p > 0.05$). The study groups were comparable in terms of age, gender, weight, body mass index (BMI), liver fat content, and serum concentrations of total cholesterol, LDL-C, HDL-C, FBS, and AST at baseline ($p > 0.05$), yet baseline serum levels of TG ($p = 0.027$), HbA1c ($p = 0.001$), and ALT ($p = 0.042$) were significantly higher in the curcumin compared with the placebo group. Baseline characteristics of the study groups are illustrated in Table 1.

## Lipids and glucose

Supplementation with curcumin was associated with significant reductions in weight and BMI ($p < 0.001$), but no significant change was observed in the placebo group ($p > 0.05$). There were also significant reductions in HbA1c levels in the curcumin ($p < 0.05$) but not placebo group ($p > 0.05$). Fasting serum glucose did not change significantly in the curcumin group ($p > 0.05$) but rose in the placebo group ($p = 0.018$) (Table 2). Between-group comparisons revealed significant reductions in weight ($p < 0.001$), BMI ($p = 0.002$), and serum levels of glucose ($p = 0.048$) and HbA1c ($p < 0.001$) compared with the placebo group (Table 3).

Comparison of baseline versus post-trial values revealed significant reductions in serum concentrations of total cholesterol ($p < 0.001$), LDL-C ($p = 0.007$), a borderline reduction in serum TG ($p = 0.055$), and elevation of HDL-C levels ($p = 0.010$) following curcumin supplementation. In the placebo group, serum levels of total cholesterol ($p = 0.027$) and LDL-C ($p = 0.015$) were increased while those of TG and HDL-C remained unaltered by the end of trial ($p > 0.05$) (Table 2). Comparison of change values between the study groups revealed reductions in serum concentrations of total cholesterol ($p < 0.001$), LDL-C ($p < 0.001$), and TG ($p = 0.014$) in the curcumin versus placebo group. However, comparison of the changes in serum HDL-C levels did not reach statistical significance ($p > 0.05$) (Table 3).

## Liver ultrasonography and transaminase levels

Comparison of liver ultrasonographic findings revealed a significant improvement in the curcumin versus placebo group ($p < 0.001$). Ultrasonographic findings
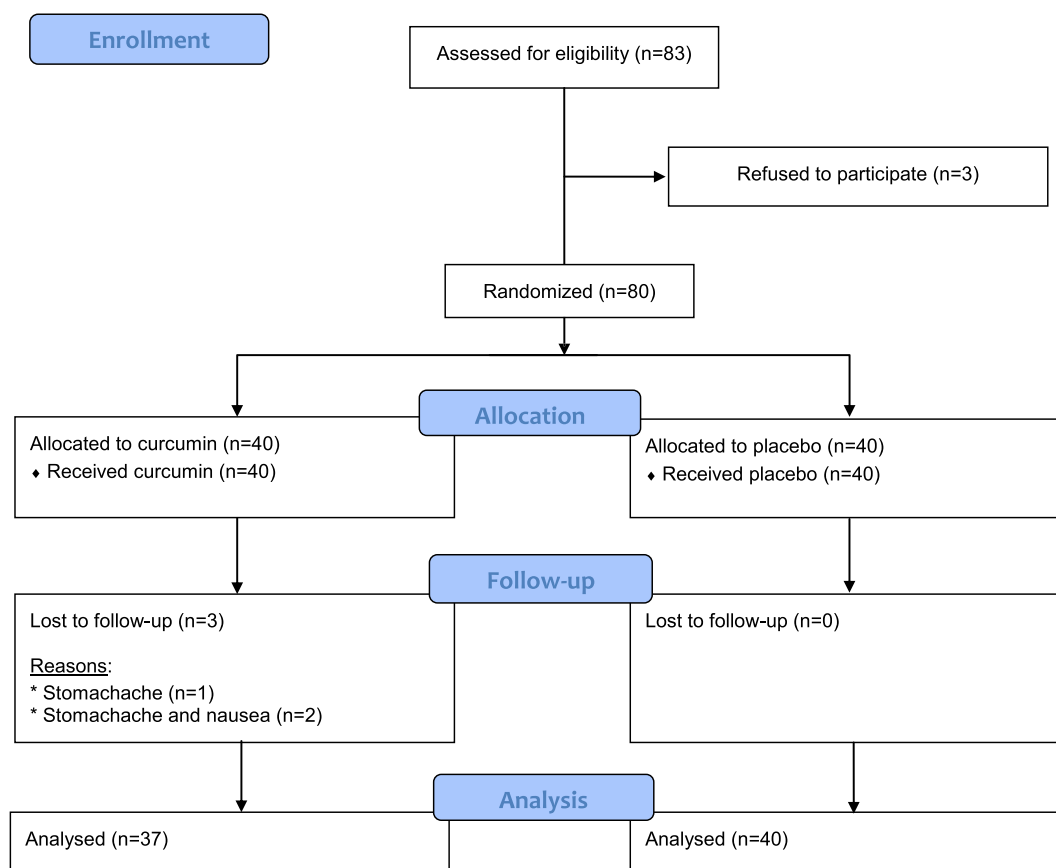
**Figure 2.** Flowchart of the trial.

**Table 1. Comparison of baseline characteristics between curcumin and placebo groups**

|  |  | Curcuminoids | Placebo | *p*-value |
|---|---|---|---|---|
| Age |  | 46.37 ± 11.57 | 48.95 ± 9.78 | 0.286 |
| Gender (M/F) |  | 19/21 | 19/21 | 1.000 |
| Height |  | 162.39 ± 8.14 | 160.48 ± 11.26 | 0.387 |
| Total cholesterol |  | 198.59 ± 41.76 | 187.78 ± 32.95 | 0.375 |
| LDL-C |  | 107.06 ± 31.36 | 115.57 ± 22.30 | 0.124 |
| HDL-C |  | 44.26 ± 11.83 | 42.62 ± 6.67 | 0.535 |
| Triglycerides |  | 199.68 ± 91.46 | 160.20 ± 61.94 | 0.027 |
| BMI |  | 30.84 ± 4.45 | 31.35 ± 5.67 | 0.758 |
| Weight |  | 81.14 ± 12.13 | 80.20 ± 13.82 | 0.717 |
| ALT |  | 39.07 ± 19.79 | 30.35 ± 13.97 | 0.042 |
| AST |  | 28.88 ± 10.60 | 32.05 ± 17.64 | 0.339 |
| FBS |  | 111.65 ± 34.64 | 116.90 ± 47.66 | 0.922 |
| HbA1c |  | 6.31 ± 1.62 | 7.37 ± 1.33 | 0.001 |
|  | Grade 0 | 0% | 0% | — |
|  | Grade 1 | 25.6% | 32.5% | 0.322 |
| NAFLD severity | Grade 2 | 48.7% | 55.0% | — |
|  | Grade 3 | 25.6% | 12.5% | — |

LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FBS, fasting blood sugar; HbA1c, glycated hemoglobin; NAFLD, non-alcoholic fatty liver disease.

were improved in 78.9% of subjects in the curcumin group, while the rate of improvement in the placebo group was 27.5%. There was no case of increased liver fat content in the curcumin group, but 17.5% of subjects in the placebo group had their liver fat content increased (Table 4). Consistent with the findings of liver ultrasonography, serum levels of AST and AST were reduced by the end of trial in the curcumin group ($p < 0.001$) while none of them was significantly altered in the placebo group ($p > 0.05$) (Table 2). The effect of curcumin in reducing serum AST ($p = 0.002$) and ALT ($p = 0.001$) levels was also significant in the between-group comparison (Table 3).

**Bivariate correlations**

Assessment of bivariate correlations between the biochemical parameters revealed significant correlations between changes in transaminases (AST and

**Table 2. Within-group comparison of biochemical parameters between curcumin and placebo groups**

| | Curcuminoids | | | Placebo | | |
|---|---|---|---|---|---|---|
| | Before | After | $p$ | Before | After | $p$ |
| Total cholesterol | 198.59 ± 41.76 | 174.38 ± 39.56 | <0.001 | 187.78 ± 32.95 | 196.82 ± 37.04 | 0.027 |
| LDL-C | 107.06 ± 31.36 | 95.59 ± 28.22 | 0.007 | 115.57 ± 22.30 | 125.00 ± 24.23 | 0.015 |
| HDL-C | 44.26 ± 11.83 | 46.68 ± 10.98 | 0.010 | 42.62 ± 6.67 | 46.72 ± 15.38 | 0.079 |
| Triglycerides | 199.68 ± 91.46 | 173.43 ± 95.44 | 0.055 | 160.20 ± 61.94 | 153.58 ± 50.12 | 0.398 |
| BMI | 30.84 ± 4.45 | 30.11 ± 4.39 | <0.001 | 31.35 ± 5.67 | 31.37 ± 5.33 | 0.915 |
| Weight | 81.14 ± 12.13 | 79.34 ± 12.10 | <0.001 | 80.20 ± 13.82 | 80.69 ± 13.66 | 0.095 |
| ALT | 39.07 ± 19.79 | 36.08 ± 46.58 | <0.001 | 30.35 ± 13.97 | 28.72 ± 10.93 | 0.409 |
| AST | 28.88 ± 10.60 | 23.84 ± 7.83 | <0.001 | 32.05 ± 17.64 | 34.07 ± 18.73 | 0.284 |
| FBS | 111.65 ± 34.64 | 107.57 ± 28.34 | 0.441 | 116.90 ± 47.66 | 118.18 ± 47.35 | 0.018 |
| HbA1c | 6.31 ± 1.62 | 5.53 ± 1.27 | <0.001 | 7.37 ± 1.33 | 7.53 ± 1.43 | 0.358 |

LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FBS, fasting blood sugar; HbA1c, glycated hemoglobin; NAFLD, non-alcoholic fatty liver disease.

**Table 3. Between-group comparison of biochemical parameters between curcumin and placebo groups**

| | Curcuminoids | Placebo | p-value |
|---|---|---|---|
| Total cholesterol | −24.22 ± 38.10 | 9.05 ± 24.98 | <0.001 |
| LDL-C | −11.46 ± 24.18 | 9.43 ± 23.50 | <0.001 |
| HDL-C | 2.42 ± 5.40 | 4.10 ± 14.39 | 0.344 |
| Triglycerides | −26.24 ± 80.61 | −6.62 ± 49.07 | 0.014 |
| BMI | −0.74 ± 0.85 | 0.02 ± 1.18 | 0.002 |
| Weight | −1.81 ± 2.01 | 0.49 ± 1.83 | <0.001 |
| ALT | −2.99 ± 47.38 | −1.62 ± 12.30 | 0.001 |
| AST | −5.04 ± 6.49 | 2.02 ± 11.79 | 0.002 |
| FBS | −4.08 ± 19.42 | 1.27 ± 13.31 | 0.048 |
| HbA1c | −0.78 ± 1.11 | 0.16 ± 1.09 | <0.001 |

LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FBS, fasting blood sugar; HbA1c, glycated hemoglobin; NAFLD, non-alcoholic fatty liver disease.

ALT) with those of weight and HbA1c. A significant association between changes in AST and fasting glucose concentrations was also found. Other significant correlations between the assessed biochemical parameters are shown in Table 5.

### Regression analysis

Binary logistic regression analysis was performed to adjust the ultrasonographic findings – as the primary efficacy measure – for baseline levels of TG, HbA1c, and ALT as well as changes in body weight during the trial as potential confounders of treatment response. Selection of confounders was based on the baseline difference between curcumin and placebo groups. The effect of curcumin in reducing liver fat remained significant ($p = 0.001$) after adjustment for the previously mentioned confounders (Table 6).

### Safety

Curcumin was safe and well tolerated in this trial. There was no case of severe adverse events. There were only one case with stomachache and two cases with combined stomachache and nausea.

### DISCUSSION

The present trial suggested a significant benefit of curcumin supplementation in improving liver fat content, biochemical, and anthropometric indices of patients with NAFLD. To the best of authors' knowledge, this is the second proof-of-concept study on the anti-steatotic effects of curcumin in humans. In the only available report, positive effects of a phytosomal preparation of curcumin (500 mg equivalent to 100-mg curcumin) were shown in patients with NAFLD following a supplementation period of 8 weeks (Panahi *et al.*, 2016c).

The present findings are in agreement with those of several experimental studies confirming the beneficial

**Table 4. Comparison of NAFLD severity within and between the study groups**

| | | Curcuminoids | | | Placebo | | | p-value[a] |
|---|---|---|---|---|---|---|---|---|
| | | Before | After | $p$ | Before | After | $p$ | |
| NAFLD severity | Grade 0 | 0% | 15.8% | <0.001 | 0% | 0% | 0.622 | <0.001 |
| | Grade 1 | 25.6% | 71.1% | | 32.5% | 35.0% | | |
| | Grade 2 | 48.7% | 13.2% | | 55.0% | 60.0% | | |
| | Grade 3 | 25.6% | 0% | | 12.5% | 5.0% | | |

[a]Comparison of change values between the study groups.
NAFLD, non-alcoholic fatty liver disease.

**Table 5. Bivariate correlations between changes in the evaluated biochemical parameters**

| | Weight | | BMI | | HbA1c | | FBS | | ALT | | AST | | LDL-C | | HDL-C | | TG | | TC | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | r | ρ | r | ρ | r | ρ | r | ρ | r | ρ | r | ρ | r | ρ | r | ρ | r | ρ | r | ρ |
| TC | 0.38 | 0.001 | 0.27 | 0.016 | 0.14 | 0.207 | −0.04 | 0.727 | 0.107 | 0.353 | 0.1 | 0.909 | 0.70 | <0.001 | −0.06 | 0.586 | 0.29 | 0.012 | — | — |
| TG | 0.12 | 0.280 | 0.08 | 0.481 | 0.12 | 0.315 | 0.13 | 0.268 | 0.21 | 0.065 | 0.21 | 0.066 | 0.22 | 0.049 | −0.35 | 0.002 | — | — | — | — |
| HDL-C | −0.13 | 0.240 | −0.21 | 0.067 | −0.06 | 0.577 | 0.09 | 0.437 | −0.24 | 0.036 | −0.18 | 0.107 | −0.17 | 0.136 | — | — | — | — | — | — |
| LDL-C | 0.23 | 0.040 | 0.18 | 0.115 | 0.16 | 0.166 | 0.07 | 0.548 | 0.15 | 0.206 | 0.08 | 0.494 | — | — | — | — | — | — | — | — |
| AST | 0.28 | 0.012 | 0.17 | 0.146 | 0.31 | 0.006 | 0.39 | 0.001 | 0.41 | <0.001 | — | — | — | — | — | — | — | — | — | — |
| ALT | 0.25 | 0.027 | 0.19 | 0.097 | 0.25 | 0.027 | 0.05 | 0.656 | — | — | — | — | — | — | — | — | — | — | — | — |
| FBS | 0.20 | 0.075 | 0.13 | 0.0257 | 0.25 | 0.031 | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| HbA1c | 0.14 | 0.224 | 0.12 | 0.299 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| BMI | 0.76 | <0.001 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Weight | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |

LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FBS, fasting blood sugar; HbA1c, glycated hemoglobin.

**Table 6. Binary logistic regression analysis for the effect of curcumin supplementation on liver fat content after adjustment for potential confounders**

| Variables entered in the model | Odds ratio | 95% CI | p-value |
|---|---|---|---|
| Baseline TG | 1.00 | 1.00, 1.01 | 0.336 |
| Baseline HbA1c | 1.07 | 0.74, 1.56 | 0.705 |
| Baseline ALT | 0.99 | 0.96, 1.02 | 0.415 |
| Weight change | 0.92 | 0.68, 1.23 | 0.572 |
| Curcumin treatment | 9.04 | 2.44, 33.54 | 0.001 |

TG, triglycerides; HbA1c, glycated hemoglobin; ALT, alanine aminotransferase.

role of curcumin in animal models of NAFLD/NASH. Additional to NAFLD, curcumin has been shown in several experimental studies to mitigate the hepatic injury induced by several known chemical hepatotoxic agents including heavy metals, carbon tetrachloride, thioacetamide, anti-tuberculosis agents, and iron overload. The mechanisms underlying these hepatoprotective effects of curcumin are the ability of this compound to inhibit oxidative stress and nuclear factor kappa-light-chain enhancer of activated B cells (NF-kB), both having causative roles in promoting liver injury (Rivera-Espinoza and Muriel, 2009). In rodent models of diet-induced steatosis and steatohepatitis, curcumin supplementation has been reported to reduce hepatic contents of TG and non-esterified fatty acids, activities of matrix metalloproteinases, and NF-kB and down-regulate pro-inflammatory cytokines, cyclooxygenase-2, type 1 collagen-a-1, intracellular adhesion molecule-1, and inducible nitric oxide synthase. In addition, curcumin has been shown to enhance hepatic activity of acyl-CoA oxidase and blunt lipid peroxidation through increasing hepatic glutathione levels and glutathione reductase activities, while reducing the levels of hydroperoxide and lipoperoxide and thiobarbituric acid reactive species (Shapiro and Bruck, 2005; Zingg et al., 2013). Antioxidant and antiinflammatory properties of curcumin have also been the subject several clinical trials in different patient populations (Panahi et al., 2015b; Panahi et al., 2015c; Panahi et al., 2014c; Panahi et al., 2012b; Sahebkar, 2014a; Sahebkar et al., 2013), showing significant reductions in circulating concentrations of pro-inflammatory cytokines, C-reactive protein and lipid peroxidation products and enhancement of the circulating levels of enzymatic and non-enzymatic antioxidants. Recently, curcumin has been shown to up-regulate the expression and activity of hepatic antioxidant enzymes in aflatoxin B1-intoxicated rats and attenuate histopathological features of liver damage (El-Bahr, 2015). Another important hepatic effect of curcumin is induction of apoptosis in hepatic stellate cells, and inhibition of hepatic stellate cell activation, which is a key step in the progression of steatosis to NASH and hepatic fibrosis (Kang et al., 2002).

Insulin resistance is regarded as an underlying mechanism linking NAFLD to types II diabetes, obesity, and metabolic syndrome (Gariani et al., 2013). Therefore, increasing insulin sensitivity and glycemic control is an effective approach for the management of

NAFLD, NASH, and other closely related comorbidities (Gariani *et al.*, 2013). An interesting finding of the present study was significant reductions observed in FBS and HbA1c levels found after supplementation with curcumin. These reductions may imply an improvement of insulin sensitivity with curcumin. Similar positive effects of curcumin on glycemic control have been previously reported. In prediabetic subjects, 9-month supplementation with curcumin reduced and delayed the occurrence of type 2 diabetes, enhanced β-cell function, and improved insulin sensitivity as well circulating concentrations of FBS, HbA1c, C-peptide, and adiponectin (Chuengsamarn *et al.*, 2012). The same effects in diabetic patients have been reported in both fasting (Ramirez *et al.*, 2013) and postprandial state (Wickenberg *et al.*, 2010). In another study in obese children with risk factors of metabolic syndrome, 4-week supplementation with curcumin (500 mg/day) was reported to attenuate insulin resistance, as well as circulating concentrations of resistin and fetuin-A. There was also a significant reduction in visceral fat content (assessed using abdominal ultrasonography) and a trend towards reduced body weight (Ismail *et al.*, 2014).

Dyslipidemia is another important metabolic risk factor of NAFLD. The prevalence of NAFLD in dyslipidemic subjects has been estimated to be as high as 50% (Assy *et al.*, 2000). Thus, correcting dyslipidemia, particularly hypertriglyceridemia, is within the overall frame work of management of NAFLD/NASH according to the guidelines (Chalasani *et al.*, 2012). Hitherto, several randomized controlled trials have shown efficient reductions in plasma TG concentrations with curcumin in populations with type 2 diabetes (Chuengsamarn *et al.*, 2014), metabolic syndrome (Panahi *et al.*, 2014a), obesity (Mohammadi *et al.*, 2013), and also in healthy subjects (DiSilvestro *et al.*, 2012). Additional to its hypotriglyceridemic effects, mechanistic studies have shown that curcumin may modulate LDL metabolism through up-regulating LDL-receptor expression – which results in enhanced plasma removal and biliary excretion of cholesterol – and inhibiting the Niemann–Pick C1-Like 1 (NPC1L1) protein, which plays a major role in the intestinal absorption of cholesterol (Sahebkar, 2014c). Moreover, curcumin could down-regulate several enzymes and receptors involved in lipogenesis, including sterol regulatory element-binding protein-1, apolipoprotein B-100, fatty acid synthase, acetyl-CoA carboxylase, acyl coenzyme A : cholesterol acyltransferase, 3-hydroxy-3-methylglutaryl-coenzyme A reductase, peroxisome proliferator-activated receptor-α, cluster of differentiation 36, and adenosine monophosphate-activated protein kinase (Sahebkar, 2014b). All these mechanistic findings can explain the favorable impact of curcumin on serum lipid levels observed in the present study and support the potential utility of this natural product in preventing the cardiovascular complications of NAFLD/NASH.

Aside from improvement of biochemical features of NAFLD and liver fat accumulation, curcumin supplementation was found to be associated with significant loss. Anti-obesity effects of curcumin have been reported in several previous pre-clinical studies and attributed to diverse mechanisms. Curcumin can dose dependently inhibit early stages of adipocyte differentiation, thereby reducing the number of adipocytes and fat content in the adipose tissue (Bradford, 2013). At the molecular level, this effect of curcumin has been attributed to the reduction of microvessel density in the subcutaneous adipose tissue, stimulation of monophosphate-activated protein kinase and fatty acid oxidation and reduction of glycerol lipids synthesis (Ejaz *et al.*, 2009; Lee *et al.*, 2009). Moreover, curcumin can suppress two important transcription factors involved in adipogenesis, namely, peroxisome proliferator-activated receptor γ and CCAAT (cytosine-cytosine-adenosine-adenosine-thymidine) enhancer binding protein α, through a β-catenin-dependent mechanism (Kim *et al.*, 2011).

Curcumin is known to have low oral bioavailability that has been suggested to be, at least in part, due to the low aqueous solubility of this compound. Low oral bioavailability of curcumin has prompted extensive research to develop bioavailable products with the aim of enhancing the pharmacological effects of curcumin (Liu *et al.*, 2016). One useful approach is to convert the crystalline structure of curcumin to the amorphous form. This conversion will result in higher water solubility and absorption from the intestinal brush border, as the crystalline structure requires a high energy for breaking and dissolution (Murdande *et al.*, 2011). Therefore, the amorphous form of curcumin used in this study might have helped to achieve a higher systemic absorption and bioavailability and possibly greater clinical effects.

Curcumin was safe and well tolerated in this trial. The safety of curcumin supplementation in human has been confirmed in numerous trials, and doses as high as 8000 g/day have been shown to be tolerated without any serious adverse event (Cheng *et al.*, 2001). Such a safety profile further supports the value of curcumin as an adjunct to standard treatment regimens of patients with cardiometabolic diseases such as NAFLD.

The present study is subject to a number of limitations. First, this study was designed as a single-dose trial, and thus, any dose-effect association for the anti-steatotic effects of curcumin remains unclear. Because some of the effects of curcumin are known to be dose dependent (Hasan *et al.*, 2014; Sahebkar, 2015), it remains unclear if higher doses could introduce a stronger clinical effect or vice versa. Second, a relatively small proportion of the studied population had stage 3 of the disease that may represent steatohepatitis. Therefore, additional studies are required to ascertain if the beneficial effects of curcumin could be replicated in patients with severe NAFLD or NASH.

In conclusion, findings of the present proof-of-concept study confirmed the efficacy of curcumin supplementation in improving lipid and glycemic profile and educing liver fat content of subjects with NAFLD. Owing to the numerous pleiotropic actions and the safety of curcumin on the one hand and the paucity of effective treatments for NAFLD/NASH on the other, the use of this phytochemical in patients with NAFLD is recommended. However, future studies are warranted to elucidate if the anti-steatotic properties of curcumin are dose dependent, and also the value of curcumin in reducing hepatic endpoints such as cirrhosis and hepatocellular carcinoma. Finally, future clinical trials should conform to the standards reported in the consolidated standards of reporting trials (CONSORT) guidelines as recently reported (Izzo *et al.*, 2016) and assess potential interactions between curcuminoids, diet, and drugs.

## Acknowledgements

## Conflict of Interest

None.

## REFERENCES

Adams LA, Sanderson S, Lindor KD, Angulo P. 2005. The histological course of nonalcoholic fatty liver disease: a longitudinal study of 103 patients with sequential liver biopsies. *J Hepatol* **42**: 132–138.

Angulo P. 2002. Nonalcoholic fatty liver disease. *N Engl J Med* **346**: 1221–1231.

Asgharian A, Askari G, Esmailzade A, Feizi A, Mohammadi V. 2016. The Effect of Symbiotic Supplementation on Liver Enzymes, C-reactive Protein and Ultrasound Findings in Patients with Non-alcoholic Fatty Liver Disease: A Clinical Trial. *Int J Prev Med* **7**: 59. DOI:10.4103/2008-7802.178533.

Assy N, Kaita K, Mymin D, Levy C, Rosser B, Minuk G. 2000. Fatty infiltration of liver in hyperlipidemic patients. *Dig Dis Sci* **45**: 1929–1934.

Bradford PG. 2013. Curcumin and obesity. *Biofactors* **39**: 78–87.

Chalasani N, Younossi Z, Lavine JE, *et al.*. 2012. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology* **55**: 2005–2023.

Cheng AL, Hsu CH, Lin JK, *et al.*. 2001. Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. *Anticancer Res* **21**: 2895–2900.

Chuengsamarn S, Rattanamongkolgul S, Luechapudiporn R, Phisalaphong C, Jirawatnotai S. 2012. Curcumin extract for prevention of type 2 diabetes. *Diabetes Care* **35**: 2121–2127.

Chuengsamarn S, Rattanamongkolgul S, Phonrat B, Tungtrongchitr R, Jirawatnotai S. 2014. Reduction of atherogenic risk in patients with type 2 diabetes by curcuminoid extract: a randomized controlled trial. *J Nutr Biochem* **25**: 144–150.

DiSilvestro RA, Joseph E, Zhao S, Bomser J. 2012. Diverse effects of a low dose supplement of lipidated curcumin in healthy middle aged people. *Nutr J* **11**: 79.

Dupont WD, Plummer WD Jr. 1998. Power and sample size calculations for studies involving linear regression. *Control Clin Trials* **19**: 589–601.

Ejaz A, Wu D, Kwan P, Meydani M. 2009. Curcumin inhibits adipogenesis in 3 T3-L1 adipocytes and angiogenesis and obesity in C57/BL mice. *J Nutr* **139**: 919–925.

El-Bahr SM. 2015. Effect of curcumin on hepatic antioxidant enzymes activities and gene expressions in rats intoxicated with aflatoxin B1. *Phytother Res: PTR* **29**: 134–140.

Esmaily H, Sahebkar A, Iranshahi M, *et al.*. 2015. An investigation of the effects of curcumin on anxiety and depression in obese individuals: a randomized controlled trial. *Chin J Integr Med* **21**: 332–338.

Foroughi M, Maghsoudi Z, Ghiasvand R, Iraj B, Askari G. 2014. Effect of Vitamin D Supplementation on C-reactive Protein in Patients with Nonalcoholic Fatty Liver. *Int J Prev Med* **5**: 969–975.

Gariani K, Philippe J, Jornayvaz FR. 2013. Non-alcoholic fatty liver disease and insulin resistance: from bench to bedside. *Diabetes Metab* **39**: 16–26.

Hasan ST, Zingg JM, Kwan P, Noble T, Smith D, Meydani M. 2014. Curcumin modulation of high fat diet-induced atherosclerosis and steatohepatosis in LDL receptor deficient mice. *Atherosclerosis* **232**: 40–51.

Ismail NA, Ragab S, Abd El Baky ANE, Hamed N, Ibrahim ASA. 2014. Effect of oral curcumin administration on insulin resistance, serum resistin and fetuin-A in obese children: randomized placebo-controlled study. *Res J Pharm Biol Chem Sci* **5**: 887–896.

Izzo AA, Hoon-Kim S, Radhakrishnan R, Williamson EM. 2016. A critical approach to evaluating clinical efficacy, adverse events and drug interactions of herbal remedies. *Phytother Res: PTR* **30**: 691–700.

Kang HC, Nan JX, Park PH, *et al.*. 2002. Curcumin inhibits collagen synthesis and hepatic stellate cell activation in-vivo and in-vitro. *J Pharm Pharmacol* **54**: 119–126.

Kim CY, Le TT, Chen C, Cheng JX, Kim KH. 2011. Curcumin inhibits adipocyte differentiation through modulation of mitotic clonal expansion. *J Nutr Biochem* **22**: 910–920.

Lee YK, Lee WS, Hwang JT, Kwon DY, Surh YJ, Park OJ. 2009. Curcumin exerts antidifferentiation effect through AMPKalpha-PPAR-gamma in 3 T3-L1 adipocytes and antiproliferatory effect through AMPKalpha-COX-2 in cancer cells. *J Agric Food Chem* **57**: 305–310.

Liu W, Zhai Y, Heng X, *et al.*. 2016. Oral bioavailability of curcumin: problems and advancements. *J Drug Target* 1–9.

Liu ZL, Xie LZ, Zhu J, Li GQ, Grant SJ, Liu JP. 2013. Herbal medicines for fatty liver diseases. *Cochrane Database Syst Rev* **8**: CD009059.

Mohammadi A, Sahebkar A, Iranshahi M, *et al.*. 2013. Effects of supplementation with curcuminoids on dyslipidemia in obese patients: a randomized crossover trial. *Phytother Res: PTR* **27**: 374–379.

Murdande SB, Pikal MJ, Shanker RM, Bogner RH. 2011. Aqueous solubility of crystalline and amorphous drugs: Challenges in measurement. *Pharm Dev Technol* **16**: 187–200.

Panahi Y, Alishiri GH, Parvin S, Sahebkar A. 2016a. Mitigation of systemic oxidative stress by curcuminoids in osteoarthritis: results of a randomized controlled trial. *J Diet Suppl* **13**: 209–220.

Panahi Y, Badeli R, Karami GR, Sahebkar A. 2015a. Investigation of the efficacy of adjunctive therapy with bioavailability-boosted curcuminoids in major depressive disorder. *Phytother Res: PTR* **29**: 17–21.

Panahi Y, Ghanei M, Bashiri S, Hajihashemi A, Sahebkar A. 2015b. Short-term curcuminoid supplementation for chronic pulmonary complications due to sulfur mustard intoxication: positive results of a randomized double-blind placebo-controlled trial. *Drug Res* **65**: 567–573.

Panahi Y, Ghanei M, Hajhashemi A, Sahebkar A. 2016b. Effects of curcuminoids-piperine combination on systemic oxidative stress, clinical symptoms and quality of life in subjects with chronic pulmonary complications due to sulfur mustard: a randomized controlled trial. *J Diet Suppl* **13**: 93–105.

Panahi Y, Hosseini MS, Khalili N, Naimi E, Majeed M, Sahebkar A. 2015c. Antioxidant and anti-inflammatory effects of curcuminoid-piperine combination in subjects with metabolic syndrome: a randomized controlled trial and an updated meta-analysis. *Clin Nutr* **34**: 1101–1108.

Panahi Y, Khalili N, Hosseini MS, Abbasinazari M, Sahebkar A. 2014a. Lipid-modifying effects of adjunctive therapy with curcuminoids-piperine combination in patients with metabolic syndrome: results of a randomized controlled trial. *Complement Ther Med* **22**: 851–857.

Panahi Y, Kianpour P, Mohtashami R, Jafari R, Simental-Mendia LE, Sahebkar A. 2016c. Curcumin lowers serum lipids and uric acid in subjects with non-alcoholic fatty liver disease: a randomized controlled trial. *J Cardiovasc Pharmacol* DOI:10.1097/FJC.0000000000000406.

Panahi Y, Rahimnia AR, Sharafi M, Alishiri G, Saburi A, Sahebkar A. 2014b. Curcuminoid treatment for knee osteoarthritis: a randomized double-blind placebo-controlled trial. *Phytother Res: PTR* **28**: 1625–1631.

Panahi Y, Saadat A, Beiraghdar F, Sahebkar A. 2014c. Adjuvant therapy with bioavailability-boosted curcuminoids suppresses systemic inflammation and improves quality of life in patients with solid tumors: a randomized double-blind placebo-controlled trial. *Phytother Res: PTR* **28**: 1461–1467.

Panahi Y, Sahebkar A, Amiri M, *et al.*. 2012a. Improvement of sulphur mustard-induced chronic pruritus, quality of life and antioxidant status by curcumin: results of a randomised, double-blind, placebo-controlled trial. *Br J Nutr* **108**: 1272–1279.

Panahi Y, Sahebkar A, Parvin S, Saadat A. 2012b. A randomized controlled trial on the anti-inflammatory effects of curcumin in patients with chronic sulphur mustard-induced cutaneous complications. *Ann Clin Biochem* **49**: 580–588.

Pezeshki A, Safi S, Feizi A, Askari G, Karami F. 2016. The Effect of Green Tea Extract Supplementation on Liver Enzymes in Patients with Nonalcoholic Fatty Liver Disease. *Int J Prev Med*. **7**: 28. DOI:10.4103/2008-7802.173051.

Ramirez CM, Goedeke L, Rotllan N, *et al.*. 2013. MicroRNA 33 regulates glucose metabolism. *Mol Cell Biol* **33**: 2891–2902.

Rivera-Espinoza Y, Muriel P. 2009. Pharmacological actions of curcumin in liver diseases or damage. *Liver Int: Off J Int Assoc Study Liver* **29**: 1457–1466.

Sahebkar A. 2013. Why it is necessary to translate curcumin into clinical practice for the prevention and treatment of metabolic syndrome? *Biofactors* **39**: 197–208.

Sahebkar A. 2014a. Are curcuminoids effective C-reactive protein-lowering agents in clinical practice? Evidence from a meta-analysis. *Phytother Res: PTR* **28**: 633–642.

Sahebkar A. 2014b. Curcuminoids for the management of hypertriglyceridaemia. *Nat Rev Cardiol* **11**: 123.

Sahebkar A. 2014c. Low-density lipoprotein is a potential target for curcumin: novel mechanistic insights. *Basic Clin Pharmacol Toxicol* **114**: 437–438.

Sahebkar A. 2015. Dual effect of curcumin in preventing atherosclerosis: the potential role of pro-oxidant-antioxidant mechanisms. *Nat Prod Res* **29**: 491–492.

Sahebkar A, Chew GT, Watts GF. 2014. Recent advances in pharmacotherapy for hypertriglyceridemia. *Prog Lipid Res* **56**: 47–66.

Sahebkar A, Mohammadi A, Atabati A, *et al.*. 2013. Curcuminoids modulate pro-oxidant-antioxidant balance but not the immune response to heat shock protein 27 and oxidized LDL in obese individuals. *Phytother Res: PTR* **27**: 1883–1888.

Shapiro H, Bruck R. 2005. Therapeutic potential of curcumin in non-alcoholic steatohepatitis. *Nutr Res Rev* **18**: 212–221.

Shi KQ, Fan YC, Liu WY, Li LF, Chen YP, Zheng MH. 2012. Traditional Chinese medicines benefit to nonalcoholic fatty liver disease: a systematic review and meta-analysis. *Mol Biol Rep* **39**: 9715–9722.

Um MY, Hwang KH, Ahn J, Ha TY. 2013. Curcumin attenuates diet-induced hepatic steatosis by activating AMP-activated protein kinase. *Basic Clin Pharmacol Toxicol* **113**: 152–157.

Vernon G, Baranova A, Younossi ZM. 2011. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther* **34**: 274–285.

Wang Y, Li J, Zhuge L, *et al.*. 2014. Comparison between the efficacies of curcumin and puerarin in C57BL/6 mice with steatohepatitis induced by a methionine- and choline-deficient diet. *Exp Ther Med* **7**: 663–668.

Wickenberg J, Ingemansson SL, Hlebowicz J. 2010. Effects of Curcuma longa (turmeric) on postprandial plasma glucose and insulin in healthy subjects. *Nutr J* **9**: 43.

Zingg JM, Hasan ST, Meydani M. 2013. Molecular mechanisms of hypolipidemic effects of curcumin. *Biofactors* **39**: 101–121.