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2 **Effects of Supplementation with Curcumin on Serum Adipokine**
3 **Concentrations: A Randomized Controlled Trial**

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Abstract

Background: Previous experimental studies have suggested curcumin as a safe phytochemical that can improve insulin resistance through effects on adiponectin and leptin. This study aimed to investigate the effect of curcumin on circulating adiponectin and leptin concentrations in patients with metabolic syndrome (MetS).

Methods: In this pilot randomized double-blind placebo-controlled trial, subjects who met the criteria of MetS according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) criteria were randomly assigned to curcumin (n=59; 1000 mg/day) or placebo (n=58) for 8 weeks. Serum adiponectin and leptin concentrations were determined before and after intervention. The pooled effect size for the impact of curcumin supplementation on serum adiponectin and leptin levels was also estimated using random-effects meta-analysis.

Results: Eight-week supplementation with curcumin was associated with a significant increase in serum adiponectin levels ($p<0.001$), and a reduction in serum leptin concentrations ($p<0.001$). Serum leptin:adiponectin ratio was also improved by curcumin ($p<0.001$). These beneficial effects of curcumin remained significant after adjustment for changes in serum lipids and glucose concentrations, and baseline differences in BMI and serum levels of glucose and HbA1c as potential confounders of treatment response. Meta-analysis suggested that curcumin supplementation can increase adiponectin levels by 76.78% (95% CI: 6.14, 147.42; $p=0.0330$), and reduce leptin by 26.49% (95% CI: -70.44, 17.46), however, this latter effect size did not reach statistical significance ($p=0.238$).

Conclusion: Curcumin can improve serum levels of adiponectin and leptin in patients with metabolic syndrome. This trial was registered at the UMIN Clinical Trials Registry (<http://www.umin.ac.jp/ctr/>) under Trial No. UMIN000018339.

Key words: Curcumin; Metabolic syndrome; Leptin; Adiponectin; Meta-analysis

Abbreviations

MetS: metabolic syndrome; LDL: low-density lipoprotein; HDL: high-density lipoprotein; NCEP-ATP III: National Cholesterol Education Program Adult Treatment Panel III; BMI: body mass index; SD: standard deviation; DBP: diastolic blood pressure; SBP: systolic blood pressure; HbA1c: hemoglobin A1c; Lp(a): lipoprotein(a); hs-CRP: high-sensitivity C-reactive protein; CI: confidence interval; RCT: randomized clinical trial; JNK: c-Jun N-terminal kinases; NFκB: nuclear factor kappa-light-chain-enhancer of activated B cells; AMPK: 5'AMP-activated protein kinase; HOMA-IR: homeostatic model assessment of insulin resistance; QUICKI: quantitative insulin sensitivity check index

Introduction

Metabolic syndrome (MetS), also known as syndrome X, is a cluster of several cardiometabolic risk factors including abdominal adiposity, hyperglycemia, hypertriglyceridemia, low HDL-C and hypertension (1, 2). MetS prevalence ranges between 10 to 84 % worldwide. According to Azimi-Nezhad et al., 55.0% of Iranian females and 30.1% of Iranian males have MetS (3). Insulin resistance and visceral adiposity are the key factors underlying MetS pathophysiology. Visceral adipose tissue acts as an endocrine organ and releases different kinds of cytokines named adipokines. These adipokines mediate multiple processes such as insulin sensitivity, oxidative stress and inflammation; hence their imbalance could contribute to the development of type 2 diabetes mellitus and atherosclerosis.

Adiponectin and leptin are the most studied adipokines, and their levels are known to be altered in patients with MetS. Adiponectin is a cardioprotective adipokine with anti-inflammatory properties that improve lipid and glucose metabolism, increase insulin sensitivity (4) and prevent atherogenesis (5). Several observational studies have reported an inverse association between circulating adiponectin concentrations and body weight, total cholesterol, triglycerides, blood pressure and insulin resistance, and a positive association with HDL-cholesterol (HDL-C) levels (6, 7). Leptin is another adipokine with a pivotal role in the regulation of energy balance in the body (8). Plasma concentrations of leptin increase with adiposity and correlate with insulin resistance (9, 10). Elevated plasma levels of leptin have been suggested as an independent risk factor for coronary artery disease (10, 11).

Curcumin is the orange-yellow pigment extracted from the famous spice turmeric. Curcumin is a unique phytochemical owing to its numerous molecular targets and diversity of biological activities. The efficacy of curcumin supplementation has been shown against a wide range of diseases including anxiety and depression (12, 13), osteoarthritis (14, 15), metabolic syndrome (16), dyslipidemia (17-19), atherosclerosis (20, 21), chronic complications due to sulfur mustard intoxication (22-25), solid tumors (26) and inflammation (27).

Interestingly, curcumin could modify almost all features of MetS (28). There is evidence indicating that curcumin lowers plasma levels of total cholesterol, LDL-cholesterol (LDL-C), triglyceride and glucose, and increases those of HDL-C (19, 29-33). Insulin-sensitizing (34-37), anti-obesity (38-40) and anti-hypertensive (41) effects of curcumin are other properties of this natural product reported in experimental studies. Experimental studies have also identified

adiponectin and leptin as targets of curcumin (42, 43). However, clinical evidence on the impact of curcumin supplementation on circulating levels of these two adipokines has been scarce. Hence, this study aimed to evaluate changes in serum levels of adiponectin and leptin, and the ratio of these two adipokines, following curcumin supplementation in patients with metabolic syndrome. A secondary aim was to pool the results of clinical trials in order to estimate the effect size for the impact of curcumin on circulating adiponectin and leptin concentrations.

Materials and methods

Subjects

This study is a *post-hoc* analysis performed on the samples obtained from our previous investigation (17). Participants were recruited from the Cardiology and Endocrinology Clinics of the Baqiyatallah Hospital (Tehran, Iran). Inclusion criteria were males and females who were not originally receiving lipid-lowering therapy, for whom a diagnosis of MetS was made according to the criteria defined by the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) guidelines as follows: ≥ 3 of the following conditions: waist circumference ≥ 102 cm (male) or ≥ 88 cm (female), blood pressure $\geq 130/85$ mmHg, triglycerides ≥ 1.7 mmol/L, HDL-C < 1.03 mmol/L (males) or < 1.29 mmol/L (females), fasting blood glucose ≥ 6.1 mmol/L (44).

Exclusion criteria were pregnancy or breastfeeding, lack of compliance with the study medication (defined as not using the medication for > 1 week), participation in a concomitant trial, hypersensitivity to the study medication, presence of malignancies and impossibility to give informed consent. The study protocol was given approval by the institutional Ethics Committee and written informed consent was obtained from participants.

Study design

This study was designed as a randomized double-blind placebo-controlled trial with a parallel-group design. Subjects who met the inclusion criteria were randomly assigned to either curcumin (Curcumin C3 Complex[®], Sami Labs LTD, Bangalore, India; $n = 59$) or matched placebo ($n = 58$) for a period of 8 weeks. Curcumin was administered at a daily dose of 1g (500 mg b.i.d.) based on the use of the same dose in our previous trial in obese individuals (45). In order to improve the bioavailability of curcumin, 5 mg piperine (Biooperine[®]; Sami Labs LTD, Bangalore,

India) was added to each 500 mg curcumin capsule (46). C3 Complex[®] preparation that was used in the present study contained three major curcuminoids i.e. curcumin, demethoxycurcumin and bisdemethoxycurcumin in a patented ratio. Placebo capsules contained the same amount of lactose plus 5 mg piperine. This trial was registered at the UMIN Clinical Trials Registry (<http://www.umin.ac.jp/ctr/>) under Trial No. UMIN000018339.

Blood Sampling

Overnight fasting blood samples were collected at baseline and at study end. The samples were allowed to clot for about 30 minutes and then centrifuged at 750 g for 10 minutes to obtain serum. Sera were aliquoted and frozen at -80°C until measurements.

Measurements

Serum concentrations of leptin and adiponectin were determined using the enzyme linked immunoassay technique with commercial kits. Weight, height, and systolic and diastolic blood pressures were measured according to standard procedures (47). BMI was calculated as weight in kilograms divided by height in meters squared (m²).

Statistical analysis

Statistical analyses were performed using the SPSS software version 11.5 (SPSS Inc., Chicago, Illinois, USA). Data were expressed as mean $\pm$ 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-square test. Bivariate correlations between changes in serum levels of leptin, adiponectin and leptin:adiponectin ratio were performed using Pearson's (for normally distributed data) and Spearman's (for non-normally distributed data) correlation coefficients. Univariate analysis of covariance (ANCOVA) using general linear model was used to adjust for the effect of potential confounders on the association between curcumin supplementation and changes in serum levels of adiponectin, leptin and leptin:adiponectin ratio.

Quantitative data synthesis

Pooled analysis was performed using the Comprehensive Meta-Analysis (CMA) V2 software (Biostat, NJ) (48). Circulating adiponectin and leptin concentrations were collated in ng/mL. Standard deviations at one time point were calculated with the formula $SD = SEM \times \text{square root } n$ (SEM: standard error of the mean, n : number of participants). Standard deviations (SDs) of the mean difference were calculated using the formula: $\text{square root } [(SD_{\text{pre-treatment}})^2 + (SD_{\text{post-treatment}})^2 - (2R \times SD_{\text{pre-treatment}} \times SD_{\text{post-treatment}})]$, assuming a correlation coefficient (R) = 0.5. Net changes in measurements (change scores) were calculated as follows: (measure at end of follow-up in the treatment group – measure at baseline in the treatment group) – (measure at end of follow-up in the control group – measure at baseline in the control group). A random-effects model and the generic inverse variance method were used owing to the heterogeneity of studies in terms of design (parallel or cross-over), dosage and formulation of curcumin, and inter-study variations in the inclusion criteria (underlying disease, age, gender and anthropometric indices).

Results

One hundred and seventeen subjects met the inclusion criteria and were assigned to either curcumin ($n = 59$) or placebo ($n = 58$). One hundred subjects completed the trial. Nine subjects in the curcumin group and eight subjects in the placebo group did not complete the study due to loss to follow-up (**Figure 1**). The number of drop-outs was not different between the study groups.

Curcumin and placebo groups were comparable at baseline with respect to age, gender, smoking frequency, systolic blood pressure (SBP) and diastolic blood pressure (DBP). However, BMI ($p = 0.002$) and serum levels of glucose ($p < 0.001$) and HbA1c ($p = 0.035$) concentrations were higher in the curcumin group (**Table 1**). There was also no significant difference between the curcumin group and placebo group in terms of baseline serum adiponectin ($p=0.795$) and leptin ($p=0.292$) concentrations and leptin:adiponectin ratio ($p=0.526$). Within-group analysis revealed a significant increase in serum adiponectin concentrations and reduction in leptin:adiponectin ratio in both curcumin ($p<0.001$) and placebo ($p<0.001$) groups. Serum leptin concentrations were reduced in the curcumin group ($p<0.001$) but did not change significantly in the placebo group ($p=0.078$) (**Table 2**).

Between-group comparison of the change values revealed a significant elevation of serum adiponectin ($p<0.001$) and a significant reduction of serum leptin concentrations ($p<0.001$) in the curcumin compared with placebo group. Likewise, serum leptin:adiponectin ratio was significantly reduced in the curcumin versus placebo group ($p<0.001$) (**Table 2**).

In order to check the effect of changes in serum lipids and glucose as potential confounders on the observed changes in adipokines, univariate ANCOVA was performed. Assignment to treatment group (yes/ no), and changes in serum levels of LDL-C, HDL-C, total cholesterol, triglycerides, Lp(a) and glucose were separately entered into the model as independent variables.

Another adjustment was also performed for baseline differences in BMI and serum concentrations of glucose and HbA1c.

According to the results, the impact of curcumin supplementation on dependent variables including changes in serum concentrations of adiponectin ($p<0.001$), leptin ($p=0.044$) and leptin:adiponectin ratio ($p=0.001$) remained statistically significant after adjustment for potential confounders. The impact of curcumin supplementation on the above-mentioned efficacy measures also remained significant after adjustment for baseline differences in BMI, serum glucose and HbA1c concentrations ($p<0.001$).

As reported in our previous report (17), curcumin were well-tolerated during the study. There were two reports of diarrhea, two reports of constipation, one report of headache, and two reports of skin rash in the curcumin group. Headache ($n=2$) and constipation ($n=1$) were reported adverse events in the placebo group. None of the drop-outs in this trial were due to the above-mentioned adverse events.

Bivariate correlations

Changes in serum adiponectin concentrations were correlated with changes in HDL-C ($p=0.002$), whilst changes in serum leptin levels and serum leptin:adiponectin ratio were not found to be associated with any of the assessed parameters. In the placebo group, there were significant correlations between changes in serum adiponectin ($p=0.010$) and leptin:adiponectin ratio ($p=0.004$) ratio with triglyceride changes. There was no significant correlation between changes in serum adiponectin and leptin concentrations in either of the studied groups (**Table 3**).

Quantitative data synthesis

Meta-analysis of data from three RCTs (including the present study) using a random-effects model showed that curcumin supplementation can increase plasma adiponectin (WMD: 76.78%, 95% CI: 6.14, 147.42; $p=0.0330$). With respect to plasma leptin concentrations, a reduction by 26.49% was calculated (95% CI: -70.44, 17.46), yet this effect size did not reach statistical significance ($p=0.238$) (**Figure 2**).

Discussion

The findings of this randomized controlled trial suggested a significant increase in serum levels of adiponectin and reduction in serum levels of leptin following 8 weeks of supplementation with curcumin in patients with metabolic syndrome. To the best of author's knowledge, this is among the very few studies dealing with the effect of curcumin on adipokines, and the first exploring this issue in patients with metabolic syndrome. In a trial among subjects with prediabetes, supplementation with curcumin (1500 mg/day) for 9 months was reported to increase plasma adiponectin concentrations by 23.5% (49). In another study in patients with type 2 diabetes, 6-month supplementation with curcumin (1500 mg/day) reduced plasma leptin levels by 65%, and increased adiponectin by 152% (50). In contrast to the above results, in another trial in patients with major depressive disorder, 8-week supplementation with curcumin (1000 mg/day) was found to increase plasma leptin levels by 23%, though this increase did not reach statistical significance (51).

Insulin resistance is defined as impairment of insulin action on glucose, lipid and protein metabolism. It is closely associated with adipose tissue. Excessive visceral and subcutaneous adipose tissue causes adipocyte dysfunction which can lead to inflammation through activation of JNK and NF κ B. Inflammation causes impaired adipokine secretion reflected as decreased adiponectin and increased leptin levels (52, 53). Adiponectin and leptin mediate insulin sensitivity through AMPK (5'-AMP-activated protein kinase) pathway. AMPK is a master switch which controls energy status in the cell, and its activation leads to enhanced β -oxidation and reduced fatty acid esterification to triglycerides (54). Moreover, several studies have suggested that leptin:adiponectin ratio could serve as a useful index of insulin resistance and atherogenic risk in both diabetic and non-diabetic populations (55-57). There are also reports showing the association between leptin:adiponectin ratio and low-grade inflammation, carotid intima media

thickness,. Arterial stiffness, first cardiovascular event and number of metabolic syndrome components (58, 59).

Some previous studies have revealed that curcumin could decrease insulin resistance by increasing fatty acid oxidation. Na et al. indicated that curcumin improves insulin resistance in skeletal muscles through activation of AMPK and fatty acid β -oxidation (60). These findings were approved in a later trial in diabetic type2 patients (61). Similarly, in an experimental study on C57BL/6J mice, it was indicated that curcumin can improve insulin resistance through inhibiting the expression of lipogenic genes and inflammation in the adipose tissue (39). In another experimental study, Weisberg et al. reported that curcumin ameliorates inflammation due to visceral adiposity, and this effect is accompanied by adiponectin elevation and mitigation of insulin sensitivity (40). There is also *in vitro* evidence indicating that curcumin blocks leptin signaling and prevents hyperlipidemia-induced oxidative stress, hepatic stellate cell activation, and liver fibrogenesis (62). The favorable impact of curcumin supplementation on adiponectin and leptin in this study is consistent with the reduction in serum glucose of the same individuals reported previously (63). In our previous report, however, no significant effect could be detected in HbA1c which might be due to the short duration of follow-up.

Aside from insulin resistance, dyslipidemia is another prevalent feature of MetS, commonly presented as low HDL-C concentrations and elevated levels of triglycerides. This phenotype is referred to as atherogenic dyslipidemia, and is a promoter of insulin resistance. The beneficial effects of curcumin on lipid indices has been reported in our previous report from the same trial, indicating reductions in LDL-C, triglycerides and Lp(a) and elevations in HDL-C (17). This lipid-modifying effect of curcumin has also been reported in some other trials (19, 50, 61).

As reported previously (16, 17), curcumin supplementation was safe in this trial. Curcumin has been approved by US FDA as a “generally recognized as safe” supplement, and its tolerability has been confirmed in several clinical studies. Therefore, owing to its safety and beneficial effects on several features of metabolic syndrome, curcumin may be suggested as a routine supplement for patients with metabolic syndrome.

Hitherto, several lines of evidence have suggested adiponectin as a key player in limiting the pathogenesis of obesity-related diseases including metabolic syndrome, non-alcoholic fatty liver disease and cardiovascular disease. The protective effects of adiponectin in reducing the risk of cardiometabolic diseases could be attributed to improvement of lipid and glucose metabolism as

well as antioxidant, anti-inflammatory, anti-thrombotic, anti-hypertensive and anti-atherosclerotic actions of this adipokine (64). These beneficial effects are mediated by the capacity of this adipokines to interact with important mediators/signaling molecules/pathways involved in cardiometabolic disturbances (65). Interestingly, curcumin has been shown to have the same multi-target capacity of action, and its capacity to interact with several key regulators such as transcription factors (eg. NFκB and activator protein 1), enzymes (cyclooxygenases, lipoxygenase and AMPK) pro-inflammatory cytokines, acute phase proteins, antioxidants, growth afctorrs, hormones, secondary messengers and nitric oxide, along with direct effects on adipokines production, could justify the beneficial cardiometabolic effects of this phytochemical (65). The present study was limited in a number of ways. First, this was a short-term trial and it is unknown if longer durations of supplementation could cause further improvements in circulating adiponectin and leptin concentrations. Second, this study tested the effects of a single dose of curcumin, hence any dose-response association for the metabolic effects of curcumin remains unclear. Finally, although circulating leptin, adiponectin and their ratio could serve as indirect biomarkers of insulin resistance, insulin resistance was not measured in this study.

In conclusion, the present trial provided the first evidence on the improvement of circulating adiponectin, leptin and leptin:adiponectin ratio in patients with metabolic syndrome. Future studies are encouraged to ascertain the impact of supplementation duration and curcumin dose on the observed beneficial effects, and also the value of improving adipokine status with curcumin in obese individuals and its plausible association with changes in body weight and fat content. Finally, evaluation of the impact of curcumin on known measures of insulin resistance, including hyperinsulinemic euglycemic clamp, homeostatic model assessment of insulin resistance (HOMA-IR), and quantitative insulin sensitivity check index (QUICKI), is greatly recommended.

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347 **Conflict of interest**

348 Muhammed Majeed is the CEO of Sabinsa Corporation and Sami Labs Ltd.

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Figure legends

Figure 1. Flow chart of the trial.

Figure 2. Forest plot detailing weighted mean difference and 95% confidence intervals for the impact of curcumin on circulating adiponectin (upper plot) and leptin (lower plot) concentrations. The pooled effect size is shown as red diamond. There was significant pooled effect of curcumin on adiponectin concentrations while for leptin meta-analysis (in which the red diamond crosses the vertical line that corresponds to the value of zero), the pooled effect did not reach statistical significance.

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586 **Table 1.** Baseline characteristics of study groups.

	Curcumin	Placebo
Age (y)	44.80 ± 8.67	43.46 ± 9.70
Female	23 (46%)	27 (54%)
Smoking	12 (24%)	8 (16%)
BMI (kg/m²)	25.46 ± 2.46	22.80 ± 5.37
SBP (mmHg)	135.56 ± 13.16	135.70 ± 14.74
DBP (mmHg)	88.34 ± 7.81	88.72 ± 8.18
Hs-CRP (g/L)	6.52 ± 2.16	7.10 ± 1.80
Adiponectin (ng/mL)	12.67±2.13	12.78±2.19
Leptin (ng/mL)	22.02±2.93	22.64±2.97
Leptin:adiponectin	1.77±0.32	1.82±0.37
LDL-C (mg/dL)	190.46 ± 20.05	157.10 ± 17.29
HDL-C (mg/dL)	31.50 ± 4.67	35.48 ± 6.54
Total cholesterol (mg/dL)	220.29 ± 37.72	184.08 ± 17.37
Triglycerides (mg/dL)	199.60 ± 23.44	185.64 ± 38.49
Lp(a) (mg/dL)	82.00 ± 7.35	84.48 ± 8.47
Glucose (mg/dL)	155.46 ± 40.89	136.98 ± 52.40
HbA1c (%)	6.69 ± 1.44	6.07 ± 1.33

587 BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; hs-CRP:
588 high-sensitivity C-reactive protein; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-
589 density lipoprotein cholesterol; Lp(a): lipoprotein(a).

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Table 2. Changes in serum adipokines concentrations during the trial.

	Curcumin				Placebo				
	Before	After	Change	<i>p</i> -value ^a	Before	After	Change	<i>p</i> -value ^a	<i>p</i> -value ^b
Adiponectin (ng/mL)	12.67±2.13	21.28±4.40	8.61±4.31	<0.001	12.78±2.19	15.97±2.69	3.19±3.36	<0.001	<0.001
Leptin (ng/mL)	22.02±2.93	17.50±2.42	-4.52±3.72	<0.001	22.64±2.97	21.56±3.82	-1.08±4.25	0.078	<0.001
Leptin:adiponectin	1.77±0.32	0.86±0.20	-0.92±0.37	<0.001	1.82±0.37	1.39±0.33	-0.43±0.48	<0.001	<0.001

^a: comparison of before vs. after values in each group; ^b: comparison of changes between the study groups.

Table 3. Bivariate correlations between changes in serum adiponectin, leptin and adiponectin:leptin ratio with serum lipids and glucose.

Curcumin group						
	Adiponectin		Leptin		Leptin:adiponectin	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
TC	0.231	0.114	-0.104	0.481	-0.186	0.205
LDL-C	0.298	0.036	-0.008	0.958	-0.220	0.124
HDL-C	0.430	0.002	-0.108	0.455	-0.250	0.080
TG	0.123	0.396	0.082	0.573	0.054	0.707
Lp(a)	0.051	0.727	-0.095	0.510	0.006	0.969
Glucose	-0.245	0.086	0.061	0.674	0.184	0.201
Placebo group						
	Adiponectin		Leptin		Leptin:adiponectin	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
TC	0.144	0.318	0.109	0.450	-0.052	0.722
LDL-C	0.022	0.881	0.007	0.962	-0.009	0.951
HDL-C	0.095	0.510	-0.044	0.760	-0.069	0.636
TG	-0.363	0.010	.165	0.252	0.397	0.004
Lp(a)	0.108	0.456	-0.010	0.943	-0.074	0.609
Glucose	0.206	0.151	0.073	0.614	-0.084	0.561

Bivariate correlations were assessed using Pearson's (for normally distributed data) and Spearman's (for non-normally distributed data) correlation coefficients. TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglycerides; sdLDL: small-dense low-density lipoprotein; Lp(a): lipoprotein(a).

Figure 1

Figure 2