Metformin restores the correlation between serum-oxidized LDL and leptin levels in type 2 diabetic patients

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Introduction: Leptin has lipid peroxidation properties in healthy individuals. Here we aimed to study the correlation between serum-oxidized low-density lipoprotein (ox-LDL) and leptin levels in patients with type 2 diabetes. We also studied the effect of metformin therapy on the correlation between serum ox-LDL and leptin levels in patients with newly diagnosed diabetes.

Methods: We performed a cross-sectional study on two groups of patients with type 2 diabetes stratified according to (1) patients with newly diagnosed diabetes and (2) patients with long-standing diabetes plus healthy controls. Patients with newly diagnosed diabetes were followed for 3 months after the initiation of metformin therapy.

Results: Patients with type 2 diabetes had a higher serum ox-LDL, ox-LDL/LDL ratio, waist circumference, fasting blood sugars (FBSs), hemoglobin A1C (HbA1C), triglyceride, homeostatic model assessment of insulin resistance (HOMA-IR) and a lower serum leptin levels than controls. Serum ox-LDL, ox-LDL/LDL ratio (0.08 (0.08 – 0.12) vs. 0.06 (0.05 – 0.08), $P < 0.001$) and HOMA-IR (3.26 ± 0.23 vs. 2.93 ± 0.32 ; $P < 0.01$) were decreased when serum leptin levels (15.9 ± 1.6 vs. 21.4 ± 2.5 , $P < 0.01$) were increased after 3 months of metformin therapy. This remained significant after multiple adjustments for age, body mass index, FBS, HbA1c, and HOMA-IR. Leptin was significantly correlated with ox-LDL/LDL ratio in controls ($r = 0.78$, $P < 0.01$), and in patients with newly diagnosed diabetes ($r = 0.4$, $P < 0.05$), after metformin therapy. There were not any correlation between leptin and ox-LDL/LDL ratio in patients with long-standing diabetes and patients with newly diagnosed diabetes before treatment.

Discussion: Metformin restores the positive correlation between serum ox-LDL and leptin levels in patients with type 2 diabetes.

Keywords: Metformin, Leptin, Oxidized LDL, Insulin resistance, Diabetes mellitus, Type 2, Oxidative stress

Introduction

Oxidative stress is a disturbance in the pro-oxidant, anti-oxidant balance in favor of the former, which leads to the potential damage.¹ Indicators of oxidative stress include measures of damaged DNA, protein oxidation, and lipid per-oxidation products.¹ Oxidized low-density lipoprotein (LDL) is a particle derived from the circulating LDL that may have peroxide and other degeneration products.² The concept that oxidative stress and oxidation of LDL might play an important role in the atherosclerosis and diabetes complications was first described over 15 years ago.³ This

idea formed from a simple observation that *in vitro* incubation of macrophages with oxidized LDL (ox-LDL), and not with native LDL led to cholesterol ester accumulation.⁴ Since then many studies established the role of ox-LDL in the pathogenesis of diabetes complications including albuminuria and coronary heart disease.^{5,6}

Leptin, an adipocyte-derived cytokine, primarily was known to involve in the regulation of food intake and energy expenditure.⁷ Leptin modulate the proliferation of monocyte/macrophages and the production of oxidative stress.⁸ Wistar rats injected with leptin had a higher plasma lipid hydroperoxide, malondialdehyde, isoprostane, and protein carbonyl content than non-treated controls.⁹ They also suggested that leptin has a proatherogenic effect.⁹

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Studies have shown a positive correlation between serum ox-LDL and leptin levels in psoriasis and healthy individuals.^{10,11} Porreca *et al.* showed a significant correlation between circulating ox-LDL and leptin levels in postmenopausal women without coronary heart disease risk factors. They suggested that leptin had a stronger effect on oxidative modification of LDL than of C reactive protein (CRP). This was because the association of ox-LDL with CRP disappeared in a multivariate model, and the change in ox-LDL concentrations, from weight loss, was predicted only by changes in leptin.¹²

Hence, we hypothesized that in chronic inflammation, like type 2 diabetes, there would be a positive correlation between serum ox-LDL and leptin levels. Studies have shown that metformin therapy improves the antioxidant status, enzymatic activity, and inflammatory parameters in type 2 diabetic patients.^{13,14} The correlation between serum ox-LDL and leptin would be altered after metformin therapy. The purposes of the present study were

1. Comparing the correlation between serum leptin and ox-LDL levels in patients with newly diagnosed diabetes, patients with long-standing type 2 diabetes, and healthy controls.
2. Studying the effect of 3 months metformin therapy on the ox-LDL and leptin correlation in the group of newly diagnosed diabetes.

Methods

We performed a cross-sectional study on the established groups of patients with type 2 diabetes defined as (1) patients with long-standing diabetes for more than 5 years; and (2) patients with newly diagnosed diabetes within recent 6 months who were not on any glucose lowering treatment other than by dietary means alone; and (3) healthy controls. Patient recruitment was from the diabetes clinic of Vali-Asr Hospital, affiliated with Tehran University of Medical Sciences. Controls were healthy volunteers from the patients' concomitants or hospital staffs. Healthy controls were selected from those without any known disease including type 2 diabetes, hyperlipidemia, ischemic heart disease, and malignancy. Patients and controls were matched according to age, sex, and body mass index (BMI).

On the basis of the results of an exploratory analysis, we designed a follow-up study of patients with newly diagnosed diabetes before and after 3 months of metformin treatment. The patients commenced on treatment immediately following diagnosis. All these patients had poorer glycemic control, sufficient to merit an oral hypoglycemic agent. Metformin was the first-line choice of glucose lowering therapy in these patients. Treatment was started with metformin monotherapy and the patients had a 3-month period

of therapy with three phases of initiation, titration, and maintenance. The average dose of metformin was 1000–1500 mg per day. Diabetes was diagnosed according to the criteria of the American Diabetes Association.¹⁵ Groups of patients and controls were matched for age, sex, and BMI. Exclusion criteria were smoking, pregnancy, creatinine >1.5 mg/dl or GFR < 70 cc/minute, glomerulonephritis, congestive heart failure, use of antioxidant, and hospital admission in recent 6 months. None of the participants had overt diabetes complications. None of the participants were on hormone replacement therapy. Demographic and anthropometric data including age, sex, duration of diabetes, height, weight in light clothing, and blood pressure in sitting position were recorded. Blood pressure was re-measured twice after 5 minutes and averaged. The BMI (kg/m²) was calculated according to the Quetelet formula. The research was carried out according to the principles of the Declaration of Helsinki. All the patients received and signed written informed consent. The local ethics review committee of Tehran University of Medical Science approved the study protocol.

Blood samples

Blood samples were collected after 12 hours of fasting, centrifuged, and kept at –70°C until analysis. Serum creatinine, fasting blood sugar (FBS), total cholesterol, triglycerides (TGs), high-density lipoprotein-cholesterol (HDL-C), LDL-cholesterol (LDL-C), and HbA1c were measured for all participants. Glucose measurements (intra-assay coefficient of variants (CV) 2.1%, inter-assay CV 2.6%) were carried out using the glucose oxidase method. Creatinine was measured using calibrated Jaffe method (Parsazmoon, Karaj, Iran). Cholesterol, HDL-C, LDL-C, and TG were determined using direct enzymatic methods. Serum samples underwent measurement of ox-LDL-beta2GPI using a commercially available sandwich enzyme-linked immunometric assays (ELISA, Cayman, Ann Arbor, MI, USA). The intra- and inter-assay coefficient of variation for the assay ranged between 6.4 and 3.4%. Insulin was measured by radioimmunoassay, using an antibody with no cross reaction against pro-insulin and C-peptide (Immunotech, Prague, Czech Republic). The intra- and inter-assay coefficients of variation were lower than 4.3 and 3.4, respectively. The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated according to; fasting insulin (μU/ml) × fasting plasma glucose (mg/dl)/405, as described by Matthews *et al.*¹⁶ Serum leptin concentration was determined by an enzyme-linked immunosorbent assay (DRG Instruments GmbH, Germany) with an intra-assay coefficient of variation of 5.9–6.9% and an inter-assay coefficient of variation of 8.6–11.5%.

Statistical analysis

The statistical package SPSS 17 for windows (Chicago, IL, USA), was used for analysis. Kolmogorov–Smirnov test was employed to test the normality of the variables in each group. Variables distributed normally are presented as mean and standard error of mean (SEM). Variables with skewed distribution are presented as median (interquartile range). One-way analysis of variance followed by Student's *t*-test and Kruskal–Wallis followed by Mann–Whitney *U* test were employed for the comparison of the normally distributed and skewed distributed variables between the studied groups, respectively.

Paired sample *t*-test and Wilcoxon signed-rank test were employed to compare the normally distributed and skewed distributed variables in patients with newly diagnosed diabetes, before and after treatment. General linear models were employed to study the changes in serum ox-LDL, ox-LDL/LDL ratio, and leptin levels, before and after metformin therapy, controlling for age, BMI, FBS, HbA1C, and HOMA-IR. Pearson correlation was employed to study the correlation between serum ox-LDL, ox-LDL/LDL ratio, leptin, HOMA-IR, and BMI levels in the group of patients with type 2 diabetes and controls.

Results

Primary characteristics of the groups of patients and controls are presented in Table 1. The drop-out rate

due to side effects following commencement of metformin therapy was (0%) in patients with newly diagnosed diabetes. The frequency of insulin therapy was (9/45; 20%) in patients with long-standing diabetes. Forty-six percent (21/45) of the patients with long-standing diabetes were on statin therapy. Leptin was significantly correlated with ox-LDL/LDL ratio in controls ($r = 0.78$, $P < 0.01$), and patients with newly diagnosed diabetes after 3 months of metformin therapy ($r = 0.4$, $P < 0.05$), when serum leptin levels were not correlated with ox-LDL/LDL ratio ($r = -0.09$, $r = -0.02$) in patients with long-standing diabetes and patients with newly diagnosed diabetes before treatment (Fig. 1).

Patients with type 2 diabetes had a higher serum ox-LDL, ox-LDL/LDL ratio, waist circumference, FBS, HbA1C, TG, and HOMA-IR levels than controls (Table 1). Likewise, ox-LDL, ox-LDL/LDL ratio, HbA1C, and HOMA-IR were higher in patients with long-standing diabetes than newly diagnosed patients (Table 1). Participants in the control group had a higher serum leptin and HDL-C levels compared to patients with type 2 diabetes (Table 1).

There was a significant decrease in serum ox-LDL (10(9.7–14.1) vs. 6(5.6–7.8); $P < 0.001$), ox-LDL/LDL (0.082 (0.080–0.12) vs. 0.06 (0.05–0.08); $P < 0.001$) (Fig. 2), HOMA-IR (3.26 ± 0.23 vs. 2.93 ± 0.32 ; $P < 0.001$), cholesterol (206.77 ± 6.161 vs. 182.97 ± 5.57 , $P < 0.01$), and LDL-C (123.96 ± 6.03

Table 1 Comparing demographic and biochemical variables among patients with long-standing diabetes, patients with newly diagnosed diabetes and healthy controls

	Control (<i>n</i> = 45)	Patients with type 2 diabetes		<i>P</i> value
		Newly diagnosed diabetes (<i>n</i> = 42)	More than 5 years diabetes (<i>n</i> = 45)	
Age (years)	50.02 ± 1.02	52.09 ± 1.77	49.97 ± 1.03	NS
Female (<i>n</i> , %)	25 (55%)	23 (54%)	25 (55%)	NS
BMI (kg/m ²)	27.16 ± 0.41	27.84 ± 0.72	27.42 ± 0.43	NS
Diastolic blood pressure (mmHg)	78.44 ± 1.16	87.51 ± 2.39	78.66 ± 1.48	NS
Systolic blood pressure (mmHg)	125.22 ± 1.55	127.94 ± 3.54	125.77 ± 2.03	NS
Creatinine (mg/dl)	0.85 ± 0.023	0.89 ± 0.033	0.82 ± 0.02	<0.01
Waist (cm)	90.77 ± 1.33*	97.28 ± 1.58	100.13 ± 1.28	<0.001
Hip (cm)	100.57 ± 1.05*	105.75 ± 1.84	101.40 ± 1.05	NS
HbA1C (%)	4.88 ± 0.06***	8.37 ± 0.36	9.51 ± 0.30**	<0.001
FBS (mg/dl)	88.62 ± 1.05***	181.90 ± 8.48	188.97 ± 8.75	<0.001
TG (mg/dl)	105.68 ± 5.51***	201.97 ± 14.86	178.53 ± 15.17	<0.001
HDL-C (mg/dl)	52.00 ± 1.64**	44.71 ± 1.85	40.91 ± 1.24	<0.001
Cholesterol (mg/dl)	205.8 ± 3.5	206.4 ± 6.3	194.6 ± 7.0	NS
LDL-C (mg/dl)	113.22 ± 1.70	123.96 ± 6.03	110.97 ± 4.11	NS
ox-LDL/LDL ratio	0.07 (0.06–0.075)*	0.082 (0.080–0.12)	0.16 (0.14–0.18)***	<0.001
ox-LDL (nmol/l)	8.1 (7.3–8.5)***	10 (9.7–14.1)	17.3 (16.4–18.2)***	<0.001
Leptin (ng/ml)	23.6 ± 2.9*	15.9 ± 1.6	15.5 ± 1.4	<0.05
HOMA-IR	1.40 ± 0.13***	3.26 ± 0.23	4.42 ± 0.58*	<0.001

Note: Normally distributed variables are expressed as mean ± SEM. Variables with skewed distribution are presented as median (interquartile range). Mann–Whitney *U* test was employed to compare variables with skewed distribution and independent sample *T*-test were employed to compare normally distributed variables.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ when comparing patients with long-standing diabetes vs. patients with newly diagnosed diabetes or controls vs. patients with newly diagnosed diabetes. The column of '*P* value' is presented when comparing patients with long-standing diabetes vs. controls.

Figure 1 Presenting correlation coefficient between serum leptin and ox-LDL/LDL ratio in A: controls, B: patients with long-standing diabetes, C: patients with newly diagnosed diabetes before treatment, and D: patients with newly diagnosed diabetes after treatment.

Figure 2 Serum A: leptin and B: ox-LDL/LDL ratio in patients with newly diagnosed diabetes, before and after 3 months of metformin therapy. The bars represent mean and the handles represent SEM.

vs. 102.171 ± 4.19 ; $P < 0.01$) in patients with newly diagnosed diabetes after treatment (Table 2). Serum leptin levels increased after metformin therapy in patients with newly diagnosed diabetes (15.9 ± 1.6 vs. 21.4 ± 2.5 ; $P < 0.01$) (Fig. 2). The observed change in serum ox-LDL (11.3 (9.6 – 13.4) vs. 6.6 (6.3 – 7.5)), $P < 0.001$, ox-LDL/LDL ratio (0.10 (0.079 – 0.12) vs. 0.064 (0.053 – 0.074), $P < 0.001$), and leptin levels

(15.2 ± 1.4 vs. 20.7 ± 2.7 , $P < 0.05$) remained significant after multiple adjustments for age, BMI, FBS, HbA1c, and HOMA-IR using general linear models.

Discussion

Our findings clearly demonstrated that there were no significant correlation between ox-LDL, ox-LDL/LDL ratio, and leptin levels in patients with diabetes,

Table 2 Characteristics of patients with newly diagnosed diabetes before and after 3 months of metformin therapy

	Before metformin treatment	After metformin treatment	P value
BMI (kg/m ²)	27.84 ± 0.72	27.6 ± 0.6486	NS
FBS (mg/dl)	188.90 ± 8.48	144.76 ± 6.25	<0.001
HbA1c (%)	8.37 ± 0.36	7.36 ± 0.59	<0.001
Creatinin (mg/dl)	0.89 ± 0.033	0.94 ± 0.03	NS
Cholesterol (mg/dl)	206.77 ± 6.161	182.97 ± 5.57	<0.01
HDL-C (mg/dl)	44.71 ± 1.85	46.06 ± 1.92	NS
LDL-C (mg/dl)	123.96 ± 6.03	102.171 ± 4.19	<0.01
Triglyceride (mg/dl)	201.97 ± 14.86	189.55 ± 12.86	NS
Leptin (ng/ml)	15.9 ± 1.6	21.4 ± 2.5	<0.01
ox-LDL (nm/l)	10 (9.7–14.1)	6 (5.6–7.8)	<0.001
ox-LDL/LDL	0.082 (0.080–0.12)	0.06 (0.05–0.08)	<0.001
HOMA-IR	3.26 ± 0.23	2.93 ± 0.32	<0.01

Note: Variables distributed normally are presented as mean ± SEM. Variables with skewed distribution are presented as median (interquartile range). The column of 'P value' is presented when comparing the studied variables between patients with newly diagnosed diabetes, before and after 3 months of metformin therapy.

when serum leptin and ox-LDL/LDL ratio were significantly correlated in controls. However, 3 months metformin therapy reestablished the positive correlation between serum leptin and ox-LDL levels in patients with newly diagnosed diabetes. Patients with type 2 diabetes had a higher serum ox-LDL, ox-LDL/LDL ratio, and a lower serum leptin level than controls. Serum ox-LDL, ox-LDL/LDL ratio, and HOMA-IR were reduced when serum leptin levels were increased after 3 months of metformin therapy. The changes in serum ox-LDL, ox-LDL/LDL ratio, and leptin levels after metformin therapy were independent of the improvement in HOMA-IR and glycemic index.

Traditionally, leptin has been regarded as a link between fat mass and food intake.¹⁷ Later on, studies showed that in humans, there are many instances where leptin dissociate from the strict role of communicating nutritional status; like decreased by testosterone and increased by estrogen.¹⁸ Leptin increases hepatic lipid peroxidation and lipolysis in skeletal muscle and adipocytes.^{19,20} Leptin increases ox-LDL phagocytosis in the monocytes of the patients.²¹ Moreover, it is increased in the situation of chronic inflammation, related to white blood cell count.¹⁸ This may be an explanation for the positive correlation between serum leptin and ox-LDL in normal subjects.^{10,11} However, we did not find any positive correlation between serum leptin and ox-LDL levels in patients with long-standing diabetes and patients with newly diagnosed diabetes before treatment. Consistent with our findings, Stringer *et al.*²² did not

report any correlation between serum leptin and ox-LDL levels in patients with type 2 diabetes. Kassi *et al.*²³ did not report any positive correlation between serum leptin and ox-LDL levels in postmenopausal women with impaired glucose tolerance test. Likewise Demirel *et al.*²⁴ did not find any correlation between leptin and ox-LDL, when leptin was significantly correlated with very LDL, lipoprotein A, and TG in patients with poly-cystic ovary (PCO) syndrome.

Why the correlation between serum leptin and ox-LDL levels fades out in diabetes. In 1998, Lord *et al.*²⁵ first reported the role of leptin as a modulator of T lymphocytes. Serum leptin levels were significantly elevated in patients with multiple myeloma and chronic lymphatic leukemia.²⁶ Earlier studies on leptin-deficient mouse have shown marked reduced number of lymphocytes with impaired humoral response.^{27,28} Lipoatrophy in HIV-infected patients is assumed to be a result of reduced leptin in these patients.²⁹ Paz-Filho *et al.*³⁰ showed that immune defects are not an obligatory feature of congenital leptin deficiency and even in the absence of significant immune defects, leptin replacement therapy enhanced T-cell responsiveness. Obese leptin-deficient ob/ob mice exhibit a low-grade chronic inflammation together with a low muscle mass.³¹ Sainz *et al.*³² showed that leptin administration down-regulates the increased expression levels of genes related to oxidative stress and inflammation in the skeletal muscle of ob/ob mice. Interestingly, it is shown that leptin modulates innate and adaptive immune cell recruitment after cigarette smoke exposure in ob/ob mice.³³

So leptin has a dimorphic action on the markers of oxidative stress^{19–21,32,33} as both leptin deficiency and excessive leptin administration induce oxidative stress. It could be hypothesized that the positive correlation between serum leptin and oxidative stress is observed only in the stable situations, when they both induce the other one, and hence we observe a linear relationship. In type 2 diabetes the inflammatory markers are produced via different pathways and biochemical cascades. We also found a lower serum leptin levels in patients with type 2 diabetes, so the positive linear relationship, which is normally observed between leptin and ox-LDL, would be confounded by other inflammatory cytokines. Interestingly, 3 months metformin therapy could rebuild the correlation between serum leptin and ox-LDL levels in patients with newly diagnosed diabetes.

The finding of a lower serum leptin level in diabetic patients confirms those from previous studies.^{34–36} Consistent with our findings, Sivitz *et al.*³⁷ showed that plasma leptin levels are reduced in untreated type 2 diabetes probably as a consequence of reduced insulin secretion. A low serum leptin levels

is a predictor of early mortality in patients on chronic hemodialysis.³⁸ Serum leptin levels are negatively correlated with serum C-reactive protein levels, in patients with chronic kidney disease.³⁹ It is also suggested that serum leptin levels increases after glycemic improvement.^{40,41} However, our findings are in contrast with some studies which have reported a higher serum leptin levels in patients with type 2 diabetes.^{42,43} Morin-Papune *et al.*⁴⁴ showed that metformin therapy reduces serum leptin levels in women with PCO after 2 months of therapy. However, serum leptin levels were increased after 4–6 months of metformin therapy in their study. We matched the patients and controls according to age, sex, and BMI, which is not usually performed in other studies. Furthermore serum leptin levels were significantly increased after 3 months of metformin therapy. It seems that the role of diabetes on serum leptin levels is not completely understood.

Consistent with our findings, studies have shown the role of metformin in reducing oxidative and nitrosative stress in patients with type 2 diabetes.^{14,45} Hyperglycemia is the leading cause of oxidative stress, inflammation, and vascular complications in patients with type 2 diabetes.⁴⁶ Hyperglycemia cause insulin resistance in skeletal muscle and damage the endothelial cells. *In vitro* and animal studies have shown a positive correlation between serum ox-LDL and insulin levels.^{47,48} The production of circulating ox-LDL is accelerated by insulin resistance in healthy individuals and diabetic patients.^{14,49} We showed that 3 months metformin therapy rebuild the positive correlation between serum leptin and ox-LDL levels.

Studies have shown the positive role of metformin on the defense mechanisms against reactive oxygen species and other reactive compounds.^{50,51} Matsumoto *et al.*⁵² suggested that hypoglycemic action of metformin does not play an important role on the vascular protection in rats with type 2 diabetes, when its antioxidative effect plays a significant role. Moreover, metformin provides protection against vascular endothelial dysfunction induced by LDL, probably with protection against oxidative stress in rats.⁵³ Metformin is shown to improve oxidative stress and preserve antioxidant function by increasing superoxide dismutase activity in patients with newly diagnosed diabetes.⁵⁴ We concluded that metformin improves serum leptin and ox-LDL levels, as two inflammatory markers, through its antioxidative properties.⁵⁵ Based on the currently available evidence the effects of metformin may also be related to the decreasing glucose levels in the patients. It has been shown that thiazolidinediones have beneficial effects on lipid oxidation and peroxidation properties,⁵⁶ which is comparable to those of metformin. However, our findings, using general

linear models demonstrated that the beneficial role of metformin in reducing serum ox-LDL levels is independent from its glucose-lowering effects.

The effects of metformin could also be related to the fact that it activates AMP-activated protein kinase (AMPK).⁵⁷ AMPK is an important inhibitor of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B).⁵⁸ NF- κ B is a protein complex that controls the transcription of DNA. NF- κ B is found in almost all animal cell types and is involved in the cellular responses to stimuli such as stress, cytokines, free radicals, and ox-LDL.⁵⁹ All these events are inhibited by the activation of AMPK, which also enhances insulin action in muscle.⁵⁷ This may explain the effect of metformin in reducing serum ox-LDL levels.

At the end, the effect of metformin can in some way be related to the immune system.⁶⁰ Oxidation of LDL is carried out by macrophages and monocytes.⁶¹ It is shown that the production of ROS by circulating monocytes in the hyperlipidemic subjects contributes to the systemic oxidative stress.⁶¹ On the other hand it is shown that metformin attenuate the inflammatory responses, at least in part, by suppressing the production of TNF- α in human monocytes.⁶⁰ The beneficial role of metformin in reducing ox-LDL, increasing leptin levels, and restoring serum leptin and ox-LDL correlation may be somehow related to its effect on the immune system.

We believe that different outcomes in different studies may be the result of differences in the inclusion criteria and differences in baseline characteristics of the patients. Future prospective studies which measure serum leptin levels with other inflammatory markers including high-sensitivity CRP, interleukin 6, and TNF- α level may elucidate the role of leptin as a negative or positive acute phase reactant in patients with type 2 diabetes. The principal limitation of the present study is its short-term follow-up duration. Furthermore we did not have placebo group, as it is considered unethical not to commence patients on treatment immediately following diabetes diagnosis. However, we took advantage of a relatively large sample size and close similarity between the groups in most of the potentially confounding clinical and laboratory variables. In conclusion, we showed a higher serum ox-LDL and a lower serum leptin level in patients with type 2 diabetes. Metformin modulated serum ox-LDL and leptin levels, independent from its glucose-lowering effect.

Acknowledgments

This study is not financially supported by any institution.

Conflicts of interest: The authors have not declared any conflicts of interest.

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