

Pro-Atherogenic Oxidized Ldl/ β_2 -Glycoprotein I Complexes in Diabetes Mellitus: Antioxidant Effect of Statins



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Abstract

Premature atherosclerotic cardiovascular disease (CVD) is a well known complication of diabetes mellitus (DM) associated with significant morbidity and mortality. The development of atherosclerosis is largely promoted by oxidative stress and chronic inflammation. Elevated low-density lipoprotein (LDL) is a known atherosclerotic risk factor but LDL must be modified to become atherogenic. Inflammatory-derived reactive oxygen and nitrogen species oxidize LDL (oxLDL) giving rise to lipid peroxides and aldehydes that favor the initiation and progression of atherosclerotic lesions. Beta-2-glycoprotein I (β_2 GPI) is a lipid binding plasma protein with pleiotropic functions that binds oxLDL via specific oxidative-derived ligands to form pro-atherogenic oxLDL/ β_2 GPI complexes and in this guise exerts a buffering effect upon LDL oxidation. Statin (Rosuvastatin) treatment lowered serum levels of oxLDL/ β_2 GPI complexes in a group of DM patients compared to statin untreated DM patient. The oxLDL/ β_2 GPI decrease was independent from the reduction of cholesterol, LDL and triglycerides but likely dependent on Rosuvastatin reduction of nitrates (NO₃⁻) suggesting that Rosuvastatin may impact on the oxidative metabolism of lipids and/or LDL. In addition, the oxLDL/ β_2 GPI complex may represent a surrogate marker of oxidative inflammation in DM.

Keywords: Diabetes; Oxidative stress; OxLDL/ β_2 GPI complexes; Statins; Atherosclerosis

Introduction

Diabetes mellitus (DM) is the fifth deadliest disease in the United States with an annual economic cost estimated over \$100 billion. Cardiovascular disease (CVD) represents the most life threatening consequence of DM accounting for the death of up to 65% of DM patients. Aggressive efforts aimed at treating and controlling the classic CVD risk factors over the last few decades have brought along a marked reduction in CVD morbidity and mortality in the US, though the morbidity and mortality attributable to CVD from DM and obesity continues to show an upward trend [1,2].

The laboratory diagnosis of DM relies on the presence of abnormal fasting glucose and/or an abnormal glucose tolerance test alongside abnormalities of lipid and protein metabolism due to defects in insulin production or activity [3]. All these metabolic abnormalities lead to a pro-atherogenic

oxidative inflammatory environment. Recent research has further unraveled the pathogenic mechanisms of CVD in DM mostly due to intrinsic rather than extrinsic factors. Because CVD remains the main cause of death in DM, there is a strong need to identify more specific mechanisms that can be acted upon to develop better CVD prevention and bend down the incidence and mortality curves [4].

Atherosclerosis is a chronic progressive disease (Figure 1) characterized by two low grade inflammatory components, one prevalently systemic that starts early in life affecting the vascular endothelium, monocytes and platelets, and another localized to the arterial wall (plaques) that develops in later adulthood [5,6]. Early identification and intervention is important to prevent disease progression. The complex inflammatory process initiates as oxidative stress (lipoprotein oxidation) and progresses with the participation of immuno-

inflammatory mononuclear cells of the innate and adaptive immune system [7,8]. The newly issued American College of Cardiology and American Heart Association (ACC/AHA-2103) Guideline on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Cardiovascular Risk in Adults took

these concepts into consideration by diverting the focus away from just measuring cholesterol into taking in consideration LDL, statin response and inflammatory biomarkers as more clinically relevant risk factors [9].

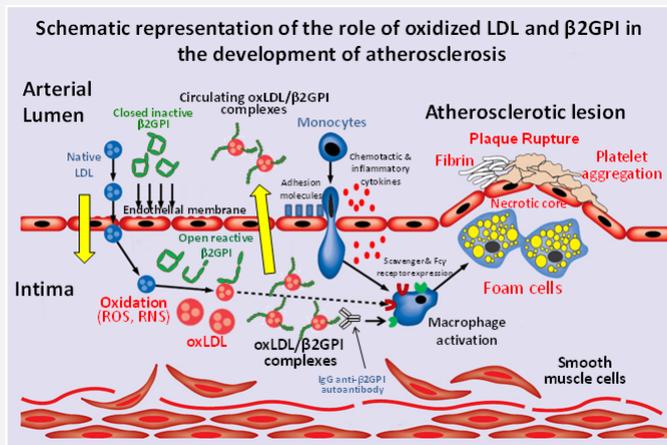


Figure 1: Schematic representation of the oxidative inflammatory events that lead to the development of atherosclerotic lesions: endothelial membrane dysfunction, increased expression of adhesion molecules with chemotactic and inflammatory cytokine production facilitate the extravascular migration of LDL, mononuclear cells and circulating inactive (closed) form of β_2 GPI into the arterial wall. An oxidative inflammatory environment (reactive oxygen species [ROS], reactive nitrogen species [RNS]) of the arterial wall promotes the following: oxidative modification of LDL, configuration change of β_2 GPI into an open and reactive form, oxLDL/ β_2 GPI complex formation with migration and activation of macrophages. These events facilitate the excessive intracellular accumulation of oxidized lipoproteins (oxLDL/ β_2 GPI complexes) via scavenger receptors. Excess oxLDL and oxLDL/ β_2 GPI complexes are released back into circulation. This process may also lead to autoantibody (anti-oxLDL, anti- β_2 GPI or oxLDL/ β_2 GPI autoantibodies) and immune complex production that further enhance macrophage lipid uptake via Fc γ receptors accelerating foam cell and plaque formation. Auto-propagation of these arterial wall events contribute to the progression of vascular changes including the proliferation of smooth muscle cells and development of unstable necrotic core in the atherosclerotic plaques making them prone to rupture into the arterial vascular space.

Oxidative stress and low grade chronic inflammation (oxidative inflammation) contribute to premature atherosclerotic CVD in DM [10]. Indeed, the abnormal lipid profile of diabetes associates biochemically with lipid peroxidation, a process whereby superoxide radical ($O_2^{\bullet-}$) released by neutrophils or endothelial cells may attack double bonds of arachidonic acid allowing the formation of oxygen containing cyclic structures termed isoprostanes [11]. Isoprostanes are recognized markers of in vivo oxidative stress and their plasma or urinary concentrations are elevated in DM [12,13]. In the course of oxidative inflammation, endothelial and mononuclear cells also generate additional reactive nitrogen species (RNS) including nitric oxide (NO^{\bullet}) that behaves as a pathogenic mediator and/or as a cytotoxic molecule [14]. However, most of NO^{\bullet} mediated pathogenicity depends on the formation of secondary intermediates such as peroxynitrite anion ($ONOO^-$) and nitrogen dioxide ($\bullet NO_2$) that are more reactive and toxic than NO^{\bullet} [15]. In the presence of superoxide radical ($O_2^{\bullet-}$), NO^{\bullet} gives rise to $ONOO^-$, a strong highly reactive oxidant with very short biological half-life producing nitrated proteins [16].

Reactive oxygen species (ROS) and RNS may exert free radical attack on low-density lipoproteins (LDL) releasing lipid

peroxides and highly reactive aldehydes (4-hydroxynonenal) that form specific adducts with lysine inducing the post-translational modification of lipoproteins, with consequent gain or loss of function. During the same process LDL becomes oxidized (oxLDL) turning into a highly pro-inflammatory and atherogenic [17,18]. Beta2-Glycoprotein I (β_2 GPI) is a lipid-binding plasma protein involved in thrombosis, fibrinolysis, apoptosis, atherosclerosis and angiogenesis [19]; it binds oxLDL via specific oxidative-derived ligands to form oxLDL/ β_2 GPI complexes [20]. Elevated plasma levels of oxLDL/ β_2 GPI complexes were initially described in patients with antiphospholipid syndrome (APS) [21] and systemic lupus erythematosus (SLE) [22], but later found in non-autoimmune chronic inflammatory diseases such as chronic nephropathies, coronary artery disease, myocardial infarction and DM [23,24]. OxLDL and β_2 GPI have been co-localized in human atherosclerotic lesions by immune-histochemical staining implying a pro-atherogenic role [25,26]. In the presence of anti- β_2 GPI antibodies, macrophages ingest oxLDL/ β_2 GPI complexes at an enhanced rate providing further support for their pro-atherogenic role [27,28]. Current experimental evidence, including in vivo imaging techniques, identified the atherosclerotic lesion as the primary site of oxLDL/ β_2 GPI complex formation [20,29].

In DM, serum levels of oxLDL/ β_2 GPI complexes were particularly elevated in patients with greater intima-media thickness (IMT) [30], but were lower in patients taking statins [24]. These observations indicate that oxLDL/ β_2 GPI complexes may behave as modifiable biomarkers and/or as risk factors for atherothrombotic complications of DM. In addition, the lower oxLDL/ β_2 GPI concentration in DM patients on statins suggested that this class of drugs may prevent or decrease the oxidative modification of LDL possibly by an antioxidant mechanism. Indeed HMG-CoA reductase inhibitors (statins) bear antioxidant properties in addition to their lipid-lowering, anti-thrombotic and anti-inflammatory effects [31,32]. We tested the hypothesis that Rosuvastatin had antioxidant effects in DM by performing an open label interventional trial and observed a significant change in serum oxLDL/ β_2 GPI concentration as the primary endpoint. In this review we discuss the role of oxidative stress in atherogenesis and the antioxidant effect of statins on oxLDL/ β_2 GPI complexes.

Oxidative Inflammation and Atherogenesis in DM

The pathogenesis of atherosclerosis in DM is multi factorial:

- i. Chronic hyperglycemia from insulin deficiency [33,34].
- ii. Chronic dyslipidemia characterized by decreased high-density lipoprotein (HDL), changes in the HDL subpopulations, raised triglycerides, and unchanged or only slightly elevated low-density lipoprotein (LDL) [35].
- iii. Metabolic syndrome characterized by obesity, dyslipidemia, hypertension and insulin resistance [36]. All three promote increased oxidative stress that initiate and perpetuate vascular damage and atherothrombotic complications [37].

Under physiologic conditions, oxidation should be well counteracted by natural enzymatic and non-enzymatic antioxidant mechanisms. In DM, oxidation overrides antioxidant mechanisms [38,39] and initiates endothelial dysfunction by favoring the expression of a pro-adhesive and pro-thrombotic surface that allow the migration of immunoinflammatory cells into the arterial wall (Figure 1). There, local pro-chemotactic and inflammatory cytokines further recruit and activate immuno-inflammatory cells that propagate lipid accumulation, oxidative inflammation and the development of the typical progressive atherosclerotic lesions (plaques) [40,41]. Moreover, early inflammation increases the expression of cell surface receptors and the intracellular accumulation of oxLDL by local arterial mononuclear cells process mediated by scavenger and Fc γ receptors [28].

Multiple efforts by several groups aimed at enhancing the antioxidant defense in DM and CVD patients. Serum and urine bio makers of systemic oxidative stress correlated with blood glucose levels and responded to anti-diabetic intervention

[42,43]. *In vivo* studies indicated that oxidative stress from hyperglycemia starts well before clinical complications become evident, underscoring the importance of glucose control to minimize long term complications of oxidative inflammation in DM. Metformin treatment lowered urinary excretion of 8-isoPGF2a and 11dhTxB2 in newly diagnosed DM patients suggesting that despite a good metabolic improvement, metformin also behaved as an antioxidant and antithrombotic agent in DM [44]. Some epidemiological studies have demonstrated a weak inverse relationship between stroke risk and ingestion of antioxidant foods. Other clinical trials have shown conflictive results regarding the protective effect of antioxidants against CVD outcomes [45,46]. Several ongoing clinical trials are assessing the effectiveness of statins from an antioxidant perspective; so far these studies have suggested a close relationship between oxidative inflammation and atherogenesis but the usefulness of antioxidant-based therapeutics on CVD remains controversial.

Atherogenic oxLDL and oxLDL Complexes

Oxidation of LDL is a key contributor to the initiation and progression of atherosclerosis [7,47] and is a complex process, in going from “minimally oxidized” to more “extensively oxidized” LDL particles induces the expression of adhesion molecules on endothelial cells and the release of chemotactic cytokines into the circulation [48]. These events allow blood monocytes to adhere to the arterial wall and to migrate into the arterial intima, where they differentiate into macrophages. In turn, these activated macrophages enhance a pro-oxidant environment of the arterial wall, causing intensive oxidative modification of LDL lipoproteins including cholesteryl esters, phospholipids and apolipoprotein B [49]. Because oxLDL becomes unrecognizable by LDL receptors, it is taken up by scavenger receptors, which facilitate a persistent intracellular accumulation of LDL by macrophages [50] transforming them into the characteristic foam cells. As the lesion evolves, these elements contribute to the morphological changes that characterize the vulnerable plaques with an unstable lipid-rich necrotic core. Advanced lesions may undergo a necrotic breakdown and plaque rupture that precipitate intra-vascular thrombosis with acute occlusion clinically expressed as unstable angina, myocardial infarction, stroke, and/or sudden cardiac death [51].

Although the oxidation of LDL occurs primarily in the vascular wall, recent studies have provided evidence for the presence of oxLDL in blood [52]. Indeed numerous studies have established oxLDL as an effective marker for the presence of atherosclerosis, detecting both subclinical disease and more advanced or severe CAD [53,54]. Because oxLDL is highly unstable with a very short half-life (30 seconds) in the systemic circulation [55], it is difficult to measure accurately by common immunoassays. In addition, some lipid binding plasma proteins such as β_2 GPI interact with circulating oxidized lipoproteins to

buffer their deleterious effects. This may cause reduced assay sensitivity and false-negative results as most of the oxLDL assays use monoclonal antibodies directed against just one or a few of the epitopes present on lipid or protein moieties. This phenomenon has hampered the use of oxLDL in CVD clinical trials and clinical laboratory to assess its predictive role in atherogenesis.

Because immune-staining of human atherosclerotic lesions co-localized β_2 GPI with oxLDL, the relationship between these molecules was further investigated [25,26]. β_2 GPI is a 50-kDa single-chain phospholipid-binding plasma protein composed of 326 amino acid residues arranged in 5 homologous repeats or domains. The fifth domain contains a positively charged amino acid patch important in anionic phospholipid and oxLDL binding [56]. Unlike native LDL, β_2 GPI binds oxLDL via specific oxidative-derived ligands to form stable and pro-atherogenic oxLDL/ β_2 GPI complexes [20,57] in an attempt what to quench in an antioxidant fashion the pro-inflammatory and pro-atherogenic effects of oxLDL. But in doing so, oxLDL/ β_2 GPI complexes also become immunogenic triggering the production of pro-atherothrombotic auto antibodies and immune complexes.

It is now recognized that the immune system plays a role in blood coagulation. Autoimmune-mediated thrombosis refers to auto antibodies that promote venous and arterial thromboembolic events in patients with systemic lupus erythematosus and antiphospholipid syndrome who develop premature atherothrombotic CVD with significant morbidity and mortality [58,59]. Endogenous pro-atherogenic oxLDL/ β_2 GPI complexes initially described in autoimmunity [22] have been associated with the development of atherosclerotic CVD in non-autoimmune diseases [23,24]. Serum levels in higher oxLDL/ β_2 GPI quartiles were associated with an geographically determined disease severity and give a 3.5 risk for adverse outcomes in acute coronary syndromes [60,61]. Interestingly, statin treatment reduced oxLDL/ β_2 GPI complexes independently from LDL-lowering effects likely

via an antioxidant mechanisms [62,63]. Thus, oxLDL/ β_2 GPI complexes meet current criteria for biomarkers of CVD risk:

- a. To have a direct mechanistic relevance to atherosclerosis (causal relationship).
- b. To be measured quantitatively with available technology that is accurate, reproducible and cost effective.
- c. To permit patient stratification for severity and outcomes.
- d. To be modified by therapeutic intervention. In summary, the endogenous or metabolically generated oxLDL/ β_2 GPI complexes seem to represent a bona fide biomarker for identifying individuals at risk for atherosclerosis and useful to develop personalized treatment and/or CVD preventive programs.

OxLDL/ β_2 GPI and its immune complexes up-regulate the macrophage expression of scavenger and Fc γ receptors, favoring enhanced oxLDL/ β_2 GPI uptake followed by its rapid accumulation in lysosomes where an immune response (innate and adaptive) may be mounted. Experiments evaluating the intracellular trafficking of β_2 GPI within macrophages showed that free β_2 GPI was poorly incorporated in late endosomes and stagnated there, whereas complexed β_2 GPI (to phosphatidylserine liposomes or oxLDL) was quickly transported to lysosomes; the addition of antibodies to β_2 GPI further accelerated this process [64]. β_2 GPI auto reactive CD4+ T cells have been identified in patients with APS that preferentially recognized a cryptic peptide (residues 276-290) in β_2 GPI domain V that contains the phospholipid-binding site. Macrophages stimulated with phospholipid-bound β_2 GPI induced an immune response to peptide 276-290 in a HLA-DR-restricted manner, while β_2 GPI or phospholipids alone did not [65]. In this respect, β_2 GPI can be viewed as a component of the innate immunity; but once bound to oxLDL, the complex may shift to the generation and maintenance of an adaptive immune response that play an important role in atherogenic inflammation via the inflammasome/IL-1B system [39].

Effect of Statins on oxLDL/ β_2 GPI Complexes

Table 1: Demographics and baseline characteristics of diabetes patients (n=111).

	DM Rosuvastatin Treatment group (n=76)	DM Control Group Without Rosuvastatin (n=35)	P value [^]
Age in years (mean±SD)	54.2 ±12.3	55.8 ±7.5	0.493
Sex (Female/Male)	53 (70%)/23 (30%)	27 (77%)/8 (23%)	0.535
Disease duration (years)	7.7 ±7.0	7.6 ±6.2	0.905
Hypertension (%)*	19 (25%)	6 (17%)	0.499
Obesity (%) ⁺	36 (47%)	23 (66%)	0.111
Oral hypoglycemics (%)	61 (80%)	30 (86%)	0.668
Insulin (%)	13 (17%)	5 (14%)	0.798
ACE Inhibitors (%)	7 (9%)	6 (17%)	0.373
Cholesterol (mg/dL)	199.7±36.3	226.7±25.9	0.0014

LDL (mg/dL)	120.0±32.3	141.4±28.3	0.0047
Triglycerides (mg/dL)	223.6±125.7	227.7±120.3	0.871
HDL (mg/dL)	45.9±9.7	45.5±9.3	0.798
Glucose (mg/dL)	157.8±80.6	137.1±67.9	0.192
HbA1c (%)	7.6±2.2	7.5±1.9	0.776
CRP (mg/dL)	0.25±0.3	0.28±0.27	0.663

^Chi-square or Fisher's exact test, *Hypertension: systolic >130mm Hg and/or diastolic >85mm Hg, +Obesity: >27 BMI for Mexican population DM: Type 2 Diabetes Mellitus; LDL: Low-Density Lipoprotein; HDL: High-Density Lipoprotein; HbA1c: Glycosilated Haemoglobin; CRP: C-reactive protein

OxLDL/ β 2GPI complexes are indicative of systemic oxidative inflammation in obese middle age men and DM, and may be used to assess pro-atherogenic pathways because circulating levels of oxLDL independently predict future CVD events [24,66,67]. It was particularly important to determine effective ways to modify oxLDL/ β 2GPI levels as these complexes have been associated with the severity and adverse outcomes of coronary disease [60,61]. The effect of statins on oxLDL/ β 2GPI complexes was studied by our group [62] on 111 type 2 DM patients (80 females, 31 males, mean age of 54.7 years). One group of 76 patients received 10mg daily for 6

weeks of oral Rosuvastatin while a control group of 35 patients did not receive Rosuvastatin. Serum samples taken at baseline and after 6 weeks were tested at the end of the study. The baseline clinical and laboratory variables of DM patients taking Rosuvastatin and control groups are shown in Table 1. DM patients in the Rosuvastatin group were stratified according to their lipid profile. In addition to oxLDL/ β 2GPI complexes, nitrite (NO₂-), nitrate (NO₃-), asymmetric dimethyl arginine (ADMA) nitrotyrosine (NT) and paraoxonase activity (PON) were measured in all samples.

Table 2: Effect of Rosuvastatin treatment on diabetes patients (n=76).

	Pre-treatment (mean±SD)	Post-treatment (mean±SD)	% change	P value^
oxLDL/ β 2GPI (U/mL)	0.79±0.49	0.53±0.36	-32.9	<0.001
Cholesterol (mg/dL)	199.5±36.1	150.3±34.8	-24.7	<0.001
LDL (mg/dL)	120.1±31.9	76.5±34.9	-36.3	<0.001
Triglycerides (mg/dL)	222.1±125.5	161.1±78.6	-27.5	<0.001
HDL (mg/dL)	46.1±9.7	45.4±8.8	-1.5	0.570
Glucose (mg/dL)	160.2±82.8	155.2±76.3	3.1	0.883
HbA1c (%)	7.6±2.2	7.6±1.9	0	0.902
CRP (mg/dL)	0.25±0.3	0.24±0.29	-4	0.946
NO ₂ - (µM)	23.5±13.8	17.5±10.5	-25.5	0.004
NO ₃ - (µM)	59.9±39.3	38.1±31.7	-36.4	<0.001
NT (nM)	9.86±10.8	8.1±9.2	-17.6	0.347
ADMA (µM)	0.59±0.10	0.56±0.11	-5.1	0.147
PON (U/L)	350.9±163.4	365.2±173.6	4.1	0.646

^Paired t-test or Wilcoxon Signed Rank Sum test

Pre-treatment: baseline measurement; Post-treatment: 6-week measurement after Rosuvastatin treatment (10mg/day); oxLDL/ β 2GPI: Oxidized Low-Density Lipoprotein/Beta2-Glycoprotein I; LDL: Low-Density Lipoprotein; HDL: High-Density Lipoprotein; HbA1c: Glycosilated Haemoglobin; CRP: C-reactive protein; NO₂-: Nitrite; NO₃-: Nitrate; NT: Nitrotyrosine; ADMA: Asymmetric Dimethylarginine; PON: Paraoxase Activity

Rosuvastatin treatment caused a significant decrease of oxLDL/ β 2GPI complexes (32.9%) along with cholesterol (24.7%), LDL (36.3%) and triglycerides (27.5%). Among the nitric oxide metabolites, Rosuvastatin treatment also decreased NO₂- (25.5%) and NO₃- (36.4%) (Table 2). The observed decrease of oxLDL/ β 2GPI complexes was more noticeable in patients with dyslipidemia (37.4%) compared to those with normal lipid profile (22.4%). Interestingly, NO₂- decreased more in dyslipidemics than in non-dyslipidemic

patients (29% vs 18.8%) while NO₃- decreased in the same way (42.9% vs 21.8%). The decrease of oxLDL/ β 2GPI complexes by Rosuvastatin treatment in these DM patients was independent of the lipid lowering effects of the statin. Further, only NO₃- was an independent predictor of oxLDL/ β 2GPI complexes (t=2.0, p=0.04).

Support to an antioxidant effect of statin indirectly assessed by a decrement of oxLDL/ β 2GPI complexes comes from very few studies.

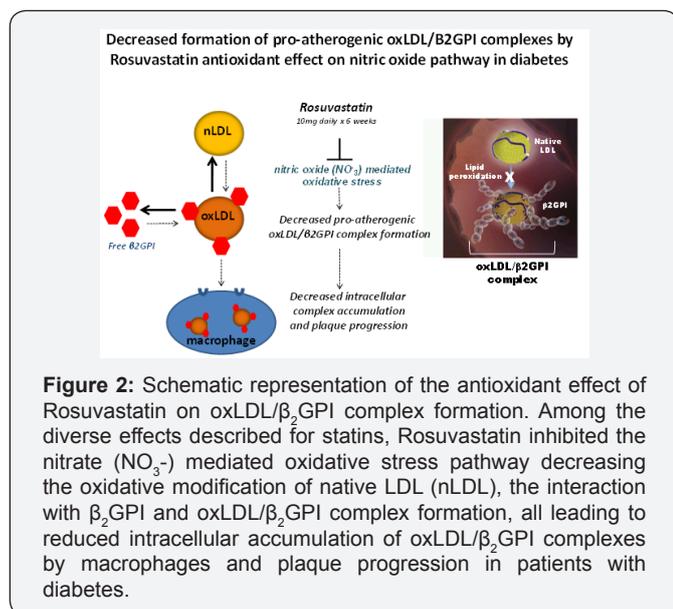
a) A randomized, double blind, placebo controlled pilot study of 37 consecutive SLE patients receiving 40mg daily atorvastatin or placebo for 12 months demonstrated a decrease of oxLDL/ β_2 GPI complexes [63]. In this study, after correction for age and disease duration oxLDL/ β_2 GPI complexes decreased by 27% ($p=0.002$).

b) Blinden et al. [68] studied the effect of statin therapy (Atorvastatin, Simvastatin, Rosuvastatin, Lovastatin, Pravastatin and Fluvastatin at doses between 5-80mg) in 186 coronary artery disease patients undergoing elective cardiac catheterization. There was a significant dose-dependent reduction of oxLDL/ β_2 GPI complexes, more noticeable at atorvastatin dose equivalents between 20-80mg.

c) Statin influence of oxLDL/ β_2 GPI levels on CVD patients have been further confirmed by Berger et al. [69] and Gurbel et al. [70]. This effect was independent and inversely associated with inflammation. These findings support the concept of a dose dependent anti-oxidant effect of statins.

Discussion

Our evaluation of the significance of oxLDL/ β_2 GPI complexes in DM demonstrated an independent association with some clinical (obesity and hypertension) and biochemical variables (nitric oxide metabolites) [62]. OxLDL/ β_2 GPI complexes were higher in males than females. This gender difference reflects the notion that in DM oxidative inflammation is enhanced [71] particularly in men [72]. With regards to biochemical variables the only independent predictor of oxLDL/ β_2 GPI was nitrate (NO_3^-). This RNS may be viewed as an “inflammatory metabolite” of $\text{NO}\cdot$ (as opposed to NO_2^- that may be viewed as the “vascular” metabolite). Thus, NO_3^- may contribute to LDL oxidation and formation of the oxLDL/ β_2 GPI complex in DM [20].



Rosuvastatin administered daily for 6 weeks caused a significant reduction of serum oxLDL/ β_2 GPI complexes (Figure 2). This reduction was accompanied by lower total cholesterol, LDL and tryglicerides, particularly in patients with dyslipidemia. However, the reduction of oxLDL/ β_2 GPI was statistically independent of any statin-mediated decrease of total cholesterol, LDL and tryglicerides. It is important to point out that oxLDL/ β_2 GPI levels were higher in DM patients with dyslipidemia, consistent with the concept that patients with elevated lipid levels may be prone to or sustain more intense oxidative damage.

Statins inhibit the enzyme HMG-CoA reductase, preventing the generation of mevalonate and the subsequent biosynthesis of cholesterol. Mevalonate is also a precursor of isoprenoid intermediates and one of these geranylgeranylated proteins (RhoA) is implicated in intracellular signaling [73,74]. Through the inhibition of protein prenylation, such as Ras and Rho, statins activate the MAPK cascade or NF- κ B pathways that induce proteins with anti-inflammatory, anti-proliferative and anti-thrombotic effects [75]. In addition, by acting on SREBP-2, statins up-regulate the expression of genes coding for paraoxonase, the enzyme that accounts for most of the antioxidant effect of HDL [76]. Thus, the inhibition of RhoA by statins have a number effects on the vasculature that could be beneficial in hypercoagulable disorders by improving nitric oxide synthase activity, regulation of angiogenesis, reduction of vascular inflammatory and prothrombotic activities and atherosclerotic plaque stabilization [77,78]. By using plasma biomarkers of oxidation such as oxLDL/ β_2 GPI, we can clinically evaluate the effect of treatment on this event.

Because the benefits of statins on the cardiovascular system are beyond those on cholesterol metabolism we speculated that Rosuvastatin may exert an antioxidant effect, either by enhancing the activity of PON and of nitric oxide synthase or by interfering with oxidative inflammatory mechanisms that promoted the generation of oxLDL and their consequent interaction with β_2 GPI [79-82].

Our studies suggest that statins would have the same antioxidant effect on oxLDL/ β_2 GPI complex formation in patients with metabolic syndrome and obesity. Fatty liver disease, particularly non-alcoholic steatohepatitis (NASH), is not only associated with insulin resistance, obesity, metabolic syndrome, liver fibrosis/cirrhosis, but also with atherosclerotic CVD [83]. It has been proposed that dyslipidemia, inflammation, oxidative stress and macrophage activation are early events in NASH, similar to atherosclerosis and perhaps they represent shared aspects of a similar disease process [84]. In this case, statins may have a more prominent therapeutic role as antioxidants are considered first line treatment for NASH.

Conclusion

These studies demonstrate that treatment with Rosuvastatin reduced serum levels of oxLDL/ β_2 GPI in DM

patients. The implications of these findings are twofold: statins independently reduce lipids and NO₃⁻ suggesting an antioxidant effect possibly mediated via lipid/nitric oxidative pathways; and that oxLDL/β₂GPI complexes may be viewed as serologic biomarkers of oxidative stress.

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