Reduced antioxidant potential & sensitivity to oxidation in plasma low density lipoprotein fraction in type 2 diabetes mellitus patients

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**Background & objectives:** The aim of this study was to investigate LDL oxidation in the ethiopathogenesis of diabetes mellitus. Diabetes mellitus a disease caused due to severe insulin dysfunction, is associated with lipid and protein metabolic disorders.

**Methods:** A total of 90 type 2 diabetes patients were grouped according to their glycated haemoglobin (HbA1c) values as regulated (<5.7%), poorly regulated (5.7 - 7.7%) and unregulated (>7.7%). Further, a healthy control group of 37 individuals was included for comparison in terms of sensitivity of low density lipoprotein (LDL) to oxidation and measurements of antioxidant potential (AOP). A heparin-citrate precipitation method was used to obtain LDL from the serum samples of patients and control groups. The LDL fractions were exposed to oxidation with CuSO4 and sensitivity to oxidation was evaluated. Ten patients each from regulated and unregulated groups, and 10 healthy controls were examined for antioxidant potential.

**Results:** The sensitivity of LDL fraction to oxidation was significantly lower in all diabetic groups compared to the control group. AOP was significantly decreased in unregulated diabetic group compared to the control group.

**Interpretation & conclusion:** We hypothesize that oxidant stress increases in diabetes mellitus and oxidant defense systems weaken during the chronic course of the illness. Due to decreased antioxidant potential, that probably shortens the LDL oxidation lag phase, the sensitivity to oxidation appears to be lower in diabetes mellitus patients.

**Key words** Diabetes mellitus - LDL oxidation

Diabetes mellitus is a metabolic disorder affecting carbohydrate, fat and protein metabolism and characterized by chronic hyperglycaemia resulting from a disorder either in insulin secretion or its action on target tissues or both. Polyneuropathy and microvascular disorders cause manifestations of chronic clinical complications of the disease such as diabetic retinopathy, diabetic nephropathy and atherosclerosis. Non-enzymatic glycation of proteins occurs with high glucose concentrations in blood,
resulting in increased formation of free radicals which in turn interact with lipid, protein, and nucleic acid molecules and disrupt their structures and functions. Free radicals are formed especially in patients with unregulated or poorly regulated diabetes mellitus because of increased oxidative activity. Transition metals play an important role in oxidative stress in diabetes mellitus, which potentially can disrupt biological structures. Glucose, polyunsaturated fatty acids (PUFA), and other chemically reductive small molecules undergo oxidation catalysed by transition metals to yield various oxidation products. Hydrogen peroxide and lipid peroxides from these reactions play a main role in oxidative stress. Free radicals can co-valently bind to membrane proteins, damaging their structural and functional properties and change the PUFA : protein ratio, thus initiating lipid peroxidation, which leads to dysfunction in cellular organelles such as increased fragility of lysosomes and changes in microsomal enzymes. These cellular dysfunctions eventually cause cell death and collagen accumulation in tissues. A typical low density lipoprotein (LDL) particle has a core which is surrounded by polar lipids. More specific proteins are located in surrounding peripheral structure. Fatty acids in LDL particle are protected against free radical attacks and oxidation by antioxidants located in the particle. LDL particles rich in antioxidants, are more resistant to oxidation and have longer lag phase in oxidation process. LDL particles with poor antioxidant capacity have shorter lag phase. Three phases are observed during LDL oxidation; lag phase, propagation phase, and decomposition phase. During lag phase, little oxidation occurs due to antioxidant defense mechanisms and presence of antioxidant molecules such as α-tocopherol and β-carotene. At the end of lag phase, antioxidant properties of LDL diminish and PUFA in LDL particle are rapidly oxidized to lipid hydroperoxides. After this propagation phase, unstable lipid hydroperoxides start to decompose and lipid peroxides concentration decreases in decomposition phase. The patients with DM had been diagnosed by using fasting blood glucose level and physical examination.

**Material & Methods**

Patients with type 2 diabetes admitted to the Endocrinology and Metabolism outpatient clinic at Ibn-i Sina Hospital, Ankara, Turkey during January to March 2004 were included in this study after obtaining informed consent. The patients were randomly divided in three groups as regulated, poorly regulated, and unregulated as per the criteria laid by Molyneaux et al. The classification uses glycated haemoglobin (HbA1c) values to evaluate the patients; <5.7 per cent was regulated, 5.7 - 7.7 per cent poorly regulated, and >7.7 per cent represented unregulated diabetes mellitus. HbA1c level was determined by using ion exchange high performance liquid chromatography (HPLC analyzers Variant, BioRad. Additionally ClinRep®, Germany). Following reference intervals were used for per cent Hb A1c range; < 6.5 normal, 6.5 - 7.5 increased, > 7.5 diabetic. In our laboratory the reference interval has been established as 4.5 - 5.7 per cent of total haemoglobin in healthy individuals and patients with regulated diabetes, 5.7 - 7.7 per cent poorly reguled diabetes and >7.7 per cent unregulated diabetes. Twenty six patients were in the regulated group (aged 55.3 ± 11.1). Twenty nine patients were in the poorly regulated group (aged 57.7 ± 13.2), and 35 in unregulated group (aged 58.3 ± 11.6). Normal healthy individuals (n=37) formed the control group (aged 40.9 ± 7.8). LDL was prepared using heparin-citrate treatment with precipitation method. Briefly, 5 ml 0.064 M Na-citrate buffer, pH 5.04 with 50000 IU/l heparine was mixed with 0.5 ml of serum, vortexed, and centrifuged at 1000 g for 10 min. The supernatant was removed and the LDL precipitate was dissolved in 1 ml 1 per cent triton-X 100. To measure the sensitivity to oxidation, malondialdehyde (MDA) levels were determined using thiobarbituric acid reactive substances (TBARS) method in copper-induced LDL samples. LDL samples were incubated at 37ºC with CuSO4, 1 mM. Malondialdehyde levels were used as an indicator of free radical generation which increased at the end of the lipid peroxidation. Coloured product generated by the reaction of thiobarbituric acid (TBA) with MDA was measured spectrophotometrically at 532 nm. The results were
expressed as nmol/mg protein, standard curve was prepared with serial dilutions of standard 1,1,3,3-tetramethoxypropane. The difference between induced and non-induced (basal) MDA levels was used to evaluate sensitivity to oxidation of the samples and results were expressed as nmol/mg/h\textsuperscript{12}. Ten regulated diabetic patients, 10 unregulated and 10 healthy controls were examined for antioxidant potential (AOP). Ten patients from unregulated group who had the highest HbA\textsubscript{1c}, 10 subjects of regulated group who had the lowest HbA\textsubscript{1c} and 10 randomly chosen from control group were tested for AOP. For the measurement of AOP, LDL samples were incubated with xanthine-xanthine oxidase system in the presence of cod liver oil. After 1 h incubation, MDA levels were measured in all samples\textsuperscript{13}. Protein determination was done by the Lowry method\textsuperscript{14}.

Statistical analysis: One way analysis of variance (ANOVA) and post-hoc Dunnet t-test were used to evaluate the sensitivity to oxidation. For antioxidant potential values, Kruskal-Wallis and Mann-Whitney U tests were used.

**Results & Discussion**

Sensitivity of LDL fraction to oxidation was significantly lower ($P<0.001$) in diabetic patients compared to controls (Table). AOP values decreased (0.94 ± 0.33 U/mg protein) significantly ($P<0.05$) unregulated diabetic group compared to the control group (1.74 ± 0.64 U/mg protein). AOP was though decreased in the regulated group (1.26 ± 0.53 U/mg protein) but the decrease was not significant.

### Table. Sensitivity of low density lipoprotein (LDL) fraction to oxidation in patients and controls

<table>
<thead>
<tr>
<th></th>
<th>Regulated DM group (n=26)</th>
<th>Poorly regulated DM group (n=29)</th>
<th>Unregulated DM group (n=35)</th>
<th>Control group (n=37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA\textsubscript{1c} (%)</td>
<td>&lt; 5.7</td>
<td>5.7 - 7.7</td>
<td>&gt; 7.7</td>
<td></td>
</tr>
<tr>
<td>Sensitivity to oxidation (nmol/mg/h)</td>
<td>2.65 ± 0.68*</td>
<td>2.57 ± 0.75*</td>
<td>2.53 ± 0.65*</td>
<td>3.26 ± 0.74</td>
</tr>
</tbody>
</table>

Values are mean ± SD  
* $P<0.001$ compared to control group (Dunnet t-test)

Increased glucose and glycated proteins lead to free radical formation by auto-oxidation resulting in increased oxidative stress. Both, increased lipid peroxidation and/or inadequate antioxidant defenses have important roles in the pathogenesis of vascular complications of diabetes\textsuperscript{3,5}.

In this study, sensitivity to oxidation was significantly lower in all diabetic groups compared with the control group. Possible factors contributing to this outcome (lower sensitivity to oxidation value in diabetic group) could be the turnover time of the oxidative process, diabetic diet and energy restriction, and anti-diabetic and supportive (vitamins and antioxidants) medication. In the process of lipid peroxidation, initially antioxidant components of LDL particles (such as $\alpha$- and $\delta$-tocopherol, ubiquinone-10) are consumed to overcome oxidative stress. We hypothesize that no end product of lipid peroxidation is formed at this stage. Therefore, regular diet and antioxidant intake in diabetic patients could maintain antioxidant defense and reduce oxidative stress. Antioxidants increase the lag time of lipid peroxidation. In diabetes, the lag phase of lipid peroxidation and $\alpha$-tocopherol levels have been reported to be significantly reduced due to increased oxidative stress\textsuperscript{15}. Plasma total antioxidant defense system has been shown to be decreased in the TRAP (total peroxyl radical trapping potential) assay in diabetic patients. Tsai et al\textsuperscript{16} showed that the lag time of copper-induced peroxidation was shorter in diabetic patients. No differences were observed in HDL and LDL peroxidation or in conjugated dien formation in diabetic patients compared to healthy subjects by Julier and his colleagues\textsuperscript{17}. Hence,
decreased lag time due to oxidative stress and diet with medication keep oxidative parameters at controlled levels. In this phenomenon, balance by antioxidant defense and oxidative stress, and the turnover of lipid peroxidation could be the main parameters to evaluate since the lag time and subsequently the turnover of oxidation increase in diabetes. Our results showed that sensitivity to oxidation and antioxidant potential values decreased in patients with type 2 diabetes. The reason could be that due to short lag time, antioxidant molecules in LDL are consumed initially and the sensitivity to oxidation and antioxidant potential are decreased.

In summary, our findings showed that patients with diabetes had decreased sensitivity to LDL oxidation and antioxidant potential because antioxidant molecules in LDL are consumed early. As low AOP probably shortens the LDL oxidation lag phase, the sensitivity of LDL to oxidation appears to be low in these patients. Studies need to be done to investigate the sensitivity of LDL to oxidation in patients before and after antidiabetic treatment.

References


16. Tsai EC, Hirsch IB, Brunzell JD, Chait A. Reduced plasma peroxyl radical trapping capacity and increased susceptibility of LDL to oxidation in poorly controlled IDDM. Diabetes 1994; 43 : 1010-4.


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