Correlation between the activities of lipoprotein lipase and paraoxonase in type 2 diabetes mellitus

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SUMMARY
In type 2 diabetes mellitus the decreased catabolism of triglyceride-rich lipoproteins as a consequence of mainly the decreased lipoprotein lipase activity results in hypertriglyceridaemia and other lipoprotein alterations promoting atherosclerosis. The high-density lipoprotein-associated enzyme, paraoxonase, prevents the oxidation of low-density lipoprotein, which is an antiatherogenic effect. Aim: to examine the relation between the activities of enzymes influencing HDL remodelling- LPL and PON- in type 2 diabetes mellitus. Methods: 56 newly diagnosed type 2 diabetic patients and 39 healthy controls were involved in the study. The serum PON activity was measured spectrophotometrically using paraoxone as substrate. PON phenotype was determined by the dual substrate method, PON mass was measured by ELISA. The determination of lipoprotein lipase activity was performed using ³H-triolein. Results: We noticed smaller PON activity decrease in our newly diagnosed diabetic subjects compared to the previous studies which investigated the alteration of enzyme activity after a longer duration of diabetes mellitus. The lipoprotein lipase activity showed a positive correlation with PON activity (r = 0.43; P < 0.02). Interestingly, the PON activity of the homozygous-low activity group did not correlate with the LPL activity, while in the heterozygous and homozygous-high activity groups there was a significantly positive correlation (r = 0.51; P < 0.05) between PON and LPL activity. Conclusion: Besides lipid alterations, the metabolic changes of type 2 diabetes mellitus influence the reduction of the antioxidant capacity of HDL by remodelling HDL and decreasing PON activity via modification of lipoprotein lipase activity, which might contribute to accelerated atherosclerosis.

Key-words: Paraoxonase · Lipoprotein lipase · Type 2 diabetes mellitus · Triglycerides.

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RéSUMÉ
Corrélations entre les activités de la lipoprotéine lipase et de la paraoxonase dans le diabète de type 2
Dans le diabète sucré de type 2 le catabolisme des particules riches en triglycérides est réduit, en raison, en premier lieu, de la diminution de l’activité de la lipoprotéine lipase. Il en résulte une hypertriglycéridémie, ainsi que différentes anomalies des lipoprotéines qui contribuent à la progression de l’athérosclérose. La paraoxonase, enzyme liée aux HDL, s’oppose à l’oxydation des LDL, et possède ainsi un effet antiathérogène.

Objectifs : Examiner les relations éventuelles entre l’activité des enzymes LPL et PON qui influencent la remodélisation des HDL chez des diabétiques de type 2.

Méthodes : Nous avons inclus dans cette étude 56 diabétiques de type 2 récemment diagnostiqués et 39 témoins. Nous avons mesuré l’activité PON du sérum par spectrophotométrie en utilisant pour substrat la paraoxone. Nous avons défini le phénytype PON par la méthode du double substrat, la PON a été mesurée par ELISA. La détermination de l’activité lipoprotéine lipase a été réalisée au moyen de la trioéline marquée de 3H.

Résultats : Chez les diabétiques, nous avons observé une diminution moins importante de l’activité PON que celle rapportée dans les études précédentes de la littérature, qui examinaient la modification de l’activité de l’enzyme après une plus longue durée d’évolution du diabète. Nous avons mis en évidence une corrélation positive entre les activités LPL et PON (r = 0,43 ; P < 0,02). De manière inattendue, l’activité paraoxonase des individus homozygotes à faible activité PON n’était pas en corrélation avec leur activité LPL, alors qu’il existait une corrélation positive (r = 0,51 ; P < 0,05) entre les activités PON et LPL des hétérozygotes et des individus homozygotes à forte activité paraoxonase.

Conclusions : Outre les modifications des paramètres lipidiques, les anomalies métaboliques observées chez les diabétiques de type 2 influencent la réduction de la capacité antioxydante des HDL en remodelant celles-ci, et en diminuant l’activité PON via des modifications de l’activité de la lipoprotéine lipase, pouvant ainsi contribuer à un athérome accéléré.

Mots-clés : Paraoxonase · Lipoprotéine lipase · Diabète sucré · Diabète de type 2 · Triglycérides.

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Cardiovascular diseases are frequent complications of diabetes mellitus [1]. The standardized mortality rate of diabetes mellitus in 2001 was 13.57 to 100 000 persons in Hungary, which is particular, regarding other European countries [2]. All of these direct attention to the fact that cardiovascular morbidity and mortality must emphatically be dealt with in the diabetic population.

In type 2 diabetes mellitus the constellation of hyperglycaemia, hyperinsulinaemia and hypertriglyceridaemia can be observed [3,4]. LPL is responsible for the catabolism of triglyceride-rich particles thus providing lipids for HDL formation [5,6]. HDL intervenes at different stages of atherosclerosis. It has partly a direct protective effect on the endothelium inhibiting the expression of adhesion molecules and the entry of monocytes into the subendothelial space [7]. On the other hand, it takes up cholesterol from peripheral cells with the help of ABCA1 transporter protein and carries it to the liver via reverse cholesterol transport [8]. The third antiatherogenic effect of HDL is the antioxidant function, which is of great significance in such illnesses as diabetes mellitus. This antioxidant effect is associated with the HDL-linked proteins [apo-AI, lecithin: cholesterol acyltransferase, platelet-activating factor acetylhydrolase (PAF-AH) and PON] [5,9,10]. The most explicit antioxidant effect belongs to the PON enzyme.

The low or high activity of paraoxonase is based on the Glu (A isoform) - Arg (B isoform) substitution at position 192 [11]. Carrying two A isoform alleles (homozygous aa phenotype) results in low enzyme activity, while one A and one B (heterozygous ab phenotype) or two B (homozygous bb phenotype) isoform alleles induces high PON activity. It is known that the antioxidant effect of HDL can be influenced not only by PON activity but the enzyme concentration as well [12]. Previous studies have demonstrated that the arylesterase activity of the enzyme shows linear relation to the amount of the enzyme [12,13].

As LPL is the crucial enzyme in triglyceride metabolism and HDL remodelling [14,15], and PON is perhaps the most important antioxidant enzyme linked to HDL, we investigated the connection between their activities in diabetic subjects. To our best knowledge this is the first study examining PON and lipase activity in newly diagnosed type 2 diabetes mellitus.

Patients and methods

Fifty-six newly (within 6 months) diagnosed patients (33 males, 23 females) with type 2 diabetes according to WHO criteria were examined. Their mean age was 52.64 ± 6.81 years, BMI: 29.82 ± 4.25 kg/m², HbA1c: 7.24 ± 0.59%. They were investigated after 6 weeks on Step 1 diet of the NCEP. The criteria for inclusion were: patients aged between 18-70 years with untreated hypertriglyceridaemia (serum triglyceride level > 2.4 mmol/l); serum cholesterol concentration < 8.0 mmol/l. Patients with liver disorders, gallstones, malignant diseases, microalbuminuria, serum creatinine > 130 µmol/l, serum cholesterol > 8.0 mmol/l, pregnancy, lactation, alcoholism, smoking, drug dependence or treatment with anticoagulants, lipid lowering agents, sulphonylurea or insulin therapy were excluded from the study. The main markers of infection (CRP, leucocyte number, sedimentation rate) were normal. Our patients did not include those suffering from retinopathy, neuropathy, nephropathy, previous stroke, myocardial infarction or atherosclerosis obliterans. All diabetic patients were on a diet.

We examined thirty-nine healthy age- and BMI-matched normolipemic volunteer control subjects (24 males, 15 females, mean age: 50.66 ± 5.75 years, BMI: 29.47 ± 3.93 kg/m²).

Ethics committee

The study protocol was approved by the Ethics Committee of the Medical and Health Science Center, University of Debrecen, Hungary. All patients signed the Informed Consent Form before being enrolled.

Blood Sampling

After overnight fasting for a minimum of 12 hours, blood was drawn for the following laboratory parameters: haemoglobin, haematocrit, white blood cell count, sedimentation rate, liver enzymes, bilirubin, urea, creatinine, uric acid, creatine kinase, fibrinogen, CRP, glycohaemoglobin, serum glucose, triglyceride, total cholesterol, HDL-cholesterol, LDI-cholesterol, apo-AI, apo-B100, lipoprotein (a) levels and serum paraoxonase activity. The determination of Hba1c was performed using Biorad HPLC (normal range: 4.2-6.1 %). Blood was centrifuged at 1500 g. Lipids were measured from fresh sera. Sera were kept at -20°C before PON activity measurements.

Abbreviations:
ABCA1: ATP-binding cassette transporter A1
ACE-INHIBITOR: angiotensin-converting enzyme inhibitor
APO-AI: apolipoprotein-AI
APO-B100: apolipoprotein-B100
APO-CII: apolipoprotein-CII
ATP: adenosyl-triphosphate
BMI: body mass index
CRP: C-reactive protein
HDL: high-density lipoprotein
HbA1c: haemoglobin A1c
LDL: low-density lipoprotein
LCAT: lecithin:cholesteryl acyltransferase
LP(A): lipoprotein (a)
LPL: lipoprotein lipase
NCEP: National Cholesterol Education Programme
PAF-AH: platelet-activating factor acetylhydrolase
PON: paraoxonase
TG: triglyceride
WHO: World Health Organisation
Lipid and apolipoprotein measurements

The Cobas Integra 700 Analyser (Roche, Basel, Switzerland) was used for lipid measurements. The LDL cholesterol fraction was calculated indirectly by using the Friedewald formula (serum triglyceride level < 4.5 mmol/l). Two patients had serum triglyceride concentration > 4.5 mmol/l. Apolipoprotein examination was performed by the immuno-nephelometric method (Ross Diagnostic kit).

Analysis of paraoxonase activity

Paraoxonase activity was determined spectrophotometrically using paraoxone substrate as was described earlier [16].

Arylesterase assay

Arylesterase activity was measured at 270 nm using phenylacetate [17].

Paraoxonase phenotype distribution

The phenotypic distribution of PON was determined by the dual substrate method [17].

Measure of PON mass

The concentration of PON enzyme was measured by ELISA [18].

Measure of lipoprotein lipase activity

Lipoprotein lipase activity was specified with the help of ³H-triolein containing phosphatidyl choline substrate which disintegrates under the influence of the enzyme in the postheparin plasma causing β-radiation [19].

Statistical methods

The statistical analysis was performed by the SAS™ for Windows™6.12 computer program (SAS Institute, Cary NC 27513 USA). Data were presented by descriptive analysis. The studied parameters had a normal distribution, except triglyceride level and paraoxonase activity. Therefore, data of these two were log-transformed. Correlation between the parameters was analysed by Pearson’s correlation test. The comparisons between groups were performed by Student’s t test. A value of P < 0.05 was accepted as a significance level.

Results

Lipid parameters

In type 2 diabetic patients compared to healthy controls significantly higher serum triglyceride (P < 0.0001), total cholesterol (P < 0.001), LDL-cholesterol (P < 0.05) and apo-B100 (P < 0.05) levels were found. At the same time, HDL-cholesterol and apo-AI concentration proved to be significantly lower (P < 0.05) (Table I).

LPL activity

The activity of LPL decreased significantly (P < 0.05) in the diabetic population in comparison with the control group (Table I).

Lipid parameters and LPL activity

The correlation analysis between triglyceride concentration and LPL activity showed an inverse significant correlation (r = -0.49) (Fig. 2a). At the same time, we noticed a positive correlation between HDL-cholesterol and LPL activity (r = 0.50) (Fig. 2b).

These relations proved to be significant (P < 0.05).

PON and arylesterase activity,

PON concentration

Measuring the PON activity a significantly lower value was detected in the patient group compared with the control population (P < 0.001) (Fig. 1a). To determine whether the altered paraoxonase activity was due to HDL-cholesterol level decrease, we standardized the enzyme activity for HDL-cholesterol concentration. The difference in PON/HDL-cholesterol ratio between the diabetic and the control groups was non-significant (Fig. 1b). To determine whether the PON activity decrease was related to the decrease in PON mass we measured the concentration and the arylesterase activity of the enzyme. Both of them showed a non-significant decrease in the diabetic patients compared to the healthy persons (PON concentration: 58.7 ± 3.7 vs. 60.4 ± 4.2 mg/ml; n.s.; arylesterase activity: 149.32 ± 47.76 vs. 164.15 ± 51.98 U/l; n.s.).

PON and lipase activity

LPL enzyme plays an important role in HDL remodelling [19-21]. It can have a consequent effect on the HDL-associated enzyme, PON activity. That is why we investigated the correlation between the activities of lipoprotein lipase and PON.

While investigating the correlation between PON and LPL activity, we found a positive significant relation (r = 0.43, P < 0.02) (Fig. 3a). The PON activity of the homozygous-low group did not correlate with LPL activity (Fig. 3b), while in the heterozygous and homozygous-high activity groups there was a significantly positive correlation (r = 0.51, P < 0.05) between PON and LPL activity (Fig. 3a).

Discussion

Previous studies have reported the key role of LPL in the catabolism of triglyceride-rich particles [14,15]. Type 2
diabetes associated lipid abnormality is mainly hypertriglyceridaemia. We sought to determine if decreased catalysis could influence high triglyceride levels besides increased production. Hypertriglyceridaemia results in a higher free fatty acid level and a consequent increase of insulin resistance [3,4]. As earlier investigations have demonstrated insulin resistance of LPL often occurs in type 2 diabetes mellitus [5,6]. The enzyme needs insulin to be active and if there is resistance despite the hyperinsulinemia, lower activity can be observed which contributes to the lipid abnormalities noticed in type 2 diabetic patients. In our recent study, LPL activity was significantly lower in the patient group compared with healthy controls. Furthermore, the enzyme activity showed a negative correlation with triglyceride concentration. Between HDL and LPL activity we found a positive relation. Earlier studies examining diabetic and healthy populations similarly found that the activity of LPL inversely correlated with

<table>
<thead>
<tr>
<th>Table I</th>
<th>Anthropometric and laboratory parameters of patients with type 2 diabetes mellitus and of controls.</th>
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<tr>
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<th>Control group</th>
<th>Diabetic group</th>
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<tbody>
<tr>
<td>n</td>
<td>39</td>
<td>56</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>24/15</td>
<td>33/23</td>
</tr>
<tr>
<td>Age (year)</td>
<td>50.66 ± 5.75</td>
<td>52.64 ± 6.81</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>29.47 ± 3.93</td>
<td>29.82 ± 4.25</td>
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<tr>
<td>Waist circumference (cm)</td>
<td>101.62 ± 14.23</td>
<td>106.45 ± 17.88</td>
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<td>Waist to hip ratio</td>
<td>1.04 ± 0.44</td>
<td>1.08 ± 0.47</td>
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<tr>
<td>Blood pressure (Hg mm)</td>
<td>129.56 ± 18.34</td>
<td>133.32 ± 19.11</td>
</tr>
<tr>
<td>LPL activity (mmol ml⁻¹ h⁻¹)</td>
<td>18.44 ± 3.01</td>
<td>12.58 ± 2.92***</td>
</tr>
<tr>
<td>Glucose (mmol/l) (fasting)</td>
<td>4.90 ± 0.80</td>
<td>8.40 ± 1.65**</td>
</tr>
<tr>
<td>HbA₁c (%)</td>
<td>4.72 ± 0.27</td>
<td>7.24 ± 0.59***</td>
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<td>Triglyceride (mmol/l)#</td>
<td>1.14; q₁ = 0.78; q₃ = 1.3</td>
<td>3.58; q₁ = 2.83; q₃ = 5.49*</td>
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<tr>
<td>Cholesterol (mmol/l)</td>
<td>4.90 ± 0.86</td>
<td>6.37 ± 1.00**</td>
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<tr>
<td>LDL-c (mmol/l)</td>
<td>3.32 ± 0.75</td>
<td>4.38 ± 0.95***</td>
</tr>
<tr>
<td>HDL-c (mmol/l)</td>
<td>1.58 ± 0.32</td>
<td>1.08 ± 0.26***</td>
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<tr>
<td>Apo-AI (g/l)</td>
<td>1.87 ± 0.29</td>
<td>1.67 ± 0.26***</td>
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<tr>
<td>Apo-B100 (g/l)</td>
<td>1.10 ± 0.27</td>
<td>1.69 ± 0.39***</td>
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Values are mean ± SD

#median/quartiles.

* P < 0.0001. ** P < 0.001. *** P < 0.05.

**Figure 1**

triglyceride concentration [20,21] and the correlation proved to be significant. The positive significant LPL-HDL correlation that we detected was confirmed by other studies [22,23]. In diabetic patients, besides insulin resistance, the causes of LPL activity decrease could be due to a mutation with inactivating effect, cofactor polymorphism, cell loading with triglyceride and gene expression influenced by peroxisome-proliferator activated receptors [24-26]. Another aspect of high triglyceride level is the accumulation of small dense LDL with increased susceptibility to oxidation which promotes the development of atherosclerosis [27]. Mackness et al. demonstrated that the HDL-linked PON had an explicit antioxidant effect inhibiting LDL oxidation and the production of lipid peroxides [28]. Earlier, both in type 1 and 2 diabetes, and in hyperlipidaemia, it was proved that PON activity decreased significantly in comparison with the healthy population [12,16,29]. We found lower PON activity in our type 2 diabetic patients, as well. We compared our PON activities (patient and control) with those of earlier studies, which also investigated diabetic patients without complications. First of all, a significantly lower enzyme activity was detected in our diabetic subjects (duration time 0-6 months) compared to healthy controls. The enzyme activity of the diabetic patients in our recent study proved to be significantly higher in comparison with the result of our earlier examination (diabetes mellitus duration time 4.2 years, no complications) [30]. Sözmen et al. also noticed significantly lower PON activity in their earlier study (diabetes duration time 4.2 years, no complications) [31,32] compared to the enzyme activity of our newly diagnosed diabetic subjects. On the basis of all these we can conclude that the activity of the antioxidant enzyme decreases parallel to the development of metabolic changes in the early part of diabetes mellitus. This enzyme activity decrease becomes more explicit with the passing of time and it can contribute to the progress of diabetic complications as well. As the patients’ HDL concentration also decreased, a question arose whether the activity of the HDL associated enzyme would be decreased as a result of the lower HDL level.

**Figure 2**


**Figure 3**

To answer this question we investigated the PON/HDL ratio which showed a non-significant decrease. This result showed that in PON activity decrease a lower HDL concentration could be a determinant. This fact led to another question which was whether the cause of PON activity decrease could be due to the decrease of paraoxonase enzyme concentration. In 1995 Durrington et al. demonstrated that in type 2 diabetes mellitus the PON mass did not change with the significant enzyme activity decrease [12]. At the same time Nevin et al. pointed out that there is a strong positive correlation between PON mass and the arylerase activity of the enzyme [29]. Measuring the arylerase activity and the concentration of PON, we found a non-significant decrease in the diabetic patients compared to healthy controls. This result means that the significant PON activity decrease is not due to the enzyme amount decrease. Since the LPL enzyme plays an important role in HDL remodelling influencing both amount and composition of HDL-c [14,15] it could have a consequent effect on the HDL linked main antioxidant enzyme, PON activity and that was why we investigated the correlation between LPL activity and PON activity. Lipoprotein lipase activity showed positive correlation with the activity of PON and the correlation proved to be significant. Nevin and his co-workers previously examined the correlation between these enzyme activities in a healthy population [29]. They demonstrated a direct significant positive relation between the activity of LPL and PON or arylerase function of low-activity PON isofrom homozgyous people. Hedrick et al. investigated the effects of incubation of human HDL under glycaemic conditions on several functions of it in vitro [33]. They found significant increases in the glycation products, and the glycated HDL had a 65% reduction in PON enzymatic activity, besides, it did not inhibit monocyte adhesion to human aortic endothelial cells in response to oxidised low-density lipoprotein in vitro. Direct glycation of purified paraoxonase protein by incubation in 25 mmol/l glucose caused a 40% reduction in enzymatic activity and the glycated enzyme did not inhibit monocyte adhesion to human aortic endothelial cells in vitro. They also measured a 40% fall in PON activity in patients with type 2 diabetes mellitus and documented coronary artery disease. In our study the cause of PON activity decrease could probably have been due to the metabolic effects of diabetes mellitus as well, but our patients were newly diagnosed type 2 diabetics with lower glucose concentration than was used for the incubation (patients’ fasting blood glucose: 8.40 ± 1.65 vs incubation concentration: 25 mmol/l) and without glycated tissues, so we do not have to consider with this kind of enzyme activity decrease. Consequently, it can be concluded that glycation supposedly has no significant role in the decrease of PON activity in newly diagnosed type 2 diabetic patients, although, the decrease in PON activity can be detected even in early stages of diabetes without complications. Interestingly, the PON activity of the homozygous-low group did not correlate with the LPL activity, while in the heterozygous and homozygous-high activity groups there was a significantly positive correlation between PON and LPL activity. We suppose that in the homozygous-low PON activity group the enzyme is less sensitive to the glucose concentration thus there is a smaller decrease in its activity. Moreover, in insulin resistant states the activity decrease of the insulin dependent enzyme, LPL, is responsible for the catabolism of triglyceride-rich particles thus providing lipids for HDL formation can also be observed. The activity decreasing LPL influences HDL remodelling in a way that accumulation of triglyceride-rich particles results in elevated cholesterol ester transport. So does the triglyceride content of HDL increase. These changes in HDL composition can also influence the activity of HDL associated enzyme PON and, consequently, increased tendency towards atherosclerosis could be detected in the diabetic population.

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