Enzymatic and genetic polymorphisms of paraoxonase-1 in the Gabonese population: the relation to lipid parameters in patients with diabetes

Introduction

Cardiovascular disease is a major cause of morbidity and mortality in developed countries. In developing countries, particularly in Africa, there is an upsurge of metabolic diseases such as obesity, type 2 diabetes and atherosclerosis, and its cardiovascular complications. All these entities partially share a common basis, namely the development of a state of imbalance between pro-oxidant and antioxidant factors. Paraoxonase-1 is a member of the three-gene family, PON 1, PON 2 and PON 3. PON 1, the best studied, is a calcium-dependent esterase and can hydrolyse organophosphates. This enzyme circulates bound to the high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, triglycerides, apolipoprotein AI, apolipoprotein B100, the latency time of oxidation of small and dense LDL, arylesterase activity and genetic polymorphism of paraoxonase-1 at positions T(-107)C, L55M and Q192R were determined and compared to healthy subjects.

Method

Population

This prospective survey was conducted from 30 October 2010 to 1 March 2011. The study was carried out in the biochemistry laboratory of the Faculty of
A questionnaire was administered to assess personal and family history, cardiovascular disease, smoking habits, diabetes and hypertension. Two blood pressure measurements were performed on each arm of the patient, after a rest of at least 15 minutes, using a usual sphygmomanometer. The two measurements were averaged. If the two readings differed (diastolic over 15 mmHg systolic or more than 25 mmHg), a third reading was taken. The average of the two closest measurements was used subsequently. Patients in whom either the systolic or diastolic was equal to, or exceeded 140 and 90 mmHg respectively, or who were taking antihypertensive therapy, were considered to be hypertensive. Subjects whose fasting glucose was above 7 mmol/l and those taking hypoglycaemic drugs were considered to be patients with diabetes. Samples were taken from patients who fasted for at least 10 hours, and were collected in tubes containing ethylene diamine tetraacetic acid (EDTA), as well as fluoride-oxalate tubes and plain tubes. After centrifugation at 3 000 revolutions per minute (rpm) for 5 minutes, plasma and sera were collected and stored at -70°C until assayed.

Conventional assays

The determination of total cholesterol and triglycerides was performed using standard enzymatic techniques based on samples taken in tubes containing EDTA. Glucose was assayed by the glucose oxidase method on the plasma obtained from samples taken in tubes containing potassium fluoride and sodium oxalate. LDL cholesterol was obtained by direct measurement from the serum, using a method based on the use of two detergents. HDL cholesterol was assayed by a method called Ultra Direct HDL Cholesterol, or Daichi’s method, on a serum sample by selective detergent methodology accelerator. Apolipoprotein AI (ApoA1) and B100 (ApoB100) were measured by immunoprecipitation in the homogeneous phase with the reagent kit supplied by Daichi’s method on a serum sample by selective detergent methodology accelerator.

Extraction and oxidation of low-density lipoprotein and small dense low-density lipoprotein

Deoxyribonucleic acid (DNA) was obtained from whole blood and extracted with phenol-chloroform. Genotype determinations were carried out by an amplification reaction [polymerase chain reaction (PCR)] in an Eppendorf® thermocycler, followed by analysis of the fragments through enzymes restriction. Sixty pmol of oligonucleotide primers, KCI 62.5 mmol/l, MgCl₂ 15 mmol/l, 50 μmol/l of deoxyxynucleoside triphosphate, 1.5 U of Taq polymerase (Amersham Pharmacia Biotech) in buffer Tris-HCl 20 mmol/l pH 8.0. The initial rate of hydrolysis was determined spectrophotometrically at 270 nm.

DNA extraction and genotyping

Two first external sequences have been used, i.e: 5’-GACGCAGGACGGATGGCAAAATGTAATG-3’ and TGGGCAGACACCGAGGCTAGGACTCTT-3’. Two first external sequences have been used, i.e: 5’-GACGCAGGACGGATGGCAAAATGTAATG-3’ and TGGGCAGACACCGAGGCTAGGACTCTT-3’. Two first external sequences have been used, i.e: 5’-GACGCAGGACGGATGGCAAAATGTAATG-3’ and TGGGCAGACACCGAGGCTAGGACTCTT-3’. Two first external sequences have been used, i.e: 5’-GACGCAGGACGGATGGCAAAATGTAATG-3’ and TGGGCAGACACCGAGGCTAGGACTCTT-3’. Two first external sequences have been used, i.e: 5’-GACGCAGGACGGATGGCAAAATGTAATG-3’ and TGGGCAGACACCGAGGCTAGGACTCTT-3’. Two first external sequences have been used, i.e: 5’-GACGCAGGACGGATGGCAAAATGTAATG-3’ and TGGGCAGACACCGAGGCTAGGACTCTT-3’. Two first external sequences have been used, i.e: 5’-GACGCAGGACGGATGGCAAAATGTAATG-3’ and TGGGCAGACACCGAGGCTAGGACTCTT-3’. Two first external sequences have been used, i.e: 5’-GACGCAGGACGGATGGCAAAATGTAATG-3’ and TGGGCAGACACCGAGGCTAGGACTCTT-3’.

Assessing the arylesterase activity of paraoxonase-1

A volume of 0.1 ml of serum was added in a final volume of 250 μl containing 1 mmol/l of phenylacetate and 2 mmol/l of CaCl₂ in Tris-HCl 20 mmol/l pH 8.0. The initial rate of hydrolis was determined spectrophotometrically at 270 nm.

Extraction and oxidation of low-density lipoprotein and small dense low-density lipoprotein

The precipitation reagent (0.1 ml) containing 150 U/ml of sodium heparin and 90 mmol/l MgCl₂ was added to 0.1 ml of a test serum, and the mixture was incubated at 37°C for 10 minutes. Samples were transferred to an ice bath for 15 minutes, then centrifuged at 15,000 rpm for 15 minutes at 4°C. The clear supernatant contained the fraction of small dense low-density lipoprotein. Thirty-two microlitres of CuCl₂ (1 mmol/l) were added to 10 μl of this supernatant. The appearance of the reaction products of oxidation, conjugated dienes, was continuously registered at 37°C, using a spectrophotometer at 234 nm.

DNA extraction and genotyping

Deoxyribonucleic acid (DNA) was obtained from whole blood and extracted with phenol-chloroform. Genotype determinations were carried out by an amplification reaction [polymerase chain reaction (PCR)] in an Eppendorf® thermocycler, followed by analysis of the fragments through enzymes restriction. Sixty pmol of oligonucleotide primers, KCl 62.5 mmol/l, MgCl₂ 15 mmol/l, 50 μmol/l of deoxyxynucleoside triphosphate, 1.5 U of Taq polymerase (Amersham Pharmacia Biotech) in buffer Tris-HCl 20 mmol/l pH 8.0. The initial rate of hydrolis was determined spectrophotometrically at 270 nm.
mentioned, a mixture to which dimethyl sulfoxide 100 ml/l was added. External primers (30 pmol each) were added to each mixture’s reaction, followed by 30 amplification cycle, performed at 94°C for 30 seconds, 64°C for 30 seconds, and 72°C for one minute, followed by a final extension at 72°C for six minutes. The PCR fragments were analysed on an agarose gel 2% with ethidium bromide staining and ultraviolet (UV) transillumination. For the PON 1 192 polymorphism, the PCR products were subjected to the action of the restriction enzyme AlwI for four hours at 37°C, then separated by electrophoresis on 3% agarose gel for 75 minutes at 60 V with ethidium bromide staining and UV transillumination. PON 1 55 polymorphism was determined after the action of the restriction enzyme Hsp92II of the amplification products, followed by electrophoresis under the same conditions as before.

Statistical method

Continuous variables were expressed as mean and standard deviation, while qualitative variables were expressed as frequency. The comparison of quantitative variables was performed using the nonparametric Kruskal Wallis test. The chi-square Mantel-Haenszel was used to compare allele frequencies between groups. Two regression models were performed, namely a linear regression model including all the variables influencing PON 1 activity, and another regression carried out step by step, to select the most relevant variables based on their importance. The null hypothesis was rejected for p-values < 0.05. The statistical analysis of data was performed using Statistica 8® US Windows for StatSoft France.

Results

The epidemiological data of the studied cases are summarised in Table I. The study population was predominantly male (64.2%), with an average age of 62.7 ± 12 years. Of the patients, 31.7% were patients with hypertension, 25.8% were patients with diabetes, 15.8% were patients with diabetes and hypertension, and 25.7% were considered to be healthy.

In this entire cohort, genotyping at position -107 showed a predominance of -107TC heterozygotes (0.440), followed by -107TT and -107CC homozygotes (Table II), corresponding to frequency of 0.540 for -107 T allele and 0.460 for -107C allele. At positions 55 and 192, we obtained a predominance of 55LL genotypes (0.510) and 192RR (0.460), corresponding to frequencies of 0.695 and 0.635 for 55L and 192R alleles, against 0.365 and 0.305 for alleles 55M and 192Q, respectively. By contrast, in the control group, we obtained a prevalence of genotype -107CC (0.560) for a frequency of 0.675 for the -107 C allele, and 0.325 for -107T allele, respectively. In subjects with diabetes, the predominant genotype was -107TT, with an allele frequency of 0.660 and 0.695 -107T respectively, as they were simply diabetic or hypertensive and diabetic (vs. 0.340 and 0.305 for allele C) at -107 position.

In the coding region at position 55, genotype 55LL was found in 66% of controls and 51% of patients with simple hypertension. This corresponded to the allele 55L frequencies of 0.745 and 0.630 in controls and hypertensive subjects, respectively (vs. 0.255 and 0.370 for allele 55M). However, in patients with diabetes, the frequency of the genotype 55LL dropped to 0.420 and 0.340 in patients with diabetes and patients with diabetes and hypertension respectively, with frequencies of 0.470 and 0.420 for allele 55L in both groups, against 0.530 and 0.580 for allele 55 M.

The Q192R polymorphism was characterised by relative constancy in the distribution of alleles 192Q and 192R in the four identified groups. Indeed, the 192Q allele was found at a frequency of 0.290 in the control group and in subjects with hypertension. Its frequency was 0.244 in subjects with diabetes and 0.230 in subjects with

| Parameter | Number (%) or medium ± SD |
|-----------|---------------------------|
| Subjects  | 600                       |
| Gender    |                           |
| Female    | 215 (35.8)                |
| Male      | 385 (64.2)                |
| Tobacco   |                           |
| Yes       | 75 (12.5)                 |
| No        | 525 (87.5)                |
| Age (years)| 62.7 ± 12.0              |
| Height (m) | 1.62 ± 0.09            |
| Body mass index (kg/m²) | 27.4 ± 5.1           |
| Male waist circumference (cm) | 104 ± 12             |
| Women waist circumference (cm) | 86 ± 8               |
| Alcohol consumption |                  |
| Yes       | 163 (27.1)                |
| No        | 437 (72.9)                |
| Sport practice | 320 (53.3)            |
| Clinical category |               |
| Control group | 160 (26.7)             |
| Patients with diabetes | 155 (25.8)           |
| Patients with hypertension | 190 (31.7)          |
| Patients with hypertension and diabetes | 95 (15.8)          |
| Average weight (kg) | 71.9 ± 9.7              |
| Average systolic blood pressure (mmHg) | 124 ± 6             |
| Average diastolic blood pressure (mmHg) | 81 ± 3              |
diabetes and hypertension (p-value = 0.432). Similarly, the 192R allele had a frequency of 0.710 in controls and subjects with hypertension. This frequency was 0.755 in subjects with diabetes and 0.770 in subjects with diabetes and hypertension (p-value = 0.652).

The distribution of enzyme activities, based on clinical category and genotypes, is summarised in Table III. Genotypes associated with increased enzyme activity were -107CC and 55LL. However, the PON 1 activity was relatively constant, regardless of the polymorphism at position 192. On the other hand, a gradual decline of enzyme activity from the control group to that of patients with diabetes and hypertension, was noticed whatever the site of polymorphism concerned.

The relationship between polymorphisms whose genotypes had an effect on enzyme activities and lipid parameters is summarised in Tables IV and V. Among the parameters studied, and irrespective of the group of people, there was no association between LDL cholesterol concentrations and the different genotypes involved in the T(-107C) polymorphism. There was a relationship between the concentrations of HDL, ApoAI and the lag time and genotypes of the promoter region studied. This was regardless of patient category. A particular decrease in concentrations of HDL cholesterol and ApoAI in the same direction was observed, from the -107CC genotype to the -107TT, with heterozygotes CT occupying an intermediate position. Vertical analysis of Table IV showed the concentration of HDL cholesterol and a high lag period, homozygous -107CC in the control group. Both parameters decreased with the onset of the allele T. Regarding the L55M polymorphism, in the control group, 55LL

| Number of subjects | Control group (160) | Patients with hypertension (190) | Patients with diabetes (155) | Patients with hypertension and diabetes (95) | Entire cohort (600) |
|--------------------|---------------------|-------------------------------|-----------------------------|---------------------------------|-------------------|
| **PON 1 -107**     |                     |                               |                             |                                 |                   |
| TT                 | 0.21                | 0.19 (0.127)                  | 0.57 (0.0001)               | 0.61 (0.001)                    | 0.32              |
| TC                 | 0.23                | 0.21 (0.131)                  | 0.18 (0.002)                | 0.17 (0.002)                    | 0.44              |
| CC                 | 0.56                | 0.60 (0.231)                  | 0.25 (0.0001)               | 0.22 (0.0001)                   | 0.24              |
| **PON 1 55**       |                     |                               |                             |                                 |                   |
| LL                 | 0.66                | 0.51 (0.01)                   | 0.42 (0.0001)               | 0.34 (0.0001)                   | 0.51              |
| LM                 | 0.17                | 0.24 (0.021)                  | 0.10 (0.001)                | 0.16 (0.234)                    | 0.37              |
| MM                 | 0.17                | 0.25 (0.001)                  | 0.46 (0.0001)               | 0.50 (0.0001)                   | 0.12              |
| **PON 1 192**      |                     |                               |                             |                                 |                   |
| QQ                 | 0.16                | 0.21 (0.067)                  | 0.11 (0.078)                | 0.11 (0.076)                    | 0.19              |
| QR                 | 0.26                | 0.16 (0.052)                  | 0.27 (0.467)                | 0.24 (0.413)                    | 0.35              |
| RR                 | 0.48                | 0.53 (0.050)                  | 0.62 (0.051)                | 0.65 (0.003)                    | 0.46              |

p-values for comparison with the control group in brackets
### Table IV: Impact of genotype on lipid parameters according to -107CT polymorphisms

|                      | CC    | CT    | TT    | p-value |
|----------------------|-------|-------|-------|---------|
| **Control group**    |       |       |       |         |
| HDL cholesterol (mmol/l) | 1.45 ± 0.04 | 1.24 ± 0.03 | 1.24 ± 0.04 | 0.0001 |
| LDL cholesterol (mmol/l) | 3.42 ± 0.04 | 3.40 ± 0.02 | 3.51 ± 0.04 | 0.851  |
| ApoAI (g/l)           | 1.14 ± 0.03 | 1.00 ± 0.02 | 1.01 ± 0.03 | 0.005  |
| Lag time (seconds)    | 90 ± 2  | 80 ± 5 | 75 ± 2 | 0.0001 |
| **Patients with diabetes** |       |       |       |         |
| HDL cholesterol (mmol/l) | 1.20 ± 0.01 | 0.91 ± 0.07 | 0.88 ± 0.06 | 0.002  |
| LDL cholesterol (mmol/l) | 4.25 ± 0.07 | 4.34 ± 0.05 | 4.42 ± 0.04 | 0.843  |
| ApoAI (g/l)           | 0.92 ± 0.02 | 0.84 ± 0.03 | 0.82 ± 0.02 | 0.006  |
| Lag time (seconds)    | 75 ± 4  | 60 ± 2 | 54 ± 4 | 0.0001 |
| **Patients with hypertension** |       |       |       |         |
| HDL cholesterol (mmol/l) | 1.40 ± 0.06 | 1.22 ± 0.04 | 1.20 ± 0.02 | 0.001  |
| LDL cholesterol (mmol/l) | 4.01 ± 0.04 | 3.95 ± 0.06 | 4.10 ± 0.05 | 0.765  |
| ApoAI (g/l)           | 1.20 ± 0.02 | 0.95 ± 0.05 | 0.90 ± 0.04 | 0.001  |
| Lag time (seconds)    | 80 ± 2  | 75 ± 4 | 70 ± 2 | 0.0001 |
| **Patients with hypertension and diabetes** |       |       |       |         |
| HDL cholesterol (mmol/l) | 0.90 ± 0.02 | 0.75 ± 0.03 | 0.72 ± 0.04 | 0.002  |
| LDL cholesterol (mmol/l) | 4.63 ± 0.07 | 4.57 ± 0.06 | 4.62 ± 0.08 | 0.742  |
| ApoAI (g/l)           | 0.84 ± 0.03 | 0.69 ± 0.02 | 0.70 ± 0.03 | 0.006  |
| Lag time (seconds)    | 62 ± 4  | 53 ± 3 | 52 ± 4 | 0.0002 |

ApoA1: apolipoprotein A1, HDL: high-density lipoprotein, LDL: low-density lipoprotein

### Table V: Impact of genotype on lipid parameters according to 55LM polymorphisms

|                      | LL    | LM    | MM    | p-value |
|----------------------|-------|-------|-------|---------|
| **Control group**    |       |       |       |         |
| HDL cholesterol (mmol/l) | 1.64 ± 0.04 | 1.42 ± 0.05 | 1.13 ± 0.04 | 0.0001 |
| LDL cholesterol (mmol/l) | 3.47 ± 0.06 | 3.42 ± 0.05 | 3.38 ± 0.07 | 0.453  |
| ApoAI (g/l)           | 1.32 ± 0.07 | 1.21 ± 0.06 | 1.10 ± 0.05 | 0.002  |
| Lag time (seconds)    | 105 ± 5 | 95 ± 2 | 90 ± 4 | 0.0001 |
| **Patients with diabetes** |       |       |       |         |
| HDL cholesterol (mmol/l) | 0.74 ± 0.05 | 0.54 ± 0.04 | 0.58 ± 0.02 | 0.0001 |
| LDL cholesterol (mmol/l) | 4.45 ± 0.05 | 4.45 ± 0.07 | 4.43 ± 0.08 | 0.732  |
| ApoAI (g/l)           | 1.11 ± 0.02 | 0.84 ± 0.08 | 0.78 ± 0.04 | 0.006  |
| Lag time (seconds)    | 82.5 ± 5 | 70 ± 4 | 65 ± 5 | 0.001  |
| **Patients with hypertension** |       |       |       |         |
| HDL cholesterol (mmol/l) | 0.94 ± 0.06 | 0.82 ± 0.04 | 0.80 ± 0.03 | 0.002  |
| LDL cholesterol (mmol/l) | 4.32 ± 0.04 | 4.28 ± 0.06 | 4.31 ± 0.07 | 0.326  |
| ApoAI (g/l)           | 0.85 ± 0.05 | 0.72 ± 0.03 | 0.69 ± 0.02 | 0.004  |
| Lag time (seconds)    | 92 ± 4  | 83 ± 2 | 75 ± 2 | 0.001  |
| **Patients with hypertension and diabetes** |       |       |       |         |
| HDL cholesterol (mmol/l) | 0.65 ± 0.05 | 0.49 ± 0.04 | 0.44 ± 0.02 | 0.004  |
| LDL cholesterol (mmol/l) | 4.19 ± 0.08 | 4.02 ± 0.05 | 4.20 ± 0.08 | 0.321  |
| ApoAI (g/l)           | 0.92 ± 0.02 | 0.54 ± 0.03 | 0.51 ± 0.02 | 0.0001 |
| Lag time (seconds)    | 72 ± 3  | 62 ± 2 | 57 ± 3 | 0.0001 |
homozygotes had a higher concentration of HDL, of 1.64 ± 0.04 mmol/l. This concentration decreased to 1.13 ± 0.04 mmol/l in 55MM homozygotes. This decrease was observed in other patient groups, with a greater depletion of HDL in patients with hypertension and diabetes. Furthermore, the lag time of subjects in the control group and 55LL homozygotes, was 105 ± 5 seconds against 90 ± 4 seconds in homozgyous 55MM in the same group, with a significant difference (p-value = 0.0001). The decrease of lag time with the appearance of the M allele was obtained with all groups of patients.

The results of the step-by-step regression analysis that was performed using all factors involved in the variability of PON1 activity are shown in Table VI. The considered variables were those that were correlated with PON1 in multivariate analysis. The main dependent variables that determined the enzyme activity were ApoAI and lag time. The non-modifiable variables that had an influence on the arylerase activity were age ($r^2 = 3.9$, p-value < 0.00001), gender ($r^2 = 0.38$, p-value < 0.006), genotype at position 55 ($r^2 = 3.0$, p-value < 0.0001), and position -107 ($r^2 = 1.84$, p-value < 0.0005), and body mass index ($r^2 = 0.55$, p-value = 0.003).

### Table VI: Step-by-step regression analysis of PON1 determinants

| Dependent parameters     | $r^2$ | p-value     |
|--------------------------|-------|-------------|
| Apolipoprotein Al         | 8.4   | < 0.0001    |
| HDL cholesterol          | 0.7   | < 0.026     |
| Lag time                 | 9.1   | < 0.0001    |
| Age                      | 3.0   | < 0.0001    |
| Genotype 55              | 2.7   | < 0.0001    |
| Genotype 107             | 1.7   | < 0.0005    |
| Triglycerides            | 0.60  | 0.035       |
| Tobacco                  | 0.59  | 0.038       |
| Body mass index          | 0.55  | 0.003       |
| Sport practice           | 0.43  | 0.005       |

### Discussion

In this paper, we studied the relationship between the arylerase activity of PON1 and lipids parameters in a group of black patients with diabetes, compared to a control group. The enzyme activity used was arylerase activity. Yet, the phenylacetate used as a substrate does not show a difference in reactivity with respect to the Q192R locus, as paraoxon. The only substrate that differentiates the activities of each of these alleles is paraoxon. The R192 isofom hydrolyses paraoxon nine times faster than the allozyme Q192.

Contrary to paraoxon, phenylacetate is easy to use and to obtain. Otherwise, the most commonly used method to measure the susceptibility of LDL to in-vitro oxidation is the determination of the lag time of the formation of conjugated dienes, initiated by a catalytic amount of transition metals. The formation of conjugated diene represents an intermediate stage of the oxidation process. As an inverse relationship between the latency, the severity and the rate of the progression of coronary atherosclerosis, the lag time is considered to be the most discriminating index of the oxidisability of LDL.

However, with this protocol, we showed that in subjects who were considered to be healthy, predominant genotypes in our population were -107CC and 192RR and 55LL. This distribution is similar to that found by Thyagarajan et al in the black patient population of the Coronary Artery Risk Development in Young Adults (CARDIA) study. The comparison of our distribution with that obtained in other populations is shown in Table VII. Distributions for 55L and 55M alleles were similar to those found in the United States and Brazil. Other distributions, especially for alleles 192Q and 192R, were different. Such a difference could be one of the factors involved in the conflicting results of epidemiological studies that have been conducted in different populations. If this hypothesis is true, then the incidence of diabetes and hypertension should be higher in Gabon, than in Korea or Thailand for example, other causes being equal. The association of PON1 genotypes, enzymatic activity and parameters of oxidation suggests a particularly high cardiovascular risk in patients with diabetes in Gabon, compared to what could be expected in Asia.

It is now suggested that the incidence of hypertension and cardiovascular mortality is higher in black patients...
living in urban zones than in Caucasians.22,23 Similarly, Hall et al have also shown a particularly significant increase of diabetes in African patients.24 However, the predominant genotypes in the control group also have the highest enzymatic activity outside the 192 position. These subjects are less likely to develop diabetes. The alleles -107T, 55M and 192R are prevalent in diabetics and are characterised by a low enzyme activity. The importance of these elements probably reflects the susceptibility of these individuals, whose PON1 activity results in a lower predisposition to developing diabetes, as suggested by the work of Martinelli.5 Therefore, the measurement of the PON 1 activity would provide supplemental information to the cardiovascular risk in this population.

In the same way, Bhattacharyya et al26 have shown a decreased incidence of cardiovascular events in subjects whose PON 1 concentration is high. In addition, the decrease of PON 1 activity in patients with diabetes is followed by a decrease in HDL concentration. This result proves that it is rather the protective potential of atherogenesis that is defective in this particular situation. LDL is present, as we have demonstrated, but with a decrease in the HDL and PON 1, a more intensive oxidative stress was obtained. This result is confirmed by Mastorikou et al28 who suggested that non-functional HDL in type 2 diabetes would be an additional factor in increased cardiovascular disease related to type 2 diabetes in metabolising 20% less membrane hydroperoxides than the HDL of controls. This reduction in the antioxidant activity of HDL in diabetes may relate to the glycation of PON 1 in these individuals and the oxidation of ApoAI and LDL. Genotypes and PON 1 activity are independently associated with concentrations of HDL and ApoAI. This relationship depends on the pathophysiological state. One interpretation of these data is that PON 1 determines the concentration of HDL and ApoAI. The generally accepted hypothesis is that PON 1 was the main beneficiary in this association. Results showing the importance of HDL in the secretion and transport of PON 1, agreed with this.27 However, the relationship between the genotype and the HDL could also reflect an impact of PON 1 on the HDL, and is consistent with the independent associations of genotypes and enzyme activity in multivariate analyses. Indeed, the oxidation of ApoAI that can inhibit PON 129 disrupts the ability of the HDL to achieve cholesterol efflux from cells. This result was confirmed by Yildiz et al,28 who showed that hypertension was accompanied by an increase in the concentration of lipid hydroperoxides, total antioxidant status, and decreased enzymatic activity of arylesterase. Gaillard et al30 also demonstrated that the nature of the HDL in subjects who did not have diabetes could be responsible for increased cardiovascular events in the black patient population, despite a higher concentration of HDL. Our study relates paraoxonase activity to the polymorphism of its gene and to lipid concentrations in different groups of study.

We have demonstrated a consistently decreasing lag time from the control group to patients with diabetes and hypertension, regardless of the genotype in question, and this diminution related to the PON 1 activity. This result corresponds with that of Nishtha et al.14 In fact, the oxidation of LDL cholesterol in an endothelial area that PON 1 can inhibit is considered to be as an important step in the development of atherosclerosis.30

Smoking is among the parameters that may influence PON 1 activity. This result confirms other work from James et al31 that has shown that increased oxidative stress is one mechanism by which smoking exerts its deleterious influence on cardiovascular risk, although Sepahvand et al32 found that smoking did not have an influence on paraoxonase activity. But this author has worked with an Iranian population where smoking habits altered the results. Finally, we have found a decrease in PON 1 activity with aging. This result is consistent with that found by other authors.33 The relationship between PON 1 activity and oxidative stress could take place according to a reduction-of-oxygen mechanism involving cysteine residues of PON 1 for example, because a reduction in PON 1 activity under oxidative stress conditions has been demonstrated.23 In this case, the system of regeneration of the reduced form of the enzyme still needs to be determined.

Relations obtained between the genotype of PON 1, PON 1 enzyme activity and other cardiovascular risk factors suggest that the Gabonese population is at particularly high cardiovascular risk. However, it seems necessary to search the haplotype with the higher enzymatic activity, and link this haplotype with the incidence of cardiovascular complications and diabetes in this population before the PON 1 activity is included in the assessment of cardiovascular risk.

**Financial interest**

The authors declare that they have no competing financial interests.

**References**

1. World Health Organization. The atlas of heart disease and stroke. Geneva: World Health Organization; 2004.
2. Kontush A, Chapman J. Functionally defective high-density lipoprotein: a new therapeutic target at the crossroads of dyslipidemia, inflammation and atherosclerosis. Pharmacol Rev. 2006;58(3):342-374.
3. Costa LG, Giordano G, Furlong CE. Pharmacological and dietary modulators of paraoxonase 1 (PON1) activity and expression: the hunt goes on. Biochem Pharmacol. 2011;81(3):337-344.
4. Gupta N, Gli K, Singh S. Paraoxonases: structure, gene and polymorphism and role in coronary artery disease. Indian J Med Res. 2009;130(4):361-368.
5. Martinelli N, Micaglio R, Consoli L, et al. Low levels of serum paraoxonase activities are characteristic of metabolic syndrome and may influence the metabolic-syndrome-related risk of coronary artery disease. Exp Diabetes Res. 2009;2012(17):98.
New Product Launch: Dyna Gliclazide SR 30 mg

Pharma Dynamics is pleased to announce the launch of Dyna Gliclazide SR 30 mg (60’s), available at R39.00 (SEP excl. VAT). This represents a cost saving of 49% versus the originator gliclazide MR 30 mg, and is comparably priced to the leading gliplazide BD formulations.

- Dyna Gliclazide SR 30 mg’s once daily dosing makes it a compelling choice in sulphonylurea treatment and offers the following benefits:
  - Improved patient compliance*
  - Sustained 24 hour glycaemic control*

No negative impact on weight

Dyna Gliclazide SR 30 mg tablets are confidently prescribed in more than 25 countries in Europe, with more than 8,3 million packs sold in 2011.

Afzal Dhanasy
Product Manager: Cardiovascular
(021) 707 7000

*References available on request