FACTORS AFFECTING SERUM PARAOXONASE 1 ACTIVITY IN MIGRANT AND RESIDENT GUJARATI SOUTH ASIANS

Jeetesh Patel,[1,2] Rebecca Mirrielees,[2] Avni Vyas,[1] Kennedy Cruickshank,[1] Dorairaj Prabhakaran,[3] Deepak Bhatnagar,[1] Bharti Mackness,[1] K. Srinath Reddy,[3] Elizabeth Hughes,[4] Paul Durrington,[1] *Michael Mackness[1,5]

1. Clinical Epidemiology & Cardiovascular Research Groups, School of Biosciences, University of Manchester, Manchester, UK
2. School of Medicine, University of Nottingham, Nottingham, UK
3. Department of Cardiology, All India Institute of Medical Sciences, Delhi, India
4. Sandwell and West Birmingham Hospitals NHS Trust, West Bromwich, UK
5. AVDA Princip D'Espanya, Tarragona, Spain
**Correspondence to Mike.mackness@gmail.com*

Disclosure: No potential conflict of interest.
Received: 30.04.14 **Accepted:** 14.07.14
Citation: EMJ Cardiol. 2014;2:87-95.

ABSTRACT

Paraoxonase 1 (PON1) protects against the development of atherosclerosis by hydrolysing damaging lipid peroxides formed in low-density lipoprotein (LDL) and cell membranes. The effect of migration on PON1 activity is unknown. We have investigated the effect of migration on serum PON1 activity by comparing an Indian Gujarati community who had migrated to Sandwell (West Midlands, UK) with people still living in their villages of origin around the town of Navsari (Gujarat, North-West India) and determined biochemical and nutritional parameters which may correlate with PON1 activity. PON1 activity was almost double in men and women living in Sandwell compared to those in Navsari. In the Spearman's Rank correlation analysis, PON1 activity was significantly negatively correlated with fasting glucose and C-reactive protein, and positively with fasting non-esterified fatty acids, homeostasis model assessment (HOMA)-insulin sensitivity, and high-density lipoprotein (HDL) in rural Indian men, positively with HDL and apolipoprotein A1 (apo A1) in migrant Indian men, negatively with HOMA--cell activity and apo A1. It was positively correlated with HDL cholesterol, mean LDL particle diameter, and oxidised-LDL (ox-LDL) in rural Indian women and positively with HDL cholesterol, apo A1, and ox-LDL in migrant Indian women. Multivariate analysis with PON1 as the dependent variable indicated significant relationships with migrant status and HDL cholesterol only (both $p<0.001$). In conclusion, in the South Asian populations studied here, PON1 activity significantly correlated with measures of insulin sensitivity and the metabolic syndrome; however, by far the strongest determinant of PON1 activity was migration, or at least environmental and dietary changes which accompany migration. We also found an as yet unexplained lack of gender difference in HDL cholesterol, which requires further investigation.

Keywords: Paraoxonase 1, migration, South Asians, coronary heart disease (CHD).

INTRODUCTION

Coronary heart disease (CHD) is the leading cause of mortality amongst Indian migrants. High CHD rates are consistently reported for populations of Indian origin across the world.[1,2] Men and women in Britain whose families originated on the Indian sub-continent have approximately 40% higher morbidity and mortality from CHD than the British population of European descent.[3,4] In India, CHD

prevalence in cities reaches similar levels to that in migrants; however, CHD rates are believed to remain low in rural India.[5]

Established risk factors such as diabetes, serum cholesterol, smoking, hypertension, and obesity are major contributors to CHD in Indian populations.[2,5] However, most previous studies attempting to explain their increase in CHD on migration have compared them with populations in the locality to which they had moved. We hypothesised that migration would adversely affect established, and perhaps reveal, novel risk factors amongst migrants to Britain when compared with contemporaries from an identical cultural and genetic background, still resident in their villages of origin in rural India.[2] To this end we compared CHD risk factors in Gujarati Indians who had migrated to Sandwell, UK, with contemporaries still living in their villages of origin in the Gujarat. We found that increased fat intake and obesity in Sandwell migrants explained the much higher prevalence of established and novel CHD risk factors found in this population.[2]

The paraoxonase (PON) multigene family comprises three members: PON1, PON2, and PON3.[6] The genes for all three members of the family are widely expressed in mammalian tissues;[7] however, PON1 and PON3 are predominantly located in the plasma associated with high-density lipoprotein (HDL) while PON2 is not found in the plasma but has a wide cellular distribution.[8] PON1, PON2, and PON3 all retard the proatherogenic oxidative modification of low-density lipoprotein (LDL) and cell membranes, and are therefore considered to be antiatherogenic.[9] PON1 is now considered to be a major factor in the antioxidative activity of HDL.[10]

The transgenic expression of human PON1, PON2, or PON3 in various mouse models of atherosclerosis (AS) has been shown to retard or reverse AS by mechanisms which include a reduction in circulating and aortic oxidised-LDL (ox-LDL), a reduction in macrophage oxidative stress and foam cell formation, an increase in reverse cholesterol transport, and a normalisation of endothelial function.[11-15]

Several studies have previously shown prospectively that PON1 activity is a risk factor for CHD development independently of HDL concentration,[16-19] although the finding is not universal.[20,21] Several case control studies have shown low PON1 to be a risk factor for CHD in various Indian populations resident on the sub-continent;[22-24] however, nothing is known regarding the effect of migration on serum PON1. The purpose of the present investigation was therefore to determine the effect of migration on serum PON1 and its relationship to other CHD risk factors in migrant and resident Gujaratis.

MATERIALS AND METHODS

Study Design, Setting, and Participants

This was a cross-sectional study of an Indian Gujarati community who had migrated to Sandwell (West Midlands, UK) from rural villages around the town of Navsari (Gujarat, North-West India) with age, gender, and caste matched contemporaries still living in those villages in India, as previously described in detail.[2] Prospective participants were contacted by letter. Clinic sessions in the UK and India ran between September 1998 and September 2001. All participants in the study gave informed consent. Ethical approval for the study was obtained both in Sandwell and Gujarat by the respective local research ethics committees.

Variables

Participants were invited to attend clinics fasting (from 10:00 pm the previous evening); trained fieldworkers administered standard, pretested questionnaires on lifestyle (including smoking and drinking habit), medical history, and place of origin. Venous blood was collected from all participants. Serum and plasma were obtained within 1 hour by centrifugation and snap-frozen to be stored at -70 °C. Venous blood samples were collected fasting and at 120 minutes after the administration of a 75 g oral glucose challenge. Waist measurements were taken (after removing upper clothing) as the narrowest circumference above the umbilicus and below the rib. The hip was measured over light clothing as the widest circumference around the buttocks. Waist and hip measurements were taken non-consecutively in duplicate (repeated where there were differences >2%). Body mass was measured on Seca 870 solar scales (Seca Ltd., Birmingham, UK). A Leicester standard rule (Seca Ltd.) was used to determine the height of the subject. Blood pressure (BP) was measured three times (using the mean of the last two for analysis), with the validated semi-automatic Omron HEM-705CP (Omron Healthcare Europe, Mannheim, Germany) with appropriate cuff sizes, after more than 5 minutes seated.

Laboratory Analyses

Venous blood was collected in EDTA from all participants and was analysed for a full blood count using haematology analysers at either the clinical haematology department, Sandwell Hospital, UK (STAK S, Beckman Coulter Corp., Hialeah, FL, USA), or the Mankodi Laboratory, India (Bayer Advia, Bayer Diagnostics, Baroda, India). Plasma glucose was determined by a glucose oxidase method using automated biochemistry analysers at Sandwell and Navsari.

Plasma and serum aliquots were stored at -70 °C and transported by air, frozen in liquid nitrogen or dry ice, from India to the UK, and analysed as a single batch for serum cholesterol (CHOD-PAP method), triglycerides (GPO-PAP method), HDL cholesterol (direct method) (ABX diagnostics, Montpellier, France), and apolipoproteins (apo) A1 and B by turbidimetry and nephelometry respectively (ABX diagnostics), all on a Cobas Mira autoanalyser (Roche, Welwyn Garden City, UK). Serum non-esterified fatty acids (NEFA) were measured by enzymatic colorimetry (WAKO chemicals, Alpha Laboratories, Eastleigh, UK). Ox-LDL was determined using a sandwich enzyme-linked immunosorbent assay technique involving a two-site immunoassay (Mercodia, Uppsala, Sweden).[25] Plasma fibrinogen and C-reactive protein (CRP) were measured by immunonephelometry on a Dade Behring BNII autoanalyser (Milton Keynes, UK). Serum B12 and homocysteine were measured by chemiluminescent immunoassay using the Access immunoassay system (Beckman Instrument Inc., Fullerton, CA, USA). Insulin was measured by immunoassay using charcoal extraction, and pancreatic β cell function and insulin sensitivity were calculated by the homeostasis model assessment (HOMA). In young, lean, and healthy reference subjects, β cell function (HOMA B) is 100% and insulin sensitivity (HOMA S) equals 1. LDL particle subclasses were determined using nondenaturing gradient gel electrophoresis.[26] Paraoxonase activity - determined by the rate of generation of *p*-nitrophenol - was determined at 405 nm, 25 °C, with the use of a continuously recording spectrophotometer (described in detail elsewhere).[27]

PON1 analysis and other biochemical analyses on frozen plasma and serum were performed in 'batch' following the recruitment of the final subjects (India and the UK) and the associated transportation of plasma samples to our UK laboratory in December 2002. Batch analysis was conducted over a period of 3 months.

Study Size, Bias, and Confounding

Of a total of 814 eligible subjects invited for the original study, 242 were recruited from the UK (67% response rate) and 294 from India (65% response rate). The required number of subjects to observe a statistically significant ($p<0.05$) correlation coefficient of at least 0.35, using a two-sided test with a power of 90%, was 81. To control for confounding variables, subsets by gender and site were developed.

Statistical Analysis

Data were analysed in SPSS v14 (SPSS Inc., Chicago, IL, USA.) using standard and non-parametric tests and Kolmogorov-Smirnov normality plots. Parametric data are presented as mean (standard deviation [SD]) and non-parametric data as medians (interquartile range [IQR]). Comparisons were made by T-test or Mann-Whitney test, as appropriate. Univariate analysis of correlations was reported with Spearman's rank correlation coefficients. Linear regression models were calculated to test the strength of association - beta (95% CI) from independent predictors. The standardised beta coefficients presented allowed a direct comparison (along a scale of 0-1) of the strength of each association within the model.

RESULTS

The demographic and biochemical characteristics of the study populations are given in Tables 1 and 2. Men and women living in Sandwell had increased body mass index (by 6 [4.5–7.4] kg/m^2 mean [95% CI]), waist circumference, systolic and diastolic blood pressure, and hypertension compared to those living in Navsari but smoked less and drank more. The prevalence of CHD presented in Table 1 also shows non-significantly higher levels in migrants compared to rural contemporaries; men and women living in Sandwell had significantly higher total cholesterol, triglyceride, apo B, apo A1, haemoglobin, fibrinogen, folate, vitamin B12, insulin, and HOMA B than those living in Navsari, but significantly lower homocysteine and HOMA S. Women in Sandwell had significantly higher HDL, iron, NEFA, and CRP than women in Navsari, but lower fasting plasma glucose and ox-LDL. PON1 activity was almost double in Sandwell men and women (Table 2).

Table 1: Demographic details of the study populations (data are mean [SD] or number [percentage]).

Characteristics of Indian Gujaratis	Men				Women				p
	Navsari (n=139)		Sandwell (n=119)		Navsari (n=155)		Sandwell (n=123)		
Age (Years)	49.1	(14.6)	49.0	(12.8)	48.5	(14.0)	49.2	(11.5)	0.18
BMI (kg/m^2)	21.0	(3.9)	25.9	(3.9)	20.8	(4.1)	26.6	(4.9)	<0.001
Waist circumference (cm)	80.0	(11.5)	91.3	(12.8)	72.9	(11.1)	82.3	(10.6)	<0.001
Systolic BP (mmHg)	121	(20)	135	(20)	111	(22)	121	(21)	<0.001
Diastolic BP (mmHg)	74.8	(11.7)	83.7	(10.5)	68.9	(10.6)	75.6	(10.5)	<0.001
Alcohol use	81	(58.3)	93	(78.2)	1	(0.6)	37	(30.1)	<0.001
Ever smoker	81	(58.3)	28	(23.5)	7	(4.5)	1	(0.8)	<0.001
Diabetes	24	(17.4)	19	(16.7)	17	(10.8)	18	(16.1)	0.36
OHT	13	(9.4)	10	(8.4)	5	(3.2)	4	(3.25)	0.005
Insulin therapy	1	(0.7)	1	(0.8)	2	(1.3)	0		0.09
Hypertension	33	(23.6)	53	(44.2)	28	(18.0)	39	(31.7)	<0.001
AHT	12	(8.6)	16	(13.4)	11	(7.1)	16	(13.1)	0.21
CHD	13	(3.4)	13	(10.9)	9	(5.8)	11	(8.9)	0.29
Stroke	1	(0.7)	2	(1.6)	0		3	(2.4)	0.02
Statin therapy	0		14	(11.8)	1	(0.6)	4	(3.3)	<0.001

SD: standard deviation; p: probability; BMI: body mass index; BP: blood pressure; OHT: oral hypoglycaemic therapy; AHT: antihypertensive therapy; CHD: coronary heart disease.

In Spearman's correlation analysis (Table 3) PON1 activity had a significant negative correlation with fasting glucose and CRP, and positive with fasting NEFA, HOMA S, and HDL in rural Indian men; positively with HDL and apo A1 in migrant Indian men; negatively with HOMA B and apo A1, and positively with HDL, mean LDL particle diameter, and ox-LDL in rural Indian women; and positively with HDL, apo A1, and ox-LDL in migrant Indian women.

Spearman's analysis of correlations of ox-LDL indicated a plethora of correlations; however, on multivariate analysis, only total cholesterol (p<0.001), haemoglobin (p=0.012), and HDL (p=0.02) remained significant (Table 4). Multivariate analysis with PON1 as the dependent variable indicated significant relationships with migrant status and HDL only (both p<0.001). Variables included in the multivariate models were repeated with an exclusion of known diabetics and statin therapy, where PON1 remained independently related to HDL and migrant status (p<0.001).

DISCUSSION

In this population of South Asians, PON1 activity correlated with a number of parameters related to insulin resistance (IR) and the metabolic syndrome (MetS), as has been described previously.[28] Recently it has been shown that human PON1 can prevent diabetes mellitus development in mice through its antioxidant properties and the stimulation of beta-cell insulin release, suggesting a possible role for PON1 in insulin biosynthesis.[29,30] PON2 also has an important role in hepatic insulin signalling[31] which may suggest a role for the PON family in energy metabolism that requires further investigation. PON1 activity in Type 1 diabetes is inversely correlated with blood glucose levels; also PON1 is lower in subjects with MetS, suggesting modulation of PON1 by factors associated with IR.[32] Similar results have recently been reported in Type 2 diabetes adding further support to this theory, but which requires more detailed molecular analysis. *In vitro* studies have indicated that the

PON1 gene is upregulated by high glucose concentrations in HepG2 hepatocytes.[33] However, PON1 is extremely susceptible to oxidative inactivation,[34] and the high levels of oxidative stress which accompany hyperglycaemia[35] may well counteract increased hepatic PON1 production. The association of PON1 with duration of diabetes, which has been reported in T2DM,[28] may also be explained by this mechanism.

The negative relationship between PON1 activity and IR offers interesting possibilities. The relationship between IR, MetS, and the subsequent progression to Type 2 diabetes could indicate that the measurement of PON1 activity may provide an early indicator of metabolic disturbances before the onset of measurable arterial changes. Further work in this area is warranted.

Table 2: Biochemical parameters of the study populations (data are mean [SD] or median and interquartile range).

	Rural Indian men	Migrant Indian men	*Differences between men (p)*	Rural Indian women	Migrant Indian women	*Differences between women (p)*
Plasma glucose (mmol/l)	5.81	-2.38	5.6	-1.8	0.81	5.41
Plasma insulin (mU/l)	8.73	(5.95, 14.10)	10.3	(7.61, 14.66)	<0.001	9.05
HOMA B (%)	103	-59,167	124	-90,198	<0.001	111
HOMA S (0-1)	0.481	(0.277, 0.809)	0.402	(0.282, 0.571)	0.002	0.521
NEFAs (mmol/l)	0.358	-0.271	0.438	(0.220)	0.027	0.269
Serum cholesterol (mmol/l)	4.84	-1.03	5.34	-1.01	<0.001	4.87
Serum triglycerides (mmol/l)	0.82	(0.64, 1.19)	1.15	(0.93, 1.57)	<0.001	0.78
HDL cholesterol (mmol/l)	1.17	-0.41	1.23	-0.31	0.51	1.15
Apo B (g/l)	0.98	-0.3	1.15	-0.29	<0.001	0.95
Apo A1 (g/l)	1.42	-0.41	1.55	-0.33	<0.001	1.48
Homocysteine (µmol/l)	15.8	(11.1, 21.9)	10.4	(7.9, 13.6)	<0.001	12.8
Serum folate (mg/l)	3.1	(2.50, 4.90)	4.9	(3.75, 6.00)	<0.001	4.15
Serum vitamin B12 (µg/ml)	148	-112, 195	181	-144, 234	0.001	142
hs-CRP (g/l)	1.17	(0.56, 2.86)	1.06	(0.69, 2.50)	0.65	0.83
PON-1 (nmol/min/ml)	140	(88, 180)	229	(186, 293)	<0.001	123
Oxidised LDL (U/l)	39	(29.0, 51.0)	41.9	(29.0, 52.0)	0.54	33

SD: standard deviation; HOMA: homeostasis model assessment; HOMA B: β-cell function; HOMA S: insulin sensitivity; NEFA: non-esterified fatty acid; HDL: high-density lipoprotein; Apo: apolipoprotein; hs-CRP: high-sensitivity C-reactive protein; PON1: paraoxonase 1 activity; LDL: low-density lipoprotein.

Table 3: Spearman's rank correlation analysis of paraoxonase 1 activity.

	Rural Indian men		Migrant Indian men		Rural Indian women		Migrant Indian women	
	R	p	R	p	R	p	R	p
Fasting glucose	-0.279	0.001						
2 hr PCG	-0.308	0.005						
Fasting NEFA	0.318	0.002						
2 hr post-challenge NEFA								
HOMA S	0.393	0.001						
HOMA B						-0.213	0.033	
HDL cholesterol	0.261	0.014	0.236		0.027	0.308	0.001	0.322
ApoB								
Apo A1			0.262	0.014		-0.269	0.003	0.37
Mean LDL particle diameter						0.221	0.017	
Ox LDL						0.182	0.05	0.272
hs-CRP	-0.295	0.005						

R: Spearman's rank correlation coefficient; PCG: post-challenge glucose; NEFA: non-esterified fatty acid; HOMA: homeostasis model assessment; HOMA S: insulin sensitivity; HOMA B: β-cell function; HDL: high-density lipoprotein; Apo: apolipoprotein; LDL: low-density lipoprotein; Ox: oxidised; hs-CRP: high-sensitivity C-reactive protein.

Table 4: Multivariate analysis of paraoxonase 1 activity and oxidised low-density lipoprotein.

Multivariate models	β (95% CI)			p
1. Dependent variable: Ox LDL (U/l)				
Serum cholesterol (mmol/l)	8	5.3	10.7	<0.001
Haemoglobin (μmol/l)	1.7	0.38	3.02	0.012
HDL cholesterol (mmol/l)	-10.8	-19.9	-1.7	0.02
2. Dependent variable: PON1 (nmol/min/ml)				
Migrant status	0.563	0.405	0.721	<0.001
HDL cholesterol (mmol/l)	0.489	0.259	0.72	<0.001

Ox: oxidised; LDL: low-density lipoprotein; HDL: high-density lipoprotein; PON1: paraoxonase-1 activity.

The increase in HDL-cholesterol (C) on migration, which we have reported previously,[2] is most probably due to the shift from a high carbohydrate diet in India to a high fat diet in the UK, leading to increased energy in the diet.[2] There was also a lack of gender differences in HDL-C concentration in either Navsari or Sandwell (and apo A1 in Sandwell). This is unusual as it is generally accepted that women have higher HDL-C than men, at least until the menopause.[36] Only 48% of the female study participants answered the question as to whether they were menopausal or not. Of those that did, 85 answered yes and 49 no. The expected differences in HDL-C were found: 1.11±0.26 mmol/l with menopause and 1.28±0.33 mmol/l without (p<0.001). It would, therefore, appear that the

lack of gender differences is due to higher than expected HDL-C in the males. The lack of gender differences in HDL-C are not explained by plasma testosterone or sex hormone binding globulin levels and require further investigation and confirmation.

Perhaps the most striking observation from this study is the relationship between PON1 activity and migrant status. PON1 activity was almost double in the Sandwell population compared to those in Navsari in both men (229 [186, 293] versus 140 [88, 180] nmol/minute/ml p<0.001) and women (216 [169, 229] versus 123 [75, 172] nmol/minute/ml p<0.001). Activity levels in Sandwell were more comparable to those reported in other European-based populations.[37] The most likely explanation for this rise in PON1 activity on migration, given that neither HDL-C or apo A1 increased to the same proportion, is that the rise in PON1 is a response to the increased blood glucose and the rise in lipids which, in turn, is likely to be due to the increase in fat and energy in the diet. This response may be to protect against an increase in oxidation and glycation due to the increased serum lipids and glucose. These findings suggest environmental rather than genetic factors to be responsible. However, as the samples were not able to be PON1 genotyped, we cannot exclude genetic differences between the two populations.

It has been found that paraoxonase functional activity is partially dependent on genotype.[38] The PON1 gene at 7q21.3 encodes both paraoxonase and arylesterase activity, and is subject to a number of polymorphisms.[39,40] Two alleles, the PON1-192 Q-isoform and PON1-192 R-isoform, have been identified as the chief determinants of paraoxonase activity.[41,42] This results in the inheritance of two PON1-192R high-activity alleles, two PON1-192Q low-activity alleles or heterozygous codominant inheritance of both, and consequently three distinct phenotypes for paraoxonase activity.[43,44] Although encoded for and by the same gene, there is no significant evidence demonstrating that arylesterase activity is affected by the PON1 genotype. It could be suggested that measurement of arylesterase activity would, therefore, produce more reliable results; however, it is not yet fully understood whether the rate of paraoxon hydrolysis affects the rate of phenyl acetate hydrolysis by a single enzymatic molecule. It was neither possible to genotype the sample populations of this study, nor to measure arylesterase activity. A further study to analyse phenotypes is needed, given that data on paraoxon as substrate alone places an important limitation on our conclusions.

Furthermore, a number of inflammatory cytokines, oxidative stress (downregulators), hypolipaemic drugs, and polyphenols such as quercetin, resveratrol, and punicalagins (upregulators) are also known to affect PON1 gene expression. This is through a variety of mechanisms including sterol regulatory element binding protein 2 and Sp1 binding to the PON1 promoter, peroxisome proliferator-activated receptor α and γ activation, stimulation of the aryl hydrocarbon receptor, and activation of c-Jun N-terminal kinase and cAMP protein kinase A signalling cascades.[45] Differences in any of these effectors in the Navsari and Sandwell populations could be responsible for the activity of differences found.

Differences in oxidative stress, a known inhibitor of PON1 activity, could also contribute.[34] The Navsari population was deficient in folate and vitamin B12 leading to increased plasma homocysteine. Homocysteine is further metabolised to homocysteine thiolactone (HTL) a potent inducer of cardiovascular disease and a natural substrate of PON1.[46] PON1 is pivotal in preventing the proatherogenicity of HTL, but HTL can also modify PON1 by N-homocysteinylation[46] inhibiting its activity, and it is conceivable that in the Navsari population, this has happened.

More recently, patients with anaemia, caused by vitamin B12 deficiency, were found to have significantly lower PON1 activity than healthy controls, or than in patients with iron deficient anaemia.[47] There was a statistically significant correlation between serum vitamin B12 concentration and PON1 activity (which was not the case in our study). PON1 activity was restored to the levels found in healthy controls after vitamin B12 therapy. Therefore, the lower PON1 activity found in the Navsari population could be secondary to the vitamin B12 deficiency found in this population. Further studies in this area are warranted.

In conclusion, in the South Asian populations studied here, PON1 activity significantly correlated with measures of insulin sensitivity and the MetS; however, by far the strongest determinant of PON1 activity was migration, or at least environmental and dietary changes which accompany migration. To reinforce our findings, a study incorporating

genotyping the PON1 gene will need to be conducted. We also found higher levels of HDL amongst migrant females relative to rural contemporaries, which could be due to alcohol intake, and also an (as yet) unexplained lack of gender difference in HDL-C, which requires further investigation.

Acknowledgements

This work was supported by project grants from the British Heart Foundation, the Sandwell Hospital NHS Trust, and the Lipid Diseases Fund, part of the Endowment Fund of the Central Manchester Healthcare Trust.

REFERENCES

1. Anand SS et al. Differences in risk factors, atherosclerosis, and cardiovascular disease between ethnic groups in Canada: the Study of Health Assessment and Risk in Ethnic Groups (SHARE). Lancet. 2000;356(9226):279-84.

2. Patel JV et al. Impact of migration on coronary heart disease risk factors: comparison of Gujaratis in Britain and their contemporaries in villages of origin in India. Atherosclerosis. 2006;185(2): 297-306.

3. Cruickshank JK et al. Heart attack, stroke, diabetes, and hypertension in West Indians, Asians, and whites in Birmingham, England. Br Med J. 1980;281(6248):1108.

4. Balarajan R. Ethnicity and variations in the nation's health. Health Trends. 1995-1996;27(4):114-9.

5. Collins VR et al. Non-insulin-dependent diabetes and 11-year mortality in Asian Indian and Melanesian Fijians. Diabet Med. 1996;13(2):125-32.

6. Primo-Parmo SL et al. The human serum paraoxonase/arylesterase gene (PON1) is one member of a multigene family. Genomics. 1996;33(3):498-507.

7. Rodríguez-Sanabria F et al. Tissue distribution and expression of paraoxonases and chemokines in mouse: the ubiquitous and joint localisation suggest a systemic and coordinated role. J Mol Histol. 2010;41(6):379-86.

8. Mackness B et al. The paraoxonase gene family and coronary heart disease. Curr Opin Lipidol. 2002;13(4):357-62.

9. Reddy ST et al. Is it just paraoxonase 1 or are other members of the paraoxonase gene family implicated in atherosclerosis? Curr Opin Lipidol. 2008;19(4):405-8.

10. Mastorikou M et al. Defective metabolism of oxidised phospholipid by HDL from people with type 2 diabetes. Diabetes. 2006;55(11):3099-103.

11. Rozenberg O et al. Paraoxonase 1 (PON1) attenuates macrophage oxidative status: studies in PON1 transfected cells and in PON1 transgenic mice. Atherosclerosis. 2005;181(1):9-18.

12. Mackness B et al. Human paraoxonase-1 overexpression inhibits atherosclerosis in a mouse model of metabolic syndrome. Arterioscler Thromb Vasc Biol. 2006;26(7):1545-50.

13. Guns PJ et al. Paraoxonase 1 gene transfer lowers vascular oxidative stress and improves vasomotor function in apolipoprotein E-deficient mice with pre-existing atherosclerosis. Br J Pharmacol. 2008;153(3):508-16.

14. Ng CJ et al. Adenovirus mediated expression of human paraoxonase 2 protects against the development of atherosclerosis in apolipoprotein E-deficient mice. Mol Genet Metab. 2006;89(4):368-73.

15. Ng CJ et al. Adenovirus-mediated expression of human paraoxonase 3 protects against the progression of atherosclerosis in apolipoprotein E-deficient mice. Arterioscler Thromb Vasc Biol. 2007;27(6):1368-74.

16. Mackness B et al. Low paraoxonase activity predicts coronary events in the Caerphilly Prospective Study. Circulation. 2003;107(22):2775-9.

17. Bhattacharyya T et al. Relationship of paraoxonase1(PON1)genepolymorphisms and functional activity with systemic oxidative stress and cardiovascular risk. JAMA. 2008;299(11):1265-76.

18. van Himbergen TM et al. Paraoxonase (PON1) and the risk for coronary heart disease and myocardial infarction in a general population of Dutch women. Atherosclerosis. 2008;199(2):408-14.

19. Ikeda Y et al. Low human paraoxonase predicts cardiovascular events in Japanese patients with type 2 diabetes. Acta Diabetol. 2009;46(3):239-42.

20. Troughton JA et al. Paraoxonase activity and coronary heart disease risk in healthy middle-aged males: the PRIME study. Atherosclerosis. 2008;197(2): 556-63.

21. Birjmohun RS et al. Both paraoxonase-1 genotype and activity do not predict the risk of future coronary artery disease; the EPIC-Norfolk Prospective Population Study. PLoS One. 2009;4(8):e6809.

22. Pati N, Pati U. Paraoxonase gene polymorphism and coronary artery disease in Indian subjects. Int J Cardiol. 1998;66(2):165-8.

23. Gupta N et al. Paraoxonase 1 (PON1) polymorphisms, haplotypes and activity in predicting cad76 risk in North-West Indian Punjabis. PLoS One. 2011;6(5):e17805.

24. Gupta N et al. Low serum PON1 activity: an independent risk factor for coronary artery disease in North-West Indian type 2 diabetics. Gene. 2012;498(1):13-9.

25. Hulthe J, Fagerberg B. Circulating oxidized LDL is associated with subclinical atherosclerosis development and inflammatory cytokines (AIR Study). Arterioscler Thromb Vasc Biol. 2002;22(7):1162-7.

26. Nichols AV et al. Nondenaturing polyacrylamide gradient gel electrophoresis. Methods Enzymol. 1986;128:417-31.

27. Abbott CA et al. Serum paraoxonase activity, concentration, and phenotype distribution in diabetes mellitus and its relationship to serum lipids and lipoproteins. Arterioscler Thromb Vasc Biol. 1995;15(11):1812-8.

28. Mackness B et al. Paraoxonase-1 is not associated with coronary artery calcification in type 2 diabetes: results from the PREDICT study. Dis Markers. 2012;33(2):101-12.

29. Rozenberg O et al. Paraoxonase 1 (PON1) attenuates diabetes development in mice through its antioxidative properties. Free Radic Biol Med. 2008;44(11):1951-9.

30. Koren-Gluzer M et al. The antioxidant HDL-associated paraoxonase-1 (PON1) attenuates diabetes development and stimulates β-cell insulin release. Atherosclerosis. 2011;219(2):510-8.

31. Bourquard N et al. Impaired hepatic

insulin signalling in PON2-deficient mice: a novel role for the PON2/apoE axis on the macrophage inflammatory response. Biochem J. 2011;436(1):91-100.

32. Kordonouri O et al. Modulation by blood glucose levels of activity and concentration of paraoxonase in young patients with type 1 diabetes mellitus. Metabolism. 2001;50(6):657-60.

33. Ikeda Y et al. High glucose induces transactivation of the human paraoxonase 1 gene in hepatocytes. Metabolism. 2008;57(12):1725-32.

34. Aviram M et al. Human serum paraoxonase (PON1) is inactivated by oxidised low density lipoprotein and preserved by antioxidants. Free Radic Biol Med. 1999;26(7-8):892-904.

35. Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res. 2010;107(9):1058-70.

36. Kwok S et al. Progestogens of varying androgenicity and cardiovascular risk factors in postmenopausal women receiving oestrogen replacement therapy. Clin Endocrinol (Oxf). 2004;61(6):760-7.

37. Mackness B et al. Paraoxonase activity in two healthy populations with differing rates of coronary heart disease. Eur J Clin Invest. 2000;30(1):4-10.

38. La Du BN et al. An improved method for phenotyping individuals for the human serum paraoxonase arylesterase polymorphism. Ann Biol Clin (Paris). 1986;44(4):369-72.

39. Camps J et al. Serum paraoxonase-1 activity and genetic polymorphisms: common errors in measurement and interpretation of results. Clin Chem Lab Med. 2010;48(6):893–4.

40. La Du BN, Eckerson HW. The polymorphic paraoxonase/arylesterase isozymes of human serum. Fed Proc. 1984;43(8):2338-41.

41. Humbert R et al. The molecular basis of the human serum paraoxonase activity polymorphism. Nat Genet. 1993;3(1):73–6.

42. Adkins S et al. Molecular basis for the polymorphic forms of human serum paraoxonase/arylesterase: glutamine or arginine at position 191, for the respective A or B allozymes. Am J Hum Genet. 1993;52(3):598–608.

43. Eckerson HW et al. The human serum paraoxonase/arylesterase polymorphism. Am J Hum Genet. 1983;35(6):1126–38.

44. Furlong CE et al. Role of genetic polymorphism of human plasma paraoxonase/arylesterase in hydrolysis of the insecticide metabolites chlorpyrifos oxon and paraoxon. Am J Hum Genet. 1988;43(3):230-8.

45. Schrader C, Rimbach G. Determinants of paraoxonase 1 status: genes, drugs and nutrition. Curr Med Chem. 2011;18(36):5624-43.

46. Jakubowski H. The role of paraoxonase 1 in the detoxification of homocysteine thiolactone. Adv Exp Med Biol. 2010;660:113-27.

47. Koc A et al. Paraoxonase and arylesterase activities in children with iron deficiency anemia and vitamin B12 deficiency anemia. Pediatr Hematol Oncol. 2012;29(4):345-53.