CIRCULATING HAEMOGLOBIN LEVELS AND THE RISK OF ATHEROSCLEROSIS IN ASIAN INDIAN POPULATIONS

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ABSTRACT

Background: The global burden of coronary heart disease (CHD) is estimated to be the highest on the Indian subcontinent. The pathophysiology of this increased risk is complex, multifactorial, and its magnitude increases with migration from India to Britain. Haemoglobin disorders, which also frequent this ethnic group, have been linked to cardiovascular disease. We investigated the impact of migration and nutritional intake on haematological parameters amongst South Asians, with a focus on their relation to molecular indices of oxidative atherogenesis.

Methods: Haematology, diet, oxidised low-density lipoprotein (LDL), and serum paraoxonase activity were measured in 230 migrant Indian Gujaratis (Britain), and 305 matched contemporaries living in rural villages (India).

Results: Median levels of haemoglobin were higher amongst migrant men (14.5 μmol/l) compared to rural men (15.0 μmol/l, P=0.004) and higher in migrant women (12.7 μmol/l) compared to rural women (11.8 μmol/l, P<0.001). Irrespective of site, haemoglobin levels in South Asians were positively associated with high blood pressure, high serum cholesterol, low high density lipoprotein (HDL) cholesterol levels, and increased CHD risk scores (P<0.001). Haemoglobin concentrations were higher amongst migrants compared to rural contemporaries (P<0.001). In rural women, red cell volume was lower, and co-ordinated with lower levels of oxidised LDL compared with migrant women (P<0.001). On multivariate analysis, haemoglobin was independently associated with oxidised LDL (P=0.001) and paraoxonase activity (P=0.025).

Conclusion: Levels of haemoglobin were independently associated with indices of atherogenesis in our populations of rural and migrant Indians. Iron availability may underline the pathogenesis for the oxidative modification of LDL in this group.

Keywords: Haemoglobin, paraoxonase, oxidised LDL, South Asian, dietary iron, coronary heart disease.

INTRODUCTION

Rates of coronary heart disease (CHD) mortality are disconcerting amongst South Asians. The burden of CHD amongst people originating from the Indian subcontinent is estimated to be the highest worldwide,[1] and there is a markedly earlier presentation of disease in this population.[2] Attention to this threat of CHD stems from the excessive disease rates reported in migrant South Asian populations,[3,4] including those in Western countries[5] where CHD is indigenously high.

Changes in diet and lifestyle promoted by the host environment (acculturation) following migration is likely to play an important role in the increased risk.[6] However, despite this higher risk, cardiovascular events in South Asians occur at clinically insignificant levels of classic CHD risk factors, as inferred from Western Societies.[7,8] For example, absolute levels of low-density lipoprotein (LDL) cholesterol are observed to be the lowest in South Asian populations compared with groups from other countries, and at a given level of LDL, the risk of CHD is higher in this group.[9] The pathophysiology of this increased CHD risk amongst South Asians is unclear and it persists in the wake of conventional antihypertensive and lipid-lowering therapy.[10]

We have reported that CHD risk amongst South Asians resident in rural India, and their contemporaries who have migrated to Britain, is underpinned by glucose intolerance, nutritional deficiency, and protein toxicity; specifically, low levels of serum folate and vitamin B_{12} and raised plasma homocysteine,[6] which are also common to other South Asian populations elsewhere.[11,12] Such a phenotype is intuitive of aberrant and increased erythropoiesis,[13-15] which may reflect the antecedents that many South Asians share for diabetes[16] and haemoglobinopathies.[17] Haemoglobin concentrations amongst South Asians may play an important role in the pathogenesis of CHD in this group, supporting established and emerging mechanisms for disease.[18] Proof of concept is supported by observations that (i) the oxidative susceptibility of LDL is increased and the protective activity of high-density lipoprotein (HDL) is reduced in thalassaemia,[19] (ii) aberrant haemoglobin metabolism can oxidise LDL,[20] and (iii) haemoglobin abnormalities in South Asians are associated with atherosclerotic disease.[21]

With an aim to understand the pathophysiology of heightened CHD risk amongst South Asians, we investigated indices of erythropoietic activity and haemoglobin levels and their relation with CHD risk. We hypothesised that an increasing gradient of haemoglobin and altered erythropoiesis facilitates an atherogenic lipid profile, increasing the oxidation of LDL and sequestration of the biological antioxidant action of HDL (paraoxonase activity).[22] In addition, we looked at the impact of the dietary transition between rural Indians and migrant contemporaries on this proposed mechanism of increased CHD risk.

METHODS

We compared a Gujarati community who had migrated to Sandwell (West Midlands, UK) from rural villages around Navsari (Gujarat, North-West India) with age, gender, and caste-matched contemporaries still living in those villages in India, as previously described in detail.[6] The ethnicity of both migrant and rural cohorts was exclusively Gujarati Indian, specifically those originating from the Kantha, near Navsari in Gujarat. Randomly sampled participants from electoral rolls were invited to clinic sessions (between 1997 and 2002) that started with venepuncture (fasting), the completion of a lifestyle questionnaire, and an analysis of dietary intake. All participants in the study gave informed consent. Ethical approval for the study was obtained both in Sandwell and Gujarat from the respective local research ethics committees.

Laboratory Methods

Details relating to the separation, storage, and transport of blood, and the analysis of lipids, lipoproteins, insulin, C-reactive protein, and homocysteine are detailed elsewhere.[6] Between 1997 and 2002 venous blood was collected in ethylenediaminetetraacetic acid (EDTA) from all participants and was analysed for a full blood count using haematology analysers at either the Clinical Haematology Department, Sandwell Hospital, West Bromwich, UK (STAK S, Beckman Coulter Corp., Hialeah, Florida, USA) or the Mankodi Laboratory, India (Bayer Advia, Bayer Diagnostics, Baroda, India). Oxidised LDL was determined using a sandwich enzyme-linked immunosorbent technique involving a two-site immunoassay (Mercodia, Uppsala, Sweden)[23] on EDTA plasma. Commercial and in-house quality controls were used and the intra-assay coefficient of variation (CV) was <8%, while the inter-assay CV was <7%. The limit of detection was 1 mU/l. 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).[24]

Key outcome measures were atherogenic indices of lipid metabolism (oxidised LDL, paraoxonase activity) and haemoglobin levels. Other cardiovascular outcome measures included C-reactive protein, serum lipids, and lipoproteins (total cholesterol, triglycerides, HDL cholesterol,

apolipoproteins A1 and B), anthropometry (body mass index [BMI], waist girth), glucose tolerance, blood pressure, and serum insulin. Comprehensive data were available in 228 participants from Sandwell and 285 from Navsari.

Cardiovascular Risk Estimation

The absolute risk (%) of developing non-fatal CHD or coronary death over the next 10 years was estimated using the algorithm derived from the Framingham Heart Study (based on the risk factors age, gender, smoking status, systolic blood pressure, total cholesterol levels, HDL cholesterol, left ventricular hypertrophy, and diabetes status).[25]

Power Calculation and Statistical Analysis

The power calculation for this study was not *a priori* and was done retrospectively. We hypothesised a relationship between haemoglobin with atherogenic indices of lipid metabolism. For a statistically significant (P<0.05, 2-sided) correlation coefficient 'r' (at least 0.20), with a power of 80%, 193 subjects were needed. Data were analysed in SPSS modeler and data mining v14 (SPSS Inc., Chicago, Illinois, USA) using standard and non-parametric tests and Kolmogorov-Smirnov normality plots. Central tendencies and variation for parametric data are presented as mean (standard deviation) or median (interquartile range [IQR]) for non-parametric data. Comparisons were made by T-test or Mann-Whitney U test, as appropriate. Univariate analysis of the association of haemoglobin and blood counts with cardiovascular risk factors was reported with Spearman rank correlation coefficients (r). Partial correlation analysis (two-tailed) was used to adjust the effects of gender and site for bivariate analysis among all subjects. Those factors that were significantly associated with haemoglobin on univariate analysis were selected for multivariate analysis. Linear regression models were calculated to test the strength of association - beta (95% confidence interval [CI]) from independent predictors of oxidative modification of lipoprotein indices. The beta coefficients presented allowed direct comparison (along a scale of 0-1) of the strength of each association within the model.

Table 1A: Concentrations of haemoglobin amongst migrant Indians living in the UK.

Risk factors, dietary intake, and energy expenditure		N	Haemoglobin (µmol/l)	Median (IQR)		P
Overall		230	13.7	(12.5,	15.1)	
Country of birth	India	50	13.7	(13.3,	15.3)	0.24
	Outside India	71	14.0	(12.6,	15.2)	
Body mass index (kg/m^2)	<27	141	13.7	(12.6,	15.1)	0.82
	≥27	88	13.7	(12.4,	15.1)	
Smoking habit in men	Non-smoker	85	15.0	(14.2,	15.6)	0.73
	Smoking history	28	15.1	(14.0,	15.6)	
Glucose tolerance	Normal	168	13.8	(12.6,	15.3)	0.33
	Impaired	13	13.8	(13.2,	14.8)	
	Diabetes	35	13.5	(13.0,	14.6)	
Systolic blood pressure (mmHg)	≤130	119	13.2	(12.0,	14.7)	0.03
	>130	74	14.2	(13.0,	15.3)	
Diastolic blood pressure (mmHg)	≤85	143	13.3	(12.3,	14.8)	0.015
	>85	50	14.2	(13.1,	15.3)	
Serum cholesterol (mmol/l)	≤4	15	12.5	(12.2,	15.0)	0.07
	>4 and ≤5	76	13.3	(12.0,	14.7)	
	>5	133	14.0	(12.8,	15.3)	
Serum triglycerides (mmol/l)	≤1.7	195	13.6	(12.4,	15.0)	0.14
	>1.7	32	14.2	(13.1,	15.3)	
HDL cholesterol (mmol/l)	≤0.9 for men and ≤1.0 for women	128	14.2	(12.9,	15.3)	<0.001
	>0.9 for men and >1.0 for women	99	13.1	(12.0,	14.7)	
Hyperinsulinaemia**	None	139	13.6	(12.4,	15.2)	0.72
	Present	90	14.0	(12.5,	15.0)	
C-reactive protein (g/l)*	≤71	53	13.5	(12.6,	15.2)	0.71
	>216	54	13.2	(12.2,	14.8)	

Table 1A continued.

Risk factors, dietary intake, and energy expenditure		N	Haemoglobin (µmol/l)	Median (IQR)		P
Homocysteine (µmol/l)*	≤9.4	88	13.1	(12.0,	14.6)	0.013
	>14.2	38	14.0	(12.9,	15.3)	
Daily energy intake (Kcal)*	≤1,410	26	13.1	(12.2,	13.6)	<0.001
	>1,960	80	14.3	(13.1,	15.3)	
Energy from dietary protein (%)*	≤12.0	50	13.3	(12.4,	14.5)	0.13
	>14.1	51	14.0	(12.8,	15.3)	
Energy from dietary fat (%)*	≤35.0	37	14.0	(12.8,	15.3)	0.14
	>40.0	58	13.2	(12.3,	15.1)	
Energy from dietary carbohydrate (%)*	≤48.6	65	14.6	(12.9,	15.5)	0.002
	>54.4	35	13.0	(12.1,	13.7)	
Daily energy expenditure (Kcal)*	≤1,580	119	13.2	(12.0,	14.7)	0.012
	>1,950	74	14.2	(13.0,	15.3)	
Cardiovascular risk score (%)*	≤4	87	13.1	(12.1,	14.3)	0.002
	>4 and ≤8	58	14.4	(12.8,	15.5)	
	>8	63	14.0	(12.7	15.0)	

IQR: interquartile range; HDL: high-density lipoprotein. * Cut-offs are tertiles (e.g. highest and lowest) calculated for the combined population. ** World Health Organization defined hyperinsulinaemia (subjects with normal glucose tolerance, but insulin levels in the upper quartile for the combined population of migrants and non-migrants).

RESULTS

Levels of haemoglobin for the combined population of 535 Indian Gujaratis were non-parametrically distributed with a median value of 13.3 (µmol/l) (IQR, 11.9-14.7). Haemoglobin was higher in men (median 14.7 µmol/l) compared to women (12.1 µmol/l), and was typically 5% higher in migrants compared with rural contemporaries (P<0.001).

Levels of Haemoglobin in Relation to Cardiovascular Risk Factors and Diet (Table 1A and 1B)

Across migrants and rural Indians combined, haemoglobin was consistently higher in those with raised blood pressure (at least 7% higher, P<0.001), raised serum cholesterol (2-6% higher, P<0.001), low HDL cholesterol (8% higher, P<0.001), and increased cardiovascular risk score (at least 7% higher, P<0.001). There were no differences in haemoglobin levels by obesity (various anthropometric variables including waist circumference). Haemoglobin differed by glucose tolerance status only amongst those in rural India, where levels were also higher in those with hyperinsulinaemia and high serum triglycerides. Amongst migrant Indians, haemoglobin levels were higher in those with raised homocysteine. Amongst male smokers in rural India, levels of haemoglobin were lower than in non-smokers. With respect to dietary intake and energy expenditure, levels of haemoglobin were highest in those with higher energy intake. Levels of haemoglobin were 5-10% higher amongst those with higher protein and 10% lower amongst those with higher carbohydrate intake. Amongst those with higher energy expenditure, levels of haemoglobin were 7% higher irrespective of site.

Haematology and Oxidative Indices of Atherogenesis

Amongst the men, levels of haemoglobin were lower and red blood cell volume was higher (non-significantly) amongst those in rural India. With respect to rural males, platelet count and oxidised LDL were all comparable to migrant men. In women, those in rural India also had lower haemoglobin, but red cell volume was also lower in comparison to migrant women. Amongst women, platelets and oxidised LDL were all significantly lower amongst rural women compared to migrant contemporaries (Table 2).

Interrelationships between Haematological, Lipoprotein-Related, and Dietary Indices

Levels of haemoglobin were positively associated with oxidised LDL (partial correlation coefficient

= 0.18, P<0.001) and paraoxonase activity (partial correlation coefficient = 0.28, P<0.001), controlling for the effects of gender and site. Amongst those with normal glucose tolerance, levels of oxidised LDL were highest in those individuals in the highest tertile of haemoglobin (gender specific).

Table 1B: Concentrations of haemoglobin amongst rural Indians living in villages in India.

Risk factors, dietary intake, and energy expenditure		N	Haemoglobin (µmol/l)	Median (IQR)		P
Overall		305	13.0	(11.3,	14.4)	
Body mass index (kg/m^2)	<27	272	13.0	(11.3,	14.4)	0.49
	≥27	32	13.1	(11.4,	15.2)	
Smoking habit in men	Non-smoker	77	14.6	(13.6,	15.7)	0.04
	Smoking history	62	14.2	(12.7,	15.3)	
Glucose tolerance	Normal	190	12.9	(11.3,	14.5)	0.03
	Impaired	54	13.0	(11.0,	14.6)	
	Diabetes	41	13.9	(11.9,	15.4)	
Systolic blood pressure (mmHg)	≤130	213	12.9	(11.2,	14.2)	0.02
	>130	57	13.8	(12.0,	15.7)	
Diastolic blood pressure (mmHg)	≤85	237	12.9	(11.1,	14.3)	<0.001
	>85	33	14.5	(12.8,	16.0)	
Serum cholesterol (mmol/l)	≤4	62	12.6	(10.1,	13.7)	0.003
	>4 and ≤5	107	12.8	(10.9,	14.4)	
	>5	126	13.4	(11.9,	14.7)	
Serum triglycerides (mmol/l)	≤1.7	272	13.0	(11.3,	14.4)	0.046
	>1.7	21	13.9	(12.1,	15.8)	
HDL cholesterol (mmol/l)	≤0.9 for men and ≤1.0 for women	132	13.4	(12.1,	14.8)	<0.001
	>0.9 for men and >1.0 for women	161	12.4	(11.0,	14.2)	
Hyperinsulinaemia**	None	187	12.8	(11.2,	14.1)	0.01
	Present	115	13.5	(11.5,	14.9)	
C-reactive protein (g/l)*	≤71	70	13.2	(10.9,	14.6)	<0.001
	>216	33	14.5	(13.0,	16.2)	
Homocysteine (µmol/l)*	≤9.4	54	12.3	(10.5,	14.6)	0.20
	>14.2	107	12.9	(11.1,	14.6)	
Daily energy intake (Kcal)*	≤1,410	69	13.0	(11.1,	14.1)	0.005
	>1,960	2	15.9	(15.7,	16.1)	
Energy from dietary protein (%)*	≤12.0	32	12.1	(10.5,	13.2)	0.018
	>14.1	31	13.4	(11.7,	14.6)	
Energy from dietary fat (%)*	≤35.0	49	12.9	(10.8,	14.1)	0.30
	>40.0	22	13.1	(11.1,	14.7)	
Energy from dietary carbohydrate (%)*	≤48.6	19	13.5	(11.1,	14.5)	0.07
	>54.4	49	12.4	(10.6,	13.5)	
Daily energy expenditure (Kcal)*	≤1,580	213	12.9	(11.2,	14.2)	<0.001
	>1,950	57	13.8	(12.0,	15.7)	
Cardiovascular risk score (%)*	≤4	111	12.7	(11.0,	14.1)	0.07
	>4 and ≤8	56	12.8	(11.1,	14.0)	
	>8	90	13.8	(13.8,	15.3)	

IQR: interquartile range; HDL: high-density lipoprotein. * Cut-offs are tertiles (e.g. highest and lowest) calculated for the combined population. ** World Health Organization defined hyperinsulinaemia (subjects with normal glucose tolerance, but insulin levels in the upper quartile for the combined population of migrants and non-migrants).

Table 2: Haematological parameters and oxidative indices of atherogenesis amongst migrant Indian Gujaratis and contemporaries in rural India.

	Rural Indian men (n=140)		Migrant Indian men (n=115)		P	Rural Indian women (n=165)		Migrant Indian women (n=115)		P
Haemoglobin (g/dl)	14.5	(13.2, 15.4)	15.0	(14.2, 15.6)	0.004	11.8	(10.5, 13.0)	12.7	(11.8, 13.3)	<0.001
Mean cell volume (fl)	87.1	(82.1, 90.7)	85.0	(81.0, 89.0)	0.06	81.6	(73.8, 85.5)	84.0	(78.8, 87.0)	0.014
Red blood cell count ($U \times 10^{12}/l$)	5.02	(4.62, 5.50)	5.20	(4.92, 5.47)	0.09	4.50	(4.16, 4.90)	4.53	(4.26, 4.97)	0.45
Platelet count ($U \times 10^{9}/l$)	224	(184, 279)	215	(193, 247)	0.11	232	(184, 281)	259	(212,313)	0.06
Paroxonase activity (nmol/min/ml)	140	(88, 180)	229	(186, 293)	<0.001	123	(75, 172)	216	(169, 299)	<0.001
Oxidised LDL (U/l)	39.0	(29.0, 51.0)	41.9	(29.0, 52.0)	0.54	33.0	(25.2, 43.8)	38.0	(30.0, 51.8)	0.002

Data are Median (IQR). LDL: low-density lipoprotein; IQR: interquartile range. * Cut-offs are tertiles (e.g. highest and lowest) calculated for the combined population. ** World Health Organization defined hyperinsulinaemia (subjects with normal glucose tolerance, but insulin levels in the upper quartile for the combined population of migrants and non-migrants).

Other haematological indices were unrelated to lipoprotein variables, and there were no interaction effects with haemoglobin in models of univariate analysis of variance. On bivariate analysis, levels of serum cholesterol and apolipoprotein B were associated with haemoglobin in men and women from both sites, and the magnitude of this association was greatest in rural India ($r=0.27$, $P<0.001$). In addition, amongst rural Indian women, haemoglobin was associated with apolipoprotein A1 levels ($r=0.25$, $P<0.05$). On multivariate analysis, levels of oxidised LDL (in a model that included haematological indices, site, gender, glucose tolerance status, smoking, systolic blood pressure, and serum lipids) were independently associated with triglycerides, total to HDL cholesterol ratio and haemoglobin levels (Table 3). Levels of haemoglobin were independently related to gender, diastolic blood pressure, oxidised LDL, and paraoxonase activity (Table 3). On regression analysis of cardiovascular risk scores across the whole population, levels of haemoglobin, oxidised LDL, BMI, and site were all independently associated.

Across the whole population, levels of oxidised LDL were associated with dietary intake of iron and vitamin B complex constituents (particularly riboflavin and thiamine), controlling for the effects of site and gender. Within rural Indian men, levels of oxidised LDL were associated with dietary niacin ($r=0.40$, $P=0.018$) and vitamin B_{12} ($r=0.33$, $P=0.05$), while in migrants, dietary intake of iron ($r\leq-0.27$, $P<0.03$) and thiamine ($r\leq-0.31$, $P<0.02$) were negatively associated with paraoxonase activity. On multivariate analysis, dietary factors were not independently associated with lipoprotein-related indices. However, in rural Indian men, there was a modest negative association between the variation of oxidised LDL levels and the percentage of energy derived from carbohydrate ($r=0.42$, $P<0.001$). Across sites, levels of homocysteine were negatively associated with paraoxonase activity ($r=-0.16$, $P=0.002$).

DISCUSSION

Amongst migrant Indians and rural contemporaries, levels of haemoglobin were associated with common risk factors for CHD, including blood pressure, lipids, and direct indices of atherosclerosis, irrespective of site and gender. Data here suggest that the molecular basis for this relationship involves the oxidative modification of LDL by haem. The potential pathway is likely to be borne from the culmination of nutritional deficiency (folate and B vitamins), toxins such as homocysteine, and (unmeasured)

genetic predisposition to bloodborne disorders and aberrant erythropoietic activity. Further work is warranted. The implication of these findings is that haemoglobin levels are intimately co-ordinated with quantitative and qualitative features of lipoproteins, and may provide insight into the increased CHD risk amongst people originating from the Indian subcontinent.

The idea that haemoglobin is a risk factor for CHD is not novel, and the Iron-Heart Hypothesis, which suggests that increased iron stores are a CHD risk factor, was presented by Sullivan in 1981.[26] Data presented here reflects a close relationship between haemoglobin levels and both quantitative and qualitative aspects of lipid metabolism. There is increasing evidence to support an interactive physiological role between lipoproteins such as LDL and HDL with haemoglobin and iron transport.[27,28] In the presence of a nutritional deficiency of folate and vitamin B_{12}, this process is likely to render HDL dysfunctional.[29] Of note, levels of paraoxonase activity in this population were closely reflected by HDL cholesterol concentrations.[30] Hence, in this population it would appear that levels of cholesterol transport on HDL also reflect other aspects of HDL functionality.

Previously we reported that migration-related changes included increases in total cholesterol, fasting triglycerides, apolipoprotein B, and blood pressure within this group of Gujarati Indians.

Table 3: Multivariate analysis of haemoglobin levels, oxidised LDL, and paraoxonase activity amongst migrant Indians and contemporaries who remained in rural villages.

Multivariate models	Beta	(95% CI)		P
1. Dependent variable: Haemoglobin (µmol/l)				
Female gender	-1.85	-2.39	-1.32	<0.001
Diastolic blood pressure (mmHg)	0.047	0.025	0.069	<0.001
Oxidised LDL (U/l)	0.019	0.005	0.033	0.01
Paraoxonase activity (nmol/min/ml)	0.56	0.07	1.04	0.025
2. Dependent variable: Oxidised LDL (U/l)				
Serum cholesterol (mmol/l)	8.0	5.3	10.7	<0.001
Haemoglobin (µmol/l)	1.70	0.38	3.02	0.012
HDL cholesterol (mmol/l)	-10.8	-19.9	-1.7	0.02
3. Dependent variable: paraoxonase activity (nmol/min/ml)				
Migrant status	0.563	0.405	0.721	<0.001
HDL cholesterol (mmol/l)	0.489	0.259	0.720	<0.001
4. Dependent variable: cardiovascular risk score (%)				
Haemoglobin (µmol/l)	0.68	0.297	1.054	0.001
Oxidised LDL (U/l)	0.05	0.008	0.094	0.021
Body mass index (kg/m^2)	0.34	0.153	0.535	<0.001
Migrant status	-3.11	-5.057	-1.156	0.002

Variables in multivariate model 1 included migrant status, homocysteine, serum cholesterol, serum triglycerides, HDL cholesterol, percentage of energy intake as carbohydrate, serum insulin, C-reactive protein, and physical activity. Model 2 included age, paraoxonase activity, diastolic blood pressure, migrant status, homocysteine, serum triglycerides, percentage of energy intake as carbohydrate, serum insulin, C-reactive protein, and physical activity. Model 3 included homocysteine, serum cholesterol, serum triglycerides, diastolic blood pressure, oxidised LDL, percentage of energy intake as carbohydrate, serum insulin, C-reactive protein, and physical activity. Model 4 included paraoxonase activity. The multivariate models were developed to include independent confounding factors (non-normally distributed variables were normalised by log-transformation).
LDL: low-density lipoprotein; HDL: high-density lipoprotein; CI: confidence interval.

However, HDL cholesterol and homocysteine were not adversely affected by migration.[6] This novel advance in CHD in this group may be explained by the findings in this analysis, which additionally reflect aberrant haemoglobinisation and erythropoiesis. Also, in the current analysis it is apparent that a greater intake of carbohydrate is associated with lower levels of oxidised LDL, underlining the deleterious effects of the nutritional transition between India and the UK for this group. These findings also suggest that increased dietary iron may pose a healthcare concern in this group, which is compounded, given that the normalisation of body iron status in response to anaemia is a common primary care intervention amongst South Asian populations.[31-33]

Patients with chronic anaemias and high erythropoietic activity (e.g. beta thalassaemia) are known to have low levels of serum and HDL cholesterol[34,35] (possibly through the increased cholesterol requirement associated with erythroid hyperplasia[36]). Also, in patients with a susceptibility to hyperlipidaemia, the presence of beta thalassaemia has an LDL cholesterol-lowering effect.[37] While there were no observations of overt anaemia within the present study, measures of lipoproteins and lipids were positively related to haematological indices. Of note, in rural India, where levels of haemoglobin were lowest, there were morphological differences in red blood cells between men and women. In rural Indian men, low levels of haemoglobin were manifest with large red blood cells (relative to migrant men), suggesting folate deficiency as a cause for differences in haematology. In rural Indian women, red blood cells were relatively smaller than for Sandwell counterparts, indicative of an iron-related deficiency as a cause for low levels of haemoglobin. In relation to iron-related deficiency, our results amongst rural Indian women suggest that this phenotype is cardio-protective, and support observations that paraoxonase activity is reduced in this cause of anaemia. However, while genetic disorders such as beta thalassaemia are endemic to India,[38] there is no evidence that this is a cause of increased erythropoeitic activity for the populations observed here. The erythropoietic demand that is generated by the development of diabetes could also represent an underlying haematological consequence amongst South Asians.

The independent association between haemoglobin levels and CHD risk scores in these populations is interesting, and may underpin novel approaches for the early identification of South Asians at increased CHD risk. Haemoglobin levels were independently associated with blood pressure levels, and one hypothesis is that the higher levels of haemoglobin in this population reflect a status of greater oxidative stress. For example, the vasodilator activity of nitric oxide (NO) is mitigated by the expression of the haem-containing NO receptor soluble guanylyl cyclase, which is impaired by the oxidative modification of its haem component.[39] In our own data, levels of haemoglobin remain associated with systolic and diastolic blood pressure, even after controlling differences in dietary intake.

The limitations of this work include the cross-sectional nature of our approach, which precludes our ability to determine cause and effect. Our stratified random sampling approach for the population was used as an attempt to minimise sources of confounding, and multivariate models were developed with the assumption that factors were normally distributed and were independent. However, we cannot rule out residual confounding and unmeasured factors related to haemoglobin, oxidised LDL, and paraoxonase activity. For example, genetic analysis of beta thalassaemia trait, haemochromatosis trait, and paraoxonase polymorphism remain unmeasured.

In summary, these findings support a link between haemoglobin levels with CHD risk factors and oxidative indices of atherosclerosis in these populations of South Asians. Iron availability may underline the pathogenesis for the increased CHD in this group and further work is warranted.

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