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Oxidative stress could precede endothelial dysfunction and insulin resistance in Indian Mauritians with impaired glucose metabolism

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Abstract

Aims/hypothesis. To measure oxidative stress, endothelial dysfunction and insulin resistance in Indian Mauritians at different stages of development of Type II (non-insulin-dependent) diabetes mellitus.
Methods. Plasma total 8-epi-$PGF_{2\alpha}$, an indicator of oxidative stress, was determined in age-matched subjects with normal glucose metabolism ($n = 39$), impaired glucose tolerance ($n = 14$), newly diagnosed diabetes ($n = 8$) and established diabetes ($n = 14$). Plasma glucose and insulin were measured at baseline and 2 h following an oral glucose tolerance test. Endothelial function was assessed by non-invasive digital pulse wave photoplethysmography.
Results. Plasma 8-epi-$PGF_{2\alpha}$ increased in subjects with impaired glucose tolerance ($p < 0.05$) compared with control subjects, and was even higher in newly diagnosed diabetic patients ($p < 0.01$) and established ($p < 0.01$) diabetic patients. A tendency towards reduced endothelial function in subjects with impaired glucose tolerance became significant in patients with newly diagnosed and established diabetes ($p < 0.01$), and was correlated with 8-epi-$PGF_{2\alpha}$ ($r = 0.36$, $p < 0.01$). Insulin resistance (homeostasis model assessment) did not change in subjects with impaired glucose tolerance compared with control subjects, but increased in newly diagnosed ($p < 0.01$) and established ($p < 0.001$) diabetic subjects. The 8-epi-$PGF_{2\alpha}$ was correlated with fasting glucose ($r = 0.50$, $p < 0.001$), triglycerides ($r = 0.40$, $p < 0.001$) and insulin resistance ($r = 0.35$, $p < 0.001$).
Conclusion/interpretation. Oxidant stress is an early event in the evolution of Type II diabetes and could precede the development of endothelial dysfunction and insulin resistance. [Diabetologia (2001) 44: 706–712

Keywords Type II diabetes, F_2-isoprostanes, oxidative stress, endothelial dysfunction, insulin resistance

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Abbreviations: $ABTS^{\cdot+}$, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical; DM, established Type II diabetes; DM-O, newly diagnosed Type II diabetes; DVP, digital volume pulse; F_2-IPs, F_2-isoprostanes; GC-MS, gas chromatography – mass spectrometry; GTN, glyceryltrinitrate; $NO^{\bullet}$, nitric oxide; $O_2^{\bullet-}$, superoxide anion; RI, reflection index; TAOS, plasma total antioxidant status.

Type II (non-insulin-dependent) diabetes mellitus is characterised by insulin resistance, pancreatic beta-cell defect, hypertension, dyslipidaemia and central obesity, and is recognised as part of a cluster of cardiovascular risk factors generally known as the Metabolic Syndrome [1]. The Indian Ocean island of Mauritius recently provided the background for a longitudinal epidemiological study (1987–1998) which is now seen as an indicator of the potential global impact of Type II diabetes [2, 3]. Mauritius has a multiethnic population of 1.2 million, including people of Asian Indian (66%), mixed European-African-Indian (31%) and Chinese (3%) descent. In 1987, the age-standardised prevalence of Type II diabetes in adults aged 30 years or more was 14.3%, increasing to

16.9 % in 1992 and 19.5 % in 1998 [4]. Current data indicate an age-adjusted prevalence of impaired glucose tolerance (IGT) in Mauritian men and women (age range 30–64 years) of 9.6–17.1 % and 19.7–21.9 %, respectively [5].

The aim of this study was to examine the relation between oxidative stress, endothelial dysfunction and insulin resistance in Mauritian subjects of Asian Indian descent with varying levels of impaired glucose metabolism, representing different stages in the development of Type II diabetes. We have proposed that oxidative stress precedes endothelial dysfunction and the subsequent development of insulin resistance [6, 7]. Oxidative stress was assessed by measurement of plasma F_2-isoprostanes (F_2-IPs), which are prostaglandin-like molecules produced by the free radical-catalysed peroxidation of arachidonic acid [8]. Plasma and urinary concentrations of one of the abundant F_2-IPs, 8-epi-$PGF_{2\alpha}$, are higher in subjects with Type II diabetes than in healthy control subjects [9] and decrease following dietary supplementation with antioxidants [10, 11]. Similar observations have been reported in animal models of diabetes [12, 13] and it is now recognised that measurement of 8-epi-$PGF_{2\alpha}$ represents a sensitive and reliable index of oxidative stress in vivo [14].

Endothelial dysfunction has been reported in patients with established Type II diabetes [15–20] and in elderly subjects with impaired fasting glucose (IFG) [21], suggesting that even a moderate disturbance in glucose metabolism could constitute a significant risk for diabetic endotheliopathy. In this study, we assessed endothelial dysfunction using non-invasive digital photoplethysmography in combination with endothelium-independent and endothelium-dependent vasodilators [22].

Subjects and methods

Subjects. Recruitment of subjects for this study was carried out at the SSR Centre for Medical Studies and Research in Mauritius. Subjects of North-Indian descent were selected from the population over a 3-month period. The majority of subjects participating in this study visited the centre for the first time but a few subjects who are involved in ongoing family studies at the SSR Centre were also included. All subjects in this study underwent a clinical examination and a resting electrocardiogram (ECG). Those with clinical evidence of ischaemic heart disease were excluded. Subjects with hypertension (systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg), high total or low density lipoprotein cholesterol (≥ 7 and/or > 5 mmol/l, respectively) and smokers were also excluded. Diabetes was diagnosed using the World Health Organisation (WHO) criteria [23]. Subjects with diabetes were not treated with either hypoglycaemic agents or insulin. None of the subjects were taking antioxidant vitamin supplements, lipid-lowering drugs or beta blockers. This study was approved by the Ethics and Scientific Advisory committees of the University of Mauritius. All participants provided written, informed consent.

Blood sampling and plasma preparation. Participants were invited to attend the SSR Centre after an overnight fast. Following registration, fasting blood samples were collected from all the subjects. To determinate of 8-epi-$PGF_{2\alpha}$, blood (10 ml) was collected from the brachial vein in polyethylene tubes containing a 3.8 % (w/v) trisodium citrate solution (blood/anticoagulant ratio of 9/1), indomethacin (as cyclooxygenase inhibitor) and butylated hydroxytoluene (BHT, as free radical scavenger) at final concentrations of 14 and 20 μmol/l, respectively. The sample was allowed to stand for 30 min at 4 °C to enable complete inhibition of cyclooxygenase enzymes. Platelet-poor plasma was obtained by centrifugation at 1120 g for 15 min at 4 °C. The was transferred to a polypropylene screw-cap tube and BHT added at a final concentration of 20 μmol/l. The sample was then stored at –80 °C until analysis. For the determination of plasma triglycerides, cholesterol, uric acid, urea and creatinine, fasting blood was collected in heparin tubes. For the measurement of plasma insulin, total antioxidant status (TAOS) and vitamin E, blood was collected in EDTA tubes. After centrifugation, plasma was transferred to screw-cap polypropylene tubes and stored at –80 °C until analysis. A second blood sample was taken 2 h following an oral glucose tolerance test (OGTT, 75 g anhydrous glucose in 250 ml water) for measurement of TAOS, insulin and glucose. For glucose determinations, fasting and 2-h blood samples were collected into tubes containing potassium fluoride. Following centrifugation, plasma glucose was assayed by midday at the latest after collection of the last 2-h sample. All plasma samples for the measurement of 8-epi-$PGF_{2\alpha}$, TAOS and insulin were stored at –80 °C and were sent to the William Harvey Research Institute by air on dry ice in two separate shipments. On arrival, samples were stored at –80 °C until analysis.

Additional subject information. As arranged when booking their appointment, participants were asked to provide a midstream early morning urine sample for the measurement of albuminuria and microalbuminuria. Prior to administration of the OGTT, anthropometric measurements were carried out with the subjects wearing light clothes and without footwear.

Analysis of 8-epi-$PGF_{2\alpha}$. Plasma samples were subjected to alkaline hydrolysis for the measurement of total (sum of free plus esterified) 8-epi-$PGF_{2\alpha}$ by gas chromatography – mass spectrometry (GC-MS), as described previously [24]. The GC-MS assay has a limit of detection of about 10 pg/ml (28 pmol/l) in plasma with intra-assay and inter-assay coefficients of variation of 4.4 and 7.6 %, respectively.

Plasma total antioxidant status measurement. The total antioxidant status of plasma was determined by its capacity to inhibit the peroxidase-mediated formation of the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical ($ABTS^{\cdot+}$), as described earlier [24]. The intra-assay and inter-assay coefficients of variation are less than 2 and less than 5 %, respectively.

Additional measurements. Plasma lipid hydroperoxides were measured by spectrophotometry at 560 nm using the ferrous oxidation-xylenol orange (FOX-2) assay [25]. Plasma vitamin E (α- and γ-tocopherol) was measured by reversed-phase high-performance liquid chromatography and fluorescence detection, as described previously [24]. Plasma glucose, triglycerides, total cholesterol, HDL-cholesterol and uric acid were determined by enzymatic colorimetric methods using a multiparametric Cobas Mira Plus auto-analyser (Roche Diagnostics Systems, Produits Roche, Neuilly-sur-Seine, France). Urea was assayed using a UV enzymatic kinetic method and creatinine by a colorimetric endpoint method. Assay reagent kits,

standards (multiparametric calibrator human serum) and control samples (two multiparametric assayed human control sera, one normal and one elevated) were obtained from Randox (Randox Laboratories, Antrim, UK). LDL cholesterol was calculated using the Friedewald formula [26]. Plasma insulin was measured using a double-antibody radioimmunoassay (Linco Research, St. Louis, Miss., USA). An estimate of insulin sensitivity was derived by homeostasis model assessment (HOMA) using the formula: fasting plasma glucose (mmol/l) X fasting plasma insulin (μU/ml)/22.5, as described previously [27]. Blood haemoglobin was determined by a colorimetric endpoint method.

Endothelial function assessment. Endothelial function was assessed in a subset of participants, using a portable photoplethysmograph (Micro Medical MP2000, Gillingham, Kent, UK) as described earlier [22]. Digital volume pulse (DVP) data were collected using a probe placed on the index finger of the right hand and in-built software was applied to provide a reflection index (RI) related to tone in small arteries. The reflection index was calculated as the height of the inflection point after the first peak of the DVP, and expressed as a percentage height of the first peak (Fig. 1).

Data collection was carried out with the subject supine in a temperature-controlled laboratory (27 ± 1 °C). Each subject was allowed to acclimatise to the laboratory temperature for 30 min before the start of measurements. Baseline recordings were carried out at 5 min intervals for a total of 15 min and a mean baseline RI was calculated ($RI_{BASELINE}$). Endothelial responses were then measured following administration of an endothelium-independent vasodilator (glyceryltrinitrate (GTN), 500 μg sublingual for 3 min) and an endothelium-dependent vasodilator (salbutamol, 400 μg by inhalation through a spacer). Each recording was carried out for 30 s and calculations based on the final 3 waveforms. In the case of GTN, the mean of measurements at 3 min was used to calculate the endothelial response (RI_{GTN}). The response to salbutamol was taken as the mean of measurements at 10 and 15 min following inhalation ($RI_{SALBUTAMOL}$). The change in RI from baseline following GTN and salbutamol was expressed as (RI_{GTN} minus $RI_{BASELINE}$) and ($RI_{SALBUTAMOL}$ minus $RI_{BASELINE}$), respectively. In situations where endothelial responses to salbutamol are normal, $RI_{SALBUTAMOL}$ is less than $RI_{BASELINE}$, resulting in a negative change in RI. In cases where endothelial responses are blunted, $RI_{SALBUTAMOL}$ is greater than $RI_{BASELINE}$ and results in a positive change in RI. Blood pressure and pulse were recorded every 5 min throughout the test. The intra-individual coefficient of variation of RI using this approach ranges from 4.4–6.4 %.

Statistical analysis. Data are expressed as means ± SEM, or as median and range where the distribution is skewed. Comparison between mean values in groups with normally distributed data was done by parametric one-way analysis of variance (ANOVA) followed by Dunnett's test, using the subjects with normal glucose tolerance as control. Non-parametric (Kruskal-Wallis) ANOVA followed by Dunn's test was applied for data with skewed distribution. A p value of less than 0.05 was considered statistically significant. Associations between the different variables were examined by determination of Pearson's correlation coefficient. Statistical calculations were carried out using Minitab Statistical Software, version 13.20 (Minitab, PA., USA).

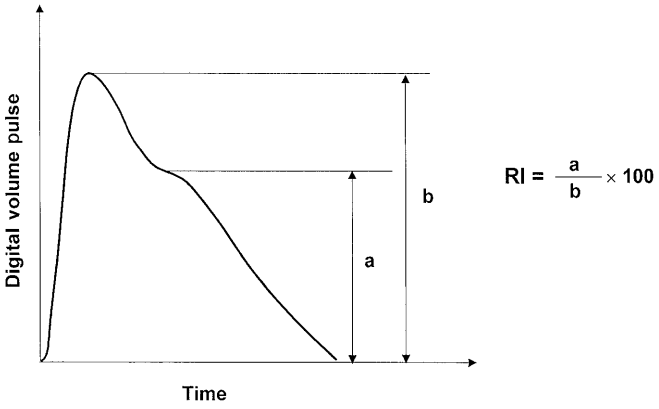

Fig. 1. The digital volume pulse (DVP) waveform. The DVP exhibits a characteristic 'notch' or inflection point in its waveform, which changes following administration of endothelium-dependent or endothelium-independent vasodilators. The amplitude of this inflection point, **a**, can be expressed as a percentage of the maximal DVP amplitude, **b**, and is termed the reflection index (RI)

Results

A total of 75 subjects (36 men and 39 women) were studied, grouped according to those with normal glucose tolerance (control, $n = 39$), impaired glucose tolerance (IGT, $n = 14$), newly diagnosed diabetes (DM-O, $n = 8$) and established diabetes (DM, $n = 14$) (Table 1).

The male:female ratio in each of the groups was different. In the case of the DM-O group, 6 subjects diagnosed with Type II diabetes had no previous record of an OGTT and were not aware of their impaired glucose metabolism prior to participating in this study. The remaining 2 subjects in this group had been classified as IGT at their previous OGTT, approximately 2 years earlier. Duration of diagnosed diabetes in the DM group ranged from 2–8 years. Fasting plasma insulin concentrations were highest in the DM-O group, representing a 1.7-fold increase ($p < 0.001$) compared to control subjects. In the DM group, fasting insulin concentrations were 1.5-fold higher compared to control subjects ($p < 0.05$). Insulin resistance determined by HOMA was increased in the DM-O ($p < 0.01$) and DM ($p < 0.001$) groups, compared to control subjects (Fig. 2A).

Post-OGTT insulin concentrations in the control, IGT, DM-O and DM groups were 6.7-, 11.3-, 9.2- and 5.3-fold higher, respectively, than the corresponding fasting concentrations. Plasma triglycerides were higher in subjects with established diabetes ($p < 0.01$) in comparison to control subjects. Although total cholesterol was similar among the different subject groups, LDL cholesterol was higher in DM-O ($p < 0.01$) and HDL cholesterol lower in IGT and DM ($p < 0.05$) groups, in comparison to the corresponding controls.

Table 1. Physical and biochemical characteristics of subjects in the study groups

	Control	IGT	DM-O	DM	*p* value
n (male/female)	22/17	6/8	0/8	8/6	
Age (years)	47.0 ± 0.8	48.7 ± 1.5	47.0 ± 1.4	48.6 ± 1	0.545
BMI (kg/m^2)	22.5 (19.4–32.9)	25.1 (22.6–30.3)	26.9 (23.1–29.3)	25.7 (20.2–38.5)	< 0.01
WHR	0.85 (0.71–0.99)	0.82 (0.70–0.96)	0.78 (0.75–0.82)	0.87 (0.70–1.00)	0.128
Systolic BP (mm Hg)	120 (90–150)	130 (110–160)	125 (100–140)	125 (110–160)	0.148
Diastolic BP (mm Hg)	80 (60–95)	83 (60–95)	80 (70–80)	80 (70–90)	0. 099
Fasting glucose (mmol/l)	5.23 (3.94–6.30)	5.37 (4.52–6.90)	5.86 (5.18–6.94)	8.26 (5.19–15.16)[c]	< 0.0001
2 h glucose (mmol/l)	5.44 (3.29–7.68)	8.71 (7.83–10.96)[c]	13.06 (11.53–16.76)[c]	15.24 (9.69–17.02)[c]	< 0.0001
Fasting insulin (pmol/l)	62 (35–442)	71 (47–250)	151 (43–239)[c]	99 (45–388)[a]	< 0.0001
2 h insulin (pmol/l)	459 (40–1494)	1013 (55–1698)[c]	1081 (508–3161)[b]	529 (283–1211)	< 0.0001
Triglycerides (mmol/l)	1.09 (0.54–3.32)	1.31 (0.91–3.10)	1.30 (0.98–1.81)	2.23 (0.50–5.98)[b]	0.001
Total cholesterol (mmol/l)	5.03 ± 0.16	4.99 ± 0.20	5.90 ± 0.23	5.33 ± 0.22	0.078
HDL cholesterol (mmol/l)	1.29 ± 0.05	1.05 ± 0.05[a]	1.15 ± 0.10	1.05 ± 0.07[a]	0.010
LDL cholesterol (mmol/l)	3.17 ± 0.13	3.25 ± 0.19	4.10 ± 0.23[b]	3.49 ± 0.15	0.015
Uric acid (μmol/l)	241 (85–395)	278 (181–463)	252 (209–317)	237 (158–406)	0.342
Urea (mmol/l)	4.45 ± 0.21	4.15 ± 0.30	4.18 ± 0.34	4.13 ± 0.38	0.791
Creatinine (μmol/l)	68.0 (27.0–112.0)	63.0 (53.0–99.0)	66.0 (59.0–69.0)	69.5 (44.0–102.0)	0.399
Haemoglobin (g/l)	138 (64–174)	134 (109–166)	125 (118–149)	135 (107–162)	0.304

Data are expressed as means ± SEM or as median (range) for skewed data. Differences between group means were tested by one-way ANOVA.
[a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$ vs control subjects

Table 2. Influence of impaired glucose metabolism on plasma markers of oxidative stress

	Control	IGT	DM-O	DM	*p* value
n (male/female)	22/17	6/8	0/8	8/6	
Lipid hydroperoxides (μmol/l)	17.9 ± 0.6	20.1 ± 1.8	19.8 ± 0.9	19.4 ± 1.0	0.317
% Inhibition of ABTS$^{\cdot+}$					
Fasting	39.1 ± 1.8	42.7 ± 1.4	42.7 ± 3.5	44.1 ± 1.8	0.300
2 h post OGTT	63.8 ± 2.9	71.7 ± 2.5	67.0 ± 9.3	69.1 ± 3.8	0.470
Vitamin E					
α-TOH (μmol/l)	24.9 ± 6.2	24.9 ± 1.8	27.5 ± 1.4	29.9 ± 3.7	0.951
γ-TOH (μmol/l)	2.09 ± 0.45	2.70 ± 0.53	2.64 ± 0.28	3.47 ± 0.51	0.317
α-TOH (μmol/mmol cholesterol)	4.57 ± 0.36	5.50 ± 0.60	4.94 ± 0.71	5.58 ± 0.84	0.456
γ-TOH (μmol/mmol cholesterol)	0.41 ± 0.10	0.60 ± 0.13	0.48 ± 0.10	0.64 ± 0.08	0.454

Data are expressed as means ± SEM. Differences between group means were tested by one-way ANOVA

Plasma concentrations of total 8-epi-$PGF_{2\alpha}$ were higher in the IGT ($p < 0.05$), DM-O ($p < 0.01$) and DM ($p < 0.01$) groups compared to control groups (Fig. 2B). Multiple correlations with fasting total 8-epi-$PGF_{2\alpha}$ as the dependent variable revealed a positive correlation with fasting glucose ($r = 0.50$, $p < 0.001$), triglycerides ($r = 0.40$, $p < 0.001$), insulin resistance (HOMA) ($r = 0.35$, $p < 0.001$) and TAOS ($r = 0.23$, $p < 0.05$). When the relation of 8-epi-$PGF_{2\alpha}$ to the fasting variables was analysed using stepwise multiple regression, only fasting glucose ($p < 0.001$) and triglycerides ($p < 0.05$) were independently significant. No significant differences between the subject groups were observed in lipid hydroperoxides, fasting and 2-h plasma TAOS, or vitamin E (α- and γ- tocopherol) (Table 2).

Interestingly, post-OGTT plasma TAOS increased approximately 1.6-fold in all subject groups following this test, compared to the corresponding fasting TAOS. To exclude a possible influence of glucose on the measurement of TAOS, glucose solutions of 5, 10 and 15 mmol/l were added directly to the assay. In addition, six control plasma samples were tested before and after addition of 15 mmol/l (final concentration) glucose. In all these cases, glucose did not interfere with the determination of plasma TAOS.

Endothelial dysfunction was assessed in a subset of the participants from the control group ($n = 25$), IGT ($n = 8$), DM-O ($n = 6$) and DM ($n = 9$) groups. Changes in RI response were determined following GTN and salbutamol administration. Baseline RI values in control subjects were similar to those observed in the other groups. At the doses used in this study, GTN resulted in a larger change in RI response than that produced by salbutamol ($p < 0.001$), although no significant differences were observed between the subject groups. The RI response following salbutamol was blunted in DM-O and DM compared to the control group ($p < 0.01$) (Fig. 2C). Finally, when the relation of 8-epi-$PGF_{2\alpha}$ to endothelial function and fasting biochemical parameters was analysed using stepwise multiple regression analysis, only fasting

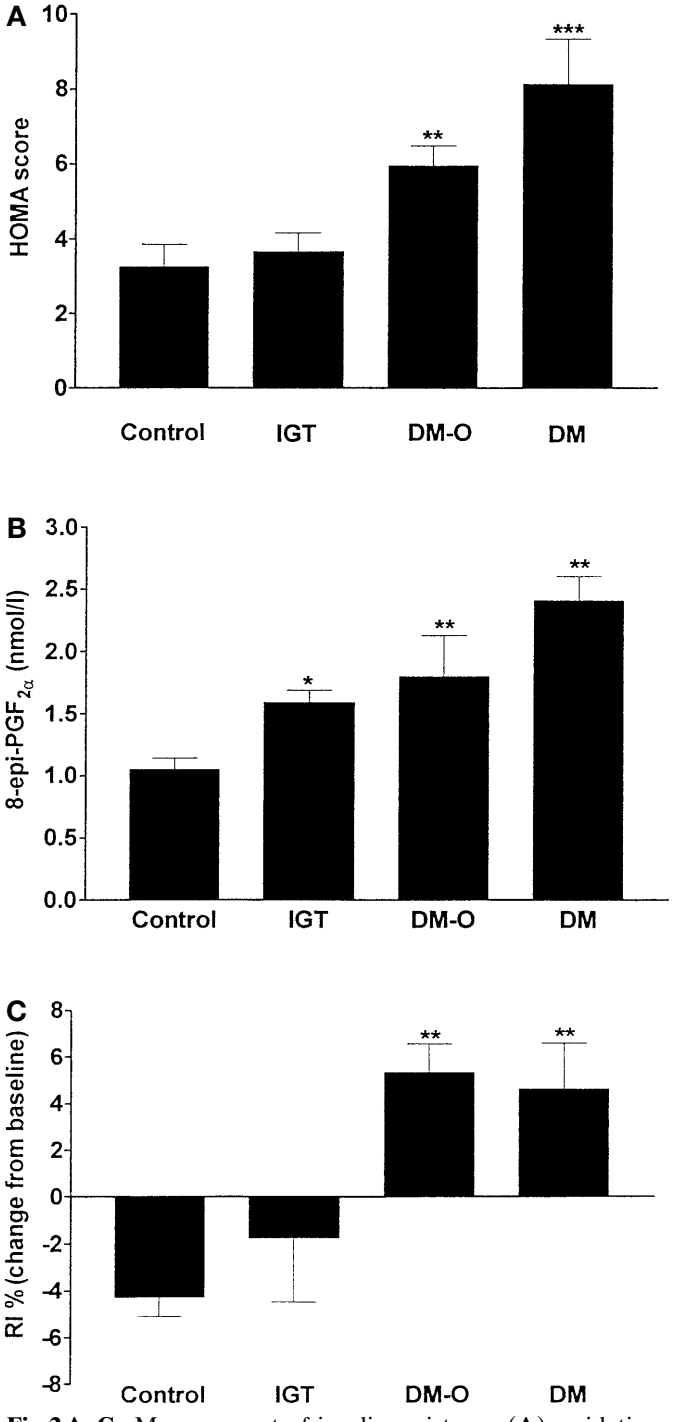

Fig. 2 A–C. Measurement of insulin resistance (**A**), oxidative stress (**B**) and endothelium-dependent endothelial response (**C**) in subjects with varying levels of impaired glucose metabolism. Insulin resistance (HOMA) and oxidative stress (plasma total 8-epi-$PGF_{2\alpha}$ measured by immunoaffinity extraction and GC-MS) were determined in control subjects ($n = 39$), and in those with IGT ($n = 14$), newly diagnosed diabetes (DM-O, $n = 8$) and established diabetes (DM, $n = 14$). Endothelial responses, expressed as a reflection index (RI) related to tone in small arteries, were measured by digital photoplethysmography following the administration of salbutamol (400 μg by inhalation) in control ($n = 25$), IGT ($n = 8$), DM-O ($n = 6$) and DM ($n = 9$) subjects. Data are expressed as means ± SEM. Differences between group means were tested by one-way ANOVA. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs control subjects

glucose ($p < 0.01$) and RI responses to salbutamol ($p < 0.01$) were independently significant.

Discussion

In this study we have shown that increased oxidative stress and endothelial dysfunction are associated with the development of Type II diabetes in Mauritians of Asian Indian descent. Circulating concentrations of 8-epi-$PGF_{2\alpha}$ (currently regarded as the most reliable index for the assessment of lipid peroxidation in humans) in the IGT, DM-O and DM groups were 1.5-fold, 1.7-fold and 2.3-fold higher, respectively, compared to the control group, indicating increasing oxidative stress with progressive dysglycaemia. A significant correlation between 8-epi-$PGF_{2\alpha}$ and fasting glucose suggests an association between glycaemia and increased lipid peroxidation among the subjects in this study. Several potential mechanisms could account for the increase in 8-epi-$PGF_{2\alpha}$ formation, including glucose autoxidation [28] and advanced glycation end products (AGE) formation [29]. Furthermore, a relatively small amount of 8-epi-$PGF_{2\alpha}$ could be formed using a cyclooxygenase-dependent pathway [30]. 8-epi-$PGF_{2\alpha}$ is a potent vasoconstrictor and is involved in the activation of intracellular signalling, suggesting that it could act as an important mediator in the manifestation of endotheliopathy in the early stages of Type II diabetes, either directly, or through the stimulated synthesis of other vasoconstrictor agents such as endothelin-1 [31, 32].

The absence of significant changes in plasma TAOS and vitamin E between the groups in our study is in agreement with a recent report [33], where no significant differences were found in plasma total antioxidant activity and vitamin E in both Caucasian and Indo-Asian subjects with Type II diabetes, compared to corresponding control subjects matched for age. It is conceivable, however, that small but significant changes in individual antioxidants were not detected in our study. In contrast to a recent report, where decreased antioxidant defences were found during the OGTT [34], we observed a 35–38% increase in the 2-h plasma TAOS, suggesting that the adaptive response of antioxidant enzymes to the short term hyperglycaemia-induced rise in oxidant stress was preserved [35].

Our data show a blunting of the endothelium-dependent response following salbutamol administration, in subjects with Type II diabetes (DM-O and DM groups). This is significantly correlated with plasma 8-epi-$PGF_{2\alpha}$ and insulin sensitivity (HOMA) and is in agreement with an earlier report [22] suggesting, in part, an impairment in the endothelial L-arginine – nitric oxide ($NO^{\cdot}$) pathway. Apart from the reduced formation of $NO^{\cdot}$, several mechanisms including impaired transport of the released $NO^{\cdot}$ and the simulta-

neous formation of vasoconstrictor mediators, could also account for the impaired endothelium-dependent responses observed in diabetic subjects. Indeed, the higher 8-epi-$PGF_{2\alpha}$ concentrations in subjects with Type II diabetes could reflect a reduced bioavailability of $NO^{\cdot}$ due to excessive production of $O_2^{\cdot -}$. The combination of $O_2^{\cdot -}$ and $NO^{\cdot}$ can potentially generate peroxynitrite and hydroxyl radicals, which could further aggravate endothelial dysfunction. The high plasma triglyceride concentrations in the DM group are consistent with a reduction in endothelial lipoprotein lipase activity and an increased flux of free fatty acids, and could contribute to endothelial dysfunction [36]. Interestingly, although impaired endothelial function was found in DM-O subjects, triglyceride concentrations were not significantly different compared to control subjects whereas LDL cholesterol concentrations were significantly higher. Higher LDL cholesterol can decrease the activity of endothelial nitric oxide synthase (eNOS) by promoting the inhibitory interaction between eNOS and caveolin, resulting in decreased $NO^{\cdot}$ formation [37]. There was a trend towards decreased endothelium-dependent responses in subjects with IGT compared to control subjects but the difference was not significant and could be related to an early compensatory mechanism in the presence of reduced $NO^{\cdot}$ availability [38].

The HOMA model has been proposed as a useful tool for the assessment of insulin sensitivity in large studies, where it is less practical to adopt more complex procedures [39]. In this study, a positive correlation was found between the HOMA score and plasma 8-epi-$PGF_{2\alpha}$, with lower insulin concentrations in the DM group compared to DM-O indicating progressive pancreatic beta-cell failure. Plasma 8-epi-$PGF_{2\alpha}$ derives to a large extent from LDL present in circulation and therefore reflects LDL oxidation in vivo. Recent clinical studies have shown inverse relation between vascular function and markers of LDL oxidation [40, 41] and suggest that elevated oxidative stress brought about by an increased proportion of oxidised LDL in circulation could contribute to the endothelial dysfunction observed in Type II diabetes. Localised excess $O_2^{\cdot -}$ could conceivably contribute to the inactivation of $NO^{\cdot}$ without influencing the total antioxidant capacity in plasma. Consequently, endothelial dysfunction could occur without detectable changes in the hydrophilic antioxidants. Indeed, endothelial dysfunction was recently reported to be independent of plasma total radical trapping capacity (TRAP), ascorbic acid, protein-bound thiols or uric acid [21].

This study, which has shown that elevated 8-epi-$PGF_{2\alpha}$ and endothelial dysfunction are associated with the development of Type II diabetes in Mauritians of Asian Indian origin, needs to be investigated further using a larger cohort. Interestingly, we have also shown that higher oxidative stress was present in the prediabetic stage (IGT), in the absence of significant changes in endothelial function and insulin sensitivity. Our data support the contention that oxidative stress could precede endothelial dysfunction and the appearance of insulin resistance, as proposed recently [42, 43].

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