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52 individuals who progressed to IGT or diabetes and 44 age, sex and BMI matched controls were studied. Progression to glucose intolerance was significantly related to baseline BCF ($p < 0.01$), but not isoprostane

levels or IS. Glucose concentrations (fasting and 2-hour) on follow-up were significantly correlated to baseline IS, baseline BCF, follow-up IS and follow-up BCF (p-values < 0.05). In multiple regression analysis, only follow-up IS and BCF (p-values \leq 0.001) independently predicted fasting glucose and 2-hour glucose levels at follow-up. Isoprostanes were not significantly associated with IS or BCF (p-values >0.1). We concluded that isoprostanes may not be causally involved in the development of glucose intolerance, insulin resistance or deteriorating BCF.

adequate and can be used in group analyses. This is stated and referenced on page 3, line 15.

5. *"...from the data any conclusion is not obtained. Therefore, this paper does not give us a few scientific information."* I am not clear what the reviewer is saying. However, it may be that he/she was not thrilled that the findings were supporting the null hypothesis and were negative. Negative findings are scientifically important and should be free from publication bias. Our findings were also similar to another study which was discussed in the Discussion section (page 8, paragraph 1).

We hope these changes meet with your approval and we eagerly look forward to your favourable reply.

Sincerely,

Michael Boyne, MD, FRCPC
Lecturer, TMRI

cc. Abi Robinson, Editorial Office

Isoprostanes, a marker of lipid peroxidation, may not be involved in the development of glucose intolerance

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Abstract

We investigated whether isoprostanes, as a marker of lipid peroxidation, may be involved in the development of impaired glucose tolerance (IGT) or diabetes. Using a nested case-control study, we tracked the changes in isoprostane levels, insulin sensitivity (IS) and beta-cell function (BCF) in Afro-Jamaicans who progressed to IGT and diabetes over 3.9 years. Anthropometry, glucose tolerance, insulin levels, blood pressure and urinary isoprostane concentration were measured at baseline and follow-up. IS and BCF were estimated by the method of homeostasis assessment.

52 individuals who progressed to IGT or diabetes and 44 age, sex and BMI matched controls were studied. Progression to glucose intolerance was significantly related to baseline BCF ($p \leq 0.01$), but not isoprostane levels or IS. Glucose concentrations (fasting and 2-hour) on follow-up were significantly correlated to baseline IS, baseline BCF, follow-up IS and follow-up BCF (p -values < 0.05). In multiple regression analysis, only follow-up IS and BCF (p -values ≤ 0.001) independently predicted fasting glucose and 2-hour glucose levels at follow-up. Isoprostanes were not significantly associated with IS or BCF (p -values >0.1). We concluded that isoprostanes may not be causally involved in the development of glucose intolerance, insulin resistance or deteriorating BCF.

Introduction

Oxidative stress has been implicated in the development of atherosclerosis and diabetic microangiopathy, but it is controversial whether it contributes to deteriorating glucose homeostasis [1]. Increased oxidative stress is associated with peroxidation of membrane lipids and LDL-cholesterol. These lipid peroxidation products can impair beta-cell function (BCF) and induce beta-cell apoptosis [2]. Peroxidation products could also theoretically impair the transfer of the insulin-receptor binding signal, thus worsening insulin sensitivity (IS).

Isoprostanes are free radical catalysed arachidonic acid derivatives that reflect *in vivo* nonenzymic lipid peroxidation [3]. They are associated with hyperglycaemia, vasoconstriction, and diabetic nephropathy [4, 5]. Improved glycaemic control and vitamin E supplementation in diabetic persons decrease isoprostanes concentrations [4]. Urinary isoprostanes (whether in pooled or spot urine samples) are thought to be the most sensitive, non-invasive index of lipid peroxidation and oxidative stress [3, 5].

We hypothesized that lipid peroxidation and thus isoprostanes may be involved in the development of glucose intolerance by adversely affecting BCF or IS. Consequently, we tracked isoprostane levels, IS and BCF in individuals who progressed to IGT or diabetes using a nested case-control study design.

Materials and Methods

Participants in the study were drawn from a cohort of 2096 randomly selected, urban-dwelling Afro-Jamaicans, age 25-74 years. The subjects were recruited for a longitudinal survey of hypertension, diabetes and chronic diseases in Spanish Town, Jamaica as described previously [6]. The baseline study was conducted from 1993 to 1996, and 932 persons were seen in the follow-up study 3.9 years (range: 2.9 - 5.2 years) later [7]. The protocol was approved by the Ethics Committee of the University of the West Indies.

Subjects had a 75-gram oral glucose tolerance test, and measurements of anthropometry and blood pressure were done using a standardised protocol [8]. Timed urine samples (range = 1.5 - 3.0 hours) were collected during the clinic visit at which time ~ 6 g of boric acid was added. The urine samples were then stored at -20°C. Individuals were categorised as having normal glucose tolerance (NGT), impaired glucose tolerance (IGT), or diabetes mellitus (DM). Individuals with incident glucose intolerance (i.e. progressed to IGT or DM) were labeled as progressors. Age, sex and body mass index (BMI) matched individuals who remained NGT or IGT (i.e. non-progressors) served as controls.

Glucose concentration was measured by the glucose oxidase method. Plasma insulin was measured using an immunometric assay (Immulite Insulin, Diagnostic Products Corporation, Los Angeles, CA, USA) which had an analytical sensitivity of 2 µIU/ml. There was no detectable cross-reactivity with proinsulin or C-peptide.

Urinary 15-isoprostane F_{2t} was measured with a commercial enzyme-linked immunoassay kit (Oxford Biomedical Research Inc, Oxford, MI, USA) with intra-assay and inter-assay CVs <10%. There was no significant cross-reactivity with $9\alpha,11\beta$ -prostaglandin $F_{2\alpha}$, $PGF_{2\alpha}$, 6-keto- $PGF_{1\alpha}$, PGE_2 , PGD_2 and arachidonic acid. The isoprostane and insulin assays were performed in 2002.

Assuming a 2-fold increase in isoprostanes in diabetes [4], a correlation coefficient of 0.40, and 80% power, a sample size of 47 would be sufficient for detecting a SD of 0.5 units between groups.

Estimates of IS (HOMA-%S) and BCF (HOMA-%B) were derived by the homeostasis model assessment (HOMA2 Calculator v2.2 downloaded from www.dtu.ox.ac.uk/homa). Isoprostane, fasting insulin, HOMA-IS and HOMA-%B were log transformed to normality. Pearson correlation coefficients were used to explore the relationships of isoprostanes, IS, BCF and glycaemia. Statistically significant correlates were used as predictors in multivariate linear regression models. Statistical significance was defined as a p-value < 0.05.

Results

There were 179 progressors (51 incident cases of DM and 128 IGT) and 432 matched nonprogressors. Our sample comprised 52 progressors (40 IGT/ 12 DM) and 44 nonprogressors who had both baseline and follow-up urine samples. There were no significant differences in age, proportion of women, or BMI between individuals who had both urine samples (and therefore used in this analysis) and the individuals who were excluded because of incomplete data.

The (mean \pm SE) age was 49.9 ± 1.3 years, BMI was 27.7 ± 0.6 kg.m⁻², 28% had hypertension and 10% were current smokers. There were no significant differences at baseline between progressors and non-progressors in BMI, age, proportion of women, waist circumference, blood pressure or tobacco use. BCF deteriorated significantly in the progressors over the study period (**Table 1**). Isoprostane concentrations at baseline were not significantly different between progressors and nonprogressors. Progression to IGT or diabetes was not significantly related to baseline isoprostane levels, IS or BCF even after adjusting for tobacco use and systolic blood pressure.

In univariate analyses, fasting glucose concentrations on follow-up were significantly correlated to baseline IS ($r = -0.30$; $p = 0.007$), baseline BCF ($r = 0.34$; $p = 0.002$), follow-up IS ($r = -0.45$; $p < 0.001$) and follow-up BCF ($r = -0.48$; $p < 0.001$). Plasma 2-hour glucose concentrations were significantly correlated with baseline BCF ($r = 0.33$; $p = 0.003$), baseline IS ($r = -0.28$, $p = 0.012$), follow-up IS ($r = -0.30$;

p=0.007) and follow-up BCF ($r = -0.24$, $p=0.03$). Isoprostane concentrations were not significantly correlated with fasting or 2-hour glucose levels, insulin, IS, BCF, BMI, age, blood pressure, or lipids (p -values > 0.1).

In regression analyses, only follow-up IS and BCF (p -values ≤ 0.01) independently predicted fasting plasma glucose (adjusted $R^2 = 0.88$) and 2-hour glucose (adjusted $R^2 = 0.28$) at follow-up.

Discussion

Although a previous cross-sectional study showed an association with urinary isoprostanes and deteriorating glucose tolerance [9], our population-based study did not demonstrate any longitudinal association. Recently, a case-control study also showed no longitudinal association of isoprostanes (as measured by GC-MS) in 26 cases of incident type 2 diabetes [10], but they did not investigate incident IGT. It may be that isoprostanes are biologically insufficient to independently induce hyperglycaemia, or alternatively, a larger sample may have more power to discriminate between the glycaemic groups.

For unclear reasons, isoprostane levels in this population were higher than that quoted by other investigators [4]. Tobacco use and severe dyslipidaemia can increase isoprostane levels, but the prevalences of these were low in this study. Our urine samples were stored at -20°C and we cannot exclude that *ex vivo* formation by autoxidation could have occurred over the years creating a systematic error. Unlike other reports, isoprostanes in our population were not associated with features of the metabolic syndrome [3], or glycaemia [5].

In conclusion, these findings do not support the hypothesis that isoprostanes and/or lipid peroxidation are causally involved in the deterioration of glucose homeostasis.

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References

1. J. W. Baynes, and S. R. Thorpe, Role of oxidative stress in diabetic complications: a new perspective on an old paradigm, *Diabetes* 48 (1999) 1-9.
2. I. Miwa, N. Ichimura, M. Sugiura, Y. Hamada, and S. Taniguchi, Inhibition of glucose-induced insulin secretion by 4-hydroxy-2-nonenal and other lipid peroxidation products, *Endocrinology* 141 (2000) 2767-2772.
3. J. D. Morrow, Quantification of isoprostanes as indices of oxidant stress and the risk of atherosclerosis in humans, *Arterioscler Thromb Vasc Biol* 25 (2005) 279-286.
4. G. Davi, G. Ciabattini, A. Consoli, A. Mezzetti, A. Falco, S. Santarone, E. Pennese, E. Vitacolonna, T. Bucciarelli, F. Costantini, F. Capani, and C. Patrono, In vivo formation of 8-iso-prostaglandin f₂alpha and platelet activation in diabetes mellitus: effects of improved metabolic control and vitamin E supplementation, *Circulation* 99 (1999) 224-229.
5. A. Mezzetti, F. Cipollone, and F. Cuccurullo, Oxidative stress and cardiovascular complications in diabetes: isoprostanes as new markers on an old paradigm, *Cardiovasc Res* 47 (2000) 475-488.
6. L. A. Sargeant, M. S. Boyne, F. I. Bennett, T. E. Forrester, R. S. Cooper, and R. J. Wilks, Impaired glucose regulation in adults in Jamaica: who should have the oral glucose tolerance test?, *Rev Panam Salud Publica* 16 (2004) 35-42.
7. M. S. Boyne, P. Gaskin, A. Luke, R. J. Wilks, F. I. Bennett, N. Younger, L. A. Sargeant, A. A. Adeyemo, R. S. Cooper, and T. E. Forrester, Energetic

- determinants of glucose tolerance status in Jamaican adults, *Eur J Clin Nutr* 58 (2004) 1666-1668.
8. S. L. Ataman, R. Cooper, C. Rotimi, D. McGee, B. Osotimehin, S. Kadiri, S. Kingue, W. Muna, H. Fraser, T. Forrester, and R. Wilks, Standardization of blood pressure measurement in an international comparative study, *J Clin Epidemiol* 49 (1996) 869-877.
 9. N. K. Gopaul, M. D. Manraj, A. Hebe, S. Lee Kwai Yan, A. Johnston, M. J. Carrier, and E. E. Anggard, Oxidative stress could precede endothelial dysfunction and insulin resistance in Indian Mauritians with impaired glucose metabolism, *Diabetologia* 44 (2001) 706-712.
 10. D. Il'yasova, J. D. Morrow, and L. E. Wagenknecht, Urinary F2-isoprostanes are not associated with increased risk of type 2 diabetes, *Obes Res* 13 (2005) 1638-1644.

Table 1. Metabolic characteristics at baseline and 3.9 years later in 52 individuals who progressed to diabetes or impaired glucose tolerance, and in 44 nonprogressors.

Data are means \pm SE

	Baseline		Follow-up	
	Nonprogressors	Progressors	Nonprogressors	Progressors
Fasting plasma glucose (mmol/l)	5.1 \pm 0.1 ^a	4.9 \pm 0.1 ^b	4.8 \pm 0.1	5.5 \pm 0.2 ^c
2-hour plasma glucose (mmol/l)	6.9 \pm 0.3	6.5 \pm 0.2 ^b	6.9 \pm 0.3	9.9 \pm 0.4 ^d
Fasting plasma insulin (μ U/ml)	7.75 \pm 0.73	8.05 \pm 0.74	7.42 \pm 0.66	8.28 \pm 0.86
HOMA-%S	128.1 \pm 9.2	117.8 \pm 8.7	129.6 \pm 8.8	112.1 \pm 7.4
HOMA-%B	92.9 \pm 6.4	108.7 \pm 7.5 ^b	100.4 \pm 6.3	88.4 \pm 5.7
Urinary isoprostane (pg/ μ mol creatinine)	598 \pm 83	585 \pm 65	829 \pm 155	638 \pm 85

Notes

^a $p < 0.05$ comparing nonprogressors at baseline and at 3.9 years

^b $p \leq 0.01$ comparing progressors at baseline and at 3.9 years

^c $p < 0.01$, comparing progressors vs. nonprogressors at 3.9 yrs

^d $p < 0.001$, comparing progressors vs. nonprogressors at 3.9 yrs