



## SEASONAL VARIATION IN UTEROTONIC ACTIVITY OF *RHOICISSUS TRIDENTATA* EXTRACTS

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**Background.** *Rhoicissus tridentata* lignotubers are widely used in southern African traditional pregnancy-related remedies.

**Objectives.** To determine the seasonal variation in contractile activity of extracts from different parts of the plant.

**Methods.** Isolated rat uterus tissue was used to compare the contractile activity of crude aqueous extracts of *R. tridentata* made from plant material harvested every 3 months over a period of 2 years.

**Results and conclusions.** The activity of the plant extracts from plants harvested in summer and autumn were 4 - 5-fold higher than extracts from plants harvested in winter or spring. The tubers stimulated the greatest degree of contractions, followed by the stems, roots and leaves. These results highlight the need to standardise the timing of harvesting *R. tridentata*.

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Decoctions of the roots and lignotubers of *Rhoicissus tridentata* (subsp. *cuneifolia*) are used by southern African women in ethnic herbal remedies for delayed childbirth and to facilitate childbirth.<sup>1,2</sup> *R. tridentata* decoctions are prepared by boiling dried milled roots or lignotubers for approximately an hour. This is done by traditional healers who sell the bottled decoctions. The plant is widely distributed throughout the eastern portion of southern Africa,<sup>3</sup> and is found in most traditional healer vending stores around Johannesburg.

Pharmacological investigations using isolated rat uterus and ileum suggest that the contractile response to hot water plant extracts may be mediated by muscarinic receptors and the stimulation of prostaglandin synthesis.<sup>4</sup> While doing preliminary work on the pharmacology of the plant extracts it became evident that there was a high degree of variability in the activity of extracts from different plant samples. The pharmacological response to *R. tridentata* has been shown to vary geographically. The plant extracts acted either agonistically or antagonistically on muscarinic receptors

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depending on where the plant was harvested.<sup>5</sup> Temporal variation is another possible source of variability in chemical constituents and biological activity of plants. This is a well-documented phenomenon.<sup>6,7</sup> The expression of genes coding for chemical constituents of plants is under the control of various factors, such as the developmental stage of the organism, duration and intensity of light, nutrient supply, triggering of internal signals and sequential expression of genes coding for secondary metabolites.<sup>8</sup> All the above factors vary temporally, which in turn causes seasonal fluctuations in the levels of both primary and secondary metabolites within plant tissues.

The aim of this study was to determine whether the contractile activity of *R. tridentata* varies seasonally.

### METHODS

*R. tridentata* subsp. *cuneifolia* plant material was collected from Suikerbosrand Nature Reserve, 60 km south of Johannesburg. Three plants were harvested every 3 months from May 1996 to April 1998. The identity of the plants was validated by L Katsoulis, and one voucher specimen for each individual plant harvested was lodged in the C E Moss Herbarium (voucher specimen numbers of the three plants: 086928, 082929 and 082930).

Milled plant material was boiled for an hour, settled, and the supernatant lyophilised. Sections of uterine tissue obtained from the central portion of the uterine horns of oestrogenised virgin Sprague-Dawley rats were mounted in 50 ml organ baths containing aerated (5% CO<sub>2</sub> in O<sub>2</sub>) Tyrode solution. The uterine tissue was maintained at 26°C to decrease spontaneous contractility. The organs were rinsed repeatedly while being allowed to return to baseline, before any investigations. Control challenges with acetylcholine were done between each test challenge. The activity of the extracts was tested by incubating the tissues with 1.3 mg/ml of the various plant extracts for 5 minutes before adding acetylcholine cumulatively. This was done to determine whether the plant extracts alter the organ's response to acetylcholine. Isotonic contractions were measured against 1 g resistance and expressed relative to the maximal response of the same tissues to acetylcholine. More details of the methods are given in the study by Veale *et al.*<sup>9</sup>

### RESULTS AND DISCUSSION

Fig. 1 illustrates how the different parts of the plants harvested in winter and spring all yielded aqueous extracts with low levels of contractile activity, whereas the extracts from different parts of the plants harvested during summer and autumn were considerably more active. During summer the tubers yielded the most active aqueous extracts, followed by the stems, roots and leaves. The autumn plants exhibited a similar pattern of

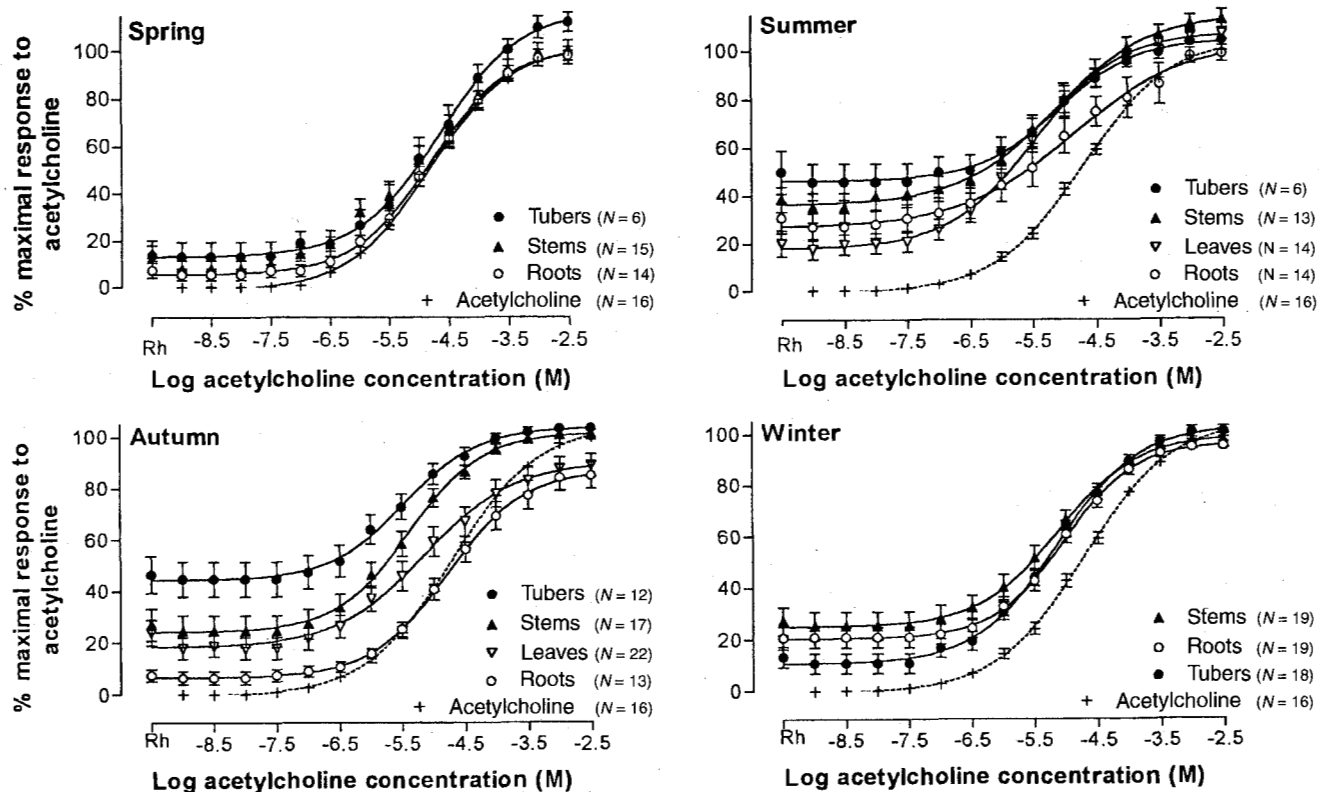


Fig. 1. Dose response curves of isolated rat uterus when pre-treated with 1.3 mg/ml aqueous extracts of all parts of *Rhoicissus tridentata* subsp. *cuneifolia* harvested during spring before the cumulative addition of acetylcholine. Each point represents the mean with the vertical bars representing the standard error of the mean (SEM).

variation except that the roots were the least active part. The autumn and summer tubers were four and five times more active than the winter and spring extracts. The stems displayed similar seasonal variability.

Statistical differences between the direct contractile activity of the plants, shown by 'Rh' on the dose response curves, are given in Table I.

All parts of the plant harvested in summer and autumn were able to stimulate contractions of the isolated uterus directly. Traditional healers usually use the lignotubers, which yield the most uterotonic extracts. The activity of the tuber extracts is highest during the growing season and decreases during the seasons when the plant is dormant.

Table I. Statistical differences between the contractile activity of extracts from different parts of *Rhoicissus* plants harvested during different seasons

Tubers	Stems	Leaves	Roots
Spring v. summer*	Spring v. summer*	Spring v. summer†	Spring v. summer†
Spring v. autumn*	Spring v. autumn*	Spring v. autumn†	Spring v. autumn†
Summer v. winter†	Summer v. winter†	Summer v. winter†	Summer v. winter†
Autumn v. winter†	Autumn v. winter†	Autumn v. winter†	Autumn v. winter†
*p < 0.05			
†p < 0.01			
‡p < 0.001			

The root and stem extracts showed a similar pattern in seasonal variation in uterotonic activity to that of the tuber extracts. That is, the activity of the extracts started increasing when plants were harvested after the first rains. The activity increased throughout the growing season (or rainy season), reaching a peak in autumn. The activity of the extracts then fell as the plants lost their leaves and became dormant during winter. The rise in activity as the rains started could have been caused by the improved uptake of minerals from the soil following the mineralisation flush after the first rains, which usually leads to a sharp rise in the nitrogen content within the plant.<sup>10</sup>

Variability in the contractile activity of the extracts could have been caused by: (i) altered concentrations of the active principle(s); (ii) a change in the quality or nature of the active principle(s); or (iii) changes in the production of compounds such as saponins which may have altered extraction of the pharmacologically active component(s).

RELEVANCE TO CONSERVATION

The stem extracts yielded a similar response to the most active part, namely the lignotubers. This is significant from an environmental perspective, as harvesting stems for the trade in traditional remedies would have a much lower impact on the



plant populations of *R. tridentata* than harvesting the lignotubers and roots. With current use of the lignotubers, the plant has to be uprooted for lignotubers to be harvested.

### CLINICAL RELEVANCE

Variability in the uterine response to the decoctions from different seasons highlights a severe flaw in the use of traditional herbal remedies. Collectors and vendors have no way of determining the chemical composition of the plant material harvested. Eight traditional healers in and around Johannesburg were questioned on whether they were aware of any seasonal variation in the efficacy of *R. tridentata*. None of them seemed to be aware of the concept.

Traditional healers were also unaware of any toxic effects of the plant (personal communication) despite reports implicating the plant in fatal poisonings.<sup>11,12</sup> This suggests that only certain plant samples may be toxic. It is possible that the toxicity of the plant also fluctuates seasonally, which would explain why traditional healers are generally unaware of the toxic potential of *R. tridentata*. Further investigation should be done to determine whether the poisonings that have occurred are concentrated at a certain time of the year.

Variability in the pharmacological activity of *R. tridentata* also reiterates the well-accepted importance of standardisation criteria that need to be established for herbal remedies. The results also support the suggestion by Tobler<sup>13</sup> that there should be a holistic approach to standardising the production of medicinal plants by controlling the entire production process for medicinal plants, starting from cultivation and continuing the control through the processing of the plant material.

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## PATHOGENIC MECHANISM OF TYPE 2 DIABETES IN GHANAIS — THE IMPORTANCE OF BETA CELL SECRETION, INSULIN SENSITIVITY AND GLUCOSE EFFECTIVENESS

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**Objective.** To assess insulin sensitivity and beta cell secretion in indigenous Ghanaian subjects with a spectrum of glucose intolerance.

**Research and methods.** We evaluated beta cell secretion, insulin sensitivity (Si) and glucose effectiveness (Sg) in three groups: group 1, 15 healthy control subjects without family history of type 2 diabetes; group 2, 11 healthy non-diabetic first-degree relatives of Ghanaian patients with type 2 diabetes; and group 3, 10 patients with type 2 diabetes living in Accra, Ghana, West Africa. A standard oral glucose tolerance test (OGTT) and frequently sampled intravenous glucose tolerance (FSIGT) test were performed for each subject. Si and Sg were measured using Bergman's minimal model method.

**Results.** The mean body mass index (BMI) and lean body mass were not different among the three groups. However, the waist-to-hip circumference ratio, total body fat as well as triceps and biceps skinfolds were significantly greater in group 3 (diabetic patients) than in group 2 (relatives) and group 1 (healthy controls). Mean fasting and postprandial serum glucose levels were not significantly different between the relatives and healthy controls during oral glucose challenge. The mean fasting and postprandial serum glucose levels were significantly higher in the group 3 diabetic patients than in the non-diabetic groups. Mean fasting serum insulin and C-peptide levels tended to be higher in group 3 than in groups 1 and 2. However, mean serum insulin and C-peptide responses after oral glucose load were significantly greater in group 2 than in the group 1 healthy controls. The insulin responses in the two non-diabetic groups after oral glucose challenge were significantly greater than in the diabetic patients. During the FSIGT, the mean serum glucose

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responses were similar in the two non-diabetic groups (groups 1 and 2). The serum glucose responses were significantly greater in group 3 than in the non-diabetic groups. Mean total and acute first and second phases of insulin and C-peptide responses were greater in group 2 than group 1. However, acute phases of insulin secretion were severely blunted in group 3 when compared with groups 1 and 2 during FSIGT in our Ghanaians. We found that the mean Si was slightly lower in group 2 ( $1.72 \pm 0.32$ ) than in the healthy controls in group 1 ( $1.9 \pm 0.55$ ,  $P = \text{NS}$ ). Mean Si was remarkably lower in the diabetic patients in group 3 ( $1.30 \pm 0.35 \times 10^{-4}/\text{min}$  ( $\mu\text{U}/\text{ml}$ )) when compared with the relatives and healthy controls, but the differences were not statistically significant. Mean glucose effectiveness at basal insulin level (Sg) was not significantly different among the relatives in group 2 ( $2.38 \pm 0.50$ ), the healthy controls in group 1 ( $2.66 \pm 0.38$ ) and the diabetic patients in group 3 ( $2.27 \pm 0.49 \times 10^{-2}/\text{min}$ ).

**Conclusions.** We conclude that (i) the pathogenetic mechanisms of type 2 diabetes in indigenous Ghanaians are characterised by severe beta cell dysfunction and moderate reduction in Si. Although the healthy relatives manifest insulin resistance with compensatory hyperinsulinaemia, our study suggests that the conversion of such subjects to type 2 diabetes is determined by deterioration in beta cell function and perhaps Si but not tissue Sg in Ghanaians. Prospective studies are needed to examine the sequential changes that lead to the development of type 2 diabetes in indigenous Ghanaians.

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The global epidemic of type 2 diabetes is of major concern to diabetologists and health care policy makers in developing and developed countries.<sup>1,3</sup> The aetiology of the disease is unknown in most cases. It is well recognised that type 2 diabetes is a genetic and familial disease.<sup>3,5</sup> The hallmark of this disease is insulin resistance and beta cell dysfunction in several populations.<sup>6,9</sup> The disease is very heterogeneous and the aetiologies may be different.<sup>9,17</sup> It is well established that the respective contributions of the pathogenetic components of hyperglycaemia and type 2 diabetes vary considerably depending on the ethnicity and race of the populations. Previous studies have suggested that the prevalence of type 2 diabetes was very low in West Africa<sup>18,19</sup> and other parts of Africa.<sup>20</sup> However, we have recently observed that the rate of type 2 diabetes in 5 000 Ghanaians residing in Accra and surrounding suburbs is approaching 5% (A B G Amoah *et al.* — unpublished observation). To the best of our knowledge, however, detailed anthropometric and metabolic characterisation of beta cell function and insulin action has not been undertaken in West Africans with and without type 2 diabetes residing in their country of origin.

Previous studies have indicated that non-diabetic first-degree

relatives of patients with type 2 diabetes and certain ethnic groups, e.g. African Americans, native Americans, etc., have an increased propensity for type 2 diabetes.<sup>12-16,21-24</sup> These studies have demonstrated that insulin resistance and hyperinsulinaemia occur in prediabetic offspring and first-degree relatives of patients with type 2 diabetes.<sup>15,16</sup> On the contrary, other authorities argue that beta cell dysfunction may be the primary disorder underlying type 2 diabetes. Therefore, we believe that metabolic and anthropometric studies of normoglycaemic, first-degree relatives could provide important insights into the pathophysiology of type 2 diabetes in Ghanaians.

The aim of the present study was, therefore, to assess beta cell function, insulin sensitivity (Si) and glucose effectiveness (Sg) in patients with type 2 diabetes, non-diabetic first-degree relatives of Ghanaian patients (who are presumably genetically at greater risk for the disease) and healthy control subjects without family history of type 2 diabetes.

## SUBJECTS, MATERIALS AND METHODS

### Subjects

The study included three Ghanaian populations, namely group 1: 15 healthy control subjects who were glucose-tolerant Ghanaians without a family history of diabetes; group 2: 11 glucose-tolerant Ghanaians who were first-degree relatives of patients with type 2 diabetes mellitus; and group 3: 10 patients with type 2 diabetes as defined by the World Health Organisation (WHO) criteria.<sup>25</sup> All the subjects lived in the greater Accra metropolitan area, with a population of about 1.2 million. The subjects were not members of the same family. The first-degree relatives were either offspring or siblings of patients with type 2 diabetes who were attending the University of Ghana Outpatient Diabetes Clinic, Korle Bu Teaching Hospital, Accra, Ghana. Patients with known type 2 diabetes were defined as: (i) individuals who developed the disease after 40 years; (ii) individuals currently receiving oral glibenclamide or metformin for glycaemic control; and (iii) individuals who had never been admitted to the hospital for diabetic ketoacidosis. Individuals who received insulin were initially treated with a sulfonylurea or metformin for several years before converting to insulin therapy. Since type 1 diabetes is generally uncommon in adult Ghanaians, we do not believe that any of our patients had type 1 diabetes. The non-diabetic healthy relatives were recruited or contacted through their parents and/or siblings followed by a personal visit to the homes of these subjects by one of the authors. This approach was adopted to ensure and ascertain that the subjects were first-degree relatives of our type 2 diabetic patients. The healthy control subjects were recruited from the same neighbourhoods and the medical complex as well as door-to-door recruitment visits. The healthy control subjects were recruited only if to the best of their knowledge none of their family members or relatives had a diagnosis of diabetes. An oral glucose tolerance test was not done in the case of parents or siblings of the healthy control subjects. Therefore, the lack



of biochemical ascertainment of parental diabetes or history of diabetes in the healthy controls could have resulted in misclassification of some of our healthy controls if their parents or siblings had pre-existing, asymptomatic, undiagnosed type 2 diabetes. Apart from the diabetic patients who were taking either a sulfonylurea or metformin, subjects taking medications known to influence glucose and insulin metabolism were excluded from the study. The oral antidiabetic medications were discontinued 3 - 5 days before the metabolic studies. In addition, subjects known to have heart, renal and liver disease by history, physical examination and appropriate laboratory testing were excluded. Informed written consent approved by the Institutional Review Board for Human Research at the University of Ghana Medical School, Accra, Ghana was obtained from each subject after the risks entailed in the study had been thoroughly explained. Clinical characteristics of the three populations are shown in Table I.

**Table I. Clinical and biochemical parameters of non-diabetic first-degree relatives of Ghanaian patients with type 2 diabetes mellitus and healthy controls (mean  $\pm$  SEM)**

Parameter	Controls	Relatives	Type 2 DM patients
Number	15	11	10
Sex (f/m,%)	37	52	59
Age (yrs)	36.5 $\pm$ 1.63	30.8 $\pm$ 1.5#	43.4 $\pm$ 1.3***
Bwt (kg)	68.6 $\pm$ 1.9	68.4 $\pm$ 2.5	71.2 $\pm$ 3.1
Height (m)	1.6 $\pm$ 0.02	1.64 $\pm$ 0.01	1.66 $\pm$ 0.02
BMI (kg/m <sup>2</sup> )	26.9 $\pm$ 2.3	25.9 $\pm$ 1.6	26.0 $\pm$ 0.03
LBM (%)	74.3 $\pm$ 1.6	73.4 $\pm$ 1.7	73.3 $\pm$ 2.1
BFM (%)	25.7 $\pm$ 1.7	26.8 $\pm$ 1.7	27.2 $\pm$ 2.4
WHR	0.86 $\pm$ 0.02	0.81 $\pm$ 0.03#	0.90 $\pm$ 0.02***
Skinfold thickness (mm)			
Triceps	16.36 $\pm$ 1.72	19.21 $\pm$ 2.01	19.65 $\pm$ 1.88
Biceps	7.53 $\pm$ 0.96	10.44 $\pm$ 1.59	11.80 $\pm$ 1.51**
Scapular	16.50 $\pm$ 1.54	17.92 $\pm$ 2.02	20.56 $\pm$ 1.89**
Subscapular	19.77 $\pm$ 1.90	23.02 $\pm$ 2.29	25.30 $\pm$ 2.27*

Bwt = body weight; BMI = body mass index, LBM = lean body mass; BFM = body fat mass; WHR = weight-hip circumference ratios; NS = not statistically significant; DM = diabetes mellitus.

\* $P < 0.05$  DM v. controls.

\*\* $P < 0.02$ , relatives v. controls

# $P < 0.05$  and \*\*\* $P < 0.001$  DM v. relatives and controls.

### Study protocol

After a 10 - 12-hour overnight fast subjects reported to the Diabetes Research Laboratory, Department of Medicine, University of Ghana Medical School. Body weight and height were measured with the subject wearing a very light gown and no shoes. Lean body mass and body fat composition were measured using the bioelectrical impedance analyser.<sup>26</sup> Body fat distribution was measured as the waist-to-hip circumference ratio. The waist circumference was measured at the level of the umbilicus (with the subject in a supine position) and the hip circumference at the level of the greater trochanter (in the

standing position). All the subjects answered a simple questionnaire on physical activity. Subjects who participated in endurance or competitive sport were excluded because of the effects of chronic strenuous exercise on insulin sensitivity.

### Metabolic studies

With the subject in the supine position, two intravenous needles ('heparin lock') were inserted into the forearm veins and kept patent with 0.9% normal saline infusion. One intravenous line was used to draw blood samples and the other to administer the intravenous glucose and exogenous insulin.

### Oral glucose tolerance test (OGTT)

Except for the diabetic patients, each subject was instructed to ingest at least 250 g carbohydrate in his or her regular meals for at least 3 days before the test. After the 10 - 12-hour overnight fast subjects ingested 75 g oral glucose load (Koladex, Baltimore, Md.) over a 2-minute period. Blood samples for serum glucose, insulin and C-peptide were obtained at baseline,  $t = 0$  minutes, and 30, 60, 90, 120, 150, 180, 210 and 240 minutes. The glucose tolerance status of the subjects was defined using the WHO criteria.<sup>27</sup>

### Frequently sampled intravenous glucose tolerance (FSIGT) test

The insulin-modified frequently sampled intravenous glucose tolerance (FSIGT) test was performed in each subject.<sup>28-30</sup> Four blood samples were obtained at  $t = -20, -10, -5,$  and 0 minutes for basal serum glucose, C-peptide and insulin concentrations. The average of the four samples was taken as the basal value. Thereafter, 0.3 g/kg glucose (50 ml of 50% dextrose water) was infused over a 1-minute period. At  $t = 19$  minutes, intravenous insulin (0.05 U/kg, Humulin, Eli Lilly, Indianapolis, Ind.) dissolved in 30 ml of 0.9% normal saline was infused over 60 seconds. Blood samples were obtained at frequent intervals at  $t = 2, 3, 4, 5, 6, 8, 10, 12, 16, 19, 22, 24, 25, 27, 30, 40, 60, 70, 90, 120, 140, 150, 160$  and 180 minutes for serum glucose, C-peptide and insulin concentrations. All the samples were centrifuged at 4°C and the sera were frozen and stored at -20°C until assayed.

### Analytical methods

Serum glucose concentrations were measured by the glucose oxidase method using a glucose autoanalyser (Beckman Instruments, Fullerton, Calif.). The serum insulin and C-peptide levels of each individual were determined by a standard double antibody radio-immunoassay technique in the same assay at the Core Laboratories of the Ohio State University Hospitals. The sensitivity of the insulin assay was 2.5 mU/l. The intra- and inter-assay coefficients of variation (CV) were 6% and 10%, respectively. The lower limit of the C-peptide assay was 0.47 ng/ml (0.02 nmol/ml) and the intra- and inter-assay CVs were 7% and 13%, respectively. The



C-peptide assay did not cross with the insulin assay. In contrast, our insulin cross-reacted 100% with proinsulin (PI). However, we have previously found (unpublished personal observation) that the per cent contribution of PI, using a specific PI assay, to the total immunoreactive insulin (IRI) is less than 10%. Therefore the absolute contribution to the total IRI in normal subjects is quantitatively very small.

### Calculations and statistical analyses

Results are expressed as mean  $\pm$  standard error of mean (SEM), unless stated otherwise. The body mass index (BMI) was calculated as weight (kg) divided by height ( $m^2$ ). Obesity was taken as BMI greater than 30 kg/ $m^2$  for females and males. The acute first ( $t = 0 - 5$  minutes) and second ( $t = 8 - 19$  minutes) phases of insulin release were calculated as the area under the curves (AUC) for insulin and C-peptide responses above the baseline values using the trapezoidal rule. Note that during routine FISGT tests the acute second phase insulin areas are often estimated as the time between  $t = 10 - 60$  minutes. In the present study this was abbreviated to 19 minutes because of the administration of exogenous insulin at  $t = 19$  minutes. The insulin sensitivity index (Si) and glucose effectiveness (Sg) were calculated using the minimal model software program MINMOD (copyright, Dr Richard Bergman).<sup>29,30</sup> Step-wise linear regression and two-way analyses of covariance (ANOCOVA) were used to adjust for the effects of age, sex, body weight and waist/hip ratio (WHR) on the various metabolic parameters. Linear regression and correlation coefficients were calculated using the least squares method. The non-parametric data were analysed using chi-square and Mann-Whitney rank tests. Statistical analyses were performed using the Student's *t*-test (unpaired) and ANOVA, where appropriate, with the Bonferroni method for *post hoc* testing. For comparison of the mean data with unequal variance, the Neuman-Keuls multiple *t*-test was used. Probability (*P*) value less than 0.05 was considered statistically significant.

## RESULTS

### Clinical characteristics

As shown in Table I, the mean age tended to be highest in the group 3 type 2 diabetic patients, intermediate in the group 1 healthy controls and lowest in the group 2 healthy relatives. The younger age of the relatives reflects the fact that most of our type 2 diabetic patients began having children in their 20s, similar to most Ghanaian women. Despite similar BMIs and lean body mass, the WHR (a surrogate of upper body obesity) and tricep and bicep skinfolds were significantly higher in the type 2 diabetic patients (group 3), intermediate in healthy controls (group 1) and lowest in the first-degree relatives (group 2). Similarly, the per cent body fat mass was highest in the type 2 diabetic patients, intermediate in the healthy relatives and lowest in the healthy controls.

### Oral glucose tolerance test

Mean fasting serum glucose levels were similar in the healthy control group and relatives ( $4.3 \pm 0.2$  v.  $4.2 \pm 0.2$  mmol/l). The mean fasting serum glucose level was  $7.2 \pm 0.6$  mmol/l in the type 2 diabetic patients (Fig. 1A, and Table II). After the oral glucose challenge the serum glucose responses and AUCs were higher in the group 3, type 2 diabetic patients than in the non-diabetic groups. Mean AUCs for serum glucose were similar in the first-degree relatives v. healthy control subjects (Fig. 1A, and Table II). Mean fasting serum insulin levels were comparable among the three groups (Table II, Fig. 1B). Following the oral glucose challenge, serum insulin levels were significantly higher ( $P < 0.05$ , and  $P < 0.001$ ) at 30 and 90 minutes in the first-degree relatives than in the healthy controls (Table II, Fig. 1B). The group 3 type 2 diabetic patients had severely blunted incremental AUC values for insulin responses when compared with groups 1 and 2. Therefore the insulin/glucose ratios were significantly lower in group 3 when compared with the non-diabetic groups. Mean serum C-peptide responses followed a trend similar to those of the insulin responses in the three groups (Table II and Fig. 1C).

Table II. Acute early serum glucose, insulin and C-peptide levels during oral tolerance in Ghanaians with varying degrees of glucose tolerance (mean  $\pm$  SEM)

Parameter	Controls	Relatives	Type 2 DM patients
Glucose (mmol/l)			
0 mins	$4.3 \pm 0.2$	$4.2 \pm 0.2$	$7.2 \pm 0.6^{\ddagger}$
30 mins	$6.3 \pm 0.4$	$6.6 \pm 0.2$	$9.8 \pm 0.6^{\ddagger}$
AUC*	$501 \pm 61$	$513 \pm 61$	$1019 \pm 87^{\ddagger}$
Insulin ( $\mu$ U/l)			
0 mins	$23.5 \pm 3.0$	$24.7 \pm 1.3$	$8.9 \pm 1.7^{\ddagger}$
30 mins	$69.0 \pm 7.8$	$77.0 \pm 6.0$	$22.7 \pm 7.5^{\ddagger}$
AUC†	$9343 \pm 1289$	$11286 \pm 1065$	$3441 \pm 643^{\ddagger}$
C-peptide (nmol/ml)			
0 mins	$0.44 \pm 0.04$	$0.48 \pm 0.03$	$0.41 \pm 0.04$
30 mins	$1.16 \pm 0.11$	$1.44 \pm 0.11$	$0.92 \pm 0.03^{\ddagger}$
AUC‡	$175 \pm 15$	$267 \pm 19^{\S}$	$115 \pm 17^{\parallel}$

\*AUC = mmol/l  $\times$  mins.

†AUC = pmol/ml  $\times$  mins.

‡AUC = nmol/ml  $\times$  mins.

§ $P < 0.05$ , relatives v. controls.

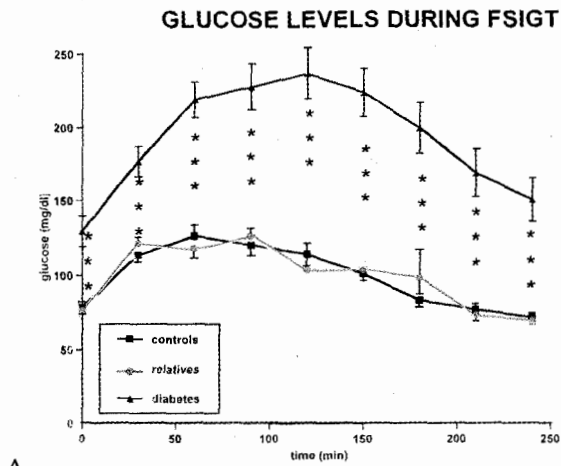
¶ $P < 0.001$ , DM patients v. relatives and controls.

|| $P < 0.001$ , DM patients v. relatives and controls.

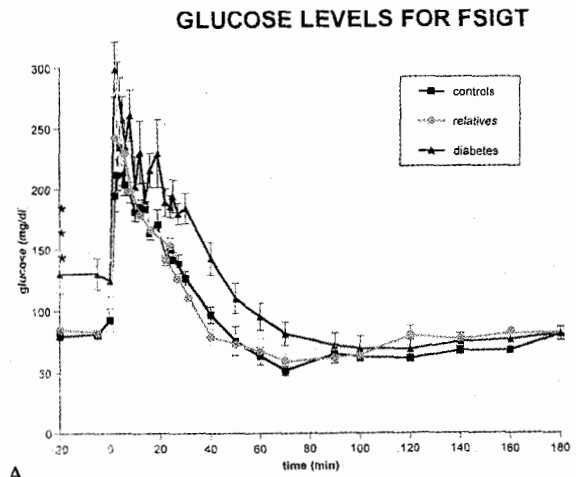
AUC = area under the curve; DM = diabetes mellitus.

### Frequently sampled intravenous glucose tolerance test

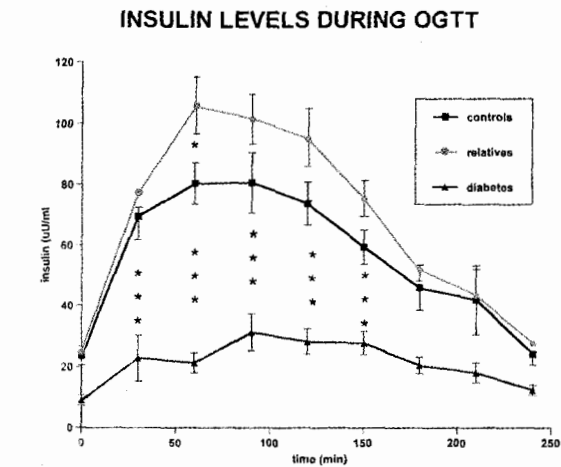
After intravenous glucose administration, mean glucose rose to peak levels of  $15.4 \pm 2.0$  and  $13.1 \pm 1.2$  mmol/l and  $15.24 \pm 1.4$  mmol/l in the relatives, healthy controls and diabetic patients, respectively (Table III, Fig. 2A). Following the



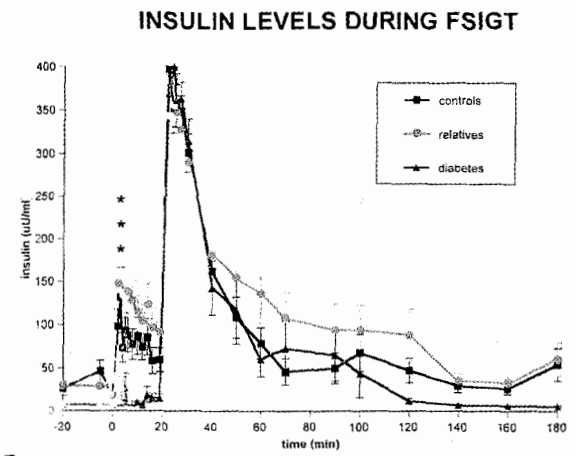
A



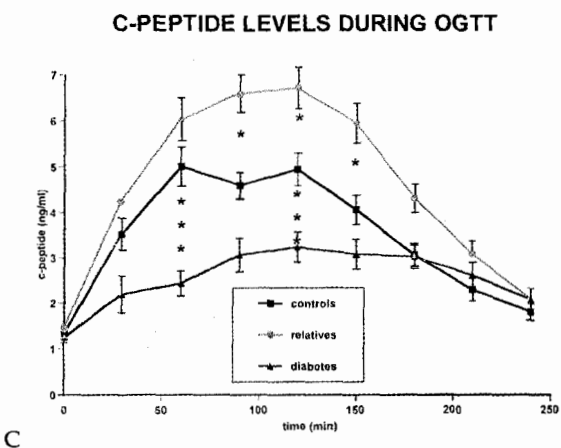
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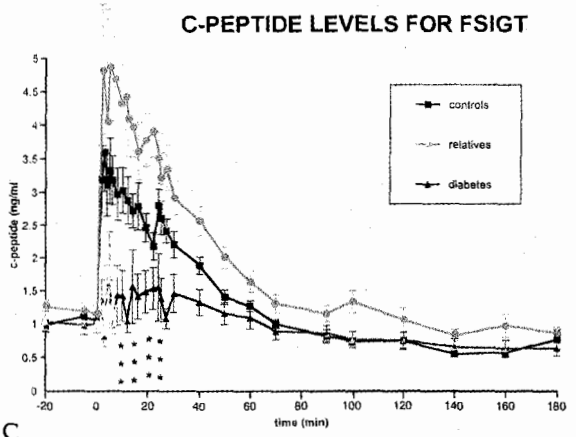
B



B



C



C

Fig. 1. Mean  $\pm$  SEM serum glucose (A), insulin (B) and C-peptide (C) levels before and during the oral glucose tolerance test in Ghanaian healthy controls with normal glucose tolerance (closed squares), first-degree relatives of patients with type 2 diabetes mellitus (closed circles), and type 2 diabetes mellitus patients (closed triangles). \* $P < 0.05$  relatives v. healthy controls, \*\*\* $P < 0.001$  diabetics v. relatives and healthy controls. (To convert insulin to pmol/ml, multiply by 6, to convert glucose to mmol/ml divide by 18, and to convert C-peptide to nmol/ml, multiply by 0.33.)

Fig. 2. Mean  $\pm$  SEM serum glucose (A), insulin (B) and C-peptide (C) levels before and during the oral glucose tolerance test in Ghanaian healthy controls with normal glucose tolerance (closed squares), first-degree relatives of patients with type 2 diabetes mellitus (closed circles), and type 2 diabetes mellitus patients (closed triangles). \* $P < 0.05$  relatives v. healthy controls, \*\*\* $P < 0.001$  diabetics v. relatives and healthy controls. (To convert insulin to pmol/ml multiply by 6, to convert glucose to mmol/ml divide by 18, and to convert C-peptide to nmol/ml multiply by 0.33.)



Table III. Acute first and second phase serum insulin and C-peptide secretion following FSIGT tests in non-diabetic first-degree relatives of Ghanaian patients with type 2 diabetes mellitus, patients with type 2 diabetes and healthy controls (mean  $\pm$  SEM)

Parameters	Controls	Relatives	DM patients	Controls v. relatives	DM patients v. controls and relatives
Acute responses (OGTT)					
Insulin (mU/l)					
0 mins	26.1 $\pm$ 3.84	28.9 $\pm$ 4.8	6.8 $\pm$ 1.0	0.095	0.001
Peak	131.2 $\pm$ 34	147.8 $\pm$ 26	27.1 $\pm$ 16	0.001	0.001
C-peptide (nmol/ml)					
0 mins	0.36 $\pm$ 0.03	0.38 $\pm$ 0.03	0.33 $\pm$ 0.04	NS	NS
Peak	1.18 $\pm$ 0.19	1.60 $\pm$ 0.15	0.56 $\pm$ 0.230	0.002	0.001
Areas under curve (AUC)					
First phase (FSIGT)					
Insulin (mU/l $\times$ mins)	122 $\pm$ 75	320 $\pm$ 11.7	7.80 $\pm$ 5.7	0.01	0.001
C-peptide (nmol/ml $\times$ mins)	7.2 $\pm$ 1.1	12.7 $\pm$ 3.27	2.66 $\pm$ 2.29	0.130	0.001
Second phase (FSIGT)					
(Insulin $\mu$ U/ml $\times$ mins)	9 8731 $\pm$ 2 025	14 656 $\pm$ 2 930	10 120 $\pm$ 1 725	NS	NS
C-peptide (nmol/ml $\times$ mins)	8.9 $\pm$ 1.3	71.2 $\pm$ 12.4	14.9 $\pm$ 1.4	0.001	0.170

OGTT = oral glucose tolerance test; FSIGT = frequently sampled intravenous glucose tolerance test; DM = diabetes mellitus; NS = not significant.

Table IV. Minimal model derived insulin sensitivity (Si) index and glucose effectiveness (Sg) at basal insulin in healthy controls and first-degree relatives of Ghanaian patients with type 2 diabetes mellitus (mean  $\pm$  SEM)

Parameters	Controls	Relatives	Type 2 DM patients	
Si ( $\times 10^{-4}$ /min/(mU/l))	1.91 $\pm$ 0.55	1.72 $\pm$ 0.32	1.30 $\pm$ 0.35	NS
Sg ( $\times 10^{-2}$ /min)	2.66 $\pm$ 0.38	2.38 $\pm$ 0.50	2.27 $\pm$ 0.49	NS

DM = diabetes mellitus; NS = not significant.

intravenous glucose load, the mean serum insulin responses (acute first and second phases) as assessed by the absolute and incremental AUCs were greater but not significantly different in the first-degree relatives and healthy controls (Table III, Fig. 2B). Both acute phases of insulin secretion were severely blunted in the type 2 diabetic patients when compared with the relatives and healthy controls. Mean serum C-peptide responses and AUCs followed patterns similar to those of the insulin profiles in the three groups (Table II, Fig. 2C).

### Minimal model parameters

As shown in Table IV, the mean Si was lowest in the type 2 diabetic patients, intermediate in the relatives and highest in the healthy controls, but the difference was not significant ( $P = NS$ ) among the relatives and healthy controls and diabetic patients receiving active antidiabetic therapy. The mean Sg was not significantly different in the type 2 diabetic patients, healthy first-degree relatives and healthy control subjects.

### DISCUSSION

Type 2 diabetes has become a global epidemic in several populations, especially in developing countries.<sup>1-3</sup> Among people of West African ancestry, the prevalence of type 2

diabetes varies depending on whether these individuals reside in urban or rural areas or Western industrialised versus developing countries.<sup>31,32</sup> Although type 2 diabetes is the commonest type of diabetes in the world, it is clearly a heterogeneous disease with varying phases of presentation. It is now clear that type 2 diabetes and its long-term complications have strong genetic and environmental components which differ among various populations. In this regard, we have recently observed increasing prevalence of type 2 diabetes and its associated long-term morbidity and mortality in our Ghanaian population.<sup>18,19</sup>

In the present study we examined the various components of hyperglycaemia in indigenous Ghanaians residing in their native country. We felt that the study could shed light on the mechanism(s) of type 2 diabetes in indigenous Ghanaians since we have previously demonstrated<sup>33</sup> that insulin secretion and insulin sensitivity in Ghanaian immigrants with and without type 2 diabetes and impaired glucose tolerance (IGT) are similar to those of African Americans residing in the USA. Furthermore, we were also interested to examine the importance of genetically mediated antecedent metabolic risk factors for type 2 diabetes in indigenous Ghanaians, which have been well described in previous studies of Caucasians and other high-risk populations.<sup>12-16</sup>





The present study demonstrated that the diabetic patients previously treated with either metformin or a sulfonylurea manifested moderately severe hyperglycaemia during fasting and after glucose challenge, as one would expect. In contrast, the serum glucose responses were similar in the relatives and healthy controls. We observed that hyperglycaemia in the Ghanaian patients with type 2 diabetes occurred at the expense of a severe beta cell dysfunction. During OGTT the serum insulin and C-peptide responses were 60% and 70% lower in the diabetic patients when compared with the healthy controls and the relatives, respectively. These findings are, however, similar to those reported in several other populations, including Ghanaian immigrants and African Americans residing in the USA.<sup>33,36</sup> Comparing the relatives and healthy controls, we found that after glucose challenge the mean stimulated absolute serum insulin and C-peptide responses were significantly greater in the relatives than in the healthy controls by ANOVA. These findings were also similar to our previous observations of healthy African Americans with and without a family history of type 2 diabetes residing in the USA,<sup>34,36</sup> and those of other ethnic groups.<sup>37,40</sup> The present metabolic findings in the relatives (albeit cross-sectional) would support genetic inheritance as the major determinant of beta cell insulin secretion in indigenous Ghanaians residing in their native country. Nevertheless, our data could also indicate that other genetic and/or environmental factors promote or induce beta cell dysfunction and precipitate the development of type 2 diabetes in Ghanaian individuals at greater risk for the disease.

Several previous studies<sup>12-16</sup> have indicated that insulin secretion is genetically inherited. Indeed, the lost or blunted acute first phase insulin secretion in response to glucose stimulation is regarded as one of the earliest lesions in the pathogenesis of type 2 diabetes in several populations.<sup>6-10,33,36</sup> We have previously demonstrated<sup>33,36</sup> loss of the acute first phase in African Americans and Ghanaian immigrants with impaired glucose tolerance and mild type 2 diabetes living in the USA. In this regard only few studies have examined the acute phases of insulin release in West Africans residing in their native country.<sup>24</sup> In the present study we found that the acute first phase of insulin secretion was blunted in the Ghanaians with type 2 diabetes. In contrast, the acute first phase was exaggerated in the first-degree relatives, demonstrating that early beta cell dysfunction is unlikely to be the primary lesion in Ghanaians with type 2 diabetes. Our present study of Ghanaian relatives is somewhat in contrast to the findings of Ezenwaka *et al.*<sup>24</sup> who found a lower acute first phase insulin secretion in the healthy, lean non-diabetic first-degree relatives of their Nigerian type 2 diabetic patients when compared with those without family history. We should note, however, that the Nigerian relatives were mostly males and had BMIs of 22 kg/m<sup>2</sup> when compared with the Ghanaians in the present study with an average BMI of 26 kg/m<sup>2</sup>. Therefore, similar to other populations, obesity appears to influence the beta cell secretion in individuals of West African ancestry. We

should also note that some studies of healthy Caucasian first-degree relatives<sup>13,22</sup> have demonstrated that the acute first phase of insulin release is either elevated or intact when compared with the first phase of those without family history of type 2 diabetes. In summary, our findings in the Ghanaians residing in their native country are consistent with those of other populations with regard to the role of beta cell dysfunction in the aetiopathogenesis of type 2 diabetes.

Previous studies have demonstrated that insulin resistance is the primary lesion underlying type 2 diabetes in these populations,<sup>6,8,9,16,41,43</sup> but these findings are not uniform.<sup>44,45</sup> Some of these previous studies<sup>15,16</sup> have confirmed that insulin resistance precedes the development of type 2 diabetes by decades. Osei *et al.*<sup>33,36</sup> have previously demonstrated insulin resistance in Ghanaian immigrants and/or African Americans residing in the USA with impaired glucose tolerance and mild type 2 diabetes when compared with healthy control subjects. Furthermore, healthy Ghanaian immigrants and African Americans residing in the same environment manifest similar degrees of Si index.<sup>34</sup> Because both populations originated from West Africa, i.e. remote and recent migrants, it was important to conduct studies among Ghanaians living in Ghana. In the present study we found that the indigenous Ghanaians with type 2 diabetes had an Si index 30% and 40% lower than the relatives and healthy controls, respectively. The lack of statistically significant difference in Si among the three groups is difficult to explain. We offer likely explanations for our findings.

First, we strongly believe that the physical activity levels and/or dietary factors (high fibre/complex carbohydrate and low-fat diet) which are known to influence Si could be involved. Second, unlike sulfonylureas, metformin has been demonstrated to improve insulin sensitivity in some previous studies, but the results have been inconsistent. Because our patients received either glibenclamide ( $N = 5$ ) or metformin ( $N = 5$ ), it could be inferred that these drugs could have a variable impact on insulin sensitivity indices in our diabetic population. Third, we have previously demonstrated that insulin sensitivity is remarkably lower in Ghanaian immigrants in the USA with untreated type 2 diabetes. We found that the average Si was  $0.6 \pm 0.4 \times 10^4 / \text{min}/(\text{uU}/\text{ml})$  in Ghanaian immigrants with untreated type 2 diabetes. Thus, we might have overestimated the severity of insulin resistance in our type 2 diabetic population in the present study of indigenous Ghanaians who had previously been treated with oral antidiabetic medications.<sup>36</sup> Fourth, obesity has been associated with insulin resistance and worsens the genetically mediated insulin resistance in individuals with and without type 2 diabetes. In this regard, Taniguchi *et al.*<sup>44,45</sup> indicated that in Japanese patients with type 2 diabetes and impaired glucose tolerance, obesity was a major determinant of insulin sensitivity as assessed by the FSIGT and Bergman's minimal model method, similar to the present study. Regarding blacks, Banergi and Lebovitz<sup>17</sup> have reported that non-obese (BMI less than 27 kg/m<sup>2</sup>) African American patients with type 2 diabetes



had a normal or preserved insulin sensitivity similar to that in the present study of Ghanaians. Indeed, in the present study the BMIs were 26.9, 25.9 and 26.0 kg/m<sup>2</sup> for the healthy controls, relatives and type 2 diabetic patients, respectively. Therefore, we believe that our Ghanaians with type 2 diabetes could perhaps represent the so-called insulin-sensitive variant of type 2 diabetes previously reported in black Americans.<sup>17</sup>

Normal glucose tolerance is achieved by both insulin-mediated and glucose-mediated glucose disposal *in vivo* in humans. This ability of glucose to mediate its own disposal is referred to as glucose effectiveness (Sg).<sup>27-29</sup> Previous investigators<sup>44,45</sup> have reported that Sg is reduced in patients with IGT and type 2 diabetes. However, previous studies from our laboratory and those of others have suggested that Sg is intact or increased in prediabetics and offspring of patients with type 2 diabetes. We have previously demonstrated<sup>43,35,36</sup> that Sg is not significantly altered in patients with IGT and type 2 diabetes in recent Ghanaian immigrants and African Americans living in the USA. In our present study we found that Sg at basal insulin was not statistically different in the three groups. It is of interest to note that Sg values in our non-diabetic and diabetic African Americans and recent Ghanaian immigrants residing in the USA were similar to those of the native Ghanaians in the present study. Therefore, Sg appears to be robust and well preserved in people of West African origin residing in diverse environments. The biological significance and the reasons for the preserved Sg in these black populations with varying degrees of glucose tolerance living in diverse populations remains to be elucidated.

In summary, the present study demonstrates that severe beta cell dysfunction and moderate insulin resistance, but not tissue glucose resistance, characterise the hyperglycaemia in Ghanaians with type 2 diabetes. Although our study was cross-sectional, we are tempted to infer from the metabolic findings in the first-degree relatives of the Ghanaian patients with type 2 diabetes that insulin resistance/hyperinsulinaemia and not beta cell dysfunction appears to be the primary or earliest lesion underlying type 2 diabetes in Ghanaians. These pathogenetic mechanisms are similar to those reported in African Americans and recent Ghanaian immigrants residing in the USA.

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