Microvascular and macrovascular complications in type 2 diabetes Ghanaian residents in Ghana and Europe: The RODAM study

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Aims: To compare microvascular and macrovascular complication rates among Ghanaians with type 2 diabetes (T2D) living in Ghana and in three European cities (Amsterdam, London and Berlin).

Methods: Data from the multicenter Research on Obesity and Diabetes among African Migrants (RODAM) study were analyzed. 650 Ghanaian participants with T2D (206 non-migrant and 444 migrants) were included. Logistic regression analyses were used to determine the association between migrant status and microvascular (nephropathy and retinopathy) and macrovascular (coronary artery disease (CAD), peripheral artery disease (PAD) and stroke) complications with adjustment for age, gender, socioeconomic status, alcohol, smoking, physical activity, hypertension, BMI, total-cholesterol, and HbA1c.

Results: Microvascular and macrovascular complications rates were higher in non-migrant Ghanaians than in migrant Ghanaians (nephropathy 32.0% vs. 19.8%; PAD 11.2% vs. 3.4%; CAD 18.4% vs. 8.3%; and stroke 14.5% vs. 4.93); and retinopathy (0.23; 0.07–25.90); CAD (2.32; 0.75–7.48); and PAD (7.48; 2.16–25.90). Except for nephropathy, the rates of microvascular and macrovascular complications were higher in non-migrant than in migrant Ghanaians with T2D. Conventional cardiovascular risk factors did not explain the differences except for nephropathy and stroke.

Conclusions: Except retinopathy, the rates of microvascular and macrovascular complications were higher in non-migrant than in migrant Ghanaians with T2D. Conventional cardiovascular risk factors did not explain the differences except for nephropathy and stroke.

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1. Introduction

Worldwide, the prevalence of diabetes is increasing, but substantial variations exist between regions.1 According to the International Diabetes Federation in 2017, about 16 million people in sub-Saharan Africa had diabetes, with the burden projected to increase to 41 million people by 2045, the highest projected increase anywhere in the world.2

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Diabetes increases the risk of macrovascular disease, including coronary heart disease (CAD), peripheral vascular disease (PAD), and stroke. Diabetes also causes specific microvascular complications such as retinopathy, nephropathy and neuropathy, and remains a leading cause of blindness, end stage kidney disease, and lower limb amputation. The microvascular and macrovascular complications of diabetes contribute to reduced quality of life, increased risk of hospitalization, disability and mortality, that pose a burden on the economies of all countries, especially the low and middle income ones.

In high-income countries, migrant populations including those from sub-Saharan Africa are disproportionately affected by diabetes, and tend to develop the disease at a younger age than the host populations. For example, the prevalence of type 2 diabetes was two to three times higher in populations of sub-Saharan Africa origin than in European host populations. Further, migrants resident in high-income countries have higher prevalence of diabetes than their counterparts in rural sub-Saharan Africa. These disparities may suggest a role of environmental factors in the development of diabetes among these populations.

In a previous Research on Obesity & Diabetes among African Migrants (RODAM) study, we observed higher diabetes prevalence in migrant-Ghanaians in three European cities compared to non-migrant Ghanaians. The prevalence of diabetes-related microvascular (nephropathy and retinopathy) and macrovascular (CAD, PAD and stroke) complications in the RODAM cohorts with type 2 diabetes in Europe and in Ghana. Furthermore, we assessed whether the rates of diabetic microvascular and macrovascular complications vary between Ghanaian migrants in Europe and their non-migrant compatriots living in Ghana.

2. Methods

2.1. Study design

The rationale, conceptual framework, design and methodology of the RODAM study have been described in detail elsewhere. For the current analysis only participants with type 2 diabetes were included in the analyses. This included data from 206 participants in Ghana and 444 Ghanaian migrants resident in Amsterdam, Berlin and London (Fig. 1). Type 2 diabetes was defined according to the World Health Organization diagnostic criteria (self-reported diabetes, documented use of glucose-lowering medication(s), fasting plasma glucose ≥ 7.0 mmol/L) or HbA1c ≥ 6.5% or ≥48 mmol/mol. Participants who met the diagnostic criteria for diabetes but who had no prior diabetes and who were not on glucose-lowering medication(s) were considered to have undiagnosed diabetes.

2.2. Participants' baseline measurements

A structured questionnaire was used to record the demographic, socioeconomic, health-related behaviors, and microvascular and macrovascular complications (retinopathy, CAD, and strokes) of the study participants. The assessment of educational status was adapted to local circumstances at the different study sites and comprised four categories: never been to school or elementary school; lower vocational schooling or

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**Fig. 1.** Flow chart of study design and inclusion in analysis.
lower secondary schooling; intermediate vocational schooling or intermediate/higher secondary schooling; and higher vocational schooling or university. Smoking was assessed as a positive reply to the question ‘Do you smoke at all?’ Alcohol intake in grams per day was estimated using standard portion sizes combined with frequencies of intake based on a standardized Food Propensity Questionnaire. Physical activity was derived for each participant using International physical activity questionnaire. Respondents were classified into three categories of total physical activity, namely low, moderate and high level. Eyesight was graded by the response to the question ‘At the present time, would you say your eyesight using both eyes (with glasses or contact lenses, if you wear them) is excellent, good, fair, poor, or very poor or are you completely blind?’

Physical examinations were performed with validated devices according to standardized operational procedures across all study sites. Weight was measured in light clothing and without shoes with SECA B77 scales to the nearest 0.1 kg. Height was measured without shoes with a portable stadiometer (SECA 217) to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m²). Waist circumference (WC) was measured in centimeters at the midpoint between the lower margin of the least palpable rib and the top of the iliac crest. Hip circumference (HC) was measured in centimeters around the widest portion of the buttocks, at the level of the greater trochanters, with the tape parallel to the floor. Waist-to-hip ratio (WHR) was determined as the ratio of WC to HC. All the anthropometrics were measured twice by the same assessor and the average of the two measurements was used for analyses. Body fat was determined using arm-leg bio-impedance measurements using a Bodystat 1500 analyzer (Bodystat Ltd., Isle of Man, UK). The body fat percentage was calculated using the African-specific formula by Kyle et al. Blood pressure (BP) was measured three times using a validated semi-automated device (Microlife Watch BP home, Widnau, Switzerland), with appropriate sized cuffs after at least 5 min rest while seated. The mean of the last two BP measurements was used for the analyses. Hypertension was defined as systolic BP ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg, and/or being on antihypertensive medication treatment. Ankle brachial index (ABI), the ratio of the resting systolic blood pressure at the ankle to the resting systolic brachial pressure at the arm, was obtained from two blood pressure measurements on the left side (leg and arm) and two on the right side (leg and arm) using the Microlife Watch BP Office ABI with appropriate sized cuffs, after at least 10 min of supine rest. The cuffs for measuring the ankle and brachial pressures were placed just above the ankle and at the upper arm, respectively.

2.3. Biochemical analyses

Fasting venous blood samples were processed and aliquoted into Sarstedt tubes after collection according to standard operation procedures, and then temporarily stored at the local research location at −20 °C. Two aliquoted blood samples and one first early morning urine sample were transported to the local research centres, where they were checked, registered and stored at −80 °C before being shipped to the laboratory at Charité–University Medicine Berlin (Berlin, Germany) for determination of biochemical variables. Shipping of the samples from European sites was carried out using Styrofoam boxes filled with dry ice and from Ghana in dry shippers filled with liquid nitrogen. Extensive quality checks were done during the biochemical analysis, including blinded serial measurements.

Fasting glucose, total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides levels were determined using the ABX Pentra 400 chemistry analyzer (HORIBA ABX, Montpellier, France). Fasting plasma glucose concentration was measured using an enzymatic method (hexokinase). Concentration of total cholesterol was assessed by using colorimetric test kits. HbA1c was measured by high-performance liquid chromatography (TOSOH G8 HPLC analyzer). Serum creatinine concentration was determined by a kinetic colorimetric spectrophotometric isotope dilution mass spectrometry calibration method (Roche Diagnostics). Estimated glomerular filtration rate (eGFR) was calculated using the 2009 CKD-EPI (CKD Epidemiology Collaboration) creatinine equation and the severity of kidney disease categorized according to the 2012 KDIGO guidelines.

Urinary albumin concentration (in mg/L) was measured by an immunnochemical turbidimetric method (Roche Diagnostics). Urinary creatinine concentration (in μmol/L) was measured by a kinetic spectrophotometric method (Roche Diagnostics). Urinary albumin–creatinine ratio (ACR; expressed in mg/g) was calculated by taking the ratio between urinary albumin and urinary creatinine and stratified according to the 2012 KDIGO classifications: A1, <3 mg/mmol (normal to mildly increased); A2, 3 to 30 mg/mmol (moderately increased); and A3, >30 mg/mmol (severely increased).

2.4. Determination of microvascular and macrovascular complications

Nephropathy was defined as albuminuria or microalbuminuria based on the report from Joint Committee on Diabetic Nephropathy. PAD was defined as ankle brachial index <0.9. Retinopathy was assessed by a positive reply to the question ‘Have you ever been told by a doctor or health care worker that you have eye disease or eye damage as a result of your diabetes (diabetic retinopathy)?’. Coronary artery disease (CAD) was assessed using the WHO Rose angina questionnaire. Possible myocardial infarction was defined as a positive reply to the question ‘Have you ever had a severe pain across the front of your chest lasting for half an hour or more?’ Angina was defined as a positive reply to the questions ‘Have you ever had any pain or discomfort in your chest?’ and ‘Do you get this pain or discomfort when you walk up hill or hurry?’ Stroke was assessed by a positive reply to the question ‘Have you ever had a stroke?’

2.5. Statistical analysis

Data were analyzed using the IBM SPSS version 22 for Windows. Data with normal distribution was presented as mean ± standard deviation whereas those not normally distributed presented as median (interquartile range). Categorical data were presented as frequencies (percentages). Differences in demographic, clinical and micro- and macrovascular function between migrants and non-migrants with type 2 diabetes were assessed by chi-square test or two-sample independent sample t-test for categorical or continuous covariates, respectively.

Multivariable logistic regression was used to build a model of factors associated with the microvascular and macrovascular complications including nephropathy, retinopathy, CAD, PAD, and stroke (dependent variables); and the sites of residence in Ghana and Europe (independent variable) with adjustments for potential covariates. Four models were used to examine the data: model 1 was unadjusted for any covariate; model 2 was adjusted for age and gender; model 3 was additionally adjusted for socioeconomic status (level of education); and model 4 was additionally adjusted for the conventional cardiovascular risk factors including, smoking; physical activity, hypertension, BMI, hypercholesterolemia, HbA1c and amount of alcohol consumed. The data as presented as odds ratios (OR) with their corresponding 95% confidence intervals (CI). The analyses were performed for Ghanaian and European sites using Europe as reference. In a sensitivity analysis, we further compared the proportions of microvascular and macrovascular complications between migrant and non-migrant Ghanaian stratiﬁed by previously known and unknown diabetes. A statistical test of significance was set at p-value <0.05.

3. Results

3.1. General characteristics

The baseline characteristics for the migrants and non-migrants with type 2 diabetes are described in Table 1. Although there was no
Difference in age, migrants were more frequently male than non-migrants. Migrants generally had a higher level of education, had a higher proportion of current smokers, and consumed more alcohol than their non-migrant counterparts. While there was no difference in the percent body fat between the two groups, the BMI and WHR were higher in the migrant group than in non-migrants. There was no difference in the duration of diabetes between migrants and non-migrants. The systolic blood pressure and diastolic blood pressure were also higher in the migrant group than in non-migrants.

The prevalence rates of microvascular and macrovascular complications for Ghanaians with type 2 diabetes living in rural and urban Ghana compared with Ghanaians living in the three European cities are shown in Table 3. After adjusting for age and gender, living in Ghana was still associated with higher odds for developing nephropathy (OR 2.01, 95% CI, 1.35–3.01), PAD (OR 3.14, 95% CI, 1.58–6.24), CAD (OR 2.31, 95% CI, 1.40–3.83) and stroke (OR 2.74, 95% CI, 1.52–4.93). Similar results were obtained after additionally adjusting for socioeconomic status. However, after further adjustments for the conventional cardiovascular risk factors, the proportion of the nephropathy and self-reported strokes was no longer statistically significant, but the differences persisted for the macrovascular complications PAD (OR 7.48, 95% CI, 2.16–25.90) and CAD (OR 2.32, 95% CI, 1.09–4.93). Adjustment for age, gender and socioeconomic status explained the differences in retinopathy diagnosed by a doctor.

The prevalence rates of microvascular and macrovascular complications were generally similar between newly diagnosed diabetes and established diabetes participants (Supplementary Table 1). When the analysis was further stratified by participants with previously known diabetes and undiagnosed diabetes (Supplementary Table 2), the proportions of nephropathy (35.8% vs. 17.1%, p < 0.001), PAD (10.6% vs. 4.3%, p = 0.028) and CAD (20.8% vs. 8.4%, p = 0.001) were higher, but self-reported diabetic retinopathy (11.0% vs. 22.2%, p = 0.022) was lower in non-migrants than in migrants among previously known diabetes participants. For participants with undiagnosed diabetes, the proportions of nephropathy, CAD and PAD were similarly higher in non-migrants than in migrants although the differences were not significant except for PAD (12.0% vs. 1.9%, p = 0.002).

4. Discussion

4.1. Key findings

Our study shows that the prevalence of both microvascular (nephropathy) and macrovascular (coronary artery disease, peripheral
artery disease and self-reported stroke) complications are higher in non-migrant than in migrant-Ghanaians with type 2 diabetes. Correction for conventional cardiovascular risk factors attenuated differences in the complication rates. However, after correction for conventional cardiovascular risk factors, the difference in PAD and CAD remained statistically significant.

4.2. Discussion of key findings

There is limited published data on community level of diabetes-related microvascular and macrovascular complications among sub-Saharan Africans with type 2 diabetes. Most studies are either diabetes clinic-based or hospital-based surveys.21-26 Moreover, on account of differences in study methodology and diagnostic criteria used in defining the diabetes-related microvascular and macrovascular complications, reports show wide variations.21-27 With the exception of CAD, our findings for non-migrant Ghanaians with type 2 diabetes compare well with these studies. The differences in the proportion of CAD could be due to the diagnostic criteria for CAD used in previous studies.26 In the study on coronary heart disease in the diabetic African, Touze et al. assessed CAD via stress test and/or coronary arteriography.26 In this study, we evaluated CAD using the WHO Rose angina questionnaire. The WHO Rose angina questionnaire has been shown to overestimate CAD prevalence, with higher sensitivity among people from lower socio-economic status.28,29 This could explain the relatively higher prevalence of CAD observed in non-migrant Ghanaians with type 2 diabetes, compared with the findings from Touze et al.

Published studies comparing the burden of microvascular complications in sub-Saharan Africans with type 2 diabetes and their migrant counterparts are limited. Choukem et al. previously compared microvascular complications between Cameroonians with type 2 diabetes living in Cameroon and those who migrated to France.30 After adjusting for the conventional cardiovascular risk factors, Cameroonians with type 2 diabetes living in Cameroon were found to have a 5.6 higher odds of nephropathy than their counterparts living in France.30 Although less marked compared with the findings by Choukem et al., this present study shows that non-migrant Ghanaians had higher prevalence of nephropathy than migrants. Our non-migrant participants with type 2 diabetes had better glycemic control than the non-migrant Cameroonians studied by Choukem et al. This could explain why we had less marked differences in the prevalence of nephropathy between migrant and non-migrant Ghanaians with type 2 diabetes.

Contrary to the other microvascular and macrovascular complications, retinopathy diagnosed by a doctor or health worker was higher in migrants than in non-migrants. This may reflect the better access to healthcare by migrants and hence increased likelihood of diagnosis of the complication. Therefore, retinopathy assessed this way may reflect diagnosis of the complication by a doctor or health care worker instead of true prevalence of the disease. In this study, when participants were asked to grade their eyesight as excellent, good, fair, poor, or very poor or completely blind, in contrast to the retinopathy previously diagnosed by a doctor or healthcare worker, non-migrant Ghanaians were more likely to report poor vision to total blind compared with their migrant peers. This highlights the need for more objective measures such as fundus photography in making diagnoses of retinopathy.

With the exception of factors related to glycemic control and blood lipids, living in Europe was associated with a worse cardiovascular risk profile in Ghanaians with type 2 diabetes. This is consistent with findings from previous studies that have compared cardiovascular disease risk factors among residents with or without type 2 diabetes living in rural and urbanized cities in their respective countries or overseas.31,32 Using data from the RODAM study, we have previously reported that European residence was associated with higher odds for elevated 10-year risk of cardiovascular disease among Ghanaian men with or without diabetes.33 It is thus interesting to observe that despite their overall poorer cardiovascular risk profiles, migrants had lower complication rates than their non-migrant counterparts. Therefore, poor glycemic control (evidenced by the higher HbA1c in non-migrant Ghanaians), instead of the other conventional cardiovascular risk factors, could be the key driving force for the development of these diabetic microvascular complications. This supports the existing pathophysiological explanation for microvascular injury that highlights chronic hyperglycemia as the central mechanism.34 Further, it underscores the importance of glycemic control in preventing diabetes-related complications and the need for implementation of strategies to improve glycemic control among people with diabetes in Ghana.

Although the higher prevalence of microvascular and macrovascular complications in non-migrant Ghanaians is likely to reflect poorer diabetes care, it remains unclear whether the observed differences is due to differences in the access to quality care or related to the microvascular and macrovascular complications rates at the time of diagnosis. While this study did not directly address the impact of healthcare on the development of microvascular and macrovascular complications, it is conceivable that access to healthcare and timely initiation of medical treatment could explain some of the differences. The attenuation of differences in microvascular and macrovascular complications after correction for conventional cardiovascular risk factors is supportive of this assumption. Indeed a previous report from Ghana had indicated limited health infrastructure and access for diabetes care.35 In people with diabetes, both lack of health care coverage and low utility of available health care service are known to be associated with poor glycemic control.36

In this study, we observed a higher proportion of undiagnosed diabetes in non-migrants compared to migrants. However, the microvascular and macrovascular complication rates in the undiagnosed and previously diagnosed diabetes groups were similar. Given the above facts, the higher proportion of undiagnosed diabetes in the non-migrant group may not explain all the observed differences in the complication rates between migrant and non-migrant Ghanaians with type

Table 3

Multivariable logistic regression models for nephropathy, retinopathy, PAD, CAD and strokes among Ghanaians living in Ghana and Ghanaians living in Europe (reference = Europe) (n = 603).

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
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<tbody>
<tr>
<td></td>
<td>OR 95% CI</td>
<td>p</td>
<td>OR 95% CI</td>
<td>p</td>
</tr>
<tr>
<td>Nephropathy</td>
<td>1.85 (1.26 to 2.73)</td>
<td>0.002</td>
<td>2.01 (1.35 to 3.01)</td>
<td>0.001</td>
</tr>
<tr>
<td>Retinopathy</td>
<td>3.42 (1.74 to 6.71)</td>
<td>&lt;0.001</td>
<td>3.14 (1.58 to 6.24)</td>
<td>0.001</td>
</tr>
<tr>
<td>PAD</td>
<td>2.29 (1.40 to 3.74)</td>
<td>0.001</td>
<td>2.31 (1.40 to 3.83)</td>
<td>0.001</td>
</tr>
<tr>
<td>Stroke</td>
<td>2.85 (1.60 to 5.07)</td>
<td>&lt;0.001</td>
<td>2.74 (1.52 to 4.93)</td>
<td>0.001</td>
</tr>
</tbody>
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Definition of abbreviations: CAD = Coronary artery disease; CI = Confidence interval; OR = odds ratio; PAD = Peripheral arterial disease.

Model 1 – unadjusted for covariates.
Model 2 – adjusted for age and gender.
Model 3 – adjusted for age, gender, and socioeconomic status.
Model 4 – adjusted for age, gender, socioeconomic status, alcohol consumption, smoking; physical activity, hypertension, BMI, total cholesterol, and HbA1c.
2 diabetes. Thus, just improving targeted screening for type 2 diabetes without appropriate treatment and management of complications may not necessarily decrease the rates of diabetes-related microvascular and macrovascular complications. Therefore care of diabetes after diagnosis as well as controlling the modifiable risk factors for vascular complications may play important roles in reducing the rates and severity of diabetes-related microvascular and macrovascular complications. This is evidenced by the lower rates of complications in migrants who reside in Europe where diabetes is likely to be diagnosed earlier and complication screening and care instituted. Previous studies have shown that the asymptomatic phase of type 2 diabetes (lasting at least 4 to 7 years) is known to be associated with increased risk of developing microvascular complications. Additionally, early diagnosis and treatment of hyperglycemia is known to prevent disease progression and delay the development of diabetes related complications.

Although the strength of association between migrant status and diabetes complications differ in the subgroups with unknown diabetes and previously known diabetes, the pattern of associations are similar. This may imply that aside hyperglycemia, other risk factors may be important in driving development of diabetic microvascular and macrovascular complications. In this study, we observed higher LDL-cholesterol concentrations and lower HDL-cholesterol concentrations in non-migrants compared to migrant Ghanaians. Diabetic dyslipidemia is known to cause or exacerbate diabetic microvascular and macrovascular complications via multiple mechanisms including alterations in the coagulation-fibrinolytic system, changes in membrane permeability, damage to endothelial cells and increased atherosclerosis. Diabetic microvascular and macrovascular complications have similar etiologic characteristics, with chronic hyperglycemia driving several metabolic and structural derangements including the formation of advanced glycation end products, activation of inflammatory pathways, and increased oxidative stress. Further, diabetics with microvascular complications are prone to accelerated atherosclerosis and subsequent macrovascular injury. It would, therefore, have been expected that the risk factors driving microvascular injury and atherosclerosis would explain the differences in the prevalence of macrovascular complications in our cohort. However, adjusting for these risk factors could not explain the differences in PAD and CAD suggesting that other unmeasured factors may play a role. It is worth noting that the time dependent effects of blood pressure, poor glycemic control and persistent dyslipidemia on microvascular and macrovascular complications were not assessed in this study due to the cross-sectional design nature of our study. More work is needed to identify other potential factors driving the differences in macrovascular complications among migrant and non-migrant Ghanaians. This could possibly inform complication prevention strategies and treatment efforts.

4.3. Strengths and limitations

Key strengths of our study are that we used a relatively homogenous multi-centered study population of Ghanaians and well-standardized study protocols across the various study sites. Additionally, we eliminated the limitation of intra-laboratory variability by using the same standard operating procedures in the same laboratory for running all samples across all sites. Our study is limited in a number of ways. First, we used both objective and subjective measures for microvascular and macrovascular complications, but both pointed to the same direction. We assessed CAD via the Rose Angina Questionnaire. Although the Rose Angina Questionnaire has moderate sensitivity, it has a high specificity to detect CAD and is valuable for screening individuals at risk of CAD in large-scale epidemiological surveys. CAD and stroke were self-reported, which could have led to reporting bias. While the question used to assess diabetic retinopathy is a very sensitive measure for proliferative retinopathy and has a high specificity for measuring the prevalence of diabetic retinopathy, it is less sensitive for non-proliferative retinopathy. Additionally, neuropathy, another microvascular complication, was not assessed. Finally, the duration of diabetes was not included as a covariate in the multivariable analysis because a large number of study participants did not provide this information.

5. Conclusion

Our study shows that Ghanaian migrants with T2D had lower prevalence of microvascular and macrovascular complications than their non-migrant counterparts. Unlike the CAD and PAD, the differences in the nephropathy and stroke were explained by poorer glycemic control in non-migrant Ghanaians. Therefore, the higher prevalence of microvascular complications in non-migrants is likely to reflect poorer diabetic care. Thus, interventions aimed at improving glycemic control among non-migrant Ghanaians may help to reduce the prevalence of some diabetic microvascular and macrovascular complications in Ghana. More work is needed to identify potential factors driving the high prevalence of microvascular and macrovascular complications among non-migrant Ghanaians to assist the prevention and treatment efforts.

Acknowledgment

We are grateful to the research assistants, interviewers, and other staff of the 5 research locations who have taken part in gathering the data and, most of all, the Ghanaian volunteers participating in the RODAM study (Research on Obesity and Diabetes Among African Migrants). We gratefully acknowledge the advisory board members for their valuable support in shaping the RODAM study methods, Jan van Straalen from the Amsterdam University Medical Centre with standardization of the laboratory procedures, and the Amsterdam University Medical Centre Biobank for their support in biobank management and high-quality storage of collected samples.

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Authorship contributions

All authors have contributed substantially to this article and approved the submission. C.H.-B., B.B., A.H.M., A.G.B.A. and C.A. conceived the idea. C.H.-B., E.B., and C.A. were responsible for data acquisition; C.H.-B., and C.A. were responsible for statistical analysis. C.H.-B., B.B., A.H.M., A.G.B.A., K.A.C.M., K.K.-G., S.B., J.S., I.D., F.M., E.B., L.S., and C.H.-B. contributed important intellectual content during article drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved. C.H.-B. takes responsibility for the fact that this study has been reported honestly, accurately and transparently, that no important aspects of the study have been omitted, and that any discrepancies from the study as planned have been explained.