Risk of Metabolic Syndrome and Diabetes Among Young Twins and Singletons in Guinea-Bissau

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OBJECTIVE—Twins in Africa may be at increased risk of metabolic disorders due to strained conditions in utero, including high exposure to infections. We studied metabolic syndrome (MS) and diabetes mellitus (DM) among young twins and singletons in Guinea-Bissau.

RESEARCH DESIGN AND METHODS—The study was cross-sectional and occurred from October 2009 until August 2011 at the Bandim Health Project, a demographic surveillance site in the capital Bissau. Twins and singleton controls between 5 and 32 years were visited at home. Fasting blood samples for metabolic measurements were collected. Zygosity was established genetically for a subset. DM was defined as HbA_{1c} ≥6.5% (48 mmol/mol) and MS by the International Diabetes Federation criteria.

RESULTS—HbA_{1c} was available for 574 twins and 463 singletons. Mean age was 15.3 years versus 15.8 years, respectively. Eighteen percent of twins were monozygotic. There were no DM cases among twins but one among singletons. A total of 1.4% (8 of 574) of twins had elevated HbA_{1c} (6.0–6.4%, 42–46 mmol/mol) compared with 2.4% (11 of 463) of singletons ($P = 0.28$). Mean HbA_{1c} was 5.3% (34 mmol/mol) for both groups. MS data were available for 364 twins and 360 singletons. The MS prevalence was 3.0% (11 of 364) among twins and 3.6% (13 of 360) among singletons ($P = 0.66$). The prevalence of fasting blood glucose (F-glucose) ≥5.6 mmol/L was 34.9% (127 of 364) for twins versus 24.7% (89 of 360) for singletons ($P = 0.003$). Median homeostasis model assessment–insulin resistance did not differ ($P = 0.34$).

CONCLUSIONS—The MS and DM prevalences among young individuals in Guinea-Bissau were low. Twins did not have a higher MS and DM burden than singletons, though elevated F-glucose was more common among twins.

Diabetes Care **36:3549–3556, 2013**

Twins are born with substantially lower birth weight than singletons (1–3). This could reflect a more adverse intrauterine environment for twins (1,3), especially for monozygotic (MZ) twins due to frequently shared placenta.

The intrauterine environment is now a well-established risk factor for metabolic disease later in life (4–7) (e.g., diabetes mellitus [DM] or metabolic syndrome [MS]). Twins may be at a higher risk of those conditions because of growth restriction in utero, as expressed by low birth weight (8). Currently, this remains unresolved, with studies pointing in different directions (8–11).

In Sub-Saharan Africa, both twinning (2) and DM (12) are common, but no metabolic twin studies are available yet. This is problematic, as the DM pathogenesis may well be different here (12–14). Moreover, African twins have very high early mortality (2,15,16) and face an altogether different pre- and postnatal environment with frequent undernutrition (15) and increased risk of infections. Hence, conclusions drawn from twin studies in high-income countries may not necessarily apply in Sub-Saharan Africa, as adverse exposures vary widely.

In this paper, we present results from a large-scale metabolic survey among twins and singletons from Guinea-Bissau. The study was conducted within the framework of a larger twin registry (17). The main objective was to determine the prevalence of MS and DM in a well-described urban population of young twins and singleton controls, as well as individual MS components.

RESEARCH DESIGN AND METHODS

Study population

The study was conducted at the Bandim Health Project (BHP) in Guinea-Bissau, West Africa. The BHP is a Health and Demographic Surveillance Site (HDSS) in the capital Bissau. The BHP covers a population of ~100,000. All individuals are registered with an identification number, age, sex, ethnicity, and socioeconomic characteristics. Twin status is recorded for all newborns. Children are intensively monitored the first 3 years of life. The surveillance database is updated through regular censuses. Recently, a diabetes notification system has been implemented.

Study cohort

The study was carried out from October 2009 until August 2011. Twins aged

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Received 20 December 2012 and accepted 24 May 2013.
DOI: 10.2337/dc12-2653
This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc12-2653/-/DC1.
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≥5 years were identified using the HDSS database (twin status variable) and visited at home. Triplets were excluded.

As BHP started collecting data on twins in 1979, no twins >32 years were included. For each twin, one singleton control with the same date of birth was selected, thereby ensuring a control group with the same age as the twins.

Survey procedures

Twins and singletons were visited by two trained field assistants and a laboratory technician. In case of absence, the house was revisited several times. Twin status was confirmed, and data regarding the cotwin was obtained. Information about chronic diseases (e.g., DM, heart disease, hypertension, asthma, etc.), family history of DM, alcohol consumption, and smoking habits was collected. Detailed questions were asked about typical DM symptoms.

Anthropometry included middle upper arm circumference (MUAC), height, weight, and waist and hip circumference with the individual wearing light clothing. MUAC was measured by nonstretchable tape and height by metallic measuring tape. Weight was obtained using standard bathroom scales, calibrated at regular intervals. Waist circumference (WC) was measured with a flexible tape at the midpoint between the lower costal margin and the iliac crest and hip circumference at the widest point in the gluteal region.

Blood pressure (BP) measurements were done using a manual sphygmomanometer with appropriate cuff size, with the person seated and at rest for at least 5 minutes. The first and fifth Korotkoff sounds were used to identify the systolic and diastolic BP, respectively. BP was measured two times at short interval, with the second value being used.

Birth weight data were available from the BHP surveillance database for 29%. The main reason for lack of birth weight was not being born in the BHP study area (half of the twins), in which case birth weight could normally not be obtained. Besides, for early BHP records, birth weight was often unavailable.

Biochemical methods

After the interview participants were advised to be fasting from late evening (after dinner) and until the following morning for the collection of blood samples. This ensured an overnight fast of at least 10–12 h. At the revisit the assistants would confirm the overnight fast. In case of nonfasting, another appointment was made.

Fasting blood glucose (F-glucose) was measured using a CareSens or Hemocue 201+ glucometer. Venous serum and EDTA blood samples were collected using Terumo tubes and within 4 hours transported to the National Public Health Laboratory in Bissau. The blood samples were immediately separated into aliquots of whole blood, plasma, and serum. Subsequently, the samples were stored at −40°C before being transported to Denmark for analyses. In Denmark, the samples were stored at −80°C.

The samples were analyzed at Odense University Hospital, Denmark, for the following markers: hemoglobin (Hb), HbA_{1c}, total cholesterol, HDL cholesterol (HDL-C), LDL cholesterol, triglycerides (TGs), and insulin.

Insulin resistance (IR) was calculated according to the homeostasis model assessment (HOMA) (18). We used the approximation HOMA-IR = (glucose [mmol/L] × insulin [mU/L])/22.5.

Zygosity

Physical similarity within twin pairs was used as a proxy for zygosity (19). Genetic analyses were carried out on a subset of randomly selected same-sex twin pairs. In this case, capillary blood was collected on filter paper. The samples were stored frozen in Guinea-Bissau and afterward transported frozen to Odense University Hospital for genetic analyses. Zygosity was established using 12 highly polymorphic microsatellite markers (19).

Definitions

DM was defined as HbA_{1c} ≥6.5% (48 mmol/mol), while HbA_{1c} between 6.0 and 6.4% (42–46 mmol/mol) was considered a high-risk category (20). As HbA_{1c} measurements were available, DM was not diagnosed based solely on glucose levels (20).

We used the International Diabetes Federation (IDF) definition of MS (21), but in accordance with the latest Joint Interim Statement, central obesity was not mandatory (22). Thus, MS in adults >15 years required at least three of the following five factors: central obesity: WC ≥94 cm for males and ≥80 cm for females; BP: systolic ≥130 mmHg or diastolic ≥85 mmHg; HDL-C: <1.03 mmol/L for males and <1.29 mmol/L for females; TGs: ≥1.7 mmol/L; and F-glucose: ≥5.6 mmol/L.

For adolescents between 10 and 15 years, MS required at least three of the following five factors: central obesity: WC ≥90th percentile according to age and sex (23); BP: ≥95th percentile according to age, sex, and height (24); HDL-C: <1.03 mmol/L; TGs: ≥1.7 mmol/L; and F-glucose: ≥5.6 mmol/L.

For children >10 years, the diagnosis of MS is not recommended (21). Hence, these children were excluded from the MS analysis, and individual MS components were not assessed in this study.

Low birth weight (LBW) was defined as birth weight >2,500 g. Anemia was defined as Hb <6.0 mmol/L.

Ethics

Written informed consent was obtained for all participants. For individuals <15 years, consent was obtained from the mother or another caretaker. The study was approved by the National Health Ethics Committee in Guinea-Bissau. The Central Ethical Committee in Denmark gave consultative approval. DM patients were referred to the Diabetes Clinic in Bissau and offered treatment.

Statistical methods

Data were entered using dBase 5.0 software (dataBased Inc., Vestal, NY). Statistical analyses were done using STATA software (Stata Corporation, College Station, TX). We expressed the MS and DM burdens as proportions. MS components were assessed both as continuous and categorical outcomes. Comparisons of categorical variables were done using Poisson regression with robust variance estimates providing prevalence ratios (PRs). Most continuous variables were normally distributed (expressed as means), with differences between twins and singletons being tested by using linear regression. The regression analyses were adjusted for intratwin-pair relationship (clustering) by adding a unique pair number to the statistical model using the cluster function.

In case of nonnormal distribution of continuous variables (expressed as medians), the Wilcoxon rank-sum test was applied (i.e., for TGs, F-insulin, and IR). In this case, adjustment for intratwin-pair relationship was not possible. Nonnormally distributed variables were also log-transformed (natural logarithm). The correlation between anemia and HbA_{1c} was explored by linear regression, while Poisson regression was applied for the association between LBW and elevated HbA_{1c}. A P value <0.05 was considered significant.

RESULTS

Inclusion

At study initiation, 883 individuals with a twin background were identified as being alive in the BHP registration database (Fig. 1), though only 52% (461 of 883) were born in the study area. Of the 883 with twin background, 678 were interviewed at home, while 205 were not located. No difference in sex distribution (P = 0.77) and mean age (P = 0.22) was observed between those interviewed and those not. By detailed questioning and reviewing of the BHP birth databases, 33 individuals were in fact singletons and therefore excluded. Fourteen triplets were also excluded. Hence, 631 confirmed twins were interviewed. Of these, 57 individuals refused having a fasting blood sample taken, or the amount of blood collected was insufficient. HbA_{1c} results were therefore available for 574 twins, including 187 twin pairs (374 individuals) and 200 single-twins.

After completing the twin cohort, 777 singletons with the same age were identified in the BHP database (Fig. 1). Singletons were not selected if date of birth did not match exactly. In case more than one singleton control was eligible, we randomly selected one singleton for each twin. Of the identified singletons, 513 were located at home, while 264 were not interviewed. No difference in sex distribution (P = 0.77) or mean age (P = 0.42) was observed between singletons interviewed and those not. Of the 513 interviewed singletons, 50 refused a fasting blood sample, or there was insufficient blood collected. HbA_{1c} results were therefore available for 463 singletons.

Zygosity

Of the 187 twin pairs, there were 123 same-sex pairs and 64 opposite-sex pairs (Fig. 2). Filter paper blood for genetic determination of zygosity was collected and analyzed for 36 of the same-sex pairs. Of these, 28% (10 of 36) were MZ. Assuming the MZ frequency would be similar for all 123 same-sex pairs, we would have 34 MZ pairs (0.28*123) in total. As all opposite-sex pairs would be dizygotic (DZ), the overall MZ frequency would be 18% (34 of 187).

Of the 36 genetically tested same-sex pairs, the field assistants were able to determine zygosity correctly in 89% of the pairs (32 of 36) by degree of similarity; four DZ pairs were misclassified as MZ.

Summary characteristics

Among the 574 twins and 463 singletons with HbA_{1c} results available, there were 46.0% (264 of 574) and 46.9% (217 of 463) males in the two groups, respectively (Table 1). The mean age was 15.3 years for twins and 15.8 years for singletons, with the slight age difference due to singletons being included after twins.

There was no difference in sex distribution between included twin pairs and single-twins (P = 0.54). The mean age was 14.6 versus 16.5 years (difference [Diff] = 1.91; CI 0.52–3.29) in the two groups, respectively.

Clinical history and examination

There was no difference in self-reported health data between twins and singletons. No difference was observed in family history of DM or tobacco or alcohol habits, defined as any alcohol intake or any smoking.

Birth weight was available for 181 twins and 119 singletons. Mean birth weight was markedly lower for twins than singletons (2.52 vs. 3.10 kg) (Diff = 0.58; CI 0.45–0.71), as was mean BMI, 17.7 versus 18.3 kg/m^2 (Diff = 0.60; CI 0.011–1.19).

HbA_{1c} and Hb

No DM cases were found among twins, while one type I DM case was observed in a young female singleton (Table 2). Her HbA_{1c} was 8.9% (74 mmol/mol); her F-glucose was 15.1 mmol/L. Insulin treatment was initiated.

Overall, 1.4% (8 of 574) of the twins versus 2.4% (11 of 463) of singletons had

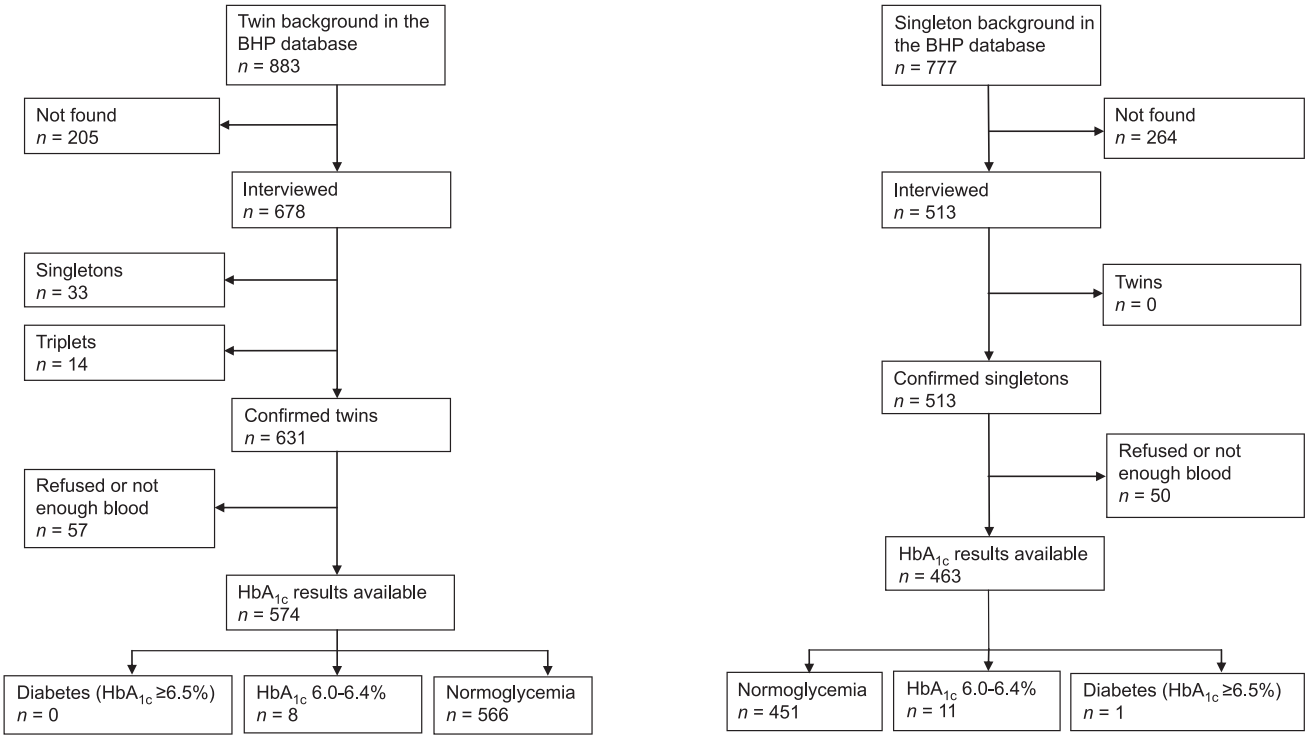

Figure 1—*Study flow chart of twins and singletons.*

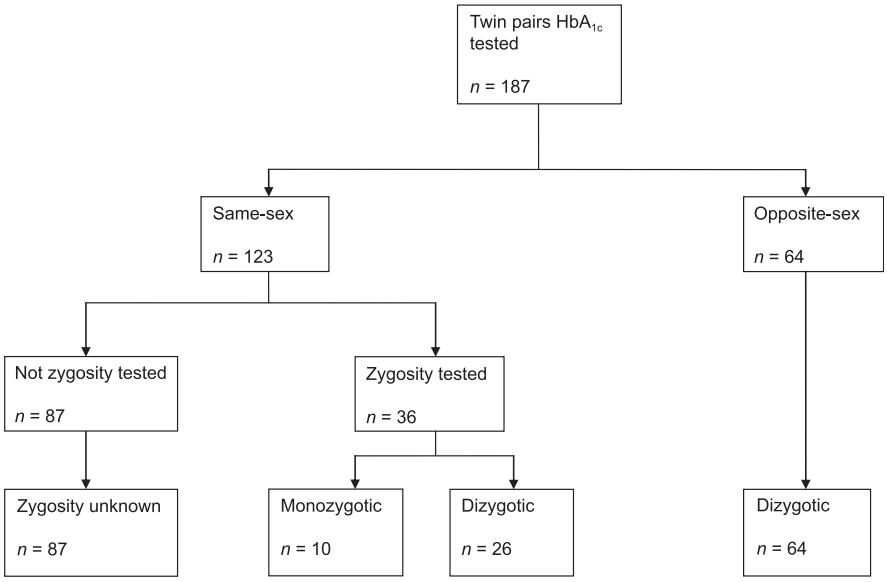

Figure 2—*Zygosity distribution among 187 twin pairs for whom HbA_{1c} results were available for both twins.*

elevated HbA_{1c} (P = 0.28) (Table 2). No difference was observed in mean HbA_{1c} (P = 0.33). Assuming zygosity could be reliably determined by physical similarity (89% correctness in our data), no difference in mean HbA_{1c} was observed between MZ and DZ twins (P = 0.56).

Mean HbA_{1c} was similar for twin pairs and single-twins (P = 0.50). The prevalence of elevated HbA_{1c} was 1.6% (6 of 374) versus 1.0% (2 of 200) in the two groups, respectively (P = 0.54).

Hb levels were similar for twins and singletons, and yet 6.9% (38 of 553) of the twins and 5.0% (23 of 463) of singletons had an unspecified Hb variant (P = 0.20). Anemia was found among 3.6% (20 of 553) of twins and 3.0% (14 of 463) of singletons (P = 0.61). Mean HbA_{1c} tended to be lower in case of anemia (P = 0.06).

MS

Complete data on WC, BP, HDL-C, TGs, and F-glucose was available for 364 twins and 360 singletons (Table 2). The mean age was slightly higher than in the overall sample, 17.8 years for twins and 18.3 for singletons. The sex distribution was similar. The MS prevalence was 3.0% (11 of 364) for twins versus 3.6% (13 of 360) for singletons (P = 0.66). Among twin pairs, the MS prevalence was 2.3% (5 of 222), while the prevalence was 4.2% (6 of 142) for single-twins (P = 0.29). Of the total 24 MS cases, 71% (17 of 24) were females.

The most common MS component was elevated F-glucose, followed by low HDL and elevated WC (Supplementary Table 1). Elevated F-glucose was the only categorical component with significant differences between twins and singletons, 34.9% (127 of 364) versus 24.7% (89 of 360) (P = 0.003), respectively.

No difference was observed in mean WC, BP, and HDL. Mean systolic BP was lower among twins than singletons, 101.8 versus 104.3 mmHg (Diff = 2.44; CI 0.72–4.15). Median TGs were higher for twins (0.90 versus 0.80 mmol/L; P = 0.03), as was mean F-glucose (5.30 versus 5.17 mmol/L) (Diff = 0.13; CI 0.03–0.23).

IR

No difference was observed in median F-insulin (P = 0.83) or median HOMA-IR (P = 0.34) between twins and singletons. After log-transformation, the data became normally distributed, yet no significant differences were observed for natural logarithm (Ln)-F-insulin (P = 0.77) or Ln-HOMA-IR (P = 0.24) in the two groups, respectively.

No difference in the comparison of F-glucose (Diff = 0.13; CI 0.03–0.22), Ln-F-insulin (Diff = 0.03; CI −0.09 to 0.15), or Ln-HOMA-IR (Diff = 0.07; CI −0.05 to 0.19) was observed after adjusting for BMI and age either.

Association between LBW and elevated HbA_{1c}

We investigated the association between LBW and elevated HbA_{1c} for 300 individuals for whom birth weight was available, though no significant association could be established (PR 2.1; CI 0.56–8.02).

Adjustment for season

As a secondary analysis, we adjusted biomarker comparisons for the rainy season (June until October). After adjustment, the difference in mean Hb between twins and singletons tended to be slightly stronger (P = 0.09), while the difference in F-glucose became nonsignificant (P = 0.23). Otherwise, the adjustment did not significantly change the estimates.

Death of cotwin

Nineteen percent (109 of 573) of twins reported that the cotwin had died. No difference was observed in elevated HbA_{1c} between those who had lost a cotwin and those who had not. Though most MS cases were observed in live twin pairs, the MS prevalence was higher when the cotwin had died (i.e., 2.1 [6 of 286] vs. 6.7% [5 of 75], respectively) (P = 0.05).

CONCLUSIONS

Main results

We found a low burden of MS (3.6% among singletons) and DM (0.2% among singletons) in urban Guinea-Bissau in the age group 5–32 years. The MS prevalence was similar for twins and singletons, and the only overt DM case was a singleton. Furthermore, we did not observe any differences in mean HbA_{1c} or median IR between twins and singletons. However, several individual MS components varied,

Table 1—*Characteristics for twins and singletons*

Summary characteristics	Twins (*N* = 574)	Singletons (*N* = 463)	*P* value	PR or Diff
Male sex	264/574 (46.0)	217/463 (46.9)	0.79	PR = 0.98 (0.86–1.13)
Age (years) (*N* = 1,037)	15.3 (7.0)	15.8 (7.3)	0.37	Diff = 0.45 (−1.44 to 0.54)
Ethnicity			0.77	*P* = 0.77
Balante	52/572 (9.1)	38/463 (8.2)		
Fula	57/572 (10.0)	53/463 (11.5)		
Pepel	192/572 (33.6)	164/463 (35.4)		
Mandinka	51/572 (8.9)	34/463 (7.3)		
Other	220/572 (38.5)	174/463 (37.6)		
Clinical history and examination				
Person feels well at the moment	541/574 (94.3)	444/462 (96.1)	0.17	PR = 0.98 (0.95–1.01)
Person has a chronic disease	27/574 (4.7)	19/457 (4.2)	0.68	PR = 1.13 (0.63–2.02)
Family history of DM	48/574 (8.4)	39/463 (8.4)	0.81	PR = 0.99 (0.64–1.54)
Tobacco smoking	9/255 (3.5)	11/215 (5.1)	0.40	PR = 0.69 (0.29–1.63)
Alcohol intake	69/254 (27.2)	49/215 (22.8)	0.30	PR = 1.19 (0.86–1.66)
Birth weight (kg) (*N* = 300)	2.52 (0.54)	3.10 (0.52)	<0.001	Diff = 0.58 (0.45–0.71)
MUAC (mm) (*N* = 1,030)	220.7 (49.9)	224.7 (50.7)	0.26	Diff = 3.98 (−2.91 to 10.9)
BMI (kg/m^2) (*N* = 1,024)	17.7 (4.1)	18.3 (4.6)	0.05	Diff = 0.60 (0.011–1.19)
HbA_{1c} and Hb				
HbA_{1c} (%) (*N* = 1,037)	5.30 (0.41)	5.28 (0.47)	0.33	Diff = 0.02 (−0.03 to 0.08)
HbA_{1c} (mmol/mol) (*N* = 1,037)	34	34	0.33	
Hb (mmol/L) (*N* = 1,016)	7.90 (1.17)	7.79 (1.05)	0.14	Diff = 0.11 (−0.03 to 0.26)
MS components				
Waist circumference (cm) (*N* = 1,031)	66.1 (12.3)	66.2 (11.7)	0.96	Diff = 0.04 (−1.68 to 1.60)
Systolic BP (mmHg) (*N* = 1,023)	101.8 (11.9)	104.3 (13.8)	<0.001	Diff = 2.44 (0.72–4.15)
Diastolic BP (mmHg) (*N* = 1,023)	63.2 (10.9)	62.1 (11.3)	0.10	Diff = 1.24 (−0.24 to 2.71)
HDL (mmol/L) (*N* = 944)	1.29 (0.31)	1.32 (0.33)	0.18	Diff = 0.03 (−0.07 to 0.01)
TG (mmol/L) (*N* = 943)*	0.90	0.80	0.03	Diff = 0.10
Ln-TG (*N* = 943)	−0.16 (0.37)	−0.10 (0.37)	0.03	Diff = 0.06 (0.01–0.11)
F-glucose (mmol/L) (*N* = 1,028)	5.30 (0.59)	5.17 (0.93)	0.01	Diff = 0.13 (0.03–0.23)
Insulin resistance				
F-insulin (pmol/L) (*N* = 852)*	28	28	0.83	Diff = 0.01
Ln-F-insulin (*N* = 852)	3.42 (0.87)	3.40 (0.87)	0.77	Diff = 0.02 (−0.10 to 0.14)
HOMA-IR (*N* = 845)*	1.10	1.08	0.34	Diff = 0.02
Ln-HOMA-IR (*N* = 845)	0.20 (0.89)	0.12 (0.89)	0.24	Diff = 0.08 (−0.05 to 0.20)

Data are *n*/*N* (%) or mean (SD) unless otherwise noted. The table includes the 574 twins and 463 singletons for whom HbA_{1c} results were available. We did not have full information on all individuals for all variables. For continuous data, we have therefore added the total number (*N*) of individuals with information. *Expressed as medians, since data are not normally distributed.

with elevated F-glucose being more common among twins.

Strengths and weaknesses

To our knowledge, this is the first study from Sub-Saharan Africa to compare the metabolic profile for twins and singletons. It has a large sample size, based upon the unique registration of twins that has been implemented at the BHP for the last 33 years. Though zygosity was primarily determined by physical resemblance, we did also analyze zygosity genetically for a subset of twin pairs and found good correlation.

We used the HbA_{1c} assay as a DM diagnostic tool as recently recommended by the International Expert Committee (20). Currently, very limited data on HbA_{1c} assay performance are available from Sub-Saharan Africa (12). As the assay may be affected by hemoglobinopathies and low Hb levels (e.g., due to malaria), caution has been suggested (25). In our study, 6% of participants had an Hb variant (unspecified), and HbA_{1c} tended to be lower in case of anemia. This calls for further evaluation of HbA_{1c} assays in Africa. Follow-up of our cohort could be important in this study.

No specific WC cutoff values exist for Sub-Saharan Africa for diagnosing MS. For adults, we used the values currently recommended by the IDF (21). However, a recent South African study suggests that modifications may be warranted (26) (i.e., in Sub-Saharan Africa, the WC cutoff for women should be higher, whereas it should be lower for men).

Our study also has more direct limitations. The MS and DM burdens were low, and we had therefore limited power to compare those phenotypes. A likely explanation is that our cohort may have been too young (mean age 15 years) to observe type II DM, in which the onset is typically at 40–60 years (12). MS may not become common until after the age of 30 years either (26). Furthermore, many type 1 DM patients may die at a young age in Guinea-Bissau (13).

Considerable effort was put into ensuring the overnight fast. Yet, it is difficult to have absolute certainty. Some participants may have been embarrassed to disclose food intake. We do, however, perceive this problem to be a minor one. Moreover, fasting

Table 2—*Distribution of elevated HbA_{1c}, DM, and MS for 574 twins and 463 singletons*

	HbA_{1c} 6.0–6.4%	DM	MS*
Twins			
Total	8/574 (1.4)	0/574	11/364 (3.0)
Male			
≤15 years	3/136 (2.2)	0/136	1/63 (1.6)
>15 years	3/128 (2.3)	0/128	5/111 (4.5)
Female			
≤15 years	2/173 (1.2)	0/173	2/79 (2.5)
>15 years	0/137	0/137	3/111 (2.7)
Singletons			
Total	11/463 (2.4)	1/463 (0.2)	13/360 (3.6)
Male			
≤15 years	5/113 (4.4)	0/113	0/66
>15 years	2/104 (1.9)	0/104	1/107 (0.9)
Female			
≤15 years	1/131 (0.8)	1/131 (0.8)	3/71 (4.2)
>15 years	3/115 (2.6)	0/115	9/116 (7.8)

Data are *n/N* (%). The age groups were divided at 15 years to fit the definition of MS criteria. *According to IDF recommendations, MS was not calculated for individuals >10 years of age.

is not a prerequisite using the HbA_{1c} assay (20).

Twin status was rigorously confirmed. However, in a few cases, confirmation was difficult, especially if the cotwin had died early. In this case, the mother might not mention the deceased cotwin and simply raise the remaining child as a singleton. Yet, for the vast majority, confirmation of twin status was possible.

Due to time constraints, twins were included first, which gave an uneven seasonal distribution between twins and singletons. As a secondary analysis, we adjusted biomarker comparisons for the rainy season, but found little difference.

About half of the included twins were not born in the study area, which could make the group less homogenous. However, most migration would likely be from the surrounding neighborhoods of Bissau, which has a highly mobile population.

Birth weight was unfortunately unavailable for the majority of the participants, particularly for those born outside the BHP study area. Also, the original twin registration by the BHP HDSS was not done with the purpose of metabolic studies, but rather to properly evaluate infant mortality. The lack of birth weight is an important limitation, as it hampers our possibility to characterize the fetal environment. Also, it limits the possibility to stratify the metabolic profile for twins and singletons by birth weight. This would be of interest, as LBW may not have the same consequences for twins and singletons (27).

Consistency with previous findings

The MS burden of 3.6% in the background population (singletons) was age- and sex-dependent; hence, MS was more common at >15 years and among females. Both findings have previously been reported among other populations (26,28). Our MS prevalence was slightly higher than in a large European multicenter study, which found MS rates of 0.2–1.4% among 10–15 year olds (28). A study from Denmark found a MS prevalence of 5.5% among young adults (29), while an American study reported a MS rate of 4% among 11-year-old children (30). A recent survey from China found a low MS burden of 0.8% among 10- to 11-year-old children (31), a fact that may reflect differences in food intake, socioeconomic factors, frequency of LBW, and other adverse exposures compared with Guinea-Bissau. From Sub-Saharan Africa, MS data are scarce, in particular for childhood MS. Available adult studies have noted highly varying MS rates (26,32–35).

Elevated F-glucose was the most prevalent MS component, with the singleton burden being similar to a recent Ethiopian survey (32). Yet, many participants had F-glucose in the 5.6–6.1 mmol/L range, which underscores the importance of the MS definition applied. Low HDL and elevated WC were also common (26,33). The prevalence of hypertension was low, which most likely reflects the young age group. In the European multicenter MS study, the prevalence of hypertension among adolescents was 1–5%.

To some extent, our findings support large-scale register studies from Northern Europe showing no difference in the DM burden between twins and singletons (9). Yet, direct comparisons could be hampered by our participants being young. Age may be important in unmasking the effect of an adverse intrauterine environment (11), and a study of older individuals would likely have yielded more cases.

As no other metabolic twin studies are available, we cannot compare our results to other parts of Sub-Saharan Africa. However, it should be emphasized that newborn twins are a very vulnerable group in our setting. In Guinea-Bissau, 22% of newborn twins currently die during the perinatal period (36), and a study from neighboring Gambia has shown high mortality and frequent malnutrition among infant twins (15). Even in childhood, twin mortality could be elevated due to higher risk of cross-infections (16). In our study, 19% of the twins reported the loss of the cotwin. Any surviving adult twin is therefore a relatively strong individual in Guinea-Bissau, which should be considered when making comparisons to settings in which twin mortality is much lower and exposure to infections such as malaria nonexisting. Our study may therefore not be directly comparable to those from high-income settings, where a healthy survivor effect for twins is less pronounced.

Although LBW twins often die early in Guinea-Bissau (i.e., survival bias), the surviving twins still had much lower birth weight than the singletons. The mean difference was 0.58 kg. According to the fetal origins hypothesis, this would predispose them to various metabolic diseases later in life. Yet, apart from elevated F-glucose among twins, we found little evidence of this. It may reflect the participants selected (i.e., young twins in a low-income country). Alternatively, it could be that twins per se do not have an increased risk and that their LBW simply reflects prematurity and spatial restrictions in utero (9). It should also be noted that twinning is only one among many reasons for low birth weight in Sub-Saharan Africa, with maternal infections such as malaria (37) and HIV (38) often being involved. Socioeconomic factors and maternal access to treatment may also play an important role. Hence, the

etiology of LBW in Africa can be altogether different.

The zygosity distribution was 18% MZ, which is very close to that of newborn twins in Guinea-Bissau (36). We were able to determine zygosity by physical similarity with 89% correctness. Though some studies have demonstrated MZ twins to be at an even higher risk of metabolic abnormalities, we found no difference in mean HbA_{1c} between MZ and DZ twins. Nor did we find any differences in HbA_{1c} or MS between twin pairs and single-twins.

Several individual MS components differed between twins and singletons. Notably, F-glucose was significantly higher for twins both as a continuous and categorical outcome. We cannot exclude the possibility that this relates to the twin fetal environment, though the fact that HbA_{1c} and F-glucose reflect different aspects of glucose metabolism may also be involved.

Implications and recommendations

Twin studies have been used extensively to differentiate between environmental and hereditary risk factors for metabolic disease (3). However, the validity of the classical twin model has been questioned (3). The argument is that twin pregnancy is a special case, and observed results may not be relevant to the general population (1,3,10). Yet, large-scale studies have failed to find differences in both mortality and DM burden between twins and singletons in adult life (9,39). Furthermore, it has also been discussed why fetal undernutrition would affect twins and singletons differently (40). Our study provides an important contribution, especially since African DM rates are rising sharply.

The most important recommendation is therefore a call for further metabolic twin studies in Sub-Saharan Africa, preferably in older age groups. A follow-up study of our cohort would be of high value there, though to facilitate similar studies elsewhere, twin status should be included as a variable in areas with demographic surveillance systems. Secondly, different metabolic outcomes should be used. Finally, longitudinal twin studies with systematic collection of metabolic measurements would provide the most definite answers.

Summary

The MS and DM burden was limited among young individuals in Guinea-Bissau. Twinning did not confer particular risk of MS and DM in this age group, though F-glucose was elevated among twins. However, our participants were living in a high-mortality and malaria-endemic setting. The results may therefore not be directly comparable to high-income countries, and further metabolic twin studies will be needed in Sub-Saharan Africa, including in older age groups.

Acknowledgments—M.B.-A. received a combined grant from Forsknings-og Innovations Styrelsen, the University of Southern Denmark, and Odense University Hospital. C.S.B. holds a starting grant from the European Research Council (ERC-2009-StG-243149). P.A. holds a research professorship grant from the Novo Nordisk Foundation. Data collection was supported by Odense University Hospital, Aase and Ejnar Danielsen's Foundation, Den Danske Forskningsfond, and Fonden til Lægevidenskabens Fremme.

No potential conflicts of interest relevant to this article were reported.

M.B.-A. designed the study, supervised the survey in Guinea-Bissau, and wrote the first draft of the manuscript. L.H. and L.C. conducted the metabolic and genetic analyses in Denmark. L.I.d.S. collected, processed, and organized the blood samples in Guinea-Bissau. L.C.J. confirmed possible diabetes cases by retesting and clinical examination. D.E.H. supervised the survey in Guinea-Bissau. P.A., C.S.B., K.C., M.S., D.M.J., and H.B.-N. designed the study. All authors commented on and approved the final manuscript. M.B.-A. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

The authors thank all of the participants for their kind cooperation, in particular Delmiro Gomes Barbosa and Moises Soares Gomes, both from the Bandim Health Project, for considerable work in the field. The authors also thank the laboratory staff at the Department of Clinical Biochemistry and Pharmacology and the Department of Clinical Genetics at Odense University Hospital, Denmark, for the contribution.

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