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

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 HbA1c ≥ 6.5% (48 mmol/mol) and by fasting glucose ≥ 7.0 mmol/L (126 mg/dL). MS prevalence was determined as the presence of at least 3 of the following: waist circumference ≥ 80 cm, triglycerides ≥ 1.7 mmol/L, HDL cholesterol < 1.0 mmol/L, systolic blood pressure ≥ 130 mmHg, or diastolic blood pressure ≥ 85 mmHg. 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 study was carried out from October 2009 until August 2011. Twins aged 5–32 years were invited to participate. Twins were included if birth weight was < 2,500 g. Height, weight, waist circumference, and blood pressure were measured in the field. Fasting blood samples for metabolic measurements were collected. Zygosity was established genetically for a subset. DM was defined as HbA1c ≥ 6.5% (48 mmol/mol) and by fasting glucose ≥ 7.0 mmol/L (126 mg/dL). MS prevalence was determined as the presence of at least 3 of the following: waist circumference ≥ 80 cm, triglycerides ≥ 1.7 mmol/L, HDL cholesterol < 1.0 mmol/L, systolic blood pressure ≥ 130 mmHg, or diastolic blood pressure ≥ 85 mmHg.

RESULTS—HbA1c was available for 534 twins and 463 singletons. Mean age was 15.3 years (± 3.9 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 HbA1c (≥ 6.5% [48 mmol/mol]) compared with 2.4% (11 of 463) of singletons (P = 0.28). Mean HbA1c 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) of twins and 3.6% (13 of 360) of singletons (P = 0.66). The prevalence of fasting blood glucose (F-glucose) ≥ 6.1 mmol/L was 34.9% (127 of 364) versus 24.7% (89 of 360) (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.

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.

From the 1Bandim Health Project, INDEPTH Network, Bissau, Guinea-Bissau; the 2Department of Infectious Diseases, Odense University Hospital, Odense C, Denmark; the 3Department of Endocrinology, Odense University Hospital, Odense C, Denmark; the 4National Public Health Laboratory, Bissau, Guinea-Bissau; the 5Department of Clinical Biochemistry and Pharmacology, Odense University Hospital, Odense C, Denmark; the 6Research Center for Vitamins and Vaccines, Statens Serum Institute, Copenhagen S, Denmark; the 7Clinical Institute, University of Southern Denmark, Odense C, Denmark; and the 8Department of Clinical Biochemistry and Pharmacology, Odense University Hospital, Odense C, Denmark.

Corresponding author: Morten Bjerregaard-Andersen, mban@dahdn.dk.

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MS and DM in twins and singletons

≥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), HbA1c, 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 HbA1c ≥6.5% (48 mmol/mol), while HbA1c between 6.0 and 6.4% (42–46 mmol/mol) was considered a high-risk category (20). As HbA1c 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 (dataBase 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 HbA1c was explored by linear regression, while Poisson regression was applied for the association between LBW and elevated HbA1c. 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. HbA1c 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 included in the study and those not. Of the 513 interviewed singletons, 50 refused a fasting blood sample, or there was insufficient blood collected. HbA1c 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 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 HbA1c 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 $\text{Diff} = 1.91; \text{CI} 0.52–3.29$) 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) ($\text{Diff} = 0.58; \text{CI} 0.45–0.71$), as was mean BMI, 17.7 versus 18.3 kg/m$^2$ ($\text{Diff} = 0.60; \text{CI} 0.01–1.19$).

HbA1c 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 HbA1c 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 the singletons had
elevated HbA1c ($P = 0.28$) (Table 2). No difference was observed in mean HbA1c ($P = 0.33$). Assuming zygosity could be reliably determined by physical similarity (89% correctness in our data), no difference in mean HbA1c was observed between MZ and DZ twins ($P = 0.56$).

Mean HbA1c was similar for twin pairs and single twins ($P = 0.50$). The prevalence of elevated HbA1c 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 HbA1c 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 HbA1c**

We investigated the association between LBW and elevated HbA1c 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 HbA1c 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 HbA1c 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 (38.3) | 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/m²) (N = 1,024)        | 17.7 (4.1)     | 18.3 (4.6)     | 0.05 | PR = 0.60 (0.011–1.19) |

HbA₁c and Hb

| HbA₁c (%) (N = 1,037) | 35.0 (41.4) | 52.8 (47.2) | 0.33 | Diff = 0.02 (–0.03 to 0.08) |
| HbA₁c (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 (0.74–4.15) |
| 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 HbA₁c 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 HbA₁c assay as a DM diagnostic tool as recently recommended by the International Expert Committee (20). Currently, very limited data on HbA₁c 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 HbA₁c tended to be lower in case of anemia. This calls for further evaluations. The MS and DM burdens were too 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).

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).
is not a prerequisite using the HbA1c 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

### Table 2—Distribution of elevated HbA1c, DM, and MS for 574 twins and 463 singletons

|                  | HbA1c 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| 13/360 (3.6) |
| Male             |                |      |      |
| ≤15 years        | 5/113 (4.4)    | 0/113| 0/66 |
| >15 years        | 2/124 (1.9)    | 0/124| 1/107 (0.9) |
| Female           |                |      |      |
| ≤15 years        | 1/131 (0.8)    | 1/131| 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.
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 HbA1c between MZ and DZ twins. Nor did we find any differences in HbA1c 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 HbA1c 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

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M.B.-A. designed the study, supervised the surveys 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 re-testing and clinical examination. D.E.H. supervised the surveys 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 the study.

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