Epigenetic-age acceleration in the emerging burden of cardiometabolic diseases among migrant and non-migrant African populations: a population-based cross-sectional RODAM substudy

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Summary

Background African populations are going through health transitions due to rapid urbanisation and international migration. However, the role of biological ageing in the emerging burden of cardiometabolic diseases among migrant and non-migrant Africans is unknown. We aimed to examine differences in epigenetic-age acceleration (EAA) as measured by four clocks (Horvath, Hannum, PhenoAge, and GrimAge) and their associations with cardiometabolic factors among migrant Ghanaians residing in Europe and non-migrant Ghanaians residing in Ghana using cross-sectional data.

Methods In this population-based cross-sectional RODAM substudy, recruitment of urban participants in Ghana was done in two cities (Kumasi and Obuasi), whereas recruitment in rural areas was done in 15 villages in the Ashanti region. In Europe, participants were recruited from the cities of Amsterdam (Netherlands), Berlin (Germany), and London (UK). The method and location of participant recruitment varied according to country and city. Participants were included in the RODAM study if they were older than 25 years, had completed the RODAM study questionnaire, were physically examined, and had blood samples taken. In the present subsample, data for DNA-methylation (DNAm) had to be available for the participants. We did not specify any exclusion criteria. We used genome-wide DNAm data from Ghanaians to quantify EAA. We assessed the correlation between DNAm-based age measures and chronological age, and then we did linear regressions to investigate the associations between EAA and body-mass index (BMI), fasting blood glucose (FBG), blood pressure, alcohol consumption, smoking status, physical activity, and one-carbon metabolism nutrients among migrant and non-migrant populations. We replicated our findings among rural–urban sibling pairs from the India Migration Study and among indigenous South Africans from the PURE-SA-NW study.

Findings Between Feb 2, 2012, and Sept 30, 2014, 736 individuals participated in the RODAM epigenetics substudy, of which 12 (2%) were excluded during DNAm quality control, and a further 12 (2%) were excluded because of genotypic and phenotypic sex discordance. 712 (97%) of 736 participants were included in the analysis; 365 (51%) of these 712 participants were migrants and 347 (49%) were non-migrants. We found that migrant Ghanaians had lower EAA than non-migrant Ghanaians (intrinsic EAA Horvath –0·34 years *vs* 0·35 years; extrinsic EAA Hannum –0·86 years *vs* 0·90 years; PhenoAge acceleration –1·68 years *vs* 1·77 years; and GrimAge acceleration –0·18 years *vs* 0·19 years). Within migrant Ghanaians, higher FBG was positively associated with EAA measures, with the adjusted regression β for intrinsic EAA being 0·30 (95% CI 0·01 to 0·59) for migrants and 0·12 (–0·04 to 0·28) for non-migrants, for extrinsic EAA being 0·31 (0·05 to 0·56) for migrants and 0·08 (–0·06 to 0·22) for non-migrants, for PhenoAge acceleration being 0·39 (0·07 to 0·71) for migrants and 0·14 (–0·01 to 0·32) for non-migrants, and for GrimAge acceleration being 0·18 (0·01 to 0·34) for migrants and 0·12 (0·03 to 0·21) for non-migrants. Within non-migrant Ghanaians, higher BMI and vitamin-B9 (folate) intake were negatively associated with EAA measures. Our findings on FBG, BMI, and folate were replicated in the independent cohorts.

Interpretation Our study shows that migration is negatively associated with EAA among Ghanaians. Moreover, cardiometabolic factors are differentially associated with EAA within migrant and non-migrant subgroups. Our results call for context-based interventions for cardiometabolic diseases among transitioning populations that account for the effects of biological ageing.

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Research in context

Evidence before this study

On Sept 22, 2020, we searched PubMed for articles published between Sept 1, 2010, and Sept 22, 2020 without language restrictions, using the MESH search terms "transients and migrants", "urbanization", "hypertension", "diabetes mellitus type 2", "DNA methylation", "body mass index", "biological clocks", and "Africa and developing countries". We did not find any study that had investigated the relationship between biological ageing (epigenetic age acceleration) and cardiometabolic traits such as obesity, diabetes, and hypertension in Africa or any other low-income countries.

Added value of this study

We provide evidence for the role of epigenetic age acceleration (EAA) in the growing burden of obesity, diabetes, and hypertension in populations going through epidemiological (health) transitions. We use powerful migration models that allow investigation of changing environmental exposures while controlling for ethnicity and early-life exposure. We also assess, to our knowledge for the first time, how one-carbon metabolism nutrients can influence EAA. We find that transitioning Ghanaian populations (migrants) have lower EAA than their non-migrant counterparts. We find among migrants that higher fasting blood glucose (linked with diabetes) is positively associated with EAA. We also find that higher body-mass index (BMI; overweight and mild obesity) and intake of folate are negatively associated with EAA in non-migrants, which could possibly be linked to the nutritional reserve that is required to repair the deleterious effects of chronic inflammation in the human body. Our results on overweight, diabetes, and folate intake were successfully replicated in independent cohorts of Indians and indigenous South Africans, which shows that most of the relationships between EAA and cardiometabolic factors are similar across populations going through epidemiological transitions.

Implications of all the available evidence

Our results show that EAA is negatively associated with epidemiological (health) transitions among Ghanaians. Clearly, the relationship between EAA and cardiometabolic factors depends on whether a population is urbanising or not, and on the prevalent environmental exposures, such as infectious disease and chronic inflammation. Harnessing the EAA effects of BMI and folate intake could possibly improve life expectancy in rural areas where nutritional deficiencies and infectious diseases might be highly prevalent. Our study calls for interventions that consider the effects of biological ageing (EAA) in the prevention and treatment of cardiometabolic diseases among Africans and other populations going through epidemiological (health) transitions.

Introduction

African populations are going through epidemiological transitions due to rapid urbanisation and international migration, often from low-income to high-income countries (HICs).[1,2] Changes in lifestyle factors upon migration (eg, tobacco smoking, poor diet, and physical inactivity) are prominent contributors to the emerging risk of cardiometabolic diseases.[3–5] Although epigenetic ageing has been linked to cardiometabolic diseases and its risk factors in populations originating from HICs, little is known about the role of epigenetic ageing in the emerging burden of cardiometabolic diseases in transitioning African populations.

Epigenetic age, which is quantified using epigenetic clocks also known as DNA methylation (DNAm)-based age estimators, is a robust biomarker for chronological age.[6] Epigenetic clocks comprise a set of cytosine-phosphate-guanine (CpGs) sites coupled with mathematical algorithms that estimate age (in years) from a DNA source.[6] The difference between chronological age and epigenetic age in an individual is termed epigenetic-age acceleration (EAA).[6] EAA can be used to predict all-cause mortality, lifespan, and incidence of chronic non-communicable diseases, including cardiometabolic diseases.[6–10] When quantified in blood, EAA can be divided into two forms, intrinsic and extrinsic. Intrinsic EAA (IEAA) captures biological ageing within each cell independently of proportions of naive or senescent cytotoxic T cells, whereas extrinsic EAA (EEAA) quantifies epigenetic ageing in immune-related components.[6] Several epigenetic clocks have been developed to measure IEAA and EEAA. The most commonly used are the Horvath clock, which measures IEAA using DNAm (HorvathAge),[11] the Hannum clock, which measures EEAA using DNAm (HannumAge),[12] the PhenoAge clock, which measures EEAA using DNAm and includes clinical characteristics in its algorithm (chronological age, albumin, creatinine, glucose, C-reactive protein, lymphocyte percentage, mean cell volume, red blood-cell distribution width, alkaline phosphatase, and white blood-cell count),[9] and the GrimAge clock, which measures EEAA using DNAm and incorporates into its algorithm chronological age, sex, seven plasma proteins (adrenomedullin, β2 macroglobulin, growth-differentiation factor 15, cystatin C, leptin, plasminogen-activation inhibitor 1, and tissue-inhibitor metalloproteinase 1), and the amount of cigarettes smoked.[13]

Previous studies have shown that physical activity, fruit and vegetable consumption, and education are negatively associated with EAA, whereas cardiometabolic traits such as obesity, diabetes, and hypertension are positively associated with EAA.[7,14,15] Given that the DNAm that underlies EAA is affected by changes in lifestyle factors

and can also directly influence cardiometabolic diseases, understanding the role of EAA in the emerging burden of cardiometabolic diseases among transitioning African populations is important.[16] Considering that migrant (urbanising) African populations have higher rates of cardiometabolic diseases than their non-migrant counterparts,[17] we hypothesise that changes in lifestyle upon migration lead to accelerated biological ageing of tissues (higher EAA), which in turn leads to higher incidence of cardiometabolic diseases. As such, we expect migrants and urbanising populations to show higher EAA than their non-migrant counterparts.

Using data from the Research on Obesity and Diabetes Among Migrants (RODAM) study, we examined differences in EAAs and their associations with cardiometabolic and lifestyle factors among migrant Ghanaians residing in Europe and non-migrant Ghanaians residing in Ghana. Considering that maintenance of DNAm relies partly on one-carbon metabolism nutrients for transmethylation (folate, choline, and betaine as methyl donors, and vitamins B2, B6, and B12 as essential factors), we included dietary intake of one-carbon metabolism nutrients as part of our lifestyle-factor measurements.[18]

Methods

Study design and participants

The RODAM study is a multicentre, cross-sectional study that was initiated in 2012 to better understand the development of obesity and diabetes among African migrants at the phenotypic, epigenetic, and genetic levels. A detailed description of the study population is provided (appendix p 2). In brief, the RODAM study enrolled 6385 participants, 2595 (40·64%) non-migrant Ghanaian men and women living in Ghana, and 3790 (59·36%) first-generation migrant Ghanaians residing in Europe. Participants were predominantly from the Akan ethnic group. In Ghana, recruitment of urban participants was done in two cities (Kumasi and Obuasi), whereas recruitment in rural areas was done in 15 villages in the Ashanti region. In Europe, participants were recruited from the cities of Amsterdam (Netherlands), Berlin (Germany), and London (UK). The method and location of participant recruitment varied according to country and city (appendix p 2). Individuals were included if they were older than 25 years, had completed the RODAM study questionnaire, and were physically examined and had blood samples taken. In the present subsample, data for DNA-methylation (DNAm) had to be available for the patients. We did not specify any exclusion criteria.

Ethics approval was obtained from the ethics committees of the relevant institutions in Ghana (Kwame Nkrumah University of Science and Technology, Kumasi; CHRPE/AP/200/12), the Netherlands (Amsterdam University Medical Centre, Amsterdam; W12–062#12.17.0086), Germany (Charité University Berlin, Berlin; EA1/307/12) and the UK (London School of Hygiene & Tropical Medicine, London; 6208) before the start of data collection.[17] All participants gave written informed consent.[17]

Phenotypic measurements

A standardised approach (appendix p 8) for questionnaires, anthropometric measurements, and venipuncture samples was used across all study sites. A detailed description of phenotypic measurements, data handling, and data-entry procedures is provided (appendix p 8). For this study, the following measurements were obtained: chronological age; sex; location of residence; level of education; alcohol consumption; smoking status; physical activity; dietary intake of vitamin B2 (mg/day), vitamin B6 (mg/day), vitamin B9 (µg/day), vitamin B12 (µg/day), and total-energy intake (kcal/day) from food-frequency questionnaires; duration of stay in the host country for migrant populations; body-mass index (BMI; kg/m²); blood pressure (in mmHg); fasting plasma glucose (FBG; mmol/L); and use of anti-diabetic medication. Education was categorised as none or elementary, primary, secondary, and tertiary. Alcohol consumption was categorised as consumed or never consumed. Tobacco smoking was categorised into current, past, and never smokers. Physical activity was categorised into low, moderate, and high according to the Global Physical Activity Questionnaire (GPAQ).[17]

DNAm processing, profiling, and quality control

Detailed procedures for DNAm processing, profiling, and quality control in the RODAM study are also reported (appendix p 9). In brief, bisulfite conversion of DNA was done with the Zymo EZ DNA Methylation kit (Zymo Research, Irvine, CA, USA). The converted DNA was amplified and hybridised on Infinium HumanMethylation450 BeadChip, which quantifies DNAm amounts of approximately 485 000 CpG sites. Quality control was done using the MethylAid package in R (version 1.4.0). Functional normalisation was applied using the minfi package (version 3.1.0.).[19] Probes annotated to X and Y chromosomes, known to involve cross hybridisation or to involve single-nucleotide polymorphisms with a minor allele frequency of at least 0·05 (5%) were removed from the dataset.[19] This resulted in a total set of 429 459 CpGs.

See Online for appendix

DNAmAge calculation and estimated blood-cell counts

DNAm-based epigenetic ages (DNAmAge) were calculated using the online DNAmAge calculator[20] from normalised DNAm data and crosschecked via the Bioconductor package Methylclock (version 0.5.0).[21] We estimated DNAm HorvathAge using 353 CpGs as specified by Horvath.[11] We obtained IEAA as residuals from the regression model that regresses HorvathAge on chronological age and blood-cell counts.[11] We estimated DNAm age with HannumAge using 71 CpGs as specified by Hannum and colleagues.[12] We obtained EEAA as residuals from a regression model that regresses

For the **DNA methylation age calculator** see http://dnamage.genetics.ucla.edu/

DNAm HannumAge aggregated with three blood-cell components (naive cytotoxic T cells, exhausted cytotoxic T cells, and plasmablasts) on chronological age.[12] We estimated DNAm PhenoAge using 513 CpGs as specified in Levine and colleagues.[9] We obtained PhenoAge acceleration (PhenoAgeAccel) as residuals of the regression models that regress DNAm PhenoAge on chronological age without adjusting for blood-cell counts.[9] We estimated DNAm GrimAge using 1030 CpG sites as specified by Lu and colleagues.[13] We obtained GrimAge acceleration (GrimAgeAccel) as residuals of the regression models that regress DNAm GrimAge on chronological age and sex, without adjusting for blood-cell counts. We estimated cell counts using a method developed by Houseman and colleagues,[22] implemented using the Methylclock package.

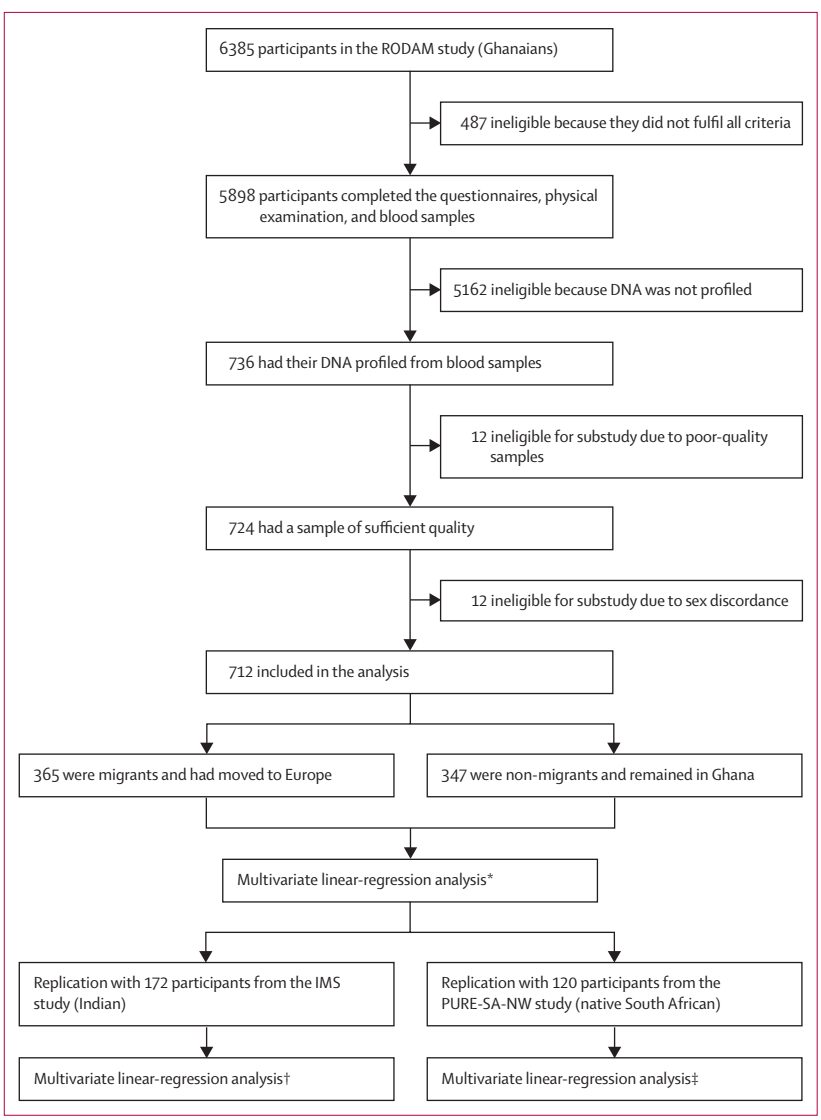

Figure 1: **Study flowchart**
Depiction of the study design and analyses. DNAmAge=DNA methylation age. *Multivariate linear-regression analysis in which outcome was DNAmAge acceleration measures, predictor categories were lifestyle factors and cardiometabolic factors, and categories were migrants compared with non-migrants. †Multivariate linear-regression analysis in which outcome was DNAmAge acceleration measures, predictor categories were lifestyle factors and cardiometabolic factors, and categories were migrants (urban) compared with non-migrants (rural). ‡Multivariate linear-regression analysis in which outcome was DNAmAge acceleration measures, predictor categories were lifestyle factors and cardiometabolic factors, and categories non-migrants only.

Sample size

For the current analyses, we used a subsample of participants from the RODAM study for whom DNAm data were available. This subsample of individuals for whom epigenetic information was obtained was originally designed in the RODAM study to detect 5% DNAm differences between participants with diabetes (n=265) compared with controls without diabetes (n=471), who were equally distributed between migrant and non-migrant groups (case–control design; power=0·80, α=0·05; appendix p 5). The final sample obtained from participants that was used in the current analyses had passed DNAm quality control and was generally representative of the overall RODAM study population (appendix p 7).

Statistical analysis

Statistical analysis was carried out using R (version 4.0.2) and Bioconductor packages. Our sample did not contain any missing data. Descriptive statistics were presented as proportions for categorical variables, as mean (SD) or as median (IQR) for skewed data. Pearson's correlations were done between chronological age and DNAmAges, and between the EAA measures. Linear regression was done between EAA measures (outcome variable) and cardiometabolic-disease traits (smoking status, alcohol consumption, physical activity, vitamin intake, duration of stay in host country for migrants, BMI, FBP, and blood pressure) among migrant and non-migrant populations separately. Linear models were adjusted for chronological age, education, sex, smoking status, alcohol consumption, physical activity, vitamin intake, total-energy intake, and duration of stay in host country among migrants.

We aimed to minimise false-positive findings in our study. Instead of using standard multiple-test corrections, we opted for other alternative methods, because cardiometabolic-disease risk factors have interconnected pathophysiological pathways and are complementary to one another in explaining our hypothesis.[23] For example, dietary intake, physical activity, obesity, and diabetes have interconnected pathophysiological pathways,[23] and moreover, changes in dietary intake, physical activity, and BMI can together explain why migrant populations have higher rates of diabetes than non-migrant populations.[17] Having significant findings in all these cardiometabolic-disease risk factors is thus possible. We therefore opted out of standard multiple-test corrections and considered detection of a similar effect in two or more EAA measures as a true finding in both the main and post-hoc analyses.

	Non-migrants* (n=347)	Migrants† (n=365)
Demographic factors		
Chronological age‡	52·36 (9·84)	49·89 (9·76)
Women	243 (70%)	166 (45%)
Men	104 (30%)	199 (55%)
Education		
No education	160 (46%)	79 (22%)
Primary school	136 (39%)	154 (42%)
Secondary school	38 (11%)	78 (21%)
Tertiary	13 (4%)	54 (15%)
Behaviour-related factors		
Any alcohol consumption	121 (35%)	152 (42%)
Smoking		
Current	5 (>1%)	18 (5%)
Never	309 (89%)	316 (87%)
Past	33 (10%)	31 (8%)
Physical activity§		
Low	131 (38%)	137 (38%)
Moderate	65 (19%)	86 (24%)
High	151 (44%)	142 (39%)
Total energy intake in kcal/day¶	2386·24 (824·96)	2894·10 (1113·31)
Median length of stay for migrants in years	NA	20·39 (12·30–25·35)
One-carbon metabolism nutrient intake‖		
Vitamin B2 (riboflavin) in mg/day	1·29 (0·51)	2·24 (1·60)
Vitamin B6 in mg/day	2·32 (0·80)	3·01 (1·29)
Vitamin B9 (folate) in µg /day	311·56 (113·22)	445·31 (203·87)
Vitamin B12 (cyanocobalamin) in µg /day	5·92 (3·81–10·17)	12·82 (7·19–34·43)
Cardiometabolic factors		
Body-mass index in kg/m²	24·74 (5·59)	28·18 (4·92)
Systolic blood pressure in mmHg	130·91 (22·27)	136·77 (18·44)
Diastolic blood pressure in mmHg	80·8 (12·32)	84·85 (11·44)
Median fasting blood glucose concentration in mmol/L	5·13 (4·73–6·18)	5·26 (4·87–7·61)
Use of medication		
Use of anti-diabetic medication**	23 (7%)	37 (10%)

Data are n (%), mean (SD), or median (IQR). *Ghanaians living in rural and urban Ghana were categorised as non-migrants. †Ghanaians living in Amsterdam (Netherlands), Berlin (Germany), and London (UK) were categorised as migrants. ‡Age provided by the participant during questionnaire interviews. §Physical activity categorised according to the global physical-activity questionnaire criteria. ¶Total-energy intake estimated via food-frequency questionnaires. ‖One-carbon metabolism nutrient intake estimated via food-frequency questionnaires. **Use of medications to treat diabetes (oral and injectables).

Table 1: **Baseline characteristics of Ghanaian participants in the RODAM study**

	Non-migrants* (n=347)	Migrants† (n=365)
Chronological age‡	52·36 (9·84)	49·89 (9·76)
DNAm HorvathAge§	50·78 (10·04)	48·00 (9·62)
DNAm HannumAge¶	44·01 (9·93)	39·82 (8·96)
DNAm PhenoAge‖	41·20 (10·77)	35·68 (10·07)
DNAm GrimAge**	39·35 (8·72)	37·07 (8·33)
IEAA, years††	0·35 (6·14)	−0·34 (5·88)
EEAA, years‡‡	0·90 (5·26)	−0·86 (5·09)
PhenoAgeAccel, years§§	1·77 (6·9)	−1·68 (6·41)
GrimAgeAccel, years¶¶	0·19 (3·79)	−0·18 (3·85)

Data are years (mean [SD]). DNAm=DNA methylation. EEAA=extrinsic epigenetic-age acceleration. IEAA=intrinsic epigenetic-age acceleration. NA=not applicable. *Ghanaians living in rural and urban Ghana were categorised as non-migrants. †Ghanaians living in Amsterdam (Netherlands), Berlin (Germany), and London (UK) were categorised as migrants. ‡Age provided by the participant during questionnaire interviews. §DNAm age obtained using the Horvath clock. ¶DNAm age obtained using the Hannum clock. ‖DNAm age obtained using the PhenoAge clock. **DNAm age obtained using the GrimAge clock. ††IEAA (within each cell) obtained using the Horvath clock. ‡‡EEAA (between different cells) obtained using the Hannum clock. §§Epigenetic-age acceleration incorporating clinical traits obtained using the PhenoAge clock. ¶¶Epigenetic-age acceleration incorporating plasma proteins obtained using the GrimAge clock.

Table 2: **Ageing estimators of Ghanaian participants in the RODAM study**

Post-hoc analyses

Post-hoc analyses (appendix pp 12–18) were done to ascertain the robustness of our findings. First, we assessed whether associations of lifestyle and cardiometabolic factors with EAA measures were also apparent in the combined sample of migrant and non-migrant populations. These associations would indicate that our findings were not substantially influenced by large differences in baseline characteristics between the two groups. Second, we assessed whether associations between FBG and EAA were influenced by usage of anti-diabetic medications. Finally, we aimed to replicate our findings among populations from Africa or other low-income and middle-income countries (LMICs) where urbanisation and health transitions are prominent. As such, we did a replication analysis in rural–urban sibling pairs from the Indian Migration Study (IMS) and among indigenous South Africans from the Prospective Urban and Rural Epidemiology study's South African, northwest province cohort (PURE-SA-NW).[24,25] Emphasis was placed on consistency in the direction of effects in the replication analyses due to smaller sample sizes in replication cohorts (low statistical power).

Role of the funding source

The study funder had no role in the study design, data collection, data analysis, data interpretation or writing of the report. The corresponding author had full access to all the data and the final responsibility to submit for publication.

Results

Between Feb 2, 2012, and Sept 30, 2014, a total of 736 individuals participated in the RODAM epigenetics substudy, of which 12 (2%) were excluded during DNAm quality control, and a further 12 (2%) were excluded because of genotypic and phenotypic sex discordance. Thus, 712 (97%) of 736 participants were included in the

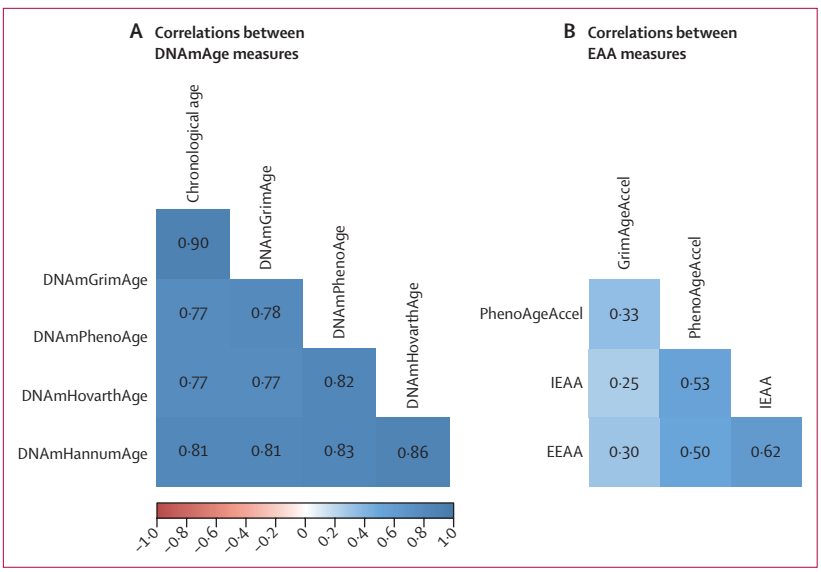

Figure 2: **Correlations between DNAmAge measures and EAA measures in the RODAM study**
Pearson's correlations between DNAmAge measures (A) and EAA measures (B). DNAmAge=DNA-methylation age. DNAmGrimAge=DNA-methylation age obtained using the GrimAge clock. DNAmHannumAge=DNA-methylation age obtained using the Hannum clock. DNAmHorvathAge=DNA-methylation age obtained using the Horvath clock. DNAmPhenoAge=DNA-methylation age obtained using the PhenoAge clock. EAA=extrinsic epigenetic age. EEAA=extrinsic epigenetic-age acceleration. GrimAgeAccel=epigenetic-age acceleration obtained using the GrimAge clock. IEAA=intrinsic epigenetic-age acceleration. PhenoAgeAccel=epigenetic-age acceleration obtained using the PhenoAge clock.

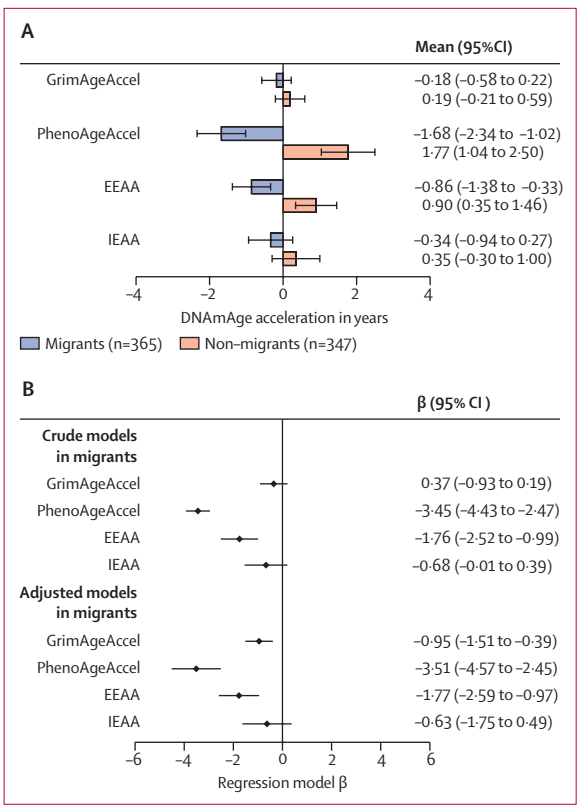

Figure 3: **Migration status and EAA measures in the RODAM study**
(A) Mean DNAmAge acceleration (years) in migrant versus non-migrant people in the RODAM study. Data are presented as mean (95% CI). (B) Linear-regression models for the associations between migration status and four EAA measures in the RODAM study, in which outcome is DNAmAge acceleration measures, predictor is migration status, and reference group is non-migrant people. The adjusted model is adjusted for age, sex, and education level. Data are presented as β coefficients (95% CI), with the red line indicating the reference for CIs. DNAmAge=DNA-methylation age. EAA=extrinsic epigenetic age. EEAA=extrinsic epigenetic-age acceleration. GrimAgeAccel=epigenetic-age acceleration obtained using the GrimAge clock. IEAA=intrinsic epigenetic-age acceleration. PhenoAgeAccel=epigenetic-age acceleration obtained using the PhenoAge clock.

analysis (figure 1). 365 (51%) of these 712 participants were migrants and 347 (49%) were non-migrants. Mean chronological age of participants who were migrants was 49·89 years (SD 9·76) and mean age of non-migrants was 52·36 years (SD 9·84). Migrants had attained higher levels of education, smoked more, and were more overweight than non-migrants. Generally, the migrant group included more male participants, and had higher blood pressure, FBG, and vitamin intake than non-migrants (table 1).

Mean DNAmAges were lower in migrants than in non-migrants (table 2). Chronological age was positively correlated with epigenetic age as estimated from all four DNAm clocks, with a correlation coefficient of 0·77 for HorvathAge estimated from DNAm, 0·81 for HannumAge estimated from DNAm, 0·77 for PhenoAge estimated from DNAm, and 0·90 for GrimAge estimated from DNAm (figure 2). These correlations are similar to those of previous studies and validate the utility of DNAmAge estimators in our study.[9,14] Correlations among EAA measurements were weaker, with correlation coefficients ranging from 0·25 (IEAA with GrimAgeAccel) to 0·62 (IEAA with EEAA). These weaker correlations between EAA measures have been previously observed and represent the uniqueness of each of the four EAA measures.[14]

We found that migrants had generally lower EAA than non-migrants (IEAA –0·34 *vs* 0·35; EEAA –0·86 *vs* 0·90, PhenoAgeAccel –1·68 *vs* 1·77, and GrimAgeAccel –0·18 *vs* 0·19 years; table 2, figure 3). Migration status was negatively associated with EEAA, PhenoAgeAccel, and GrimAgeAccel after adjusting for age, sex, and education (figure 3). However, duration of stay in the host country among migrants was not associated with any EAA measure (table 3, figures 4 and 5).

When investigating modifiable lifestyle risk factors (table 3, figures 4 and 5), adjusted linear-regression models showed that alcohol consumption was not associated with any EAA measure in migrant or non-migrant populations. Current tobacco smoking was negatively associated with GrimAgeAccel among migrants, whereas current and past tobacco smoking were negatively associated with PhenoAgeAccel among non-migrants. Higher physical activity was negatively associated with PhenoAgeAccel among migrants but not among non-migrants.

When investigating one-carbon metabolism nutrients (table 3, figures 4 and 5), adjusted linear-regression models showed that higher vitamin-B2 (riboflavin)

	IEAA (Horvath)*		EEAA (Hannum)†		PhenoAgeAccel‡		GrimAgeAccel§	
	Non-migrants¶ (n=347)	Migrants‖ (n=365)	Non-migrants¶ (n=347)	Migrants‖ (n=365)	Non-migrants¶ (n=347)	Migrants‖ (n=365)	Non-migrants¶ (n=347)	Migrants‖ (n=365)
Lifestyle factors								
Alcohol consumption								
No	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Yes (crude)**	−0·77 (−2·13 to 0·59)	−1·07 (−2·29 to 0·16)	−0·82 (−1·99 to 0·34)	−0·39 (−1·46 to 0·67)	−0·37 (−1·90 to 1·16)	−0·38 (−1·72 to 0·96)	−0·58 (−0·28 to 1·39)	0·64 (−0·17 to 1·44)
Yes (adjusted)††	−0·92 (−2·32 to 0·48)	−0·01 (−1·71 to 1·70)	−1·03 (−2·23 to 0·18)	−0·27 (−1·70 to 1·16)	−0·74 (−2·31 to 0·84)	−0·08 −1·86 to 1·71)	−0·17 (−0·97 to 0·63)	0·44 (−0·33 to 1·22)
Physical activity‡‡								
Low	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Moderate (crude)	−0·77 (−2·60 to 1·07)	−0·27 (−1·86 to 1·32)	−0·20 (−1·78 to 1·37)	−0·90 (−2·28 to 0·48)	0·96 (−1·10 to 3·03)	−0·37 (−2·10 to 1·35)	0·74 (−0·38 to 1·86)	−0·29 (−1·34 to 0·74)
Moderate (adjusted)	−0·78 (−2·67 to 1·11)	0·64 (−2·82 to 2·82)	−0·18 (−1·81 to 1·44)	−0·33 (−2·16 to 1·49)	0·71 (−1·41 to 2·85)	0·12 (−2·16 to 2·41)	−0·13 (−1·21 to 0·04)	−0·52 (−1·49 to 0·44)
High (crude)	−0·67 (−2·12 to 0·77)	−1·16 (−2·54 to 0·22)	−0·674 (−1·91 to 0·56)	−0·71 (−1·92 to 0·48)	0·41 (−1·21 to 2·04)	−1·91 (−3·41 to 0·41)	−0·53 (−1·43 to 0·35)	0·49 (−0·41 to 1·39)
High (adjusted)	−0·33 (−1·85 to 1·20)	−0·19 (−1·69 to 1·69)	−0·347 (−1·66 to 0·96)	−0·21 (−1·79 to 1·36)	0·55 (−1·16 to 2·27)	−2·16 (−4·13 to −0·20)	−1·12 (−1·98 to 0·25)	−0·15 (−1·00 to 0·69)
Tobacco smoking								
Past (crude)	1·59 (−0·61 to 3·79)	−0·35 (−0·72 to 0·03)	0·62 (−1·28 to 2·52)	−0·13 (−2·02 to 1·75)	−0·11 (−2·60 to 2·37)	−1·67 (−4·05 to 0·70)	3·14 (1·81 to 4·49)	1·89 (0·52 to 3·26)
Past (adjusted)	−3·15 (−9·09 to 2·79)	−0·26 (−0·42 to 0·42)	−1·33 (−6·44 to 3·78)	3·11 (−0·79 to 7·02)	−7·07 (−13·76 to −0·40)	1·44 (−3·4 to 6·32)	0·51 (−2·86 to 3·89)	−2·09 (−4·22 to 0·02)
Current (crude)	5·01 (−0·40 to 10·43)	−0·03 (−2·83 to 2·78)	1·07 (−3·60 to 5·75)	−1·91 (−4·33 to 0·52)	5·62 (−0·48 to 11·73)	−0·24 (−3·30 to 2·81)	1·83 (−1·43 to 5·19)	4·56 (2·79 to 6·32)
Current (adjusted)	−5·13 (−10·61 to 0·35)	−1·39 (−5·19 to 5·19)	−1·34 (−6·06 to 3·37)	2·91 (−0·28 to 6·09)	−6·31 (−12·47 to −0·15)	1·29 (−2·68 to 5·28)	−1·26 (−4·38 to 1·85)	−3·60 (−5·36 to −1·80)
Length of stay in Europe among migrants§§								
Length of stay (crude)	NA	−0·01 (−0·07 to 0·05)	NA	−0·03 (−0·07 to 0·01)	NA	−0·04 (−0·14 to 0·06)	NA	0·01 (−0·04 to 0·05)
Length of stay (adjusted)	NA	0·04 (−0·02 to 0·12)	NA	0·04 (−0·03 to 0·11)	NA	0·05 (−0·04 to 0·13)	NA	0·03 (−0·02 to 0·08)
One-carbon metabolism nutrients								
Vitamin B2 (crude)	−1·37 (−2·63 to 0·12)	−0·35 (−0·72 to 0·03)	−0·585 (−1·67 to 0·50)	−0·13 (−0·47 to 0·19)	−1·59 (−3·01 to −0·18)	0·07 (−0·34 to 0·49)	−0·54 (−1·32 to 0·24)	0·09 (−0·15 to 0·33)
Vitamin B2 (adjusted)	−1·06 (−3·39 to 1·27)	−0·26 (−0·94 to 0·42)	−1·92 (−3·94 to 0·08)	−0·12 (−0·69 to 0·45)	−3·09 (−5·72 to −0·47)	−0·29 (−1·01 to 0·41)	−1·51 (−2·75 to 0·27)	−0·06 (−0·39 to 0·26)
Vitamin B6 (crude)	−0·69 (−1·50 to 0·12)	−0·14 (−0·61 to 0·33)	0·01 (−0·69 to 0·70)	−0·05 (−0·46 to 0·36)	−0·43 (−1·35 to 0·47)	0·46 (−0·05 to 0·97)	−0·10 (−0·60 to 0·41)	0·10 (−0·20 to 0·49)
Vitamin B6 (adjusted)	−1·31 (−3·78 to 1·15)	0·66 (−2·18 to 2·18)	−0·59 (−2·72 to 1·52)	−0·08 (−1·36 to 1·19)	−2·13 (−4·90 to 0·64)	0·96 (−0·63 to 2·56)	−0·93 (−2·26 to 0·39)	0·39 (−0·32 to 1·03)
Vitamin B9 (crude)	0·00 (−0·01 to 0·00)	0·00 (0·00 to 0·00)	0·02 (0·00 to 0·01)	−0·01 (0·00 to 0·00)	0·01 (−0·01 to 0·01)	0·00 (0·00 to 0·01)	0·01 (−0·02 to 0·05)	0·00 (−0·02 to 0·02)
Vitamin B9 (adjusted)	−0·01 (0·00 to 0·03)	0·00 (−0·01 to 0·01)	−0·01 (−0·02 to −0·001)	−0·01 (−0·01 to 0·00)	−0·02 (−0·04 to −0·001)	−0·00 (−0·01 to 0·01)	−0·01 (−0·03 to −0·02)	0·00 (−0·04 to 0·03)
Vitamin B12 (crude)	0·00 (−0·04 to 0·03)	0·04 (−0·03 to 0·10)	0·02 (−0·01 to 0·05)	−0·01 (−0·06 to 0·05)	−0·01 (−0·04 to 0·04)	0·08 (0·00 to 0·14)	−0·01 (−0·02 to 0·02)	−0·02 (−0·01 to −0·03)
Vitamin B12 (adjusted)	0·00 (−0·04 to 0·04)	0·05 (−0·20 to 0·31)	0·03 (0·001 to 0·06)	0·14 (−0·09 to 0·36)	0·01 (−0·03 to 0·05)	0·13 (−0·15 to 0·41)	−0·01 (−0·03 to 0·02)	0·02 (−0·02 to 0·06)
Cardiometabolic factors								
BMI (crude)	−0·15 (−0·27 to −0·04)	−0·04 (−0·16 to 0·09)	−0·16 (−0·26 to −0·07)	−0·03 (−0·14 to 0·07)	−0·22 (−0·35 to −0·10)	−0·13 (−0·26 to 0·01)	−0·16 (−0·23 to −0·09)	−0·09 (−0·17 to 0·02)
BMI (adjusted)	−0·14 (−0·26 to −0·02)	0·00 (−0·14 to 0·14)	−0·14 (−0·25 to −0·04)	0·00 (−0·11 to 0·12)	−0·21 (−0·34 to −0·07)	−0·13 (−0·27 to 0·01)	−0·11 (−0·18 to −0·04)	−0·01 (−0·09 to 0·07)
SBP (crude)	0·01 (−0·03 to 0·04)	−0·01 (−0·05 to 0·02)	0·01 (−0·01 to 0·04)	0·00 (−0·03 to 0·03)	−0·01 (−0·04 to 0·02)	0·01 (−0·03 to 0·05)	0·00 (−0·01 to 0·02)	0·02 (−0·01 to 0·04)

(Table 3 continues on next page)

	IEAA (Horvath)*		EEAA (Hannum)†		PhenoAgeAccel‡		GrimAgeAccel§	
	Non-migrants¶ (n=347)	Migrants‖ (n=365)	Non-migrants¶ (n=347)	Migrants‖ (n=365)	Non-migrants¶ (n=347)	Migrants‖ (n=365)	Non-migrants¶ (n=347)	Migrants‖ (n=365)
(Continued from previous page)								
SBP (adjusted)	0·00 (–0·03 to 0·03)	–0·02 (–0·02 to 0·02)	0·00 (–0·02 to 0·03)	0·00 (–0·03 to 0·03)	–0·02 (–0·05 to 0·02)	0·02 (–0·02 to 0·06)	–0·01 (–0·02 to 0·02)	0·01 (–0·01 to 0·03)
DBP (crude)	–0·02 (–0·08 to 0·03)	–0·02 (–0·07 to 0·03)	0·02 (–0·03 to 0·06)	0·01 (–0·04 to 0·06)	–0·04 (–0·10 to 0·02)	0·00 (–0·06 to –0·06)	0·01 (–0·02 to 0·04)	0·07 (0·03 to 0·11)
DBP (adjusted)	–0·03 (–0·09 to 0·02)	–0·04 (–0·02 to 0·02)	0·01 (–0·04 to 0·05)	–0·01 –0·06 to 0·05)	–0·05 (–0·11 to 0·02)	0·01 (–0·06–0·07)	–0·01 (–0·04 to 0·02)	0·03 (–0·01 to 0·06)
FBG (crude)	0·12 (–0·03 to 0·28)	0·29 (0·01 to 0·58)	0·10 (–0·03 to 0·23)	0·32 (0·08 to 0·57)	0·15 (–0·03 to 0·32)	0·37 (0·05–0·68)	0·15 (0·06 to 0·25)	0·29 (0·11 to 0·48)
FBG (adjusted)	0·12 (–0·04 to 0·28)	0·30 (0·01 to 0·59)	0·08 (–0·06 to 0·22)	0·31 (0·05 to 0·56)	0·14 (–0·01 to 0·32)	0·39 (0·07 to 0·71)	0·12 (0·03 to 0·21)	0·18 (0·01 to 0·34)

Data are presented as β (95% CI), as obtained from the linear-regression model. Vitamin intake was measured in mg per day for vitamin B2 and vitamin B6, whereas vitamin B9 and vitamin B12 were measured in µg per day. BMI=body-mass index (kg/m²). DBP=diastolic blood pressure (mmHg). EEAA=extrinsic epigenetic-age acceleration. FBG=fasting blood glucose (mmol/L). GPAQ=Global Physical Activity Questionnaire. IEAA=intrinsic epigenetic-age acceleration. SBP=systolic blood pressure (mmHg). Ref=reference values. *Intrinsic epigenetic-age acceleration (within each cell) obtained using the Horvath clock. †Extrinsic epigenetic-age acceleration (between different cells) obtained using the Hannum clock. ‡Epigenetic-age acceleration incorporating clinical traits obtained using the PhenoAge clock. §Epigenetic-age acceleration incorporating plasma proteins obtained using the GrimAge clock. ¶Ghanaians living in rural and urban Ghana were categorised as non-migrants. ||Ghanaians living in Amsterdam (Netherlands), Berlin (Germany), and London (UK) were categorised as migrants. **Crude linear-regression model. ††Linear-regression model fully adjusted for age, sex, education level, smoking status, physical activity, alcohol intake, total-energy intake, and for migrants, duration of stay in host countries. ‡‡Physical activity categorised according to GPAQ criteria. §§Length of stay indicates duration of stay in Europe for migrants.

Table 3: **Lifestyle factors, cardiometabolic traits and epigenetic age acceleration in migrant and non-migrant Ghanaians in RODAM study**

intake was negatively associated with PhenoAgeAccel among non-migrants but not among migrants. Higher vitamin-B9 (folate) intake was negatively associated with EEAA, PhenoAgeAccel, and GrimAgeAccel among non-migrants but not among migrants. Such associations were not observed for vitamin B6 (pyridoxine) and vitamin B12 (cobalamin) in migrants or non-migrants.

When investigating cardiometabolic factors (table 3, figures 4 and 5), adjusted linear-regression models showed that higher BMI was negatively associated with all EAA measures among non-migrants. Such associations were not observed among migrants. Higher FBG was positively associated with all EAA measures among migrants. Higher FBG was also positively associated with GrimAgeAccel among non-migrants. Systolic and diastolic blood pressure were not associated with any EAA measure, regardless of migration status.

Results from post-hoc analyses are presented (appendix pp 20–37) and summarised (appendix p 38). In brief, our findings on BMI, FBG, and folate in the main analyses were also apparent in the combined sample of migrants and non-migrants (appendix p 20). Our results on FBG in the main analyses were also observable in linear-regression models excluding participants taking anti-diabetic medications (appendix p 21). Our findings on BMI, FBG, and folate in the main analyses were also detectable in the independent replication cohorts (appendix pp 22–39).

Discussion

In this analysis of EAA and cardiometabolic-disease risk factors among migrant and non-migrant Ghanaians, we have found that migration status is negatively associated with EAA measures. Within migrants, FBG was positively associated with EAA measures (independent of use of anti-diabetic medications). Within non-migrants, BMI was negatively associated with EAA measures. The findings among Ghanaians also show the role of folate, a one-carbon metabolism nutrient. Higher folate intake was negatively associated with EAA measures among non-migrants. Our findings on FBG, BMI, and folate among Ghanaians were observable in the combined migrant and non-migrant sample and were also successfully replicated in independent cohorts.

Our results among Ghanaians suggest that the interplay between migration, EAA, and cardiometabolic diseases is not as we hypothesised. On the basis of findings from HICs in which lifestyle factors (tobacco smoking, unhealthy diets, and increased alcohol intake) and cardiometabolic factors (obesity, diabetes, and hypertension) were positively associated with EAA, we had hypothesised that changes in lifestyle upon migration would lead to accelerated biological ageing of tissues, which would in turn be associated with cardiometabolic diseases.[7,14,15] This finding would explain why migrant and urban populations have higher rates of obesity, hypertension, and diabetes than non-migrant or rural populations.[16,17,24] However, we found in our study that migration was negatively associated with EAA measures among Ghanaians (even after adjusting for potential confounders), despite their increased risk for cardiometabolic diseases. Nevertheless, Horvath[11] reported a similar observation of decreased EAA with urbanisation or migration among African hunter gatherers and Mexicans.[26] In that study, African hunter gatherers living in forests had higher EEAA compared with those who had shifted to an agriculturist life, whereas Mexicans born outside of the USA (but living in the USA)

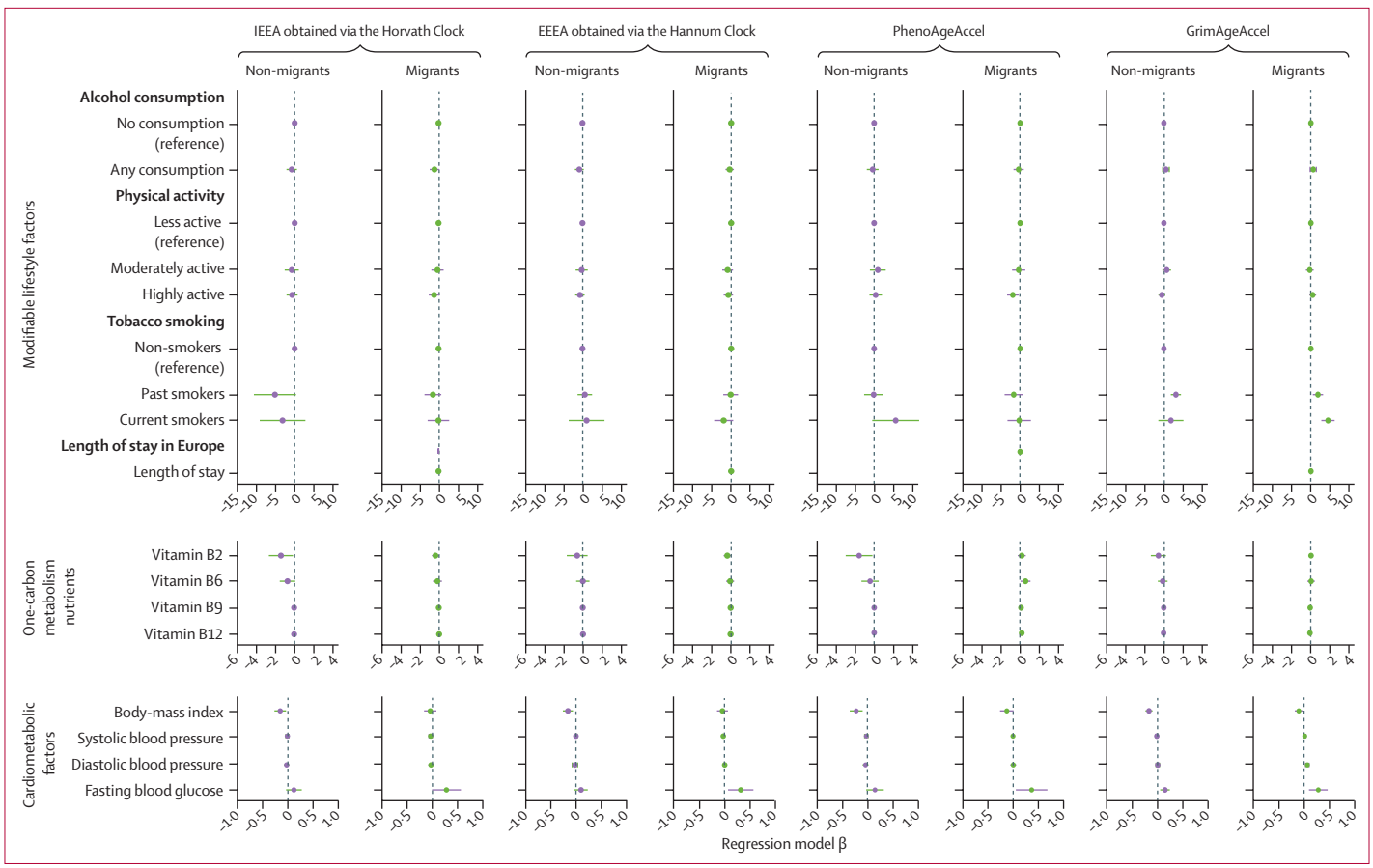

Figure 4: **Crude regression-model β relating EAA measures to cardiometabolic traits in the RODAM study**

Data are presented with forest plots as β (95% CIs) and were obtained from crude linear-regression models. 365 people were migrants and 347 were non-migrants. The red line indicates the reference for CIs. Physical activity is categorised according to the criteria of the Global Physical Activity Questionnaire. Vitamin intake was measured in mg per day for vitamin B6 and vitamin B9, whereas vitamin B9 and vitamin B12 were measured in μg per day. Body-mass index was measure in kg per m^2, fasting blood glucose in mmol per L, blood pressure in mmHg. Length of stay represented the duration of stay of migrants in Europe. Reference (comparison) groups for modifiable risk factors were as follows: reference for smoking was non-smokers; reference for alcohol consumption was no (never) alcohol consumption; reference for physical activity was low levels of physical activity. EAA=extrinsic epigenetic age. EEAA=extrinsic epigenetic-age acceleration. GrimAgeAccel=epigenetic-age acceleration obtained using the GrimAge clock. IEAA=intrinsic epigenetic-age acceleration. PhenoAgeAccel=epigenetic-age acceleration obtained using the PhenoAge clock. Ref=reference.

had higher EEAA than Mexicans born in the USA (also living in the USA).[26] Given that all measures of EAA have been shown to predict all-cause mortality, our findings of lower EAA in migrants could be related to, at least in part, the mortality advantage observed among migrant and urbanising populations (the so-called healthy-migrant effect).[27] The so-called healthy-migrant effect describes an empirically observed mortality advantage of migrants relative to the remaining population in the native country, but also to the majority in the host country. Given that previous studies have shown that migrant populations have lower all-cause mortality compared with non-migrant populations, even with higher rates of cardiometabolic diseases derived from multiple factors, including better access to health services,[27] our findings seem to provide some molecular evidence for the mortality advantage observed among some migrants.[27]

We found that FBG was positively associated with all EAA measures among Ghanaian migrants independently of anti-diabetic medication usage. Considering that migrants have higher rates of diabetes than non-migrants, this finding was expected. Epigenetic ageing represents tissue ageing, thus higher EAA was expected among individuals with higher FBG given that glucose-homeostasis mechanisms are impaired. Moreover, previous studies in HICs have also shown a positive relationship between diabetes and higher EAA.[7,14,15]

We found that BMI was negatively associated with all EAA measures among non-migrant Ghanaians. This inverse relationship between BMI and EAA is in direct contrast to previous studies from HICs that found positive associations between obesity and EAA measures.[7,14,15] The explanations for these inconsistent results are unclear. However, a study done in rural South Africa has also shown that overweight or obese individuals have a lower risk of all-cause mortality than those with a normal BMI after adjusting for potential confounders.[28]

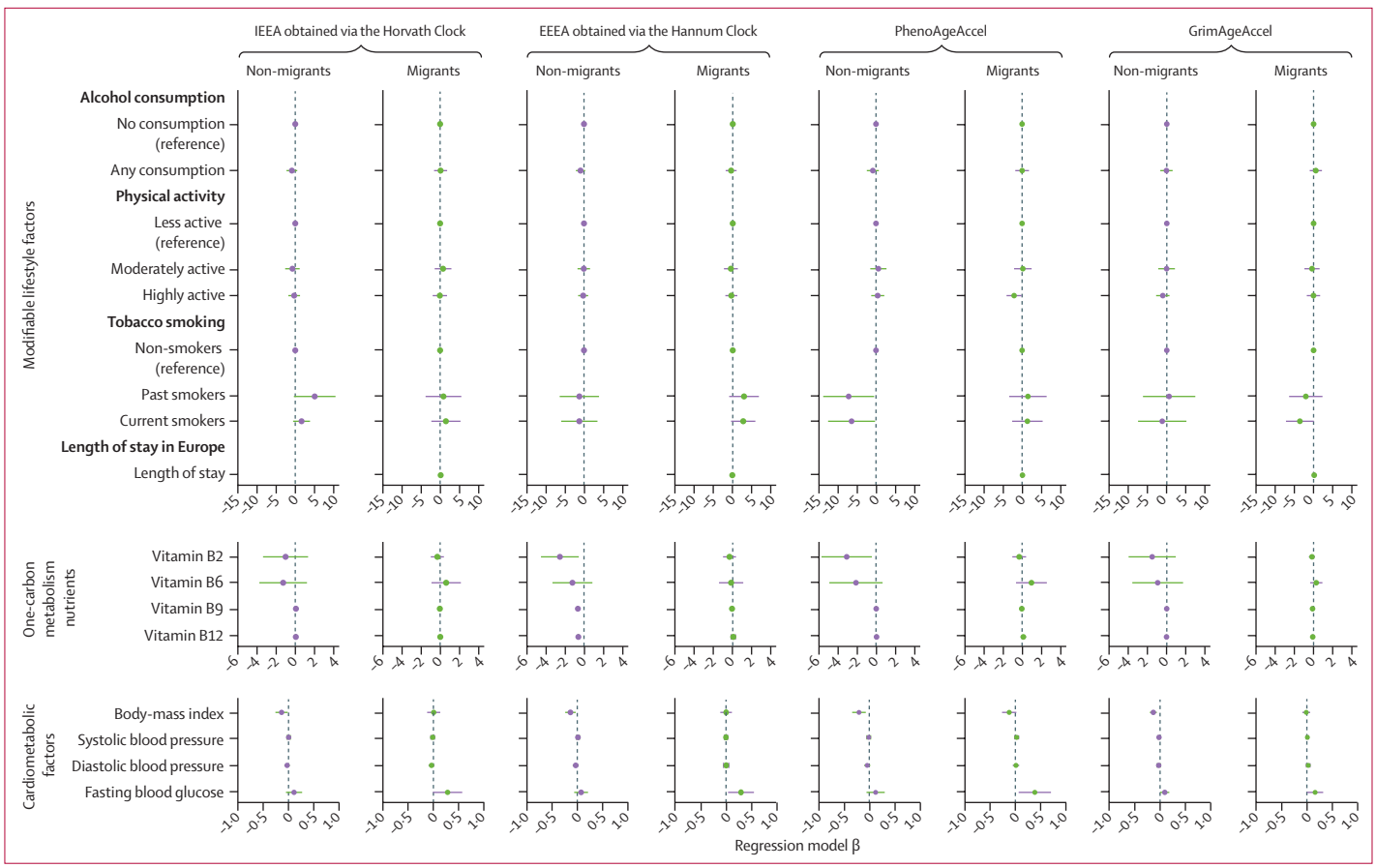

Figure 5: **Adjusted regression-model β relating EAA measures to cardiometabolic traits in the RODAM study**

Data are presented with forest plots as β (95% CIs) and were obtained from adjusted linear-regression models adjusted for age, sex, education level, smoking status, physical activity, alcohol intake, total-energy intake, and for migrants, duration of stay in host countries. 365 people were migrants and 347 were non-migrants. The red line indicates reference for CIs. Physical activity is categorised according to the criteria of the Global Physical Activity Questionnaire. Vitamin intake was measured in mg per day for vitamin B6 and vitamin B9, whereas vitamin B9 and vitamin B12 were measured in μg per day. Body-mass index was measure in kg per m^2, fasting blood glucose in mmol per L, blood pressure in mmHg. Length of stay represented the duration of stay of migrants in Europe. Reference (comparison) groups for modifiable risk factors were as follows: reference for smoking was non-smokers; reference for alcohol consumption was no (never) alcohol consumption; reference for physical activity was low levels of physical activity. EAA=extrinsic epigenetic age. EEAA=extrinsic epigenetic-age acceleration. GrimAgeAccel=epigenetic-age acceleration obtained using the GrimAge clock. IEAA=intrinsic epigenetic-age acceleration. PhenoAgeAccel=epigenetic-age acceleration obtained using the PhenoAge clock. Ref=reference.

In that study, the protective effect of overweight and mild obesity was best shown for infectious causes of death.[28] Considering the results of our study and the South African study together, both these findings possibly point towards the protective effects of BMI (overweight and mild obesity) on mortality in environments in which the infectious disease (inflammatory) load is high. For instance, infectious diseases and high chronic inflammatory load are common among less urbanised populations (non-migrant Ghanaians in our case, as compared with migrants). Such a lifetime of diverse pathogen stresses, elevated inflammation, and extensive immune activation are known to rapidly deplete naive CD4 T cells and lead to greater expression of exhausted T cells (rapid immunosenescence), and eventually to increased EAA (as also seen among non-migrant Ghanaians in our study).[26] These ongoing environmental insults might result in increased nutritional needs for cell repair.[29] As such, individuals with a nutritional reserve (moderately higher BMI) possibly have better capacity for cell repair than lean individuals in contexts with a high load of environmental insults, hence the lower EAA.

Our study showed a role for folate as a one-carbon metabolism nutrient. DNAm is dependent upon one-carbon pathways, which enables transmethylation reactions to occur.[19] Therefore, dietary intake of one-carbon metabolism nutrients could influence EAA.[19] Our findings on folate further support our hypothesis on BMI, in which EAA in environments with high inflammatory load depends on nutritional reserve for ongoing cell repair to these environmental insults (ie, nutritional reserve leads to better cell repair, which in turn leads to lower EAA). For instance, we found in the RODAM study that higher vitamin-B9 (folate) intake was negatively associated with EAA measures among non-migrant Ghanaians after adjusting for confounders. Folate has a crucial role in cell repair (especially DNA repair).[30–32] Folate is essential for the de-novo synthesis of

purines and pyrimidines, which are required during the replication and repair of DNA.[31] Thus, similarly to BMI, presence of this one-carbon metabolism nutrient could possibly enhance the capacity for cell repair in contexts with a high load of inflammation, thereby decreasing EAA among people with higher intake of folate compared with those with deficiencies.

Our goal was to validate our findings in independent cohorts. There is a scarcity of epigenetic data in populations from LMICs, especially cohorts examining effects of migration from LMICs to HICs in homogenous populations, and so, we used the IMS and PURE-SA-NW cohorts to replicate our findings.[24,25] Although the IMS study investigated rural-to-urban migration, and was in an entirely different ethnic group, changes in cardiometabolic-disease risk factors at the phenotypic level have been shown to be similar whether migrating from rural-to-urban areas within a country,[16] or internationally from LMICs to HICs (although effect sizes might differ).[17] Moreover, migrants and non-migrants in the IMS cohort were matched by age range and sex, which minimised differences in baseline characteristics, which can confound migration studies.[23] Associations between migration status and EAA did not pass the replication criteria in the IMS cohort, which could be due to a broad range of factors including type of migration (rural-to-urban *vs* LMIC to HIC), ethnicity (genetic influences on the epigenome) and other environmental factors (eg, pollution) not shared between Ghanaians and Indians.[33] Despite the unsuccessful replication of migration status among Indians, one thing that was clear was the mirroring of EAA in migrants and non-migrants. For instance, when the mean IEAA was –0·30 years in migrants, in non-migrants it would be the opposite at 0·30 years. This finding was observed in all EAA measures, and in the RODAM study. This finding is interesting because establishing EAA in one group (eg, migrants) could possibly predict an opposite EAA effect in the other group (homogenous group of non-migrants). The effects of FBG, BMI, and folate observed among Ghanaian migrant and non-migrants were also detected in the IMS study. This shared finding between Ghanaians and Indians could point to the fact that these specific effects are similar across populations undergoing health transitions, irrespective of type of migration and ethnicity.

The PURE-SA-NW cohort[25] (native South Africans) was not the ideal cohort to replicate our findings due to factors such as absence of comparison groups and absence of participants with higher BMI and FBG.[25] However, this cohort was the only African cohort with data on DNA methylation and cardiometabolic-disease risk factors. Despite the challenges, we believed that the effects of lifestyle factors on EAA among indigenous South Africans residing in Africa would be captured. These effects would then in turn be compared with those of Ghanaians also residing in Africa (non-migrants). We subsequently found that higher folate intake was negatively associated with EAA measures as had been observed in non-migrant Ghanaians. This finding validated the role of folate in EAA for populations residing in Africa in general.

Our findings have the potential to improve population health. For example, further studies could determine a cutoff point at which higher BMI has a benefit on biological ageing (improved life expectancy) and yet minimises the risk of other cardiometabolic diseases in environments with a high-inflammatory (infectious-disease) load. Moreover, with high rates of chronic undernutrition in less urbanised populations, further studies could establish whether supplementation of one-carbon metabolism nutrients such as folate (ie, correcting vitamin deficiencies) can reverse EAA in these environments. More importantly, breakthroughs in the reversal of biological clocks have been made.[34] Such future treatments will be important for specific groups of migrants and non-migrants that exhibit higher EAA.

The most important strength of our study is that we attempted replication in independent cohorts, which validates our findings. Second, the migration models used in our study are powerful because they allow for investigation of changing environmental exposures while controlling for ethnicity and early-life exposure. However, our study is not without limitations. First, we used a subsample of the overall RODAM study, which could have introduced selection bias. Nevertheless, our epigenetic subsample was generally representative of the overall RODAM population (appendix p 7). Second, we observed large baseline differences between migrants and non-migrants, which could confound our results. Although these differences represented real-life contrasts between migrants and non-migrants (with migrants being mainly young, with poor lifestyle and cardiometabolic profiles compared with non-migrants), we minimised confounding by doing sensitivity analyses in the combined migrant and non-migrant sample, in which the effect would be largely free of subgroup baseline differences (appendix pp 20–21). Third, socioeconomic status is known to influence BMI and overall health status. As such, our findings could also possibly be influenced by socioeconomic status. For instance, the lower EAA seen in migrants could be because migrants have a better socioeconomic status than non-migrants, whereas lower EAA associated with higher BMI among non-migrants might also reflect the higher socioeconomic status among those with higher BMI. We minimised this socioeconomic-status confounding by adjusting for education. Although education has been shown to be a powerful predictor for socioeconomic status,[35] it does not capture the full spectrum of socioeconomic status. Other components of socioeconomic status, such as income and occupation might possibly still influence our findings. Given that no data is available on how socioeconomic status affects EAA in Africans or other populations from LMICs, further studies are needed to evaluate in detail the effects of other

indicators of socioeconomic status on EAA in these populations. Fourth, although we adjusted for age and sex, we cannot rule out the possibility of residual confounding. Nevertheless, successful replication of most of our findings in the IMS cohort in which confounding was minimised by matching migrants and non-migrants by age and sex further substantiated our findings.[24] Fifth, we introduced criteria to minimise false positives. Some positive findings on alcohol consumption, smoking status, physical activity, and vitamin intake which did not pass the criteria (ie, were observed in only one EAA measure) might have been true findings. However, application of criteria to minimise false positives in our study enabled us to identify effects that were consistent across most EAA measures. Lastly, vitamin intake was calculated from food-frequency questionnaires, relying on the ability of participants to accurately recall frequency and number of consumed foods, which might have introduced recall bias.

In conclusion, our study among Ghanaians shows that migration is negatively associated with EAA. Moreover, cardiometabolic-disease traits are differentially associated with EAA within migrant and non-migrant subgroups. Many of these associations are also apparent in other ethnic groups. Harnessing the EAA effects of BMI and folate intake to improve life expectancy in rural areas where nutritional deficiencies and infectious diseases might be highly prevalent has potential. Our study therefore calls for interventions that consider the effects of biological ageing (EAA) in the prevention and treatment of cardiometabolic diseases among Africans and other LMIC populations.

Contributors

FPC, PH, and CA conceived and designed the study. FPC, HRE, HTC analysed the data. PH, CA, GKW, and MP verified the underlying data. FPC wrote the paper with PH and CA. All authors participated in the interpretation of the data, drafting of the manuscript, or revising and reviewing the manuscript for content.

Declaration of interests

We declare no competing interest.

Data sharing

Individual participant data from the RODAM study used in the current analyses will be deposited to the European Genome-Phenome Archive by June, 2021, in a deidentified or anonymised format. The study protocol and statistical analysis plans were previously published.[17] Data will be shared with researchers submitting a research proposal and requesting access to data. Data will be made available for analyses as approved by the data access committee. Data from the IMS study is available upon request; details can be found on the study webpage. Data from the PURE-SA-NW study is available with permission of the Health Research Ethics Committee of North-West University and the principal investigator of the PURE-SA-NW study, Prof Lanthe M Kruger (lanthe.kruger@nwu.ac.za) andProf Marlien Pieters (marlien.pieters@nwu.ac.za).

For the **European Genome-Phenome Archive** see https://ega-archive.org/

For the **study webpage** see https://apcaps.lshtm.ac.uk/related-studies/ims/

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