Title:

Childhood ecology influences salivary testosterone, pubertal age and stature of Bangladeshi UK migrant men

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Abstract:

Male reproductive investment is energetically costly, and measures of human reproductive steroid hormones (testosterone), developmental tempo (pubertal timing) and growth (stature), correlate with local ecologies at the population level. It is unclear whether male reproductive investment in later life is "set" during childhood development, mediated through adulthood, or varies by ethnicity. Applying a life-course model to Bangladeshi migrants to the UK, here we investigate plasticity in human male reproductive function resulting from childhood developmental conditions. We hypothesised that childhood ecology shapes adult trade-offs between reproductive investment and/or other fitness-related traits. We predicted correspondence between these traits and developmental timing of exposure to ecological constraints (Bangladesh) or conditions of surplus (UK). We compared: (i) Bangladesh sedentees (*n*=107), (ii) Bangladeshi men who migrated in childhood to the UK (*n*=59), (iii) migrants who arrived in adulthood (*n*=75), (iv) second-generation UK-born and raised children of Bangladeshi migrants (*n*=56), and (v) UK-born ethnic Europeans (*n*=62). Migration before puberty predicted higher testosterone and an earlier recalled pubertal age compared to Bangladeshi sedentees or adult migrants, with more pronounced differences in men who arrived before age eight. Second-generation Bangladeshis were taller with higher testosterone than sedentees and adult migrants, and higher waking testosterone than Europeans. Age-related testosterone profiles varied by group, declining in UK migrants, increasing in sedentees, and having no significant relationship within UK-born groups. We conclude that male reproductive function apparently remains plastic late into

56 childhood, is independent of Bengali or European ethnicity, and shapes physiological
57 trade-offs later in life.

58

1 Introduction

Globally, men in wealthy, developed regions generally have higher testosterone than those living in less affluent ones[1–3]. While some researchers link such variation to "ethnic", "racial" or genetic traits[4–6], ecological and behavioural variables associated with energy availability like abundant nutritional intake, pathogen load, and sedentary lifestyles also potentially contribute to inter-population differences in reproductive phenotypes[7–12]. Developmental exposure to energetic variables during childhood may further explain adult variation in reproductive steroid hormones. Evidence supporting this "developmental hypothesis" connects early infancy, pre-birth or childhood experience with sex steroid levels in later infancy[13,14], developmental timing as measured by adult height and pubertal age[15–18], or adult reproductive function[3,18–21].

Migration studies support the developmental hypothesis. Children migrating from less to more affluent regions show rapid postnatal growth and earlier sexual maturation[19,22,23]. Levels of salivary progesterone, ovulation rates, and menopausal age of Bangladeshi women who reached adulthood in more ecologically constrained environments were lower compared to those who migrated to a less challenging one[19,24,25], and early childhood migration (age 0-8 versus 9-16 years) was associated with more robust ovarian function [19,23].

We lack comparable migrant studies among men but, based on the above findings, we predict that men with different life histories would express varying degrees of reproductive investment depending on differential developmental conditions. We

81 expect that males encountering improved ecologies before or during developmental
82 transitions would invest in more costly reproductive effort associated with
83 competition and/or sexual signalling, mediated by testosterone[26–28]. Based on
84 ecological developmental histories, we presume that individual trade-offs between
85 testosterone-mediated traits and other energetic demands would lead to population-
86 level differences. Considering male variation in reproductive function, hormonal
87 variations in non-clinical populations are unlikely to impact fecundity[29,30], but instead
88 relate to trade-offs between traits associated with survivorship and reproductive
89 effort[2,3,31–34].

90 We therefore designed a cross-cultural study to distinguish whether global variations
91 in male reproductive phenotypes (measured by salivary testosterone levels, pubertal
92 age and stature) reflect: a) developmentally plastic, organizational responses to
93 childhood ecology, or b) current, activational responses to local ecology. We
94 selected a generally homogenous, ethno-cultural group of Bangladeshis of Bengali
95 ethnic origin, some of whom migrated from a less to more affluent region
96 (specifically, Sylhet, northeast Bangladesh, to London, UK).

97 We assume fewer ecological constraints upon males in the UK compared to
98 Bangladesh. Despite improvements, Bangladesh still ranks globally among the
99 poorest quartile of countries, with high indicators of maternal undernutrition and
100 stunting (36%) among children aged <5 years[35,36]. However, the Bangladeshi
101 populations studied here originate from the land-owning, middle-class not normally
102 subject to nutritional or energetic constraints. Instead, they contrast with migrants in

developmental exposure to infectious and/or parasitic diseases and environmental instability (i.e., political unrest, periodic flooding, poor public health provision and sanitation)[37,38], which cross social and economic boundaries[37,39–42]. Limitations on energy availability during growth can lead to trade-offs in reproductive function[7,11,12,24,25]. In London, migrants join other British Bangladeshis, 65% of whom are classified in income poverty, higher than other UK ethnic groups[43].

We selected timing of migration as a dependent variable (encompassing developmental exposure to environments with abundant resources) to predict hormonal (salivary testosterone) and maturational/growth (recall of pubertal timing, standing height) markers of adult reproductive function. Developmental milestones of birth, middle childhood (concurrent with adrenarche: the pre-pubertal adrenal secretion of androgens), and puberty informed our hypotheses. Groups comprised: 1) “sedentees” (men who never left Sylhet); 2) “adult migrants” (British-Bangladeshis from Sylhet who migrated as adults (post-puberty) to London, UK); 3) “child migrants” (British-Bangladeshis who migrated to the UK as children (aged ≤19)); 4) “second-generation migrants” (UK-resident Bangladeshis born and raised in the UK, with parents originating from Sylhet); and 5) “British European” (UK-born men of European ethnicity who grew up in the UK and reside in similar neighbourhoods and socioeconomic conditions as the migrants).

We tested two childhood developmental hypotheses: 1) men who experienced fewer ecological constraints prior to puberty express greater adult reproductive investment than men with more constrained childhood experience. The second hypothesis

refined the first, by focusing on early childhoood prior to age 9, proposing: 2) fewer ecological constraints experienced between 0-8 years would lead to greater adult reproductive investment compared to men with more constrained early childhood experience. Both hypotheses predicted that, compared to adult migrants and sedentees, men who grew up in the UK would have: a) significantly higher levels of salivary testosterone; b) earlier recalled age markers of puberty[44], and c) greater stature.

Our third, non-childhood developmental hypothesis proposed that: 3) adult male reproductive traits (e.g., salivary testosterone) remain plastic during the life-course, reflecting either cumulative exposure to adult ecological conditions, or responses to current, local ecology. This predicted: a) significantly higher salivary testosterone in adult migrants compared to sedentees; and b) correlation within adult migrants between number of years spent in the UK and salivary testosterone, adjusting for age.

Our final, non-developmental hypothesis proposed: 4) biological and cultural traits associated with ethnicity explain inter-population variations in reproductive traits. This predicted: a) higher salivary testosterone; and b) an earlier age at puberty and taller stature in UK-born British-Europeans compared to second-generation, British-Bangladeshis.

Results:

Table 1 presents descriptive statistics for all groups. Adult migrants were significantly older than all other groups averaging 48.4 years (95%CI=44.6, 52.3). Second-

147 generation men were the youngest, averaging 24.5 years (95%CI=22.5, 25.8). Age
148 at migration and recruitment correlated for child (r=0.44, *t*=3.3, df=46, *p*=0.001), but
149 not adult migrants (r=0.13, *t*=1.0, df=62, *p*=0.3). Men who arrived <9 years were
150 younger at recruitment than those who arrived aged 9-19 (28.0 and 36.9 years,
151 respectively, *t*=3.1, df=33.6, *p*=0.004).

152 Compared to sedentees, British-Europeans and all migrants were significantly taller
153 (except adult migrants) and heavier with higher BMIs (table 1). Age at recruitment
154 negatively predicted height of child migrants (n=40) compared to sedentees (β=-
155 0.379 SD, 95%CI=-0.749, -0.008, n=106, *p*=0.045), while no significant secular
156 trends for height were observed within other residence groups (figure 4). Across all
157 groups, older men recalled reaching puberty later (β=0.28 SD, 95%CI=0.155, 0.410,
158 n=237, *p*=0.00002). After correcting for age at recruitment, men with higher salivary
159 testosterone recalled reaching puberty at earlier (waking: β=-0.172 SD, 95%CI=-
160 0.301, -0.044, n=219, *p*=0.01; evening: β=-0.130 SD, 95%CI=-0.258, -0.003, n=220,
161 *p*=0.047; supplemental table 10). However, no such relationship was observed when
162 restricting the same analysis to Bangladeshis resident in the UK or only to
163 sedentees. All intergroup regressions included age at recruitment and all salivary
164 testosterone analyses included BMI as covariates with established predictive
165 relationships with adult testosterone[34,45–49]. Testosterone regressions confined to
166 child migrants included BMI imputed at the population mean (23.9 for n=8, 24% of
167 cases), and were replicated with complete cases.

168 Regression findings supported both childhood developmental hypotheses and their
169 associated predictions with few exceptions, while the third and fourth hypotheses
170 based on adult ecology or ethnicity were not supported (table 2). The experience of
171 UK ecological conditions prior to adulthood led to higher testosterone, an earlier age
172 at puberty and taller stature compared to men who experienced similar conditions
173 after puberty. Second-generation men who spent all of their childhood in the UK had
174 the highest waking (153.5 pg/mL, 95%CI=133.8, 173.2, n=25) and evening (119.4
175 pg/mL, 95%CI=98.3, 140.5, n=28) salivary testosterone of any group (figure 1),
176 significantly higher than adult migrants and sedentees, respectively (waking=90.7
177 pg/mL, 95%CI=80.7, 100.7, n=53 *p*=0.0001; 100.9 pg/mL, 95%CI=91.4, 110.4,
178 n=103, *p*=0.0002, and evening=75.0 pg/mL, 95%CI=65.5, 83.5, n=53 *p*=0.03; 76.2
179 pg/mL, 95%CI=68.3, 84.2, n=102, *p*=0.007). Child migrants had the second highest
180 salivary testosterone, higher for waking (141.4 pg/mL, 95%CI=119.2, 163.5, n=26)
181 and evening (100.1, 95%CI=84.6, 115.7, n=27) samples than sedentees (*p*=0.002;
182 0.02) or adult migrants (*p*=0.0003; 0.07).

183 Age at migration predicted an earlier recalled age at puberty for migrants who
184 arrived before completing puberty (age 19), but not for those who migrated as adults
185 (figure 3); however, this relationship was not significant in child migrants after
186 including recruitment age in the model (β=1.10 SD, 95%CI= -0.106, 2.30, n=19,
187 *p*=0.071). Both child migrants (15.8, 95%CI=14.5, 17.1, n=19) and second-
188 generation men (14.2, 95%CI=13.5, 14.8 years, n=21) recalled earlier ages at
189 puberty compared to sedentees (16.1, 95%CI=15.7, 16.5, n=103), and adult
190 migrants (16.4, 95%CI=15.9, 16.9, n=49), but these differences were only significant

for the second-generation (*p*=0.003; 0.02; table 2). Similarly, second-generation men (n=49) averaged 8.6cm (95%CI=6.6, 10.7) taller than sedentees and 7.1cm (95%CI=4.0, 10.2) taller than adult migrants (n=106, 65; *p*=4^-15, 0.0001), while child migrants (n=44) averaged 4.3cm (95%CI=2.2, 6.5; *p*=0.0007) taller than sedentees, and a non-significant 2.8cm (95%CI=-0.4, 6.0; *p*=0.12) taller than adult migrants.

Ecological conditions in the UK predicted higher testosterone, an earlier age at puberty and taller stature if experienced during early childhood (ages 0-8) compared to men only exposed to these conditions in middle childhood and puberty (>9). Within child migrants, age at migration negatively predicted evening salivary testosterone independently of number of years spent in the UK (waking β=-0.553 SD, 95%CI=-1.136, 0.029, n=33, *p*=0.07; evening β= -0.930 SD, 95%CI=-1.480, -0.380, n=34, *p*=0.003; supplemental table 2) and infant or early childhood migrants had significantly higher evening salivary testosterone compared to late childhood migrants (9th-19th year) (figure 2, supplemental figure 1), although the difference was not significant if adjusting for recruitment age (supplemental table 4). Migration between birth and age 9 (n=8) predicted earlier recalled age at puberty compared to migration >19 years (β=-0.858 SD, 95%CI=-1.637, -0.079, n=40, *p*=0.034; supplemental table 6), but not compared to migration between 9-19 (β=-0.919 SD, 95%CI=-1.96, 0.121, n=20, *p*=0.10). Combining second-generation and child migrants, exposure to UK conditions before birth to age 8 (n=29) predicted an earlier age of puberty compared to UK migrants who moved between ages 9-19 (β=-0.969

SD, 95%CI=-1.675, -0.264, *p*=0.004) or after age 19 (β=-0.909 SD, 95%CI=-1.495, -0.322, *p*=0.003).

Child migrants were taller if they migrated earlier. Age of migration predicted adult height of child migrants after adjusting for number of years in the UK (β=-0.719 SD, 95%CI=-1.22, -0.217, n=37, *p*=0.009; supplemental table 11), although there were no significant predictors of height if adjusting instead for recruitment age (supplemental table 12).

The experience of ecological conditions in the UK at any point during adulthood did not lead to higher testosterone. Instead, waking salivary testosterone of adult migrants was significantly lower than sedentees, suggesting fixation of this trait in relation to ecological conditions at some point prior to adulthood, or even an opposite directional effect from that seen in child migrants (table 1). Moreover, the number of adult years spent in the UK correlated negatively with salivary testosterone (waking β=-0.019 SD, 95%CI=-0.034, -0.003, n=53, *p*=0.03; evening β=-0.024 SD, 95%CI=-0.036, -0.011, n=56, *p*=0.0005), while age at adult migration failed to show a relationship with testosterone (waking β=-0.061 SD, 95%CI=-0.443, 0.320, *p*=0.8; evening β=-0.093 SD, 95%CI=-0.392, 0.207, *p*=0.5; supplemental table 7).

Characteristics distinctive to European ethnicity failed to predict higher salivary testosterone or earlier age at puberty compared to Bengalis sharing similar developmental histories. Instead, waking testosterone of British-Europeans (n=44) was marginally lower than second-generation migrants (β=-0.78 SD, 95%CI=0.102,

1.46, n=25, *p*=0.02) and no higher than sedentees, child or adult migrants at waking or evening (figure 1, table 2, supplemental table 1). Recalled age at puberty did not differ between British-European and second-generation British-Bangladeshi men, but was significantly earlier in Europeans compared to all other groups (table 2). While British-Europeans were 5.6cm taller than second-generation migrants (95%CI=8.1, 2.4; *p*=0.00002), this difference was smaller in comparison to the other ethnic Bengali groups.

Across populations, waking and evening salivary testosterone declined by age at recruitment. Adjusting for BMI, the decline was -0.79 (95% CI=-1.22, -0.357, n=251, *p*=0.001) and -0.55 (95% CI=-0.949, -0.156, n=254, *p*=0.01) pg/mL per year. The relationship between age and salivary testosterone varied by residence group, declining in child and adult migrants, non-significant in both UK-born groups, and increasing in sedentees (supplemental figure 1, supplemental table 9). Within UK resident groups, child migrants (n=34) showed a more pronounced age-related decline in waking salivary testosterone than Europeans (β=-0.50 SD, 95%CI=-0.952, -0.042, n=44, *p*=0.01). As a pooled group, men born in the UK significantly declined in waking -1.22 (95%CI= -2.01, -0.435, n=76, *p*=0.002) but not evening -0.91 (95%CI= -1.839, 0.020, n=77, *p*=0.055) pg/mL salivary testosterone per year. Waking salivary testosterone still remained 34.1 (95%CI= 0.0769, 68.21, *p*=0.049) pg/mL higher in second-generation migrants (n=25) compared to Europeans after adjusting for this UK-born decline.

Reanalysis performed on men aged ≤40 years at recruitment and replication of child migrant regressions applying multiple imputation methods for BMI yielded substantially similar findings to those performed with mean imputation either supported or failed to contradict findings within the full cohort (Supplementary section 5 details both reanalyses).

Discussion:

Both childhood developmental hypotheses and predictions were supported: Bangladeshi men who migrated from Sylhet (with greater ecological risks, higher exposure to infectious diseases and poorer healthcare) to London during childhood had higher levels of adult salivary testosterone, an earlier age at puberty and taller stature compared to men who completed their childhood in Sylhet. Differences were particularly marked if individuals migrated in early childhood, aged 0-8 years, and most pronounced for second-generation British-Bangladeshis. We conclude that variations in male reproductive phenotype are explained, in this case, by exposure to less constrained ecological conditions during childhood. Male reproductive function apparently remains plastic into late childhood and more plastic in early than late childhood.

In contrast, adult exposure to less constrained ecological conditions did not positively influence salivary testosterone. Instead, adult migrants to the UK had *lower* waking salivary testosterone while evening levels were not significantly different from non-migrant sedentees. Additionally, number of adult years in the UK did not positively affect salivary testosterone.

We found partial and contradictory evidence relating male reproductive investment to biological and cultural traits specific to the two ethnicities studied. Neighbouring British-Europeans with similar developmental histories and socioeconomic position to resident Bangladeshis did not show greater investment in male reproductive traits compared to second-generation, British-Bangladeshi men. Instead, salivary testosterone was not significantly higher in men of European origin compared to any Bengali group, and waking samples were even marginally lower than second-generation migrants. This unexpected result potentially relates to research linking male testosterone to dominance ranking of primates, as well as human status interactions, perceived social position, competition and provisioning or caregiving[50–58]. While testing for such relationships falls outside the scope of analyses here, further exploration of social hypotheses[59,60] forms the basis of future study (Magid et al., in prep).

While recalled age of puberty was earlier for British-Europeans compared to groups born in Bangladesh, this did not differ from their UK-born, Bengali counterparts. European men were taller than all ethnic Bengali groups, but this difference was smallest when comparing second-generation migrants, suggesting a generational trend toward matching local averages in height, a well-documented phenomenon following migration[61,62]. This supports the assumption that UK conditions are more conducive to childhood growth than Bangladesh.

Patterns of male reproductive ageing varied with childhood and adult conditions. The relationship between age and testosterone was: a) significantly different between

men who shared childhood but not adult conditions (negative for adult migrants and positive for sedentees), b) between men who shared adult but not childhood conditions (steeper for child than adult migrants), and c) not different between men sharing both sets of conditions (no significant pattern in second-generation migrants and British Europeans). Such variability adds to evidence of the non-universality of male age-related testosterone decline, particularly in non-industrialized societies, possibly representing developmental responses to energetic conditions[2,8,33,34].

The lack of a robust age-related decline in salivary testosterone within the British European group and comparatively shallow -1.43 pg/mL decline per year of UK-born men remains unexpected considering male ageing effects widely documented elsewhere[45,46]. The serum testosterone decline reported in a large, longitudinal study of middle-class Caucasian (87%) men in the USA[46] equates to -2.13 pg/mL/year of salivary testosterone[63]. Characteristics of the relatively small, socioeconomically poor, urban population of UK-born men may explain differences between our findings and large-scale epidemiological studies.

Our findings support the conclusion that developmental reproductive responses to ecological conditions are most distinctly expressed in early adulthood[2,64] and diurnally at waking[65]. Differences in salivary testosterone between groups were greatest in early adulthood, but trend towards convergence around age 40 (supplemental section 2). Migrants experiencing decreased ecological constraints during childhood development had the steepest age-related decline, suggesting

early-life improvements led to adjustment of male reproductive function in early adulthood accompanied by rapid decline at later ages.

The above findings lend further support to the "developmental hypothesis" whereby pre-birth, early infancy or childhood conditions influence reproductive development in later infancy[13,14], developmental transitions to adulthood[15–18,66], and adult reproductive and senescent traits in both women and men[18–21]. Migration during childhood to a less constrained ecology leads to increased investment in two proximate measures of male reproductive function: salivary testosterone and age at puberty. Ecological conditions in the UK during all or part of childhood also lead to greater childhood growth evidenced by taller stature (figure 4). Child migrants recruited at younger ages were taller, likely reflecting developmental effects on growth combined with cohort effects of peak migration, discussed below.

The association of childhood development in the UK with increased male reproductive investment across the life course mirrors results from migrant studies of Bangladeshi women that found higher salivary progesterone, higher rates of ovulation, an earlier age at adrenarche and menarche, later menopause, and slower reproductive ageing among women who migrated during childhood[19,23–25].

We interpret population differences in these reproductive traits as evolved strategies to balance lifetime investment in reproduction against demands of growth, immunity and maintenance[67]. From this life history theory perspective, ecological conditions during critical phases in the organization of hormonal axes and somatic tissues shape investment in reproductive effort at a population level in ways that are

343 expressed throughout adulthood. While we selected age 8 as an important childhood
344 biosocial threshold[68,69] when early male hormonal organisation becomes set,
345 sufficient plasticity persists into late childhood such that migration to the UK prior to
346 sexual maturity apparently promotes greater investment in phenotypic measures of
347 reproductive effort[3].

348 The differences seen here between child migrant cohorts do not argue against
349 gradual linear transitional stages, as opposed to punctuated thresholds of sensitivity
350 to ecology at middle childhood and adolescence[70,71]. We split early and late
351 childhood migrants according to a chronological, not a physiological marker, and
352 ecology likely influences the timing of physiological transitions as documented in
353 migrant girls from Bangladesh to the UK[23], and as seen in self-report of puberty in
354 this population. Moreover, cohorts were separated at thresholds when we expected
355 completion of pre-adrenarche or pre-pubertal development, meaning they likely
356 contain individuals who were peri-adrenarcheal or peri-pubescent at the time of
357 migration, with associated linear trends suggesting diminishing organizational effects
358 by chronological age at migration.

359 The selected Bangladeshi communities share dietary, physical and cultural practices
360 and the migrant populations are uniquely homogenous in socioeconomics and
361 geography. While these attributes reduce potential sources of variation in male
362 reproductive function, cohort differences between migrant groups also limit our
363 findings. Demographically, migration of adult Bangladeshi men to the UK peaked in
364 the 1970s, while wives and children of adult migrants typically followed in the

1980s[72]. The average recruitment age of adult migrants reflects the 1970s peak. The correlation between age at migration and recruitment in child migrants reflects the 1980s peak. The timing of UK family unification limits the maximum age of British-born offspring. While we included age as a covariate in all models testing for inter-group differences, we remain limited in our ability to contrast ecological influences on reproductive function of older males. Moreover, despite screening for family members of migrants among sedentees, we cannot exclude the possibility of a selection bias in our migrant groups.

Retrospective measures of pubertal timing are open to recall error which is likely to be exacerbated by ageing[73,74]; however, in cross-sectional life-course research, combined recall instruments of this kind provide limited but internally consistent estimates of relative maturational rates with low test-retest variation[44,75,76]. Older men recalled later ages at puberty, which may represent a secular trend independent of our cohort differences in childhood ecology or systematic recall/response bias. Relative differences in developmental cohorts, however, remained evident after including age at recruitment as a covariate and restricting our analysis to men <40 years (supplemental section 2). Finally, while we propose immunological aspects of ecology as a primary explanation for inter-population differences observed above, we cannot exclude the possibility that recalled age of puberty, as well as biomarkers of reproductive investment, result exclusively from social components of acculturation, stress from discrimination, or perceived threats to status unique to growing up as a minority (such as identifying as part of an outgroup)[77] or social stimuli from interactions within peer groups.

Based on our study design, we conclude that variation in biomarkers of reproductive function within Bangladeshi men relate to inconsistencies in their ecologies. We consider childhood exposure to disease within Bangladesh, as well as the experience of migration itself, as the most plausible causes of the observed variation. Results from British Europeans suggest limits to the biological and cultural traits associated with ethnicity in predicting adult male reproductive function, and potential differences in the influence of social position on testosterone of migrant and non-migrant men. These findings have implications for life history interpretations of reproductive disease and aetiology[78], by relating early life conditions to prostate cancer or disease[79], incorporating ecological variations to documented health outcomes of age-related change in testosterone[46,80], trends in pubertal timing[81,82], and global clinical definitions of "normal" ranges in androgen supplementation[83] therapies.

METHODS

Study population: Bangladeshis in London and Sylhet form a homogeneous ethnic group, originating from an affluent socioeconomic position, share consistency in dietary, religious and social practices, and are subject to limited physical work and nutritional stress. Following migration to the UK, access to Bangladeshi foods and community cohesion within a geographically condensed region preserves much of this homogeneity[84]. In 2004-2010, we recruited 359 healthy male volunteers aged 17-78 at completion of study, screened to exclude thyroid conditions or diabetes. First-order relatives were excluded from participation to avoid closely shared genetic or immediate environmental confounders. Participants were divided into the following

groups: 1) Bangladeshi sedentees (n=107) born and still resident in the Sylhet City District, northeast Bangladesh; 2) first generation migrants from Sylhet (n=75) who moved to the UK after reaching puberty determined from our data at >19 years (adult migrants); 3) first-generation migrants from Sylhet (n=59) who moved to the UK prior to completing puberty (aged ≤19) (child migrants); 4) second-generation British-Bangladeshi men (n=56) born to parents who had themselves migrated to the UK from Bangladesh; and 5) London residents of British-European ethnicity (n=62) recruited from similar neighbourhoods and of similar socioeconomic status to the migrant groups.

Migrants were classified as adults if they arrived in the UK post-puberty, based on a self-recalled, composite age at puberty (measures detailed below). Of 68 migrants who provided age at migration and recalled pubertal age, 19 reported arriving before, and 49 after puberty. The remaining first-generation men were classified as adult migrants if they arrived after the mean composite age of puberty +2SD: 15.75+(2*2.09)=19.93. To ensure that the sedentee population reflected a comparable ethnic and socioeconomic group to migrants with sufficient means to emigrate, participants in Sylhet were screened for relatives who had migrated to the UK, mainland Europe, or the North American continent and were recruited using local networks and snowballing techniques. Participants in London were recruited from community centres, mosques, fitness centres/clubs, or from internet and newspaper advertisements.

Questionnaires: We collected demographic, migration, reproductive, nutritional, recalled pubertal markers, and health information using previous methods employed in a study of Bangladeshi migrant women[19,85]. Native English speakers were given the option of completing a slightly shortened questionnaire online via a protected portal.

Saliva sampling: A total of six saliva samples were collected over two non-consecutive days from each participant. To capture diurnal patterns of hormonal profiles that included later analyses of salivary cortisol, one sample was requested immediately upon waking, one approximately 30 minutes post-waking, and one immediately before retiring to bed. For purposes of salivary testosterone analyses, we only report here the first waking and evening samples. Participants were asked to record the exact times of sampling each day; all reported giving their first sample within 30 minutes of waking. Salivary testosterone was measured in duplicate by radioimmunoassay without extraction[86]. Antiserum was prepared, and all analyses performed between 2006-2010 in the laboratory of co-author RTC at Northwestern University, Chicago USA. Inter-assay CVs were within 15% for high (100pg/mL), low (50pg/mL), and internal (pooled saliva sample) quality controls, while recovery of spiked samples was 97.1% ±18.2 SD. Sensitivity was 0.028 nmol/L and average intra-assay CV was 2.01%. Duplicate readings of two samples were excluded as both exceeded the limits of detection of the high standard of the assay, four samples were based on single readings due to limited sample or laboratory error of the second reading. Seven outlying samples with z-scores above 3.29 were recoded to +2 SD of the population mean of salivary testosterone for that time point.

455 *Anthropometry*: Standing height and weight were collected according to
456 standardized methods[87]. Eight child migrants lacked anthropometric data. In order to
457 preserve sample size in analyses of migration effects, testosterone regressions
458 within this group only were performed with BMI imputed at the population mean and
459 also replicated with complete cases only and with multiple imputation methods (see
460 supplemental section 2).

461 *Pubertal measures:* A composite age at puberty was adapted from The Adolescence
462 Scale (AS-ICSM) retrospective self-assessment of puberty milestones[44]. Age at
463 puberty was estimated by averaging when men recalled, where possible, four
464 markers of male secondary sexual development: i) voice breaking; ii) appearance of
465 facial hair or start of shaving; iii) first appearance of pubic and underarm hair; and iv)
466 first nocturnal emission. Questions were phrased “Do you remember how old you
467 were when…?” Participants were asked to respond yes or no, and following this,
468 were asked: “If you remember, how old were you?”. This response was open ended,
469 and if the age in years was unknown, respondents were free to estimate by other
470 measures such as year of school or other historical events. Men responding with
471 estimates spanning two years, e.g., “12-13” were coded at midpoint, 12.5 years.

472 *Statistical Analyses:* We tested all hypotheses by multiple linear regression analysis.
473 A full description of all variables used and statistical tests performed can be found in
474 supplemental section 3. All intergroup regressions included age at recruitment as a
475 covariate to adjust for cohort differences, potential effects of male reproductive
476 ageing[45,46] and demographic or secular trends unrelated to our hypotheses. Salivary

testosterone analyses included BMI as a covariate with an established predictive relationship with adult testosterone[34,47–49].

To test for evidence that contrasting ecological conditions prior to puberty relate to dependent measures of adult reproductive function, we performed multiple linear regressions with age at recruitment, BMI (in testosterone regressions only) and residence group included as covariates. To test for evidence that contrasting ecological conditions during early childhood relate to dependent measures of adult testosterone, we performed multiple linear regressions with covariates being either age at recruitment or number of years spent in the UK since migration, imputed BMI, and two cohorts of child migrants split by age of migration before and after their 9th year. As inclusion of both age at recruitment and number of years in the UK in the same model exceeded limits of collinearity (variance inflation factor >10)[88], for salivary testosterone number of years in the UK was considered a combined measure of influences of exposure to adult and current ecological conditions, and age at recruitment. Results including only complete cases for BMI and reanalysis applying multiple imputation techniques are included in supplemental materials.

Second-generation men and child migrants exposed to UK conditions from before birth to age 8 were combined into a single cohort in a linear regression contrasting pubertal recall with cohorts of later childhood (9-19 years at migration) or adult migrants (aged >19 years at migration). Age of recruitment was also included as a control for secular demographic trends for puberty regressions where cumulative influences of environment were expected to become fixed at adulthood. In addition,

we tested for linear relationships between dependent variables and age at migration within either the child migrant or adult migrant group only, and included age at migration and either age at recruitment or number of years in the UK covariates in addition to BMI or imputed BMI in testosterone regressions. To limit confounding between effects of ageing/senescence and exposure to ecological conditions in the UK in adult migrants, we ran the above regressions separately within two age cohorts (≤40 and >40 at recruitment), split at a conventional point of inflection for male life course studies of sex hormones[89].

Post-hoc analysis of the regressions where "group" or "cohort" was an independent variable, with Tukey correction of all-pair multiple comparison using the R package *multicomp* tested for evidence for ethnic or developmental cohort differences.

To test for differences in age-related trends in salivary testosterone, we ran both linear regression and ANCOVA including an interaction effect between each residence group and age at recruitment on transformed and untransformed values. Between-group differences in the slope of age-related declines in testosterone were tested in post-hoc analysis as described above. Within UK-born men, we performed an additional regression with measured salivary testosterone offset by multiplying number of years after 22, an established point of male age-related decline[46], by the UK-born population trend as the dependent variable and age at recruitment, BMI and ethnic group as covariates. Between-group differences in descriptive variables were tested using linear regressions and post-hoc analysis of differences between residence groups.

Prior to running the models, salivary testosterone measures were transformed by natural logarithm to correct for skewed normality of distribution, and all measures were z-transformed to a mean of zero and a standard deviation of 1, except calculations for age-related effects on salivary testosterone (supplemental table 9) which were left untransformed for comparison to published rates of decline. All analyses were performed using R statistical software v.3.3.1[90] with packages detailed in analysis code.

Data availability: Code and source data for all analysis and figures generated during the current study are included in this published article (supplemental sections 4 and 5) and are also available in the GitHub repository at: https://github.com/kessonovitch/BHAI_Data/

Ethics: Ethical approval was granted by the UCL Research Ethics Committee (ID: 0144/002), and the Osmani Medical College in Sylhet. All participants provided written consent and were compensated for their time upon completion of the study. Data were stored in accordance with the Data Protection Act (UK).

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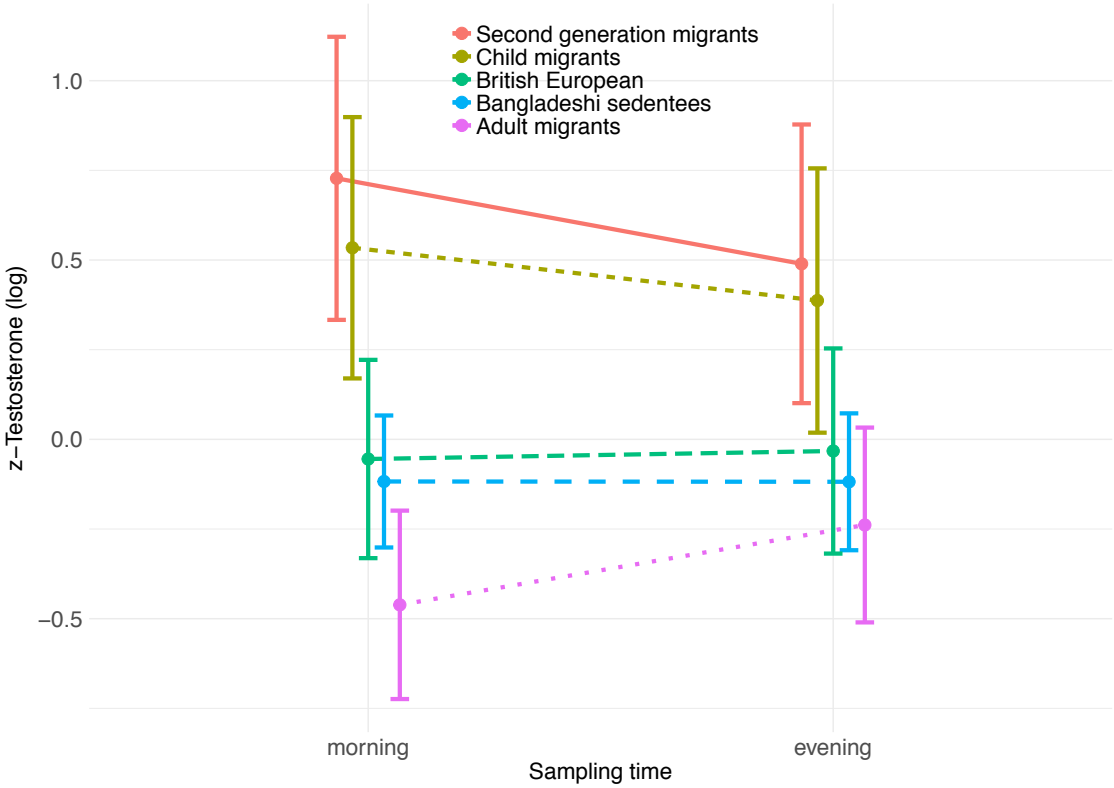
Author Contributions

KSM and GRB designed the study and drafted the manuscript, KSM carried out all data and laboratory analysis, KSM and FUA supervised and performed data collection, RTC designed, advised and assisted in laboratory analysis.

Competing Interests statement

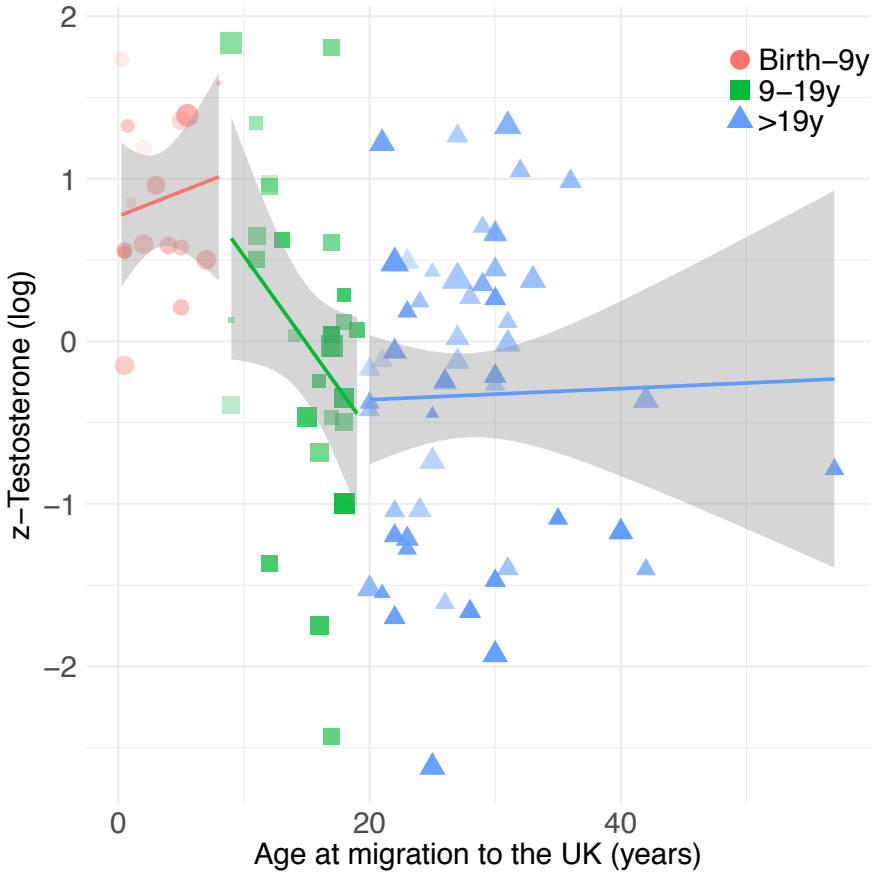
The authors declare no competing interests.

Figure 1. Resident group least square means of salivary testosterone, adjusted for age, BMI.



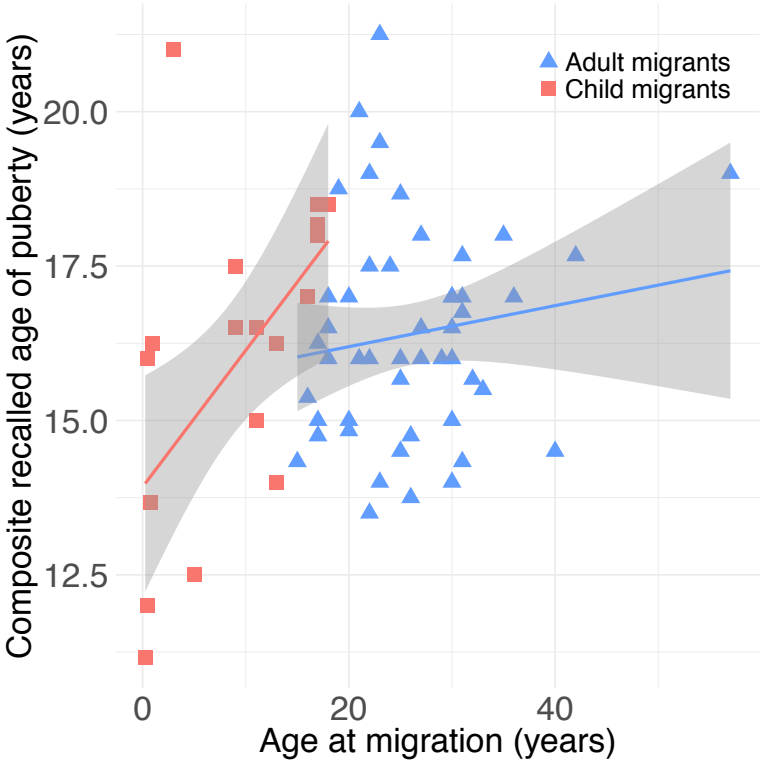
Morning and evening salivary testosterone, log transformed and in SD units. Each point indicates the residence group least squares mean adjusted for age and BMI. Error bars indicate 95% CI. Child age of migration split at ≤19 years. LS mean values (±CI) calculated using the R package *lsmeans*, and sample sizes (n=morning, evening if different): Second-generation: 0.728 (0.333, 1.122), 0.489 (0.101, 0.878), n=25, 28; Child migrants: 0.534 (0.17, 0.898), 0.387 (0.019, 0.756), n=26, 27; British European: -0.055 (-0.331, 0.222), -0.032 (-0.319, 0.254), n=44; Bangladeshi sedentees: -0.117 (-0.301, 0.067), -0.118 (-0.309, 0.073), n=103, 102; Adult migrants: -0.461 (0.724, -0.199), -0.239 (-0.51, 0.033), n=53.

Figure 2. Daily average salivary testosterone by age at migration



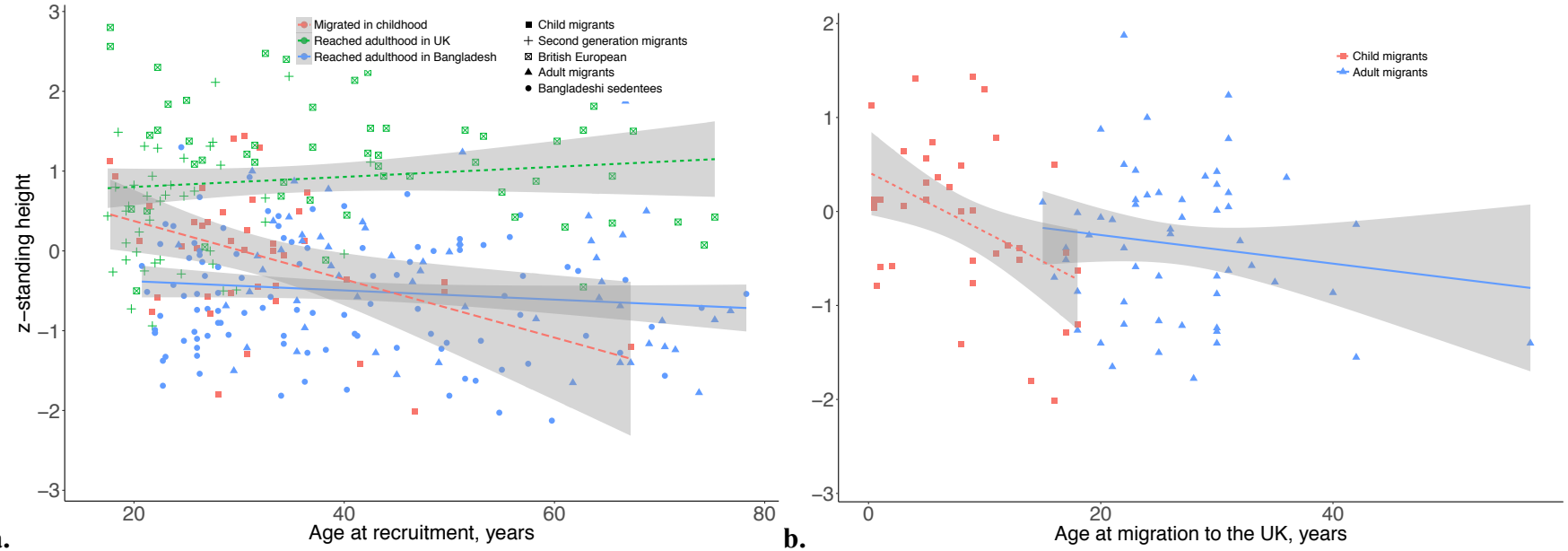
Log transformed SD units salivary testosterone of migrants arriving in the UK in early childhood (Birth-9y, n=26), late childhood (9-19y, n=33), and adulthood (19y+, n=55). Age at recruitment is indicated by darkness of point (older=darker), and point size indicates BMI imputed at population mean=24.07 for n=6, 8, 7, respectively. Line indicates linear regression with standard error. Linear regression differences in daily mean salivary testosterone adjusted for age at recruitment and imputed BMI between >19 and 0-9y migrants: 0.529 SD, 95%CI= 0.044, 1.141, p=0.035, and between >19 and 9-19y migrants: 0.141 SD, 95%CI= -0.253, 0.536, p=0.48. Each point indicates mean salivary testosterone sampled on two non-consecutive days from a single individual at waking and before bed, samples analysed by radioimmunoassay in duplicate.

Figure 3. Composite recalled age at puberty by age at migration



Migrants split into cohorts at based on whether they migrated before ("Child") or after ("Adult") composite recalled age at puberty, or ≤19 years at migration if not recalled. Linear regression of z-transformed values of composite puberty by age at migration in ≤19 (β=1.22 SD, 95%CI= 0.311, 2.13, n=19, *p*=0.01; with age at recruitment as covariate: β=1.10 SD, 95%CI= -0.106, 2.30, n=19, *p*=0.071) >19 years with age at recruitment as covariate (β=0.159 SD, 95%CI= -0.209, 0.527, n=49, *p*=0.39) line indicates linear regression with standard error, points indicate averaged remembered age at four developmental milestones.

Figure 4. Linear regression of standing height by (a) age at recruitment (b) age at migration



a. **b.**

Standing height in SD units. Lines indicate linear regression with SE of: (a) age at recruitment, cohorts separated by childhood conditions. Significant correlation between age at recruitment for child migrants (-0.483 SD, 95%CI=-0.840, -0.125, n=40, *p*=0.009), non-significant secular trends in men who reached puberty in the UK (0.123 SD, 95%CI=-0.033, 0.279, n=96, *p*=0.12) or Bangladesh (-0.078 SD, 95%CI=-0.20, 0.05, n=162, *p*=0.22); (b) age at migration, separated by migration after self-reported age at puberty, or age <19. Significant correlation between age at migration for child migrants (-0.713 SD, 95%CI: -1.235, -0.191; n=37, *p*=0.009), but not adult migrants (-0.171 SD, 95% CI= -0.491, 0.148, n=56, *p*=0.28). Linear regression including both age at recruitment and migration was non-significant for both child and adult migrants for all covariates (all p>0.1)

19 *Table 1. Descriptive statistics, mean (sd) by residence group*

	N	Age, y	Height, cm	Weight, kg	BMI	Waking, pg/mL	Evening, pg/mL	Recalled age at puberty, y
Bangladeshi sedentees	107	38.7 (14.1)	162.8 (5.6)	60.0 (9.2)	22.6 (3.2)	100.9 (49)	76.2 (40.8)	16.2 (1.9)
Adult migrants	75	48.4 (15.6)	164.3 (6.6)	67.8 (9.2)	25.1 (2.9)	90.7 (40.8)	75.0 (33.9)	16.4 (1.7)
Child migrants	59	32.1 (10.8)	167.1 (6.4)	69.0 (12.2)	24.6 (3.6)	141.4 (67.3)	100.1 (48)	15.8 (2.7)
Second-generation migrants	56	24.2 (5.6)	171.4 (5.5)	71.2 (12.6)	24.2 (3.8)	153.5 (54.6)	119.4 (59.6)	14.2 (1.4)
British European	62	41.4 (16.1)	177.1 (6.3)	76.8 (10.7)	24.5 (3.2)	114.5 (52.6)	92.1 (64.9)	14.2 (1.4)
All groups	359	38.0 (15.3)	167.5 (8)	67.4 (12)	23.9 (3.4)	112.0 (55.3)	86.8 (49.7)	15.7 (2)

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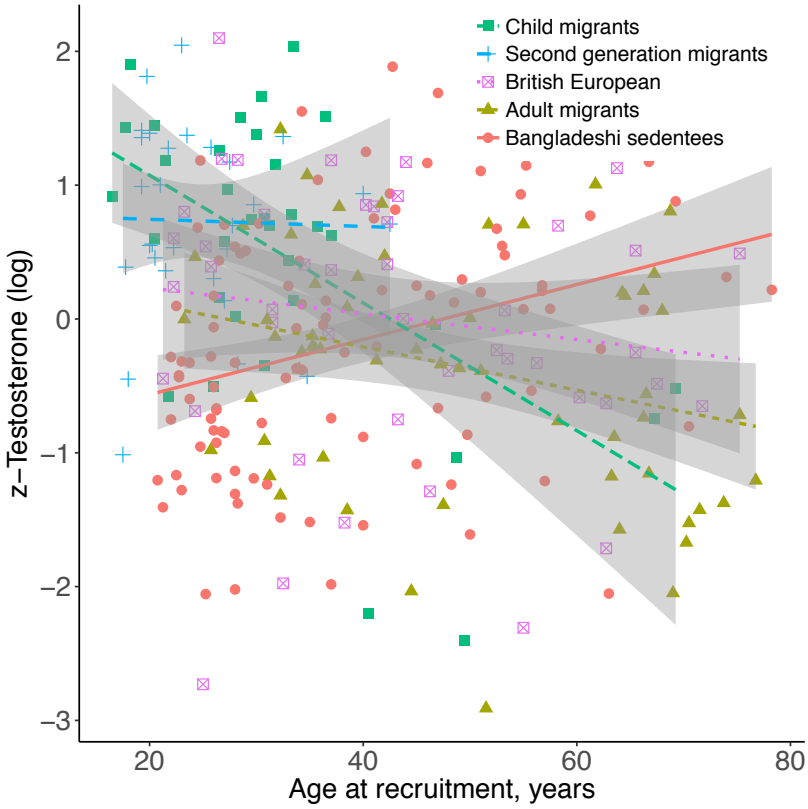
Table 2. Multiple linear regression of salivary testosterone and composite age at puberty by residence group

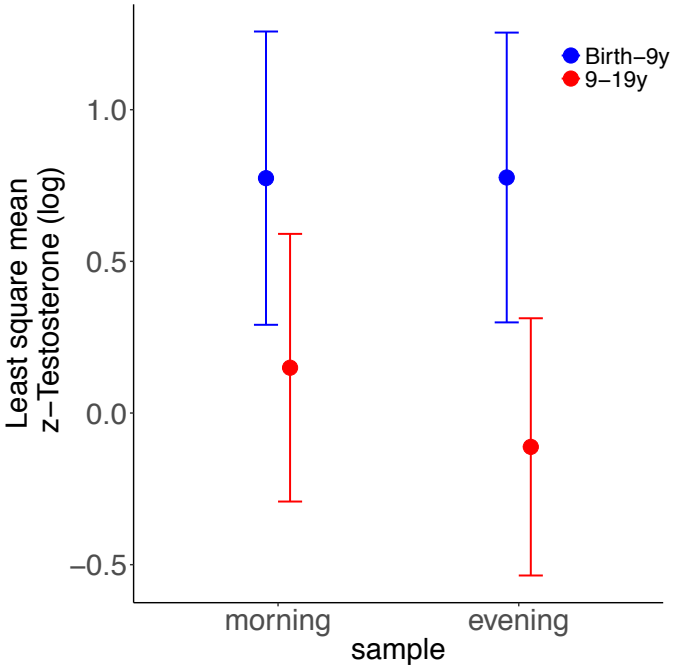
	measure		
	Salivary testosterone		*Composite age at puberty*
	sample time		
	Waking	Evening	
Constant	-0.113 (-0.297, 0.072)	-0.114 (-0.306, 0.078)	0.257** (0.087, 0.427)
	p = 0.234	p = 0.246	p = 0.004
Age(log)	-0.031 (-0.169, 0.106)	-0.021 (-0.164, 0.121)	0.229*** (0.100, 0.358)
	p = 0.657	p = 0.771	p = 0.001
BMI	0.173** (0.051, 0.294)	0.128* (0.004, 0.252)	N/A
	p = 0.006	p = 0.045	
Adult migrants	-0.344* (-0.667, -0.021)	-0.120 (-0.454, 0.213)	-0.060 (-0.371, 0.251)
	p = 0.039	p = 0.480	p = 0.707
Child migrants	0.652** (0.242, 1.061)	0.505* (0.089, 0.922)	-0.110 (-0.544, 0.323)
	p = 0.003	p = 0.019	p = 0.620
Second-generation migrants	0.845*** (0.407, 1.283)	0.608** (0.171, 1.044)	-0.791*** (-1.222, -0.360)
	p = 0.0002	p = 0.007	p = 0.0004
British European	0.063 (-0.272, 0.398)	0.086 (-0.260, 0.432)	-1.003*** (-1.312, -0.694)
	p = 0.715	p = 0.628	p = 0.0000001
Observations	251	254	237
R^2	0.177	0.088	0.242
Adjusted R^2	0.156	0.066	0.225
Residual Std. Error	0.917 (df = 244)	0.948 (df = 247)	0.880 (df = 231)
F Statistic	8.731*** (df = 6; 244)	3.995*** (df = 6; 247)	14.723*** (df = 5; 231)
Note:			*p<0.05; **p<0.01; ***p<0.001
			all values are z-transformed SD units, age and testosterone also log transformed. Reference category: Bangladeshi sedentees

Supplementary Information from Magid K., Chatterton RT, Ahamed FU, Bentley GR, "Childhood ecology influences salivary testosterone, pubertal age and stature of Bangladeshi UK migrant men"

Supplemental Section 1. Supplemental figures and tables



Supplemental figure 1. Regression of waking salivary testosterone by age at recruitment. Waking salivary testosterone (log), SD units of residence groups by age at recruitment. Lines indicate line.ar regression with SE, see supplemental table 9 for all slope coefficients. After adjusting for BMI, significant difference by linear regression between age trend of Bangladeshi sedentees (n=103) and that of Adult migrants -0.609, 95%CI=-0.963, -0.255, n=53, p=0.0008), Child migrants (-0.931, 95%CI=-1.428, -0.433, n=26, p=0.0003), and British Europeans (-0.478, 95%CI=-0.822, -0.133, n=44, p=0.007), but not Second-generation migrants (-0.339, 95% CI=-0.934, 0.256, n=25, p=0.26). Within UK resident groups only, Child migrant trend differed from British Europeans (β=-0.75, 95%CI=-1.32, -0.18, p=0.01). Each point indicates mean salivary testosterone sampled on two non-consecutive days from a single individual at waking, samples analysed by radioimmunoassay in duplicate.



Supplemental figure 2. Least square mean salivary testosterone by childhood age at migration, adjusted for number of years in the UK and BMI. Salivary testosterone of early and late childhood migrants by time of sampling. Boxes indicate the LS mean adjusted for number of years in the UK and BMI (imputed at population mean=24.07 for n=9). Error bars indicate the 95% confidence interval. Migrant groups differences at both time points in linear regression analysis (morning β=0.625 SD, 95%CI= -0.031, 1.28, n=33, *p*=0.061; evening 0.888 SD, 95%CI= 0.247, 1.53, n=34, *p*=0.008). Morning, evening LS mean values (95%CI) of log transformed testosterone in SD units calculated using the R package *lsmeans*: Birth-9y: 0.774 (0.291, 1.26), 0.777 (0.299, 1.25), n=15; 9-19y: 0.149, (-0.291, 0.591), -0.112 (-0.536, 0.312), n=18, 19.

Supplemental table 1. Post-hoc multiple comparison of table 2: Multiple linear regression of salivary testosterone by residence group with estimates of age and BMI, Tukey correction of all-pair multiple comparison (contrasts shown between non-sedentee groups only)

	Salivary testosterone							
	Waking, pg/mL				Evening, pg/mL			
	Estimate	Std. Error	*t*	*p*	Estimate	Std. Error	*t*	*p*
Child migrants - Adult migrants	**1.00**	**0.23**	**4.25**	**0.0003**	0.63	0.24	2.62	0.07
Second-generation migrants - Adult migrants	**1.19**	**0.25**	**4.71**	**0.0001**	**0.73**	**0.25**	**2.88**	**0.03**
British European - Adult migrants	0.41	0.19	2.16	0.19	0.21	0.19	1.06	0.82
Second-generation migrants - Child migrants	0.19	0.26	0.75	0.94	0.10	0.26	0.40	0.99
British European - Child migrants	-0.59	0.23	-2.52	0.09	-0.42	0.24	-1.75	0.39
British European - Second-generation migrants	**-0.78**	**0.25**	**-3.15**	**0.02**	-0.52	0.25	-2.09	0.22

Supplemental table 2. Multiple linear regression of salivary testosterone, effect of number of years in the UK and age of migration, child migrants, imputed BMI

	sample time	
	Waking	Evening
Constant	1.052 (0.035, 2.068)	-0.044 (-1.004, 0.916)
	p = 0.052	p = 0.930
Number of years in the UK	-0.046* (-0.079, -0.013)	-0.019 (-0.050, 0.013)
	p = 0.012	p = 0.254
BMI (imputed)	0.133 (-0.196, 0.462)	-0.085 (-0.378, 0.207)
	p = 0.436	p = 0.573
Age at migration	-0.553 (-1.136, 0.029)	-0.930** (-1.480, -0.380)
	p = 0.073	p = 0.003
Observations	33	34
R^2	0.293	0.313
Adjusted R^2	0.220	0.244
Residual Std. Error	0.919 (df = 29)	0.868 (df = 30)
F Statistic	4.012* (df = 3; 29)	4.549** (df = 3; 30)
Note:		*p<0.05; **p<0.01; ***p<0.001
		all values are z-transformed SD units, age and testosterone also log transformed
		BMI imputed for n=8 at population mean 23.9

Supplemental table 3. Multiple linear regression of salivary testosterone, effect of number of years in the UK and age of migration, child migrants, complete cases only

	sample time	
	Waking	Evening
Constant	1.218 (-0.008, 2.445)	-0.154 (-1.350, 1.043)
	p = 0.066	p = 0.804
Number of years in the UK	-0.048* (-0.094, -0.003)	-0.003 (-0.047, 0.041)
	p = 0.050	p = 0.897
BMI	0.145 (-0.207, 0.498)	-0.127 (-0.449, 0.196)
	p = 0.429	p = 0.449
Age at migration	-0.461 (-1.112, 0.189)	-0.747* (-1.382, -0.112)
	p = 0.180	p = 0.031
Observations	25	26
R^2	0.230	0.209
Adjusted R^2	0.120	0.101
Residual Std. Error	0.903 (df = 21)	0.881 (df = 22)
F Statistic	2.091 (df = 3; 21)	1.940 (df = 3; 22)
Note:		*p<0.05; **p<0.01; ***p<0.001
	all values are z-transformed SD units, age and testosterone also log transformed.	

Supplemental table 4. Multiple linear regression of salivary testosterone, effect of age of migration (split at age 9) of child migrants, imputed BMI

	sample time	
	Waking	Evening
Constant	0.156 (-0.275, 0.586)	-0.092 (-0.485, 0.301)
	p = 0.485	p = 0.650
Age(log)	-0.604* (-1.044, -0.163)	-0.425 (-0.839, -0.011)
	p = 0.012	p = 0.054
BMI (imputed)	0.120 (-0.211, 0.451)	-0.078 (-0.374, 0.217)
	p = 0.484	p = 0.607
Infancy or early childhood migrants (birth-8 years)	0.152 (-0.579, 0.882)	0.556 (-0.126, 1.238)
	p = 0.688	p = 0.121
Observations	33	34
R^2	0.278	0.304
Adjusted R^2	0.203	0.234
Residual Std. Error	0.929 (df = 29)	0.874 (df = 30)
F Statistic	3.718* (df = 3; 29)	4.367* (df = 3; 30)
Note:		*p<0.05; **p<0.01; ***p<0.001
	all values are z-transformed SD units, age and testosterone also log transformed. BMI imputed for n=8 at population mean 23.9. Reference category: Late childhood migrants (9-19 years)	

Supplemental table 5. Multiple linear regression of salivary testosterone, effect of age of migration (split at age 9) of child migrants, complete cases only

	sample time	
	Waking	Evening
Constant	1.376* (0.283, 2.470)	0.251 (-0.890, 1.392)
	p = 0.023	p = 0.671
Number of years in the UK	-0.052* (-0.097, -0.008)	-0.006 (-0.052, 0.040)
	p = 0.032	p = 0.800
BMI	0.149 (-0.193, 0.491)	-0.134 (-0.471, 0.203)
	p = 0.403	p = 0.445
Infancy or early childhood migrants (birth-8 years)	0.640 (-0.054, 1.334)	0.650 (-0.066, 1.365)
	p = 0.085	p = 0.089
Observations	25	26
R^2	0.273	0.141
Adjusted R^2	0.169	0.024
Residual Std. Error	0.878 (df = 21)	0.918 (df = 22)
F Statistic	2.622 (df = 3; 21)	1.209 (df = 3; 22)
Note:		*p<0.05; **p<0.01; ***p<0.001
	all values are z-transformed SD units, age and testosterone also log transformed. Reference category: Late childhood migrants (9-19 years)	

Supplemental table 6. Multiple linear regression of recalled age at puberty, effect of age of migration cohort (split at age 9) of child migrants

	sample time
	Composite age at puberty
Constant	0.308 (-0.023, 0.639)
	p = 0.072
Age at recruitment (log)	0.130 (-0.116, 0.376)
	p = 0.303
Late childhood migrants (9-19 years)	0.061 (-0.430, 0.552)
	p = 0.809
Infancy or early childhood migrants (birth-8 years)	-0.858* (-1.637, -0.079)
	p = 0.034
Pre-birth (Born in UK)	-0.929** (-1.547, -0.312)
	p = 0.005
Observations	89
R^2	0.275
Adjusted R^2	0.241
Residual Std. Error	0.892 (df = 84)
F Statistic	7.982*** (df = 4; 84)
Note:	*p<0.05; **p<0.01; ***p<0.001
	all values are z-transformed SD units. Reference category: Adult migrants >19 years

Supplemental table 7. Multiple linear regression of salivary testosterone, effect of age of migration in adult migrants >19y only

	sample time	
	Waking	Evening
Constant	0.021 (-0.576, 0.619)	0.347 (-0.113, 0.807)
	p = 0.945	p = 0.146
Number of years in the UK	-0.019* (-0.034, -0.003)	-0.024*** (-0.036, -0.011)
	p = 0.026	p = 0.0005
BMI	0.092 (-0.194, 0.378)	0.132 (-0.092, 0.356)
	p = 0.532	p = 0.253
Age at migration	-0.061 (-0.443, 0.320)	-0.093 (-0.392, 0.207)
	p = 0.755	p = 0.548
Observations	53	53
R^2	0.111	0.256
Adjusted R^2	0.057	0.210
Residual Std. Error (df = 49)	0.891	0.690
F Statistic (df = 3; 49)	2.039	5.609**
Note:		*p<0.05; **p<0.01; ***p<0.001
	all values are z-transformed SD units, age and testosterone also log transformed.	

Supplemental table 8a. Multiple linear regression of salivary testosterone by number of years in the UK and age of migration, adult migrants, aged ≤40 years at recruitment

	sample time	
	Waking	Evening
Constant	-0.075 (-1.549, 1.399)	0.565 (-0.588, 1.718)
	p = 0.922	p = 0.351
Number of years in the UK	-0.014 (-0.114, 0.086)	-0.073 (-0.150, 0.003)
	p = 0.791	p = 0.079
BMI	-0.116 (-0.521, 0.289)	0.188 (-0.136, 0.511)
	p = 0.583	p = 0.272
Age at migration	0.083 (-1.104, 1.270)	-0.068 (-1.003, 0.867)
	p = 0.893	p = 0.889
Observations	20	21
R^2	0.032	0.284
Adjusted R^2	-0.150	0.158
Residual Std. Error	0.790 (df = 16)	0.634 (df = 17)
F Statistic	0.174 (df = 3; 16)	2.250 (df = 3; 17)
Note:		*p<0.05; **p<0.01; ***p<0.001
	all values are z-transformed SD units, age and testosterone also log transformed	

Supplemental table 8b. Multiple linear regression of salivary testosterone by number of years in the UK and age of migration, adult migrants, aged >40 years at recruitment

	sample time	
	Waking	Evening
Constant	0.044 (-1.331, 1.418)	1.225* (0.282, 2.167)
	p = 0.951	p = 0.017
Number of years in the UK	-0.020 (-0.051, 0.011)	-0.041*** (-0.062, -0.020)
	p = 0.220	p = 0.001
BMI	0.221 (-0.192, 0.633)	0.038 (-0.256, 0.331)
	p = 0.303	p = 0.803
Age at migration	-0.071 (-0.600, 0.457)	-0.359 (-0.738, 0.019)
	p = 0.794	p = 0.073
Observations	33	32
R^2	0.107	0.350
Adjusted R^2	0.015	0.281
Residual Std. Error	0.980 (df = 29)	0.687 (df = 28)
F Statistic	1.158 (df = 3; 29)	5.031** (df = 3; 28)
Note:		*p<0.05; **p<0.01; ***p<0.001
	all values are z-transformed SD units, age and testosterone also log transformed	

Supplemental table 9. Age at recruitment effects on waking and evening salivary testosterone (pg/mL), linear regression coefficients, divided by residence group.

	Salivary testosterone							
	Waking, pg/mL				Evening, pg/mL			
	Estimate	95% CI	r^2	*p*	Estimate	95% CI	r^2	*p*
Bangladeshi sedentees	**1.098**	**0.452, 1.745**	**0.10**	**0.001**	**1.044**	**0.515, 1.573**	**0.132**	**<0.001**
Adult migrants	-0.644	-1.317, 0.03	0.058	0.061	**-0.912**	**-1.435, -0.388**	**0.173**	**0.001**
Child migrants	**-2.659**	**-4.283, -1.035**	**0.258**	**0.002**	**-1.901**	**-3.204, -0.599**	**0.211**	**0.006**
Second-generation migrants	-0.602	-3.789, 2.585	0.006	0.701	-2.639	-5.838, 0.561	0.092	0.102
British European	-0.704	-1.721, 0.313	0.041	0.17	-0.359	-1.682, 0.965	0.007	0.588
All groups	**-0.666**	**-1.077, -0.255**	**0.036**	**0.002**	**-0.474**	**-0.852, -0.096**	**0.021**	**0.0141**
UK-born groups only	**-1.223**	**-2.012, -0.435**	**0.114**	**0.003**	-1.801	-4.452, 0.849	0.037	0.178

Supplemental table 10. Multiple linear regression of salivary testosterone as predictor of composite recalled age at puberty

	sample time	
	Waking	Evening
Constant	-0.068 (-0.196, 0.059)	-0.055 (-0.182, 0.072)
	p = 0.296	p = 0.399
Age(log)	0.283*** (0.151, 0.415)	0.298*** (0.165, 0.430)
	p = 0.00004	p = 0.00002
Salivary testosterone	-0.172** (-0.301, -0.044)	-0.130* (-0.258, -0.003)
	p = 0.010	p = 0.047
Observations	219	220
R^2	0.115	0.100
Adjusted R^2	0.107	0.091
Residual Std. Error	0.947 (df = 216)	0.954 (df = 217)
F Statistic	14.056*** (df = 2; 216)	12.009*** (df = 2; 217)
Note:	*p<0.05; **p<0.01; ***p<0.001	
	all values are z-transformed SD units, age also log transformed.	

Supplemental table 11. Multiple linear regression of standing height by number of years in the UK and age of migration, Child migrants ≤19y

	Dependent variable:
Constant	-0.304 (-1.236, 0.628)
	p = 0.527
Number of years in the UK	-0.019 (-0.051, 0.014)
	p = 0.269
Age at migration	-0.719** (-1.221, -0.217)
	p = 0.009
Observations	37
R^2	0.209
Adjusted R^2	0.163
Residual Std. Error	0.768 (df = 34)
F Statistic	4.505* (df = 2; 34)
Note:	*p<0.05; **p<0.01; ***p<0.001
	all values are z-transformed SD units.

Supplemental table 12. Multiple linear regression of standing height by age at recruitment and age of migration, Child migrants ≤19y

	Dependent variable:
Constant	-0.656* (-1.217, -0.095)
	p = 0.029
Age at recruitment	-0.219 (-0.678, 0.241)
	p = 0.358
Age at migration	-0.536 (-1.163, 0.092)
	p = 0.104
Observations	37
R^2	0.201
Adjusted R^2	0.153
Residual Std. Error	0.772 (df = 34)
F Statistic	4.263* (df = 2; 34)
Note:	*p<0.05; **p<0.01; ***p<0.001
	all values are z-transformed SD units.

Supplemental Section 2. Supplemental analysis within age at recruitment subsets and comparative missing data techniques for BMI of child migrants. From Magid K., Chatterton RT, Ahamed FU, Bentley GR, "Childhood ecology influences salivary testosterone, pubertal age and stature of Bangladeshi UK migrant men"

Supplemental section 2.1 Restricting analysis to men aged ≤40 years at recruitment:

Introduction/methods:

In order to reduce variation due to potential male ageing effects on testosterone or recall bias for self-report of age at puberty, analysis was replicated on subsets of younger men. Men 40 years or younger at the time of recruitment were included in the analysis, all statistical methods were replications of those performed in the body of the paper. Regressions of testosterone by age at recruitment were replicated within subsets of men ≤40 years and >40 years.

Results:

After restricting the dataset to men ≤40 years, adult migrants remained significantly older, and second-generation migrants significantly younger than the average age of sedentees, British Europeans and Child migrants. Child migrants and Europeans were not significantly different.

Supplemental table 13 reports descriptive statistics after restricting the dataset to men ≤40 years. Adult migrants remained significantly older than all other groups with an average age of 33.3 years (95%CI=31.6, 35), while the second generation were the youngest group, averaging 23.8 years (95%CI=22.3, 25.2). Age at migration and mean age at recruitment of men ≤40 were not significantly correlated in either child

(r=0.37, t = 2.0, df = 25, p = 0.056), or adult migrants (r=0.28, t = 1.8, df = 37, p = 0.08).

Compared to sedentees, British Europeans and all migrant groups were significantly taller (except adult migrants) and heavier with higher BMIs (Supplemental table 13). Secular trends for height were not observed when restricting analysis to ≤40 years at recruitment. Across groups ≤40, older men recalled reaching puberty at a later age (β=0.51 SD, 95%CI=0.203, 0.819, n=139, p=0.001). After correcting for age at recruitment, men with higher waking but not evening salivary testosterone recalled reaching puberty at an earlier age (waking: β=-0.206 SD, 95%CI=-0.377, -0.035, n=127, p=0.02; evening: β=-0.143 SD, 95%CI=-0.302, -0.015, n=129, p=0.076).

Within men by men ≤40 years at recruitment, we found similar support for our childhood ecology hypotheses as within the full cohort. Ecological conditions experienced in the UK prior to adulthood led to higher testosterone levels, an earlier age at puberty and taller stature when compared to men who experienced similar conditions only after puberty (supplemental table 14, 15). Child and second-generation men who spent the entirety of their childhood in the UK had the highest levels of waking and evening salivary testosterone of any group studied (supplemental table 15). These levels were significantly higher than those of adult migrants and sedentees.

As with the full cohort of all migrants (not divided by childhood conditions), within migrants ≤40y at recruitment, age of migration remains a significant predictor of evening testosterone β= -0.77 SD (95%CI: -1.33, -0.2 n=44; p=0.009) after adjusting

for number of years in the UK, but differed from the full cohort with a non-significant effect of age at migration on waking salivary testosterone β= -0.215, (95%CI: -0.773, 0.343; n=42; p=0.44).

When limiting the population to men ≤40, age at migration was not a significant predictor of composite age at puberty in child migrants, (1.17 SD 95%CI= -0.133, 2.47; n=15, p=0.07). Both child migrants and second-generation men recalled an earlier composite age at puberty compared to sedentees, although these differences were not significant compared to adult migrants.

We did not find evidence that experience of ecological conditions in the UK at any point during adulthood before the age of 40 leads to higher testosterone. When adult migrants were divided into two cohorts by age of recruitment at 40 years, men below age 40 did not show a relationship between number of adult years in the UK and waking or evening salivary testosterone (waking β=-0.014 SD, 95%CI=-0.114, 0.086, n=20, *p*=0.8; evening β=-0.73 SD, 95%CI=-0.150, 0.003, n=21, *p*=0.08; supplemental table S8a). In contrast to the full cohort, within this subset waking salivary testosterone of adult migrants was not lower than sedentees β=0.45 SD (95%CI: -0.033, 0.940; p=0.07; table S5.2). For adult migrants above age 40, evening salivary testosterone was lower in men who spent more adult years in the UK (waking β=-0.020 SD, 95%CI=-0.051, 0.011, n=33, *p*=0.22; evening β=-0.041 SD, 95%CI=-0.062, -0.020, *p*=0.001; supplemental table S8b).

When comparing men ≤40 of contrasting ethnicities experiencing similar ecological conditions and similar socioeconomic status, waking testosterone of second

generation migrants was not different from British Europeans (in contrast to the full population), however within this subset child migrant waking salivary testosterone was marginally higher than British Europeans. As with the full population, recalled age at puberty was not different between British European and second generation British-Bangladeshi men, but was significantly earlier in Europeans compared to all other groups.

The overall population decline within men ≤40 was closer to that reported elsewhere in longitudinal studies[1]. The published rate of decline of serum testosterone in that cohort, adjusted for our methods is 2.133 pg/ml per year. We find significant negative relationship of salivary testosterone with age at recruitment within men ≤40, adjusting for BMI for both waking β=-2.27pg/mL per year (95%CI: -3.87, -0.68) pg/mL per year and a sig. negative decline in evening salivary testosterone of -1.82 pg/mL per year (95%CI: -3.14, -0.513). We also observe something closer to this within men >40 after adjusting for BMI: -1.313 (95%CI: -2.23, -0.39) pg/mL per year and a sig. negative decline in bed salT of -1.88 (95%CI: -2.80, -0.96) pg/mL per year. When confining analysis to a 'middle aged' cohort between age 30-50 at recruitment, there was not a significant relationship between relationship between age and testosterone observed non-significant decline in waking salivary testosterone of -0.7941 pg/mL per year (95%CI: -2.56, 0.970) and a non-significant positive trend for evening salivary testosterone of 0.80 pg/mL per year (95%CI: -0.859, 2.47). We found no evidence of a difference between residential groups in age related decline, except for child migrants in their evening samples.

Conclusions:

When restricting analysis to men ≤40 results support or do not contradict the findings with the full cohort. There are few indications of differential effects of childhood ecology on salivary testosterone depending on whether a man is in early adulthood, as compared to the effects seen in men at all ages. Potential cohort or bias relating to age still appears to influence recalled age at puberty. Concerning the patterns of ageing in men, when subset at 40 years at recruitment, our findings suggest that the significant age-related declines are most pronounced at older and younger ages in this population. Based on the lack of differences in slope between groups when confining analysis to men <40y, we conclude that age-related variations seen across groups of contrasting ethnicity and ecology are particular to the whole of the lifecourse, that differences in the slopes of decline are steepest when comparing men at younger or older ages, and not as steep during middle age.

Supplemental table 13. Descriptive statistics, subset of men ≤40y at age of recruitment

	N	Age, y	Height, cm	Weight, cm	BMI	Waking, pg/mL	Evening, pg/mL	Recalled age at puberty, y
Bangladeshi sedentees	64	28.8 (5.2)	163.5 (5.5)	58.5 (9.5)	21.8 (3.1)	84.5 (37.6)	62.2 (30.5)	16.1 (2)
Adult migrants	27	33.3 (4.3)	166.2 (5.5)	68.4 (10)	24.7 (3.2)	99.5 (39.4)	82.4 (31.7)	16.1 (1.3)
Child migrants	42	28.2 (5.5)	168.4 (5.9)	69.5 (12.7)	24.4 (3.7)	160.5 (54.3)	109.6 (46.7)	15.6 (2.8)
Second generation migrants	45	23.8 (4.9)	171.4 (5.7)	72.4 (13.1)	24.6 (3.9)	153.6 (53.1)	120.4 (55.3)	14.3 (1.3)
British European	29	28.0 (6.4)	177.8 (6.8)	74.2 (11.1)	23.5 (3.3)	118.2 (61.2)	82.0 (53.3)	13.7 (1.2)
All groups	207	28.1 (5.9)	168.4 (7.5)	66.8 (12.8)	23.5 (3.6)	116.1 (56.4)	86.3 (47.4)	15.4 (2)

Supplemental table 14. Multiple linear regression of salivary testosterone and composite age at puberty by residence group, ≤40y age at recruitment

	measure		
	Salivary testosterone		*Composite age at puberty*
	sample time		
	Waking	Evening	
Constant	-0.432** (-0.699, -0.165)	-0.506*** (-0.799, -0.214)	0.435** (0.158, 0.712)
	p = 0.002	p = 0.001	p = 0.003
Age(log)	-0.071 (-0.372, 0.230)	-0.169 (-0.497, 0.158)	0.382* (0.077, 0.688)
	p = 0.644	p = 0.313	p = 0.016
BMI	0.128 (-0.014, 0.270)	0.082 (-0.072, 0.236)	-0.163 (-0.623, 0.298)
	p = 0.079	p = 0.298	p = 0.490
Adult migrants	0.227 (-0.221, 0.675)	0.453 (-0.033, 0.940)	-0.232 (-0.726, 0.263)
	p = 0.322	p = 0.070	p = 0.361
Child migrants	1.163*** (0.746, 1.580)	0.846*** (0.397, 1.294)	-0.738** (-1.195, -0.282)
	p = 0.00000	p = 0.0004	p = 0.002
Second generation migrants	1.131*** (0.686, 1.576)	0.897*** (0.424, 1.369)	-1.166*** (-1.585, -0.747)
	p = 0.00001	p = 0.0003	p = 0.00000
British European	0.421 (-0.025, 0.866)	0.330 (-0.160, 0.820)	0.435** (0.158, 0.712)
	p = 0.067	p = 0.189	p = 0.003
Observations	146	151	141
R^2	0.323	0.191	0.260
Adjusted R^2	0.294	0.157	0.232
Residual Std. Error	0.833 (df = 139)	0.917 (df = 144)	0.877 (df = 135)
F Statistic	11.051*** (df = 6; 139)	5.666*** (df = 6; 144)	9.470*** (df = 5; 135)
Note:	*p<0.05; **p<0.01; ***p<0.001		
all values are z-transformed SD units, age and testosterone also log transformed. Reference category: Bangladeshi sedentees			

Supplemental table 15. Post-hoc multiple comparison of table 2a: Multiple linear regression of salivary testosterone by residence group with estimates of age and BMI, age of recruitment ≤40y. Tukey correction of all-pair multiple comparison (contrasts shown between non-sedentee groups only)

	Salivary testosterone							
	Waking, pg/mL				Evening, pg/mL			
	Estimate	Std. Error	*t*	*p*	Estimate	Std. Error	*t*	*p*
Child migrants - Adult migrants	**0.94**	**0.27**	**3.53**	**0.01**	0.39	0.29	1.37	0.64
Second generation migrants - Adult migrants	**0.90**	**0.29**	**3.15**	**0.02**	0.44	0.31	1.44	0.59
British European - Adult migrants	0.19	0.28	0.70	0.96	-0.12	0.30	-0.41	0.99
Second generation migrants - Child migrants	-0.03	0.25	-0.13	1.00	0.05	0.27	0.19	1.00
British European - Child migrants	**-0.74**	**0.26**	**-2.82**	**0.04**	-0.52	0.29	-1.80	0.37
British European - Second generation migrants	-0.71	0.27	-2.60	0.07	-0.57	0.29	-1.93	0.30

Supplemental section 2.2: Missing BMI values in salivary testosterone regressions within child migrants. Comparison of multiple imputation, replacement by population mean and listwise deletion techniques

Introduction:

The percentage of nonmissing anthropometric values for BMI by residence group ranged from Bangladeshi sedentees 99.1, Adult migrants 86.7 and British European 90.3, while sample size was lowest for Child migrants 74.6 and Second-generation migrants 87.5. This was primarily due to recruitment locations, where the first three groups were primarily recruited at locations where anthropometric equipment could be used at the point of recruitment, non-respondents from the latter two groups were primarily recruited in locations requiring follow up visits to obtain physical data.

Methods:

While listwise deletion for missing data was practiced in all inter-group analysis, for intra-group analysis confined to child migrants we addressed the exclusion of up to 24% of responses by missing data methods of replacement by population mean as well as multiple imputation techniques. For multiple imputation of BMI in intragroup child migrant regressions, a four-chain, 30-iteration multiple imputation of BMI was performed the R package "*mi*" on the complete data set. Imputation was based on eight relevant variables (residence group, age at recruitment, height, weight, three measures of physical activity and ethnicity). Four replications of each childhood age at migration regression with these imputed datasets are reported below, with results pooled using the package *"mitools"* according to Rubin's rules[2].

Results:

Age at recruitment was a significant negative predictor of waking salivary testosterone in all imputed regressions and for evening regressions including early versus middle childhood birth cohorts (supplemental tables 16, 18). Number of years in the UK was a significant negative predictor of waking salivary testosterone in all imputed and complete case regressions, and early childhood migration or age at migration were significant predictors of evening salivary testosterone in all regressions including number of years in the UK regardless of imputation or listwise deletion (supplemental tables 17, 19).

Conclusion:

We conclude that multiple imputation methods did not yield substantially different values or results for main effects or covariates from those found with the mean replacement method, and both imputation methods compare similarly with listwise deletion results.

Supplemental table 16. Multiple linear regression coefficients (a) waking and (b) evening salivary testosterone of child migrants, estimates including BMI by multiple imputation (MI), population mean imputation and listwise deletion methods

(a) waking

	Pooled MI (n=34)		Population mean imputed BMI method (n=34)		Complete cases (n=26)	
	β (95% CI)	p range	β (95% CI)	p	β (95% CI)	p
Constant	0.199 (-0.436, 0.834)	0.41-0.61	0.179 (-0.473, 0.831)	0.58	0.308 (-0.46, 1.075)	0.41
Age at recruitment	**-0.616 (-1.103, -0.129)**	**0.01-0.03**	**-0.63 (-1.141, -0.119)**	**0.02**	-0.591 (-1.311, 0.129)	0.1
BMI	0.106 (-0.257, 0.47)	0.17-0.92	0.129 (-0.219, 0.476)	0.46	0.125 (-0.259, 0.51)	0.51
Age at migration	-0.04 (-0.798, 0.718)	0.8-0.9	-0.044 (-0.821, 0.734)	0.91	0.03 (-0.892, 0.952)	0.95
(b) evening						
Constant	-0.358 (-0.94, 0.223)	0.22-0.26	-0.369 (-0.97, 0.231)	0.22	-0.199 (-0.921, 0.522)	0.57
Age at recruitment	-0.339 (-0.79, 0.111)	0.13-0.18	-0.322 (-0.793, 0.15)	0.17	-0.069 (-0.748, 0.609)	0.83
BMI	-0.029 (-0.321, 0.262)	0.53-0.95	-0.076 (-0.378, 0.225)	0.61	-0.123 (-0.463, 0.217)	0.46
Age at migration	-0.636 (-1.328, 0.057)	0.07-0.1	-0.662 (-1.38, 0.055)	0.07	-0.69 (-1.56, 0.18)	0.11

Note: all values are z-transformed SD units, age and testosterone also log transformed
MI: 4 chain, 30 iteration imputation performed with R package *mi*
Population mean BMI imputed for n=8 at 23.9

Supplemental table 17. Multiple linear regression coefficients (a) waking and (b) evening salivary testosterone of child migrants, estimates including BMI by multiple imputation (MI), population mean imputation and listwise deletion methods

(a) waking

	Pooled MI (n=34)		Population mean imputed BMI method (n=34)		Complete cases (n=26)	
	β (95% CI)	p range	β (95% CI)	p	β (95% CI)	p
Constant	1.067 (0.04, 2.094)	0.03-0.06	1.052 (-0.008, 2.113)	0.05	1.218 (-0.083, 2.519)	0.07
Number of years in the UK	**-0.046 (-0.079, -0.012)**	**0.01-0.02**	**-0.046 (-0.081, -0.011)**	**0.01**	**-0.048 (-0.097, 0)**	**0.05**
BMI	0.124 (-0.221, 0.469)	0.17-0.79	0.133 (-0.209, 0.476)	0.43	0.145 (-0.229, 0.519)	0.43
Age at migration	-0.534 (-1.126, 0.058)	0.07-0.12	-0.553 (-1.16, 0.055)	0.07	-0.461 (-1.152, 0.229)	0.18
(b) evening						
Constant	-0.007 (-0.977, 0.963)	0.92-0.98	-0.042 (-1.043, 0.959)	0.93	-0.154 (-1.42, 1.112)	0.8
Number of years in the UK	-0.02 (-0.052, 0.012)	0.2-0.27	-0.019 (-0.052, 0.014)	0.25	-0.003 (-0.05, 0.044)	0.9
BMI	-0.028 (-0.324, 0.267)	0.55-0.97	-0.082 (-0.387, 0.222)	0.58	-0.127 (-0.468, 0.214)	0.45
Age at migration	**-0.917 (-1.473, -0.36)**	**0.002-0.003**	**-0.93 (-1.504, -0.356)**	**0.002**	**-0.747 (-1.419, -0.076)**	**0.03**

Note: all values are z-transformed SD units, age and testosterone also log transformed
MI: 4 chain, 30 iteration imputation performed with R package *mi*
Population mean BMI imputed for n=8 at 23.9

Supplemental table 18. Multiple linear regression coefficients (a) waking and (b) evening salivary testosterone of child migrants, estimates including BMI by multiple imputation (MI), population mean imputation and listwise deletion methods

(a) waking

	Pooled MI (n=34)		Population mean imputed BMI method (n=34)		Complete cases (n=26)	
	β (95% CI)	p range	β (95% CI)	p	β (95% CI)	p
Constant	0.176 (-0.256, 0.607)	0.36-0.46	0.156 (-0.292, 0.605)	0.48	0.204 (-0.353, 0.762)	0.45
Age at recruitment	**-0.59 (-1.029, -0.152)**	**0.01-0.02**	**-0.604 (-1.063, -0.144)**	**0.01**	-0.5 (-1.129, 0.13)	0.11
BMI	0.097 (-0.263, 0.457)	0.19-0.97	0.121 (-0.224, 0.466)	0.48	0.104 (-0.273, 0.481)	0.57
Infancy or early childhood migrants (birth-8 years)	0.144 (-0.598, 0.886)	0.61-0.85	0.15 (-0.612, 0.913)	0.69	0.222 (-0.657, 1.102)	0.6
(b) evening						
Constant	-0.092 (-0.488, 0.305)	0.63-0.69	-0.092 (-0.502, 0.317)	0.65	0.1 (-0.423, 0.622)	0.7
Age at recruitment	**-0.438 (-0.852, -0.024)**	**0.04-0.06**	**-0.426 (-0.857, 0.006)**	**0.05**	-0.245 (-0.859, 0.37)	0.42
BMI	-0.034 (-0.343, 0.275)	0.47-0.94	-0.076 (-0.384, 0.232)	0.62	-0.107 (-0.457, 0.243)	0.53
Infancy or early childhood migrants (birth-8 years)	0.533 (-0.158, 1.224)	0.11-0.17	0.555 (-0.156, 1.266)	0.12	0.478 (-0.371, 1.327)	0.26

Note: all values are z-transformed SD units, age and testosterone also log transformed; Reference category: Late childhood migrants (9-19 years)
MI: 4 chain, 30 iteration imputation performed with R package *mi*
Population mean BMI imputed for n=8 at 23.9

Supplemental table 19. Multiple linear regression coefficients (a) waking and (b) evening salivary testosterone of child migrants, estimates including BMI by multiple imputation (MI), population mean imputation and listwise deletion methods

(a) waking

	Pooled MI (n=34)		Population mean imputed BMI method (n=34)		Complete cases (n=26)	
	β (95% CI)	p range	β (95% CI)	p	β (95% CI)	p
Constant	1.3 (0.403, 2.198)	0.005-0.01	1.297 (0.368, 2.227)	0.01	1.376 (0.216, 2.536)	0.02
Number of years in the UK	**-0.048 (-0.081, -0.015)**	**0.005-0.01**	**-0.049 (-0.083, -0.014)**	**0.01**	**-0.052 (-0.099, -0.005)**	**0.03**
BMI	0.118 (-0.237, 0.472)	0.16-0.86	0.135 (-0.206, 0.476)	0.42	0.149 (-0.214, 0.512)	0.4
Infancy or early childhood migrants (birth-8 years)	0.602 (-0.036, 1.241)	0.06-0.09	0.624 (-0.032, 1.28)	0.06	0.64 (-0.096, 1.376)	0.08
(b) evening						
Constant	0.483 (-0.402, 1.368)	0.25-0.35	0.457 (-0.454, 1.368)	0.31	0.251 (-0.956, 1.458)	0.67
Number of years in the UK	-0.025 (-0.057, 0.008)	0.12-0.2	-0.023 (-0.057, 0.011)	0.17	-0.006 (-0.055, 0.043)	0.8
BMI	-0.044 (-0.37, 0.283)	0.42-1	-0.095 (-0.413, 0.222)	0.54	-0.134 (-0.491, 0.223)	0.44
Infancy or early childhood migrants (birth-8 years)	**0.873 (0.249, 1.498)**	**0.01-0.01**	**0.888 (0.246, 1.53)**	**0.01**	**0.65 (-0.107, 1.407)**	**0.09**

Note: all values are z-transformed SD units, age and testosterone also log transformed; Reference category: Late childhood migrants (9-19 years)
MI: 4 chain, 30 iteration imputation performed with R package *mi*

Supplemental references

1. Harman, M. S., Metter, J. E., Tobin, J. E., Pearson, J. & Blackman, M. R. Longitudinal effects of aging on serum total and free testosterone levels in healthy men. *J. Clin. Endocrinol. Metab.* **86,** 724–731 (2001).
2. Rubin, D. B. & Schenker, N. Multiple imputation for interval estimation from simple random samples with ignorable nonresponses. *J. Am. Stat. Assoc.* **81,** 366–374 (1986).

Supplemental section 3. Descriptions of variables used and statistical tests performed in Magid K., Chatterton RT, Ahamed FU, Bentley GR, "Childhood ecology influences salivary testosterone, pubertal age and stature of Bangladeshi UK migrant men"

Supplemental table 20. Definitions of variable names in the dataset for Magid K., Chatterton RT, Ahamed FU, Bentley GR, "Childhood ecology influences salivary testosterone, pubertal age and stature of Bangladeshi UK migrant men"

Variable name	Definition
PartNum	Participant ID code
residence19pub	categorical variable separating men by residential, developmental and ethnic characteristics: "Bangladeshi sedentees"(SED), "Adult migrants"(ADU), "Child migrants"(CHI), "Second generation migrants"(2NG), "British European"(EUR). Adult migrants are classified by age at migration after self-reported age at puberty or <19 years.
AgeMigUK	Age at migration (years)
NumYearsUK	Number of years in the UK (AgeMigUK - AgeRecruit)
AgeRecruit	Age at recruitment (years)
Height	Height (cm)
Weight	Weight (kg)
BMI	Body Mass Index: kg/m^2
MssBMI	for BMI missing data, imputed from the overall population mean BMI value (24.07)
MeanS1D1D2	Average waking salivary testosterone, sampled <30min following waking over two non-consecutive days
MeanS3D1D2	Average evening salivary testosterone, sampled prior to bed over two non-consecutive days
PubVoice.n	Recalled age (years) at which voice first broke
PubShave.n	Recalled age (years) at which shaving first began
PubPub.n	Recalled age (years) at which pubic hair first appeared
PubNE.n	Recalled age (years) at which first nocturnal emission
pub.compos	Average of recalled pubertal milestones (years)
age.8.19.mig	categorical variable separating child migrants by age at migration cohorts: >19y; 9-18y; Birth-9y; Pre-Birth (Born UK)

age.8b.19.mig	categorical variable separating child migrants by age at migration cohorts: >19y; 9-19y; Pre-Birth (Born UK)-9y
ukbd.born.adu	categorical variable separating men by where they reached adulthood (as defined by the variable “residence19pub”): "Reached adulthood in Bangladesh", "Reached adulthood in UK", "Migrated in childhood"
z.log.meanS1D1D2	natural logarithm of MeanS1D1D2 in SD units
z.log.meanS3D1D2	natural logarithm of MeanS3D1D2 in SD units
z.log.meanS1S3D1D2	natural logarithm of daily average salivary testosterone, in SD units
z.log.age	natural logarithm of AgeRecruit, in SD units
z.AgeMigUK	Age at migration (SD)
z.height	Height (SD)
z.bmi	BMI (SD)
z.mssbmi	MssBMI (SD)
z.pub.voice	PubVoice.n (SD)
z.pub.shave	PubShave.n (SD)
z.pub.pub	PubPub.n (SD)
z.pub.ne	PubNE.n (SD)
z.pub.compos	pub.compos (SD)

Supplemental table 21. Description of hypotheses tested in the dataset for Magid K., Chatterton RT, Ahamed FU, Bentley GR, "Childhood ecology influences salivary testosterone, pubertal age and stature of Bangladeshi UK migrant men"

Hypotheses/tests	Prediction/design	Dependent variable	Covariates
1	*Cohorts separated by ethnicity and developmental exposure to ecological conditions will differ in salivary testosterone*		
1.1		z.log salivary testosterone	Residence groups (CHI ≤16), z.age, z.bmi
1.2		z.log salivary testosterone	z.age at migration, z.log.age, z.bmi
2	*Childhood migration leads to differences in salivary testosterone (analysis restricted to CHI ≤16)*		
2.1	*Continuous within CHI, including age and BMI*		
2.1.1		z.log salivary testosterone	z.age at migration, z.log.age, z.bmi
2.1.2		z.log salivary testosterone	z.age at migration, z.log.age, z.bmi(imputed)
2.2	*Continuous within CHI, including number of years in the UK and bmi*		
2.2.1		z.log salivary testosterone	z.age at migration, NumYearsUK, z.bmi
2.2.2		z.log salivary testosterone	z.age at migration, NumYearsUK, z.bmi(imputed)
2.3	*Cohorts split at age migration 8years, including age at recruitment and BMI*		
2.3.1		z.log salivary testosterone	CHI ≤8 versus CHI 9-16, z.log.age, z.bmi
2.3.2		z.log salivary testosterone	CHI ≤8 versus CHI 9-16, z.log.age, z.bmi(imputed)
2.4	*Cohorts split at age migration 8years, including number of years in the UK and BMI*		
2.4.1		z.log salivary testosterone	CHI ≤8 versus CHI 9-16, NumYearsUK, z.bmi
2.4.2		z.log salivary testosterone	CHI ≤8 versus CHI 9-16, NumYearsUK, z.bmi(imputed)
3	*Adult migration leads to differences in salivary testosterone (analysis restricted to ADU)*		
3.1	*Continuous within ADU, including number of years in the UK and BMI*		
3.1.1		z.log salivary testosterone	NumYearsUK, z.bmi
3.2	*Continuous within ADU split at age migration 40 years, including number of years in the UK and BMI*		
3.2.1		z.log salivary testosterone	ADU(<40 only)NumYearsUK, z.bmi
3.2.2		z.log salivary testosterone	ADU(>40 only)NumYearsUK, z.bmi

4	*Residence group characteristics lead to differences in salivary testosterone aging profile. Note: untransformed salivary testosterone to assess slopes in pg/ml per year, results with transformed units not shown, but not different in interpretation*			
4.1	*Age at recruitment continuous predictor of salivary testosterone across all populations*			
4.1.1		salivary testosterone	age at recruitment	
4.2	*Age of recruitment by salivary testosterone slopes significantly differ between residence groups*			
4.2.1		salivary testosterone	Residence groups, age at recruitment	
4.3	*Age at recruitment continuous predictor of salivary testosterone within residence groups (separate analysis within each group)*			
4.3.1		salivary testosterone	age at recruitment (repeated for SED, ADU, CHI, 2NG, EUR)	
4.4	*Age of recruitment by salivary testosterone slopes significantly differ between residence groups showing decline (all UK residence groups)*			
4.4.1		salivary testosterone	age at recruitment (analysis restricted to ADU, CHI, 2NG, EUR)	
4.5	*is there a sig effect of aging on salivary testosterone in UK-born men, when not considering residence group? If so, does this explain differences in waking salT found between 2NG and EUR*			
4.5.1		salivary testosterone	age at recruitment (analysis restricted to 2NG, EUR)	
4.5.2		salivary testosterone (adjusted for age-decline)	age at recruitment (analysis restricted to 2NG, EUR)	
5	*Cohorts separated by ethnicity and developmental exposure to ecological conditions will differ in recalled markers of age at puberty, including age at recruitment to adjust for demographic trends or recall bias*			
5.1	*Age at puberty differs by residence group*			
5.1.1		Recalled age at puberty measures	Residence group, z.log.age	
5.2	*Continuous age at childhood migration leads to differences in recalled age at puberty (analysis restricted to CHI ≤18)*			
5.2.1		Recalled age at puberty measures	AgeMigUK, z.log.age	
5.3	*Age at childhood migration leads to differences in recalled age at puberty between cohorts of CHI ≤18, split at age migration 8years*			
5.3.1		Recalled age at puberty measures	CHI ≤8 versus CHI 9-16, AgeMigUK, z.log.age	

5.4	*Continuous age at adult migration (<18.4) leads to differences in recalled age of migration, including age at recruitment (analysis restricted to ADU <18.4)*			
5.4.1		Recalled age at puberty measures	AgeMigUK, z.log.age	
6	*Men with higher adult salivary T recall earlier age at puberty, including age at recruitment to adjust for demographic trends or recall bias*			
6.1	*Across all groups, without separation by ethnicity or developmental exposure to ecological conditions*			
6.1.1		Recalled age at puberty	salivary testosterone, z.log.age	
7.1.1	*Restricting analysis within Bangladeshi men resident in the UK*			
7.1.2		Recalled age at puberty	salivary testosterone, z.log.age	
8.1.1	*Restricting analysis within men resident in Bangladesh*			
8.1.2		Recalled age at puberty	salivary testosterone, z.log.age	
9	Childhood age at migration is a predictor of adult height			
9.1	*Restricting analysis within child migrants*			
9.1.1		z.standing height	z.AgeMigUK	
9.1.2		z.standing height	z.AgeMigUK, z.log.age	
9.1.3		z.standing height	z.log.age	
9.1.4		z.standing height	NumYearsUK, z.AgeMigUK	
9.2.1		z.standing height	age.8.19.mig	
9.2.2		z.standing height	age.8.19.mig, z.log.age	
9.2.3		z.standing height	age.8.19.mig, NumYearsUK	
10	Adult age at migration is a predictor of adult height			
10.1	*Restricting analysis within adult migrants*			
10.1.1		z.standing height	z.AgeMigUK	
10.1.2		z.standing height	z.AgeMigUK, z.log.age	
10.1.3		z.standing height	z.log.age	