Rural to Urban Migration and Female Adolescent Stress in Mali

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Abstract

Rural to urban migration is a growing trend in much of the developing world; often this migration is accompanied by increases in blood pressure and other negative health outcomes. These changes are often attributed to the adoption of a "Western lifestyle" in urban areas. The aim of this study was to examine the possible effects of rural to urban migration on stress, and to explore potential contributions of stress to negative health changes seen after migration. This study focused on female adolescents undergoing rural to urban migration, an understudied population in migration studies. Subjects were adolescents enrolled in a 25-year prospective cohort study in the Dogon of Mali, some of whom remained in rural Bandiagara, and some of whom had migrated to the Malian capitol, Bamako. Hair cortisol concentrations, Perceived Stress Scale (PSS) scores, and systolic blood pressure (SBP) were used as stress and health-related outcomes, with measures of body mass index (BMI), percent body fat, age, breast stage, and wealth as predictor variables. Females living in Bamako had higher hair cortisol concentrations and SBP than females living in Bandiagara, but there was no significant relationship between rural or urban residence and PSS scores. Higher hair cortisol concentrations were also significantly associated with breast stage, while higher SBP was significantly associated with BMI and negatively associated with hair cortisol. These findings add to the literature concerning rural to urban migration, and support further investigation into the role of rural to urban migration on adolescent stress, particularly in regard to cortisol production.

Keywords: rural to urban migration, hair cortisol, blood pressure, stress

1. Introduction

In the developing world, it has become increasingly common for adolescents and families to leave rural areas to seek work, education, or other opportunities in urban centers. Urbanization is growing rapidly worldwide – in 1950, more than two-thirds of the global population lived in rural areas, but by 2050 this distribution is predicted to reverse, with two-thirds of the global population projected to live in urban areas (United Nations Population Division, 2014). In Mali, 39% of the population resides in urban areas, and this number is expected to rise to 60% by 2050 (United Nations Population Division, 2014). Urban living is often accompanied by greater education and literacy, better access to healthcare, longer life expectancy, poverty reduction, and greater economic opportunity, but rapid urbanization also poses many challenges for infrastructure, health and social services, policy-making, and sustainability (United Nations Population Division, 2014).

In addition, migration to urban centers is also often accompanied by a nutritional transition to more westernized diets, high in saturated fats, simple sugars, energy-dense foods, and low fiber, as well as a decrease in physical activity (Misra & Ganda, 2007). These transitions can increase risk for high blood pressure, cardiovascular disease, obesity, metabolic syndrome, and diabetes (Misra & Ganda, 2007). In addition to changes in diet and activity, many studies have attributed increasing blood pressure with migration, in part, to psychosocial stresses related to acculturation and loss of social support (Steffen, Smith, Larson, & Butler, 2006).

Changes in stress can have far-reaching impacts, from chronic and cardiovascular diseases to illness susceptibility and mental health (Cohen & Williamson, 1991; Misra & Ganda, 2007; Staufenbiel, Penninx, Spijker, Elzinga, & van Rossum, 2013; Steffen et al., 2006). As such, understanding potential changes in stress within a rapidly urbanizing population can offer valuable information for future public health efforts. It is especially important to understand the effects of urbanization on adolescents to prevent or mediate future negative health outcomes before they escalate.

This study aims to further elucidate the effects of rural to urban migration on stress and also bridge gaps in the literature regarding the effects of this migration on adolescents and females specifically. This study is novel in that it involves a longitudinal study of adolescents from the same region and ethnic group, allowing direct comparisons between health measurements of these same adolescents before and after migration to urban centers. While these analyses reflect preliminary results from the first field year of this study, direct comparisons over time of individuals who have undergone this migration will provide much more insightful results than cross-sectional studies can provide. Through analysis of hair cortisol concentrations, perceived stress, and systolic blood pressure, this study examines the effects of rural to urban migration on Dogon adolescent females in rural Bandiagara and urban Bamako, Mali.

2. Materials and Methods

2.1 Study Population and Collection Sessions

This study was conducted as part of a prospective cohort study of health and human biology by Dr. Beverly I. Strassmann in the Dogon of Mali. This study began in May 1998, enrolling all children under the age of 5 years in 9 rural villages, as well as all children born in these villages from 1998-2000 (Strassmann, 2011). Data used in this analysis was collected in urban Bamako, Mali and rural Bandiagara, Mali in 4 data collection sessions in the 2013-2014 field year. 566 females between the ages of 13 and 20 years of age and 634 males between the ages of 13 and 21 years of age appeared for data collection sessions, some appearing for collection at multiple sessions. At these data collection sessions, participants provided a variety of measurements, including height, weight, triceps, iliac and subscapular skin folds, waist circumference, hair and saliva samples, reproductive and lifestyle surveys, and the Global Perceived Stress Scale. More ethnographic information concerning the study population and methods of data collection throughout this prospective study can be found in Strassmann 2000 and Strassmann 2011.

In these analyses, only data from female subjects were used. Because this was the first field year in which hair samples were collected, and hair sample collection began late in the field year, due to the schedule of sample collection far fewer hair samples from males in Bamako were collected compared to females in Bamako. Thus in order to use hair cortisol concentrations in these analyses, only female subject data were used.

2.2 Collection Methods and Measurements

Global Perceived Stress Scale

The 14-Item Perceived Stress Scale (PSS) was first validated by Cohen et al. in 1983, and was later modified to the 10-Item PSS when it was determined that the 10-Item PSS showed more internal reliability (Cohen, Kamarck, & Mermelstein, 1983; Cohen & Williamson, 1988). This self-reporting, standardized questionnaire is used to measure the degree to which individuals perceive their lives as "unpredictable, uncontrollable, and overloaded" (Cohen & Williamson, 1988). The PSS has been previously translated into, and administered in, at least 19 languages with good success (Lesage, Berjot, & Deschamps, 2012). For this study, Cohen's 10-Item PSS was translated into French and then into Dogon by Dr. Strassmann and her field team. The scale was administered verbally in French or Dogon (depending on subject preference) and modified to a "Yes" or "No" answer format instead of the usual 0-4 Likert Scale to avoid confusion, especially among non-literate study participants.

Parent Wealth Z-Score

This variable was used to control for subject wealth. Villages were censused in 2000 and again in 2011 (the 2011 score was used for this analysis), as family wealth remains very stable from year to year. The score was determined by four to five members from each village, who independently ranked each family in their own village in three relative categories: 1 (wealthy), 2 (average), and 3 (poor). They then split each category into two further groups to form six total wealth groups. Each "judge" ordered each family in the village from wealthiest to poorest, including his or her own family. Rankings were very similar between judges, and any discrepancies were addressed in a meeting of all judges from the same village to

arise at a consensus ranking. These consensus rankings were then transformed into Z-scores to normalize the data.

Anthropometric Data

Subjects were weighed to an accuracy of 0.1 kg using the Tanita BWB-800A scale. Percent body fat was measured through bioelectrical impedance using the Tanita SC-3331S Body Composition Monitor to an accuracy of 0.01%. Triceps, iliac and subscapular skinfolds were measured to an accuracy of 1 mm using the Complete Medical Supplies SKU 3175 large skinfold caliper. Buttocks and waist circumference were measured to an accuracy of 1 cm using a flexible tape measure, with waist circumference measured at the smallest circumference below the last rib. Height was measured to an accuracy of 0.01 cm using the Perspective Enterprises PE-AIM-101 portable measuring unit, and was measured in triplicate with repeated measures averaged.

Blood Pressure

Systolic and diastolic blood pressure were measured using recommendations from the National Institutes of Health's Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents (The Fourth Report 2005). Blood pressure was measured on the right arm using Omron blood pressure monitors, Littman pediatric and adult stethoscopes, and four different cuff sizes. Ambient temperature was recorded at the time of each measurement.

Hair Sample Collection

Hair samples of up to five strands of hair from the cranial vertex and/or nape of the neck were collected from each participating subject. Hair was cut with scissors as close as possible to the scalp, and from this sample up to 3 cm from the scalp was preserved. These samples were placed in numbered test tubes and frozen at -20 °C in the field, and in the laboratory at -80 °C until analysis.

2.3 Hair Cortisol Assay

The cortisol extraction protocol used was adapted from a protocol described by Meyer, et al (J. Meyer, Novak, Hamel, & Rosenberg, 2014). In addition, to ensure consistency between samples, only hair samples from the cranial vertex were assayed in this analysis (J. S. Meyer & Novak, 2012).

Hair samples were thawed at room temperature for 30 minutes. After thawing, samples were transferred to 2 mL microcentrifuge tubes and washed in 667 μL HPLC grade isopropanol using constant inversion on the Labnet Revolver Adjustable Rotator (H5600-02) for 3 minutes. The isopropanol was decanted, samples were washed again using the same process, and isopropanol was again decanted. Samples were dried for 1 hour on medium heat using the Fisher Savant DNA Speedvac (DNA120-115). Hair was then transferred to pre-weighed 2 mL microcentrifuge tubes reinforced for bead beating and cut finely with scissors. Tubes containing these cut hair samples were then re-weighed to obtain sample weights and 3 3.2 mm chrome steel beads were added to each tube. Samples were ground for 3 minutes in the BioSpec Mini-Beadbeater 16 (Cat. No. 607); if hair appeared to be insufficiently ground, samples were ground for an additional 1.5 minutes. 1.5 mL HPLC grade methanol was added to each tube containing ground hair, and tubes were capped and incubated for 20-24 hours at room temperature using constant inversion on the Labnet rotator. Tubes were then centrifuged at 10,000 rpm using the Fisher accuSpin Micro 17 (13-100-

675) for 10 minutes at room temperature. 1.0 mL of the supernatant was transferred to a clean 1.5 mL microcentrifuge tube, taking to care to avoid disturbing the pellet. The methanol in the supernatant was then dried down for 1 hour on medium heat in the Fisher Speedvac, or until all methanol had evaporated.

After methanol removal, the cortisol extract was reconstituted in 0.1 mL assay diluent from Salimetrics' Salivary Cortisol ELISA Kit (1-3002-5) followed by strong vortexing for 10 seconds or until the pellet appeared to dissolve. Reconstituted samples were placed in a water bath at 37 °C for 30 minutes, followed by vortexing to ensure the sample was thoroughly mixed. After returning to room temperature, samples were frozen at -80 °C for later analysis. Before assays, samples were thawed to room temperature for 1 hour and then re-mixed using the vortex and water bath process previously described. The ELISA assay was performed using Salimetrics' kit instructions, using the Labline Titer Plate Shaker (4625), Molecular Devices EMax Microplate Reader, and Molecular Devices SoftMax Pro 6 Software (SMP6). Samples were run in duplicate, with standard curves run antiparallel on either end of the plate. Plates were read at 450 nm with a 490 nm reference filter, in accordance with kit recommendations, and concentrations were calculated using a 4 parameter curve fit. Using the calculation described by Meyer, et al., assay output was converted to amount of cortisol per unit weight of hair using the following formula (Meyer et al. 2014):

$$\frac{A}{B} \times \frac{C}{D} \times E \times 10{,}000 = F$$

Where A = assay output (in µg cortisol/dL sample), B = weight of hair ground (in mg), C = volume of methanol added to powered hair (1.5 mL), D = volume of methanol recovered from extraction and dried down (1.0 mL), E = volume of assay diluent used to reconstitute cortisol extract (0.1 mL), and F = hair cortisol concentration (in pg cortisol/mg hair).

2.4 Statistical Analyses

All analyses were performed using IBM's Statistical Package for the Social Sciences (SPSS) software (Version 22). ANOVA analyses were conducted with rural or urban status as a fixed factor, and hair cortisol concentrations, PSS Scores, and systolic blood pressure (SBP) as dependent variables. Other variables tested as covariates include age, percent body fat, waist circumference, BMI, parental wealth, and Tanner Breast Stage. All continuous covariates were centered by subtracting the 2013 mean for each variable from each subject's observed measurement. P-values < .05 were considered statistically significant. Backward regressions were performed until the model with highest predictive quality and normality (as determined by adjusted R squared values and Q-Q plots of studentized residuals, respectively), and which lacked high collinearity, was found. Collinearity was determined using VIF scores; variables with VIFs > 2.5 were considered collinear.

3. Results

3.1 Hair Cortisol Concentrations

Of all females in this study for whom hair cortisol concentrations were obtained (N=158), the mean cortisol concentration was 51.22 pg cortisol/mg hair (SD=23.32) with a range of 10.52 pg cortisol/mg hair - 161.54 pg cortisol/mg hair. In an ANOVA analysis with parent wealth, Tanner Breast Stage (as a marker of level of puberty), and perceived stress (as determined by the PSS) covariates, females living in rural areas had significantly lower levels of cortisol (p=0.008) than females living in Bamako (Figure 3A). In this model, residence in Bamako was accompanied by a 13.996 pg cortisol/mg hair rise in cortisol concentrations (Figure 3A). Tanner Breast Stage was also a significant predictor of cortisol levels (p=0.009). Variables including age (p=0.694, VIF=2.548), BMI (p=0.470, VIF=2.861), waist circumference (p=0.784, VIF= 2.885), and percent body fat (p=0.975, VIF=1.694) were also considered in the model, but were removed due to lack of significance and/or collinearity. Additional models for hair cortisol concentration can be found in Supplementary Figure 1.

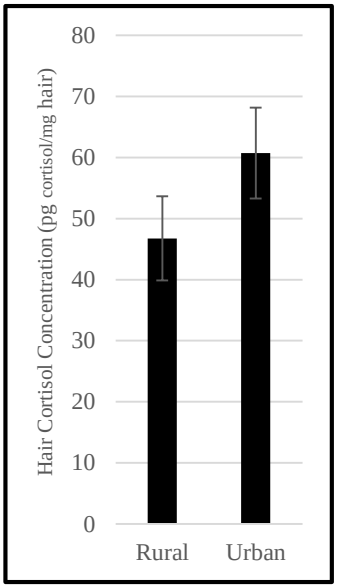

Figure 1. Comparison of the Mean Hair Cortisol Concentrations in Rural and Urban Female Populations. Error bars represent 95% confidence interval.

3.2 Global Perceived Stress Scale Scores

PSS scores range from 0-10, with higher scores indicating higher levels of perceived stress. The mean PSS score for all females in this analysis (N=157) was 3.31 (SD=1.88), with a range of 0-10. In this analysis, breast stage (p=0.456, VIF=2.476) as well as BMI (p=0.704, VIF=2.508) and percent body fat (p=0.464, VIF=1.526) were removed for lack of significance and/or collinearity, but rural or urban residence, centered cortisol levels, parental wealth, and age were included in the model. Rural or urban residence did not have a significant effect on PSS scores in this model, although centered age was found to be a significant predictor, showing slightly lower PSS scores with increasing age (p=0.007) (Figure 3B). Additional models for PSS scores can be found in Supplementary Figure 2.

3.3 Systolic Blood Pressure

SBP for females in this study (N=155) ranged from 73 - 154 mm Hg with a mean SBP of 105.62 mm Hg (SD=13.53). Measurements used in this study tended to be more predictive of SBP; in regressions with hair cortisol concentration, PSS scores, age, parent wealth, temperature during blood pressure measurement, and BMI covariates, females living in rural areas had significantly lower SBP (p=0.001) than females living in Bamako (Figure 3C). SBP in the urban group was 10.798 mm Hg higher than in the rural group (Figure 3C). In addition, hair cortisol concentration (p=0.013) and BMI (p=0.001) were also significant predictors of SBP (Figure 3C).

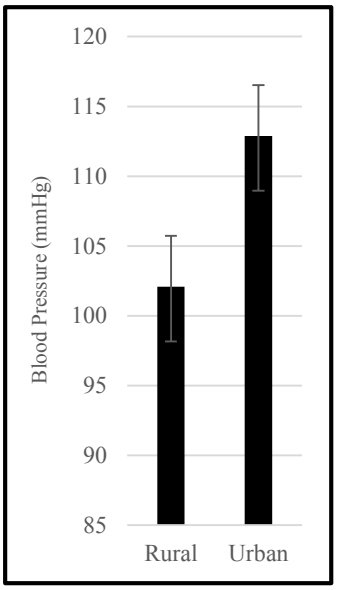

Figure 2. Comparison of the Mean Blood Pressures in Rural and Urban Female Populations. Errors represent 95% confidence interval.

Breast stage (p=0.511, VIF=2.800) and percent body fat (p=0.940, VIF=2.647) were also considered, but were removed due to lack of significance and high collinearity. Additional models for SBP can be found in Supplementary Figure 3.

Figure 3. Analyses of Hair Cortisol Concentration, PSS Scores, and SBP between Rural and Urban Female Populations

A. Dependent Variable: Cranial vertex hair cortisol concentration (pg cortisol/mg hair) in 2013.

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	38.624	9.012	4.286	.000	20.720	56.528
Centered PSS Score	1.339	1.454	.921	.360	-1.550	4.228
Tanner Breast Stage	6.124	2.298	2.664	.009	1.558	10.690
Parent Wealth	3.051	3.017	1.011	.315	-2.943	9.044
Not Living in Bamako	-13.966	5.166	-2.704	.008	-24.228	-3.703
Living in Bamako (ref)	0	.	.	.	.	.

R Squared = .153 (Adjusted R Squared = .115)

Total N = 95, Living in Bamako = 44, Not Living in Bamako = 51

B. Dependent Variable: Global Perceived Stress Scale scores in 2013.

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	3.295	.326	10.111	.000	2.647	3.942
Centered Cortisol Concentration	.005	.007	.676	.501	-.010	.019
Centered Age	-.293	.106	-2.768	.007	-.503	-.083
Parent Wealth	-.328	.216	-1.519	.132	-.756	.101
Not Living in Bamako	.069	.439	.158	.875	-.803	.942
Living in Bamako (ref)	0	.	.	.	.	.

R Squared = .153 (Adjusted R Squared = .115)

Total N = 95, Living in Bamako = 44, Not Living in Bamako = 51

C. Dependent Variable: Systolic blood pressure in mm Hg in 2013.

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	109.378	2.452	44.599	.000	104.502	114.255
Centered Cortisol Concentration	-.116	.045	-2.544	.013	-.206	-.025
Centered PSS Score	.003	.652	.005	.996	-1.293	1.299
Centered Age	.919	.805	1.141	.257	-.682	2.519
Parent Wealth	2.303	1.365	1.687	.095	-.411	5.018
Centered Temperature	.852	.520	1.636	.105	-.183	1.886
Centered BMI	1.279	.489	2.615	.011	.307	2.252
Not Living in Bamako	-10.798	3.086	-3.499	.001	-16.933	-4.662
Living in Bamako (ref)	0	.	.	.	.	.

R Squared = .476 (Adjusted R Squared = .433)

Total N = 93, Living in Bamako = 44, Not Living in Bamako = 49

4. Discussion

Although the effects of rural to urban migration on adolescent stress are not very well studied, some results of this study support a number of findings in the literature for adolescent and adult stress, and other results proved surprising. While further work is currently underway to expand the initial findings of this study, these preliminary results raise important questions for adolescent health with increasing urbanization.

4.1 Effects of Urban Environments on Female Adolescent Stress and Blood Pressure

Adolescent females living in the capitol of Mali had significantly higher concentrations of hair cortisol, and also had significantly higher blood pressure, than their counterparts living in rural Mali. However, rural or urban residence appeared to have no significant effect on PSS scores.

Current Literature

Few studies have examined the effects of urban or rural residence on adolescent cortisol specifically, although some studies have looked at this interaction in adult populations. A recent prospective, cross-sectional study by Kann et al. found that the Ovahimba people of Namibia living in urban areas have higher salivary cortisol than Ovahimba people living in rural areas, and hypothesized that psychosocial stress caused by urbanization and loss of social support, as well as behavioral, nutritional, and lifestyle changes, may have contributed to these differences (Kann et al., 2015). Similar results were found in cross-sectional studies of blood and salivary cortisol in rural and urban males in South Africa and Botswana, although one study of South African males and females showed higher cortisol in rural females compared to urban females (Decker, 2006; Huisman et al., 2002; Malan et al., 2012). While the results of our study seem to support some similar findings in the literature, it is clear that more work needs to be done to understand the relationship between cortisol levels and rural or urban residence, especially among females and adolescents. In addition, these studies were all cross-sectional and involved potentially more heterogeneous populations in comparison to our prospective, longitudinal study, allowing our study to offer more controlled results.

The relationship between rural and urban residence and blood pressure is much more widely studied. Six studies comparing rural and urban Nigerians, Namibians, Inuit, Samoans, South Africans, Peruvians, and Cameroonians all found higher SBP in urban women, compared to rural women (Bjerregaard, Jørgensen, Lumholt, Mosgaard, & Borch-Johnsen, 2002; Hanna, 1996; Kann et al., 2015; Kaufman, Owoaje, Rotimi, & Cooper, 1999; Miranda, Gilman, & Smeeth, 2011; Sobngwi et al., 2002; van Rooyen et al., 2000). One cohort migration study in Tanzania did find decreased SBP in urban migrant women, although subjects were only monitored for three months after migration, which could limit their findings (Unwin et al., 2010). One study in Guatemala found no significant change in SBP between rural and urban migrants (Torun et al., 2002). However, none of these studies were conducted specifically within an adolescent population. A review by Hernandez, et al. found that nine out of twelve studies of rural to urban within-country migration cardiovascular risk found higher SBP in migrants than in their rural counterparts, with five out of six studies finding SBP lower in migrants than in born-and-raised urban dwellers (Hernandez, Pasupuleti, Deshpande, Bernabe-Ortiz, & Miranda, 2012). Another larger review of 125 studies, conducted by Steffen, et al., investigated the relationship between acculturation to western society (including western language acquisition, migration, and/or increased exposure to “industrialization,

modernization, and urbanization") and blood pressure. This review found a consistent relationship between increasing acculturation and higher blood pressure, independent of world region (Steffen et al., 2006). While this relationship is fairly well-studied and the data predict an increase in blood pressure with rural to urban migration, our study is novel in its focus on adolescents.

While the PSS is widely used in psychological research, comparisons of rural and urban perceived stress are rare in the literature. A study by Kaufman et al. in 1999 did use this scale in an analysis of blood pressure determinants in rural and urban Nigerian populations. His team found mean PSS scores were higher in the urban population than in the rural population (Kaufman et al., 1999). Another study of South African adolescents in an urban secondary school showed that adolescents who had been raised in rural environments had lower levels of stress than adolescents who had been raised in a city (Spangenberg & Henderson, 2001). While the relationship between the PSS and rural to urban migration specifically is not well studied, current literature predicts that females in this study living in Bamako would have higher perceived stress. However, levels of perceived stress showed no significant relationship to rural or urban residence in our study; this may provide an interesting new perspective into perceived stress in adolescents undergoing rural to urban migration, although more analysis is needed to determine whether our unique method of PSS administration accurately represented perceived stress in this population.

How Could Urban Environments Increase Cortisol Levels?

Cortisol is an indicator of both psychosocial and physiological stress, so increases in hair cortisol in urban environments could be attributed to multiple stressors (Staufenbiel et al., 2013). Possible contributing factors to increased cortisol in urban environments include increased psychosocial stress, increased body fat, and increased exposure to pollution and infectious disease. This study examined the effects of rural to urban migration on increased body fat as well as some aspects of psychosocial stress, and this section will explore the relationship between cortisol and other potential stressors as well.

Although we did not study rates of infection or exposure to pollution, these environmental factors could contribute to cortisol levels. Cortisol levels rise with infection, and large urban environments increase risk of infection through increased contact rates, mobility, and "heterogeneity of health" among people living in urban areas (Alirol, Getaz, Stoll, Chappuis, & Loutan, 2010; Silverman, Pearce, Biron, & Miller, 2005). Population density is also a significant predictor of emerging infectious diseases (K. E. Jones et al., 2008). In addition, the relationship between cortisol and pollution has also been explored. One study found that policemen who worked outdoors in an urban environment and were exposed to more pollutants had higher levels of cortisol than policemen who worked indoors and were exposed to less pollutants (Rosati et al., 2011). A recent study found inhalable particulate matter concentrations in Bamako exceeded international twenty-four hour air quality limits for 58-95% of days (depending on the guideline used) in a nine month period between 2012 and 2013, which may put Bamako residents at risk of inflammation or disease (Garrison et al., 2014). However, in rural Mali, poor water quality and decreased access to healthcare can also put Bandiagara residents at risk of infection as well, so quantifying the comparative risk of pollution and infection between Bamako and Bandiagara is difficult (Plate, Strassmann, & Wilson, 2004). It is possible that in the city, exposure to new diseases, increased probability of exposure to disease, and increased exposure to pollutants may increase cortisol levels of females living Bamako, although the comparative risks between these environments are not easy to determine.

Rural to urban migration is often seen with increases in BMI and body fat, a trend that is also seen in this study population. After controlling for age, females in Bamako have significantly higher BMI, body fat, and waist circumference ($p<0.001$ for all measures) compared to females living in Dogon villages (Supplementary Figure 4). Surprisingly, we did not find that body fat, BMI, or waist circumference were significant predictors of cortisol levels. The current literature offers a contradictory view of the relationship between anthropometric measures and cortisol. Cortisol is secreted by the adrenal glands, under control of the hypothalamic-pituitary-adrenocortical (HPA) axis, but can also be generated in adipose tissue (especially visceral adipose tissue, or abdominal fat), where inert cortisone can be cleaved to form cortisol (Stimson et al., 2009). Thus it would be expected that increasing body fat may increase cortisol levels.

Some studies have found a relationship between anthropometric measures and cortisol, with Stalder et al. finding a positive relationship between BMI and cortisol in adults, and Keiss et al. finding this same relationship with both body weight and BMI in children (Kiess et al., 1995; Stalder et al., 2012). However, in another study by Weigensburg et al., no significant relationship was found between waist circumference and cortisol in overweight adolescents (Weigensberg, Toledo-Corral, & Goran, 2008). To confuse this relationship further, both Moyer et al. and Epel et al. found that an increased waist-to-hip ratio increased cortisol in women in response to stressful lab tests, but Jones et al. and Rutters et al. found the inverse relationship between cortisol and body fat distribution in response to stress (Epel et al., 2000; A. Jones et al., 2012; Moyer et al., 1994; Rutters, Nieuwenhuizen, Lemmens, Born, & Westerterp-Plantenga, 2010).

While this relationship between cortisol and anthropometric measures is still not well understood, a recent review by Tchernof and Després suggests that it may be a sort of positive feedback relationship. Mouse studies, as well as studies of patients with hypercortisolism, shows that increased cortisol can lead to increased body fat accumulation (Tchernof & Després, 2013). This increase in adiposity causes an increase in the enzymatic activity of 11β-HSD-1, which cleaves cortisone to produce more cortisol; this continues to drive increased fat accumulation and maintains this positive feedback loop (Tchernof & Després, 2013). It may be that the relationship between cortisol and body fat is not immediate, especially in young, lean subjects. Only 29 out of 558 females in this study in 2013 (5.2%) had a BMI greater than 25, the CDC's cutoff for overweight or obesity classification (Centers for Disease Control and Prevention, 2003). While there may not have been a significant relationship between cortisol and body fat in this population now, increased cortisol levels seen in females in Bamako may contribute to increasing body fat over time. Increasing fat over time may start this feedback loop of increasing cortisol and increasing fat in urban populations. In addition, cortisol is linked to the accumulation of metabolically unhealthy visceral fat, which could set up risk for metabolic syndrome in these urban populations as well (Tchernof & Després, 2013). Although the results of this study did not further clarify the relationship between cortisol and adiposity, follow up studies over time may add valuable insight to this relationship in the long term.

A final aspect of the urban environment that may contribute to increased cortisol levels is psychosocial stress. Noise, crowding, acculturation, changes in social support, occupational or educational stresses, etc. are all potential causes of increased psychosocial stress in urban environments. Although this analysis showed no relationship between cortisol and PSS scores, some chronic psychosocial stressors like noise, occupational or educational stresses have been shown to increase cortisol levels in other studies (Evans,

Lercher, Meis, Ising, & Kofler, 2001; Ising, Lange-Asschenfeldt, Moriske, Born, & Eilts, 2004; Staufenbiel et al., 2013). More on the relationship between the PSS and cortisol levels will be discussed in Section 4.3.

In conclusion, exposure to pollutants and infectious disease, body fat, and psychosocial stress could all contribute to increased cortisol levels seen in urban females in this study. More analysis should be done to study exposure to pollution, rates of disease, exposure to noise, and other potential stressors to further elucidate the relationship between the urban environment and increased cortisol levels in adolescent females.

How Could Urban Environments Raise Blood Pressure?

The factors which contribute to higher blood pressure among urban dwellers are far better studied. Our study examined the relationship between SBP and BMI, hair cortisol concentration, and PSS scores, although other factors that could contribute to increased blood pressure in urban environments include changes in physical activity and diet as well as other forms of psychosocial stress.

A positive relationship between BMI and blood pressure is often observed, as was also found in our study (van Rooyen et al., 2000). Although the mechanism by which increased BMI increases blood pressure is not entirely clear, it is thought to involve altered kidney function, resulting in changes in natriuresis, thereby increasing cardiac output and blood pressure (Must & Strauss, 1999; van Rooyen et al., 2000).

Migration to urban environments is often also accompanied by decreased levels of physical activity, which can both increase BMI and raise blood pressure. Exercise can lower blood pressure even in normotensive individuals, and in studies where levels of activity in rural areas are high (as is the case in this population), decreased activity in urban environments has proven to be a significant predictor of increasing blood pressure and/or BMI (Kaufman et al., 1999; Sobngwi et al., 2002; Whelton, Chin, Xin, & He, 2002). Although information about levels of physical activity were not collected for both rural and urban females in the 2013 field year, collection of this data is currently underway in the field.

Certain changes in diet have also been linked to increasing blood pressure, especially pertaining to electrolyte intake. Consuming high levels of sodium, as is common in a Western diet, have been linked to increased blood pressure (Appel et al., 2006; van Rooyen et al., 2000). Consuming high levels of potassium works counter to sodium consumption and is linked to reduced blood pressure; this effect has been especially prominent in people of African descent (Appel et al., 2006). Sodium and potassium consumption are difficult to track and measure, but can be monitored through urine excretion, as was done by Kaufman et al. in a Nigerian rural-urban comparison (Kaufman et al., 1999; van Rooyen et al., 2000). This study found that the sodium/potassium ratio was higher in urban populations, largely due to decreased consumption of fruits and vegetables in urban populations (Kaufman et al., 1999). However, a study of rural to urban migration in Tanzania found lower blood pressure and higher consumption of fruits and vegetables in urban migrants (Unwin et al., 2010). It is likely that food availability in urban environments and food preferences of urban migrants play an important role in blood pressure, and should be further studied in adolescents undergoing rural to urban migration.

Increasing psychosocial stress not only increases cortisol levels, but also contributes to rising blood pressure. Urbanization is linked to increased activity of the sympathetic nervous system, likely a result of

psychosocial stress, which can increase blood pressure (van Rooyen et al., 2000). Social support has been found to play an important role in blood pressure levels, especially in women, in rural-urban comparison studies of Nigerian, Samoan, and South African populations (Hanna, 1996; Kaufman et al., 1999; van Rooyen et al., 2000). Social relationships have been linked to decreasing stress, potentially through emotional or material support, but can also prove stressful if these relationships are strained (Hanna, 1996). Studies of young Samoan migrants by Hanna have revealed that young women are more responsive to emotional and material support than their male counterparts, and that social support and relationships are important in lowering blood pressure in migrant and acculturating women (Hanna, 1996). Relocation to an urban environment can shrink social contact networks, and urban populations are often associated with smaller networks and increased individualism, which can contribute to stress levels (Steffen et al., 2006). Although social relationships and support were not examined in this study, further investigation into the dynamics of social relationships in urban and rural environments for these adolescents should be conducted.

Cortisol was also a significant predictor of SBP in this study. Exposure to high levels of stress and HPA axis activity can create a prolonged biological stress response, which results in high blood pressure and heart rate (Barksdale, Woods-Giscombe, & Logan, 2013). Cortisol can increase in urban environments due to many factors discussed above, and contribute to increased blood pressure. Surprisingly, this study found that increased cortisol concentration in hair actually predicted lower SBP, although this difference was small ($B= -0.116$, $p=0.013$, $N=93$). These results are surprising, although a recent review found that, while one study found a positive correlation between hair cortisol concentration and blood pressure, two other studies found no significant correlation (Wosu, Valdimarsdóttir, Shields, Williams, & Williams, 2013). This study therefore contributes valuable information to the understudied relationship between hair cortisol concentrations and SBP, and analysis of more hair samples from this population in the future will further elucidate this relationship.

In conclusion, increased BMI, physical activity and dietary changes, and increased psychosocial stress could all contribute to the increase in SBP seen in rural to urban migrants in this study. Further investigation into possible dietary changes between rural and urban Dogon populations, as well as levels of social support in each of these environments, could enhance understanding of the changes in blood pressure seen with rural to urban migration in these adolescents.

How Is Rural to Urban Migration Related to Perceived Stress?

As discussed above, the literature has yet to provide a clear answer to this question. The results of this study found no significant difference between adolescent female PSS scores in Bamako and Bandiagara, with only age predicting PSS scores. While not directly studying the PSS, a review by Bhugra explored the relationship between migration and stress. This review found that migration is often accompanied by psychosocial stress, but is an individual experience mediated by support systems, education, age, occupation, and individual psychology and coping mechanisms (Bhugra, 2004). Other studies have also cited the importance of social support in mediating acculturation and migration-related stress responses as well, especially in female subjects (Hanna, 1996; van Rooyen et al., 2000) It is likely that perceptions of the migration experience are individual, and are mediated by personal experiences, resources, opportunities, and psychology. It is also possible that, in this population, rural to urban migration may not be perceived as stressful. There was little difference between the mean PSS scores of the rural and urban

groups in our study, so it is possible that the perceived stress of all of these subjects are much more strongly affected by factors other than rural or urban residence. However, it is also possible that the method of PSS administration used in this study may not have provided the most accurate measure of perceived stress in this population.

Conclusions

In sum, there are many factors which could contribute to the increases in hair cortisol concentration and SBP seen following rural to urban migration in this study. Increased body fat and BMI could be contributing factors to cortisol levels and blood pressure respectively. While the relationship between cortisol and body fat is still not clear, we did not find a significant relationship in this study, and follow up studies may provide more insight into this relationship. BMI is commonly positively linked to blood pressure, as was also seen in this study.

Psychosocial stress is also a contributing factor to both cortisol levels and blood pressure. While we did not see a relationship between PSS scores and these outcomes, other possible stressors including noise, crowding, loss of social support, acculturation, etc. have been linked to increasing cortisol and blood pressure, and could be studied in the future. No significant relationship between PSS scores and rural or urban residence was found in our study. These results may indicate that this migration is not perceived as stressful by this population, that other factors are far more important in determining levels of perceived stress in these adolescents, and/or that the dichotomous format or verbal PSS administration used in this study did not accurately measure perceived stress.

Hair cortisol concentration was also a significant predictor of SBP, however increasing cortisol predicted slightly lower SBP, which is not seen in the literature. Analyzing hair samples from this population in future years will help to determine whether this effect persists, and if so may prove an interesting contribution to the hair cortisol literature.

Other factors that may contribute to cortisol, perceived stress, and blood pressure in urban environments and were not included in the study include exposure to pollution and infectious disease and dietary changes. Further investigation into these factors could also add to understanding of the effects seen in this study.

4.2 The Effects of Age and Puberty on Female Adolescent Stress and Blood Pressure

In this analysis, breast stage was a significant predictor of hair cortisol concentration in Dogon adolescent females, with increasing breast stage associated with increasing hair cortisol concentration. This positive association with breast stage around puberty has support in the literature, although all of the studies that could be found measured either salivary or urinary free cortisol (Kang et al., 2014; Kiess et al., 1995; Legro, Lin, Demers, & Lloyd, 2003). Both Kang et al. and Kiess et al. studied salivary cortisol levels, in a premenarcheal pubertal Korean population and in an infant to adult German population respectively; both found increasing salivary cortisol levels with increasing pubertal stage (Kang et al., 2014; Kiess et al., 1995). Legro et al. studied urinary free cortisol in perimenarcheal Caucasian American adolescents, and also found increasing cortisol concentration/body surface area with each Tanner Stage (Legro et al., 2003). However, Netherton et al. studied salivary cortisol in British adolescents and did not find a significant difference in cortisol levels between girls in early stage (Tanner Breast Stage < III) and mid-

late stage (Tanner Breast Stage > II) of puberty (Netherton, Goodyer, Tamplin, & Herbert, 2004). No studies examining the effect of puberty on adolescent female cortisol levels in developing countries were found, and no studies examining the effects of puberty on hair cortisol concentrations were found. This study is unique in this regard; because hair cortisol concentration offers insight into more chronic, rather than current, HPA axis activity, hair cortisol may provide a more nuanced readout of HPA axis activity throughout puberty.

Age was the only significant predictor of PSS scores in this study. The findings of this study are inconsistent with findings in a recent review of the PSS, which found lower PSS scores among younger subjects (Lee, 2012). As occupational and familial responsibilities increase with age, it is intuitive that perceived stress would also increase. However it may be that coping mechanisms also increase with age, and/or that older girls in this study perceive stressors as less stressful than younger girls. More work should be done to determine the causes of this trend. Age was not a significant predictor of SBP in this study, but is an important control. Increasing blood pressure is linked to growth and maturation, although these subjects were of similar ages, which may have made the effect of age less important than other factors such as BMI (Kotchen, McKean, & Kotchen, 1982).

In summary, level of puberty is a significant predictor of hair cortisol concentrations in this population, and other studies of salivary or urinary free cortisol have largely found similar effects. However, this study is unique in its use of hair cortisol and also in its focus on adolescents in developing countries. Age was a significant predictor of PSS scores, although the results of this study found a relationship inverse to that found in the literature. However, age was not a significant predictor of SBP, likely due to a small subject age range and increased importance of other factors in determining blood pressure.

4.3 The Relationship between Perceived Stress and Cortisol and PSS Measure Validation

The use of hair cortisol as a measure of chronic stress is an emerging technique, first studied in 2004; it provides a measure of long-term stress exposure and is less susceptible to individual differences in circadian rhythm (Staufenbiel et al., 2013). A recent review by Staufenbiel et al. found that, of eleven studies examining the relationship between PSS scores and hair cortisol concentrations, two found a positive correlation, seven found no significant relationship, and two found a negative correlation (Staufenbiel et al., 2013). While more studies need to be conducted, the relationship between these two measures appears to be weak.

It may be that hair cortisol and the PSS are both valid indicators of stress, but the two measures assess different aspects of individual stress. The PSS asks subjects about their perception of stress in the past month, while hair cortisol offers a view of cortisol levels over a longer time period. Hair grows approximately one centimeter per month, so a three centimeter sample could potentially provide a measure of hair cortisol levels over the past three months (Staufenbiel et al., 2013). An individual's current stress level may be very different in one month compared to his or her stress levels over a longer period. In addition, because discrepancies have also been found between salivary and plasma cortisol and perceived stress, it has been proposed that there could be a "lack of psychoendocrine covariance" (J. S. Meyer & Novak, 2012). This hypothesis suggests that there could be a difference in HPA axis responses to stress and the subjective stress experience (J. S. Meyer & Novak, 2012). Used together, the PSS and hair cortisol concentrations could offer a more holistic view of stress: recent and long-term, subjective and biological.

This study administered the PSS in a unique way, with answers in a yes/no format and verbal response to accommodate varying levels of subject literacy. Although subjects were taken aside to answer the PSS questions, it is possible that the verbal format may have prompted some subjects to give more restrained answers. However, in this study no significant correlation was found between hair cortisol and PSS scores in female subjects in 2013 (Pearson correlation=0.007, p=0.930, N=157). These results echo what has been found in the majority of the current literature, so this unique method of PSS administration may be a valid and useful way to assess perceived stress in younger populations with diverse levels of literacy. Other methods of perceived stress measurement, including focus groups or life events surveys, could be used to determine the accuracy of PSS scores for reported levels of perceived stress. However, hair cortisol is still an emerging technique, and its relationship to perceived stress should be further studied.

4.4 Implications for Health

As more and more adolescents continue to migrate from rural to urban environments, the negative health outcomes observed in this study could contribute to future health problems in this population. Adolescence is an important time for growth and development, and chronic stress during this period especially can affect personality development and/or lead to "disorders related to growth and development, thyroid function, reproduction, metabolism, gastrointestinal function, immune function," and individual psychology (De Vriendt, Moreno, & De Henauw, 2009). Following the effects of migration in this population over time will contribute valuable insight into the long-term effects of rural to urban migration during adolescence, and will potentially inform public health efforts as these adolescents age.

In addition, a recent paper found that urban upbringing may change HPA axis functioning. This study found that individuals with urban upbringing produced more cortisol in response to stress but had blunted cortisol awakening responses, in comparison to those raised in a rural environment (Steinheuser, Ackermann, Schönfeld, & Schwabe, 2014). As populations continue to migrate to, and grow up in, urban areas, effects of urban upbringing on children and adolescents may alter HPA activity and lead to negative changes in psychopathology and other health outcomes for greater sections of the population.

Childhood and adolescent blood pressure tends to track to adulthood, and high blood pressure increases risk for cardiovascular disease (Chen & Wang, 2008; Ezzati, Lopez, Rodgers, Vander Hoorn, & Murray, 2002). High BMI also increases risk of cardiovascular disease, diabetes, and some cancers (Ezzati et al., 2002). Increases in blood pressure and BMI seen in this study following rural to urban migration may also have long term health consequences for these adolescents as they age. The most recent Global Burden of Disease Study, using information from 2013, found that the risk of death from cardiovascular disease for women ages 15-49 in Mali is 2.34% compared to the all-cause age-sex-specific total risk of death of 16.06%; this is higher than the average for women in this age group worldwide (0.87%) and in the Western Sub-Saharan Africa region (0.98%) (GBD 2013 Mortality and Causes of Death Collaborators, 2015). For women in Mali ages 50-74, the risk of death from cardiovascular disease is higher than the risk of death from any other cause (17.19% where total age-sex-specific all-cause total risk is 53.05%), and is far higher than this age-gender specific risk worldwide (10.68%) and in Western Sub-Saharan Africa (14.15%) (GBD 2013 Mortality and Causes of Death Collaborators, 2015). It is clear that cardiovascular disease is substantially affecting the health of women in Mali, and more studies into the causes of high

blood pressure and other risk factors for cardiovascular disease can inform prevention to decrease mortality in the future.

4.5 Limitations and Future Directions

While these analyses do offer interesting results, it is important to note that the models for both hair cortisol concentration and PSS scores had fairly low adjusted R squared values, with only 11.5% of the variance explained by the variables examined. The model for blood pressure had a much better adjusted R squared value, with 43.3% of the variance explained. Further analyses of all samples from the 2013 and subsequent field years will increase sample size and further elucidate the relationships seen thus far. In addition, exploring additional potential contributing factors to changes in stress and health, such as changes in diet and physical activity, social relationships, pollution, disease, etc. could all expand understanding of the effects of rural to urban migration on adolescent stress and health.

As rural to urban migration becomes more and more prevalent worldwide, gathering information about the consequences of this shift for health and wellbeing will become increasingly important for public health efforts. More studies examining the effects of migration should be conducted, especially for understudied populations including women, children, and adolescents, in various regions of the world to increase age, culture, and sex-specific knowledge of migration impacts.

5. Conclusion

In sum, rural to urban migration has an effect on hair cortisol concentrations and SBP in female adolescents in Mali, with higher cortisol levels and SBP found in females who have migrated to urban centers. PSS scores appear to have no significant association with this migration. Although the models in this study were fairly limited for hair cortisol concentration and PSS scores, additional exploration of factors such as changes in social support, diet, physical activity, pollution and disease exposure could all expand the breadth of this study. In addition, this study is currently collecting more samples and data from the field, which will expand these findings and offer a more long-term view of the effects of rural to urban migration on adolescent stress and health.

However, it is clear that both cortisol and SBP are higher in Dogon adolescent females who have undergone rural to urban migration, which could have an effect on their future health and place them at higher risk for cardiovascular disease. Cardiovascular disease is currently a substantial health problem for women in Mali, and increasing urbanization could exacerbate this issue. Further studies into possible prevention or health interventions for young rural to urban migrants may help to mitigate potential negative health outcomes related to this migration.

6. Supplementary Figures

Supplementary Figure 1. Additional analyses of hair cortisol concentration including age, BMI, waist circumference, and percent body fat in 2013. For all models, dependent variable is cranial vertex hair cortisol concentration (pg cortisol/mg hair).

A. Including age.

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	37.297	9.660	3.861	.000	18.103	56.492
Centered PSS Score	1.235	1.485	.832	.408	-1.716	4.185
Tanner Breast Stage	6.835	2.931	2.332	.022	1.011	12.660
Parent Wealth	2.865	3.067	.934	.353	-3.229	8.960
Centered Age	-.770	1.954	-.394	.694	-4.652	3.112
Not Living in Bamako	-15.430	6.382	-2.418	.018	-28.111	-2.749
Living in Bamako (ref)	0	.	.	.	.	.

R Squared = .145 (Adjusted R Squared = .095)
Total N=95, Living in BKO=44, Not Living in BKO=51.

B. Including BMI.

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	39.278	9.243	4.249	.000	20.906	57.651
Centered PSS Score	1.259	1.464	.860	.392	-1.650	4.168
Tanner Breast Stage	7.015	2.919	2.404	.018	1.214	12.816
Parent Wealth	2.650	3.072	.863	.391	-3.457	8.756
Centered BMI	-.870	1.199	-.725	.470	-3.252	1.513
Not Living in Bamako	-17.581	7.314	-2.404	.018	-32.118	-3.044
Living in Bamako (ref)	0	.	.	.	.	.

R Squared = .149 (Adjusted R Squared = .100)
Total N=93, Living in BKO=44, Not Living in BKO=49.

C. Including waist circumference.

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	40.478	9.263	4.370	.000	22.068	58.889
Centered PSS Score	1.343	1.467	.915	.363	-1.573	4.259
Tanner Breast Stage	5.271	2.906	1.814	.073	-.505	11.046
Parent Wealth	2.538	3.076	.825	.412	-3.576	8.652
Centered Waist Circumference	.141	.510	.276	.784	-.873	1.155
Not Living in Bamako	-12.444	7.402	-1.681	.096	-27.156	2.267
Living in Bamako (ref)	0	.	.	.	.	.

R Squared = .145 (Adjusted R Squared = .095)
Total N=93, Living in BKO=44, Not Living in BKO=49.

D. Including percent body fat.

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	40.249	9.609	4.189	.000	21.147	59.351
Centered PSS Score	1.203	1.492	.806	.422	-1.763	4.168
Tanner Breast Stage	5.763	2.524	2.283	.025	.745	10.781
Parent Wealth	2.431	3.093	.786	.434	-3.717	8.579
Centered Percent Body Fat	-.015	.476	-.032	.975	-.961	.931
Not Living in Bamako	-14.168	6.276	-2.258	.027	-26.644	-1.692
Living in Bamako (ref)	0	.	.	.	.	.

R Squared = .146 (Adjusted R Squared = .096)
Total N=92, Living in BKO=44, Not Living in BKO=48.

Supplementary Figure 2. Additional analyses of PSS scores including Tanner Breast Stage, BMI, and percent body fat in 2013. For all models, dependent variable is PSS score.

A. Including Tanner Breast Stage.

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	3.752	.693	5.417	.000	2.376	5.129
Centered Cortisol Concentration	.006	.008	.832	.408	-.009	.021
Centered Age	-.228	.137	-1.664	.100	-.500	.044
Parent Wealth	-.319	.217	-1.475	.144	-.750	.111
Tanner Breast Stage	-.160	.214	-.749	.456	-.586	.265
Not Living in Bamako	.189	.468	.403	.688	-.741	1.119
Living in Bamako (ref)	0	.	.	.	.	.

R Squared = .124 (Adjusted R Squared = .075)
Total N=95, Living in BKO=44, Not Living in BKO=51.

B. Including BMI.

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	3.370	.405	8.315	.000	2.564	4.175
Centered Cortisol Concentration	.005	.008	.723	.472	-.009	.020
Centered Age	-.264	.129	-2.043	.044	-.520	-.007
Parent Wealth	-.304	.223	-1.364	.176	-.748	.139
Centered BMI	-.031	.081	-.381	.704	-.192	.130
Not Living in Bamako	.005	.506	.009	.993	-1.001	1.010
Living in Bamako (ref)	0	.	.	.	.	.

R Squared = .118 (Adjusted R Squared = .068)
Total N=93, Living in BKO=44, Not Living in BKO=49.

C. Including percent body fat.

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	3.573	.488	7.326	.000	2.603	4.542
Centered Cortisol Concentration	.005	.007	.677	.500	-.010	.020
Centered Age	-.283	.110	-2.562	.012	-.503	-.063
Parent Wealth	-.327	.219	-1.493	.139	-.763	.108
Centered Percent Body Fat	-.023	.032	-.735	.464	-.087	.040
Not Living in Bamako	-.141	.490	-.287	.775	-1.114	.833
Living in Bamako (ref)	0	.	.	.	.	.

R Squared = .126 (Adjusted R Squared = .075)
Total N=92, Living in BKO=44, Not Living in BKO=48.

Supplementary Figure 3. Additional analyses of SBP including Tanner Breast Stage and percent body fat in 2013. For all models, dependent variable is SBP (mm Hg).

A. Including Tanner Breast Stage.

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	111.722	4.318	25.872	.000	103.134	120.309
Centered Cortisol Concentration	-.109	.047	-2.317	.023	-.202	-.015
Centered PSS Score	-.027	.656	-.041	.968	-1.330	1.277
Centered Age	1.174	.895	1.311	.193	-.606	2.954
Parent Wealth	2.285	1.370	1.668	.099	-.440	5.009
Centered Temperature	.834	.523	1.596	.114	-.205	1.874
Centered BMI	1.429	.541	2.643	.010	.354	2.504
Tanner Breast Stage	-.969	1.467	-.660	.511	-3.886	1.949
Not Living in Bamako	-9.650	3.551	-2.717	.008	-16.711	-2.588
Living in Bamako (ref)	0	.	.	.	.	.

R Squared = .479 (Adjusted R Squared = .429)
Total N=93, Living in BKO=44, Not Living in BKO=49.

B. Including percent body fat.

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	109.485	3.040	36.014	.000	103.439	115.532
Centered Cortisol Concentration	-.116	.046	-2.523	.014	-.208	-.025
Centered PSS Score	-.040	.669	-.060	.952	-1.372	1.291
Centered Age	.861	.842	1.023	.309	-.813	2.535
Parent Wealth	2.245	1.397	1.607	.112	-.534	5.025
Centered Temperature	.882	.534	1.651	.102	-.180	1.945
Centered BMI	1.337	.653	2.046	.044	.037	2.636
Centered Percent Body Fat	-.020	.260	-.076	.940	-.537	.497
Not Living in Bamako	-10.915	3.162	-3.452	.001	-17.203	-4.626
Living in Bamako (ref)	0	.	.	.	.	.

R Squared = .474 (Adjusted R Squared = .424)
Total N=92, Living in BKO=44, Not Living in BKO=48.

Supplementary Figure 4: Analyses of waist circumference, percent body fat, and BMI (controlling for age) for all females in the study in 2013 (N=558).

Dependent Variable: Waist circumference (cm)

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	71.439	.518	138.021	.000	70.422	72.455
Centered Age	2.141	.125	17.157	.000	1.895	2.386
Not Living in Bamako	-3.699	.599	-6.171	.000	-4.877	-2.522
Living in Bamako (ref)	0	.	.	.	.	.

R Squared = .466 (Adjusted R Squared = .464)

Dependent Variable: Percent body fat

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	23.984	.478	50.187	.000	23.045	24.922
Centered Age	.744	.115	6.473	.000	.519	.970
Not Living in Bamako	-4.839	.553	-8.748	.000	-5.925	-3.752
Living in Bamako (ref)	0	.	.	.	.	.

R Squared = .255 (Adjusted R Squared = .252)

Dependent Variable: BMI

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	21.439	.219	97.840	.000	21.009	21.870
Centered Age	.840	.053	15.976	.000	.737	.944
Not Living in Bamako	-1.937	.253	-7.650	.000	-2.435	-1.440
Living in Bamako (ref)	0	.	.	.	.	.

R Squared = .460 (Adjusted R Squared = .458)

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