



Dental enamel defects predict adolescent health indicators: A cohort study among the Tsimane' of Bolivia

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Abstract

Objectives: Bioarchaeological findings have linked defective enamel formation in preadulthood with adult mortality. We investigated how defective enamel formation in infancy and childhood is associated with risk factors for adult morbidity and mortality in adolescents.

Methods: This cohort study of 349 Amerindian adolescents (10-17 years of age) related extent of enamel defects on the central maxillary incisors (none, less than 1/3, 1/3 to 2/3, more than 2/3) to adolescent anthropometrics (height, weight) and biomarkers (hemoglobin, glycated hemoglobin, white blood cell count, and blood pressure). Risk differences and 95% confidence intervals were estimated using multiple linear regression. Enamel defects and stunted growth were compared in their ability to predict adolescent health indicators using log-binomial regression and receiver operating characteristics (ROCs).

Results: Greater extent of defective enamel formation on the tooth surface was associated with shorter height (−1.35 cm, 95% CI: −2.17, −0.53), lower weight (−0.98 kg, 95% CI: −1.70, −0.26), lower hemoglobin (−0.36 g/dL, 95% CI: −0.59, −0.13), lower glycated hemoglobin (−0.04 %A_{1c}, 95% CI: −0.08, −0.00008), and higher white blood cell count (0.74 10⁹/L, 95% CI: 0.35, 1.14) in adolescence. Extent of enamel defects and stunted growth independently performed similarly as risk factors for adverse adolescent outcomes, including anemia, prediabetes/type II diabetes, elevated WBC count, prehypertension/hypertension, and metabolic health.

Conclusions: Defective enamel formation in infancy and childhood predicted adolescent health outcomes and may be primarily associated with infection. Extent of enamel defects and stunted growth may be equally predictive of adverse adolescent health outcomes.

1 | INTRODUCTION

Low birth weight and growth stunting can be markers of adverse prenatal and early childhood experiences (Barker, 1994; Barker, Osmond, Winter, & Margetts, 1989; Foster et al.,

2005; Kuh & Ben-Shlomo, 2004; McDade et al., 2005; Newnham & Ross, 2009; Pickles, Maughan, & Wadsworth, 2007; Wells, 2007; Wintour & Owens, 2006). In turn, these two anthropometric biomarkers have been shown to be predictive of obesity, type II diabetes, hypertension, and cardiovascular



disease in adulthood (Barker & Osmond, 1986; Barker et al., 1989; Barker, 1994; Newnham & Ross, 2009). They have also been the basis for extensive research on the developmental origins of health and disease (DOHaD) (Barker et al., 1989; Barker, 1994; Newnham & Ross, 2009; Wells, 2007).

The advancement of DOHaD research and a clearer understanding of other potential underlying mechanisms is hindered by difficulty in obtaining reliable measures of early childhood exposures and by the poor specificity of existing anthropometric biomarkers (Kuzawa & Quinn, 2009; Kuzawa, 2005a; Newnham & Ross, 2009). Markers such as low birth weight may not only reflect information on early life stressors, but may also reflect inherited characteristics (Hujoel, Masterson, & Bollen, 2017). Therefore, identification of alternative measures of early childhood experience (beyond birth weight and growth stunting), particularly those that capture the source and timescale of early childhood exposures, are a priority for research on the DOHaD (Kuzawa & Quinn, 2009; Kuzawa, 2005b; Newnham & Ross, 2009).

Developmental enamel defects may offer one such sensitive and specific measure of childhood exposures (e.g., infection, micronutrient deficiency), which predicts long-term adverse health outcomes (Armélagos, Goodman, Harper, & Blakey, 2009). Dental enamel calcifies incrementally in utero and during early childhood, and is sensitive to physiological stress which can manifest itself as permanent defects in the enamel. Defective enamel does not repair during the life course, making it a permanent marker of early-life stressors (Goodman & Rose, 1990; Pindborg, 1982; Sarnat & Schour, 1941). The potential advantages to using developmental enamel defects as a biomarker of early childhood experience are multiple. Developmental enamel defects can be measured in a field setting (Golkari et al., 2011), the measure is not reliant on the existence of childhood medical records, it is not subject to recall bias, and it may have the ability to capture the timing of childhood exposures, thus permitting retrospective estimation of the age at which an individual experienced a physiological stressor. Developmental enamel defects may also capture specific information on the nature of adverse early life exposure in two ways. First, certain early life exposures disrupt formation of the dental enamel and lead to a pattern and distribution of defects that may enable one to determine the actual stressor (e.g., vitamin D deficiency). Second, dental enamel composition can be analyzed to directly determine absorption of environmental chemicals into the enamel as it formed during early life (e.g., heavy metals, fluorides, pesticides, and drugs) (Andra, Austin, & Arora, 2015, 2016; Arora & Austin, 2013).

Bioarchaeological findings have consistently associated defective enamel formation in early life with premature adult mortality (Armélagos et al., 2009; Duray, 1996; Goodman & Armélagos, 1988; Goodman, 1996; Rose, Lallo, & Armélagos, 1978; White, 1978). This predictive ability of enamel defects formed in infancy and childhood for adverse

adolescent anthropometrics and disease risk markers have been rarely evaluated in living, human populations. The limited existing literature indicates that enamel defects in the permanent dentition are associated with subsequent (after enamel formation is complete) stunted growth (Rugg-Gunn, Al-Mohammadi, & Butler, 1997; Santos & Coimbra, 1999) and several syndromes and systemic diseases (Seow, 1991, 2014). Screening for celiac disease has been recommended in patients with enamel defects (El-Hodhod, El-Agouza, & Abdel-Al, 2012; Pastore et al., 2008; Rashid, Zarkadas, & Anca, 2011). We investigated whether enamel defects in the permanent dentition predicts adverse adolescent anthropometrics and health indicators in an Amerindian population in Bolivia.

2 | METHODS

2.1 | Study design and sample

This cohort study was conducted among adolescent participants ($n = 336$) identified from the Tsimane' Amazonian Panel Study (TAPS) (Leonard et al., 2015). TAPS was a study conducted among an Amerindian group of approximately 17 000 people who live across more than 100 small, remote communities, accessible by dirt roads and/or canoe in Bolivia's Amazonian Basin (Instituto Nacional de Estadística [INE], 2012). Criteria for inclusion in the study included being 10–17 years of age at the time of data collection (so that the central maxillary incisors were fully erupted), enrollment in the 9-year TAPS for at least one year when aged 5 years or less, and residence in one of 15 communities visited by the 2015 data collection team. Approval for data collection and use was obtained from the local tribal government, the *Gran Consejo Tsimane'* (GCT, Tsimane' Grand Council), and the University of Washington (UW) Human Subjects Division (HSD).

2.2 | Exposure

The exposure of interest was enamel defects in the permanent central maxillary incisors. Enamel defects were captured through digital intraoral photographs with a macro lens and ring flash, detected using Photoshop software, and quantified per the DDE Index/Modified DDE Index (Clarkson & O'Mullane, 1989; International Dental Federation [FDI], 1992). Enamel defects were measured on the two central maxillary incisors because these teeth were determined to be the most reliable for abstracting enamel defect data from digital photographs in this study sample. Intra-examiner reliability for these teeth was classified as "very good" based on kappa = 0.77 (Landis & Koch, 1977). This study focused on the most prevalent enamel defect pattern observed in the study sample, which was a hypoplasia characterized by an orange peel texture on a large central depression (Masterson, Fitzpatrick, & Enquobahrie, 2017). Enamel defects were classified by extent of tooth surface affected as

none, less than 1/3, 1/3 to 2/3, or more than 2/3. Enamel defect measurement was based on the left central maxillary incisor or—if the left was missing or severely decayed—on the right central maxillary incisor. There was 93.4% concordance for enamel defects occurrence for the two central maxillary incisors.

2.3 | Outcomes

Evaluated outcomes include height (measured in cm), weight (measured in kg), hemoglobin (Hb, measured in g/dL), glycosylated hemoglobin (HbA_{1c}, measured as %A_{1c}), white blood cell count (WBC, measured in 10⁹/L), and systolic and diastolic blood pressure (measured in mm Hg). A stadiometer was used to measure height and an electric scale was used to measure weight. Anthropometric thresholds were based on World Health Organization (WHO) guidelines (de Onis et al., 2007). Biomarker outcomes were collected in the field using portable, point-of-care devices and a digital blood pressure cuff. Point-of-care measure thresholds were based on standard clinical care for adolescents. American Diabetes Association thresholds were used to classify HbA_{1c} measures as “prediabetic” (%A_{1c} between 5.7 and 6.5) and “type II diabetes” (%A_{1c} > 6.5) (National Diabetes Education Initiative [NDEI], 2015). Standard age- and sex-based reference values for clinical practice were used to determine thresholds for anemia based on Hb and elevated WBC count (Mayo Clinic, 2015). National Institute of Health’s age and sex specific parameters for blood pressure were used to classify “hypertension” and “prehypertension” (National Institutes of Health, 2005). An index (0–3) was created to indicate an additional outcome, “poor metabolic health,” by summing the number of the following conditions: overweight, prediabetes/type II diabetes and prehypertension/hypertension status. Study sample size was reduced for a few adolescent outcomes due to point-of-care device errors in the field: hemoglobin ($n = 335$), glycosylated hemoglobin ($n = 319$), white blood cell count ($n = 256$).

2.4 | Covariates

Covariates included age (measured in years), sex (male/female), childhood household socioeconomic status (SES), and childhood exposure to parasitic helminth infection. Household SES was characterized using total wealth, a locally-developed sum of 23 traditional and modern capital measured by value in the local currency (bolivianos, Bs) that reflects economic standing (Leonard et al., 2015). Helminth infection was determined based on fecal samples taken on a subset ($n = 113$) of study participants (Tanner, Leonard, & McDade, 2009).

2.5 | Statistical analyses

Distributions of adolescent outcomes were stratified by extent of enamel defects. Multiple linear regression analyses

with robust standard errors were fit to evaluate whether defective enamel formation in infancy and early childhood was associated with adolescent anthropometrics and health. Trends were assessed based on the type III sum of squares from general linear models. We then compared enamel defects to stunted growth in adolescence (HAZ < -2.0) (de Onis et al., 2007), in terms of their respective ability to predict adolescent biomarkers. To do so, we evaluated the strength of each as a risk factor for adolescent biomarkers using log-binomial regression with robust standard errors and presented prevalence ratios and corresponding 95% confidence intervals. We also compared the performance of enamel defects and stunted growth as classifiers of adolescent health status using receiver operating characteristics (ROCs). We evaluated the accuracy of each predictor at classifying measures of adolescent health outcomes based on areas under the curve (AUCs) (Pepe et al., 2008). AUCs and corresponding 95% confidence intervals are reported numerically and displayed in bar graphs.

Secondary analyses additionally adjusted for SES and for adolescent HAZ separately. We also additionally adjusted for parasitic helminth infection during childhood, a potential cause of enamel defects (Masterton et al., 2017), in a subsample that had this measure available ($n = 113$).

All models were adjusted for age and sex. Analyses were conducted using SAS Version 9.4 for Windows (SAS Institute Inc., Cary, North Carolina).

3 | RESULTS

3.1 | Study sample description

The analytic study sample included 336 participants, aged 10–17 years. The sample was approximately half male and participants were on average 13 years old. Three hundred and eleven (92.3%) of the participants had a particular enamel defect pattern, characterized by an orange peel texture on a large central depression on the labial surface of the central maxillary incisors, which is the focus of this analysis (Figure 1). The extent of the defective enamel among our study participants was most commonly 1/3 to 2/3 of the tooth surface (47%) (Table 1).



FIGURE 1 Enamel defect pattern observed in Tsimane’ adolescents, characterized by an orange peel texture and large central depression on the labial surface of the permanent central maxillary incisors

TABLE 1 Description of adolescent demographics, anthropometrics and biomarkers by extent of enamel defects ($n = 336$)

Measure	Extent of enamel defects				Overall <i>n</i> (%) or mean \pm SD
	No defects ($n = 26$) <i>n</i> (%) or mean \pm SD	< 1/3 ($n = 80$) <i>n</i> (%) or mean \pm SD	1/3 to 2/3 ($n = 147$) <i>n</i> (%) or mean \pm SD	> 2/3 ($n = 83$) <i>n</i> (%) or mean \pm SD	
Sex, % male	10 (38.5%)	38 (47.5%)	83 (56.5%)	43 (51.8%)	174 (51.8%)
Age (years)	13.1 \pm 2.2	13.1 \pm 2.1	12.8 \pm 2.0	13.2 \pm 2.4	13.0 \pm 2.2
Household wealth (Bs) ^a	3957 \pm 2852	3634 \pm 2899	3280 \pm 2144	3327 \pm 1948	3425 \pm 2344
Anthropometrics					
Height (cm)	145 \pm 13	143 \pm 12	142 \pm 11	141 \pm 12	142 \pm 12
Weight (kg)	43 \pm 12	40 \pm 12	39 \pm 10	39 \pm 11	40 \pm 11
Height-for-age (z-score)	-1.4 \pm 0.7	-1.7 \pm 0.9	-1.6 \pm 0.9	-2.0 \pm 1.0	-1.7 \pm 0.9
Short stature (HAZ < -2.0)	5 (19.2%)	29 (36.3%)	46 (31.3%)	35 (42.2%)	115 (34.2%)
BMI-for-age (z-score)	0.42 \pm 0.83	0.20 \pm 0.76	0.26 \pm 0.77	0.22 \pm 0.73	0.25 \pm 0.76
Overweight (BAZ > +1.0)	8 (30.8%)	12 (15.0%)	19 (12.9%)	13 (15.7%)	52 (15.5%)
Health outcomes					
Hemoglobin (g/dL) ^b	11.2 \pm 2.0	11.5 \pm 1.8	11.3 \pm 2.0	10.4 \pm 1.7	11.1 \pm 1.9
Anemia	18 (72.0%)	59 (73.8%)	102 (69.9%)	72 (86.8%)	251 (75.2%)
Glycated hemoglobin (%A _{1c}) ^b	5.39 \pm 0.3	5.35 \pm 0.3	5.31 \pm 0.3	5.26 \pm 0.3	5.31 \pm 0.3
Prediabetes	5 (21.7%)	7 (9.1%)	14 (9.9%)	5 (6.5%)	31 (9.8%)
Type II diabetes	0 (0%)	2 (2.6%)	1 (0.7%)	0 (0%)	3 (1.0%)
White blood cell count (10 ⁹ /L) ^b	10.0 \pm 2.8	10.0 \pm 2.8	10.9 \pm 2.8	11.8 \pm 2.8	10.9 \pm 2.9
“Elevated”	9 (52.9%)	33 (57.9%)	71 (64.6%)	54 (76.1%)	167 (66.5%)
Systolic blood pressure (mm Hg)	115 \pm 11	111 \pm 11	111 \pm 10	112 \pm 9	112 \pm 10
Diastolic blood pressure (mm Hg)	65 \pm 8	63 \pm 9	63 \pm 7	65 \pm 9	63 \pm 8
Prehypertension	2 (8.0%)	4 (5.0%)	9 (6.1%)	4 (4.8%)	19 (5.7%)
Stage 1 hypertension	2 (8.0%)	3 (3.8%)	4 (2.7%)	4 (4.8%)	13 (3.9%)
Poor metabolic health^{b,c}					
0	12 (46.2%)	56 (70.0%)	106 (72.1%)	59 (71.1%)	233 (69.3%)
1	11 (42.3%)	20 (25.0%)	37 (25.2%)	22 (26.5%)	90 (26.7%)
2	3 (11.5%)	4 (5.0%)	3 (2.0%)	2 (2.4%)	12 (3.6%)
3	0 (0%)	0 (0%)	1 (0.7%)	0 (0%)	1 (0.3%)

^aSum of 23 animal, trade and modern capital, measured by value in the local currency (Bs, bolivianos).

^bReduced sample due to point-of-care device errors: hemoglobin $n = 335$, glycated hemoglobin/metabolic health $n = 319$, WBC count $n = 256$.

^cCount of overweight, prediabetes/type II diabetes, prehypertension/stage 1 hypertension.

One third of the study sample (33%) had stunted vertical growth in adolescence, according to WHO standards (HAZ < -2.0) (de Onis et al., 2007). Fifty-two participants (16%) were “overweight” by WHO standards (WAZ > 1.0). Over half of the sample had anemia (74%) and an elevated WBC count (66%). Smaller proportions were diabetic/

prediabetic (10.8%) or hypertensive/prehypertensive (9.6%). Nearly one third of all participants (30.7%) had at least one marker of poor metabolic health (Table 1). Study participants with enamel defects were less likely to be overweight ($P = .02$) and less likely to have prediabetes ($P = .04$) than those without defects.

After adjustment for age and sex, extent of enamel defects was predictive of shorter height and lower weight in adolescence ($P = .001$ and $P = .008$, respectively) (Table 2). A linear trend across defect extent was also observed ($p < 0.01$ for height and $P = .01$ for weight). Compared to those with no defects or minimal defects ($< 1/3$ of the tooth surface affected), extensive enamel defects (more than $2/3$ of the tooth surface affected) predicted shorter adolescent height (3.22 cm on average; 95% CI: 1.21 to 5.23; $P = .002$) and lighter weight (2.13 kg on average; 95% CI: 0.36 to 3.90; $P = .02$).

Extent of enamel defects also predicted lower hemoglobin, lower glycosylated hemoglobin and higher WBC count ($P = .002$, $P = .0496$, $P = .0002$, respectively) (Table 2). A linear trend across the extent of defects was detected only for hemoglobin and WBC count ($P < .01$ and $P < .01$, respectively). Compared to those with either no defects or minimal defects ($< 1/3$ of the tooth surface affected), extensive enamel defects ($> 2/3$ of the tooth surface affected) predicted lower hemoglobin (1.00 g/dL on average; 95% CI: 0.46 to 1.53; $P < .001$) and higher WBC count ($1.79 \times 10^9/L$ on average; 95% CI: 0.87 to 2.71; $P < .001$) in adolescence. Enamel defects were not associated with systolic or diastolic blood pressure ($P = .48$ and $P = .73$, respectively).

We performed three secondary analyses exploring the roles of several potentially interconnected factors. First, to more carefully evaluate the potential confounding role of SES (in local currency, bolivianos (Bs)) in the relationship between enamel defect extent and adolescent outcomes, we carried out a secondary analysis that adjusted the linear regression analyses for SES. This adjustment only slightly attenuated the effect sizes of enamel defect extent predicting adolescent height, weight, hemoglobin, glycosylated hemoglobin, or WBC count. The association between enamel defect extent and glycosylated hemoglobin was no longer statistically significant after adjusting for SES. Next, we analyzed the enamel defect-adolescent health outcome relationship adjusting for adolescent HAZ to assess the ability of enamel defects to predict health outcomes above and beyond the role of adolescent body size. This adjustment similarly did not have a strong influence on the ability of enamel defect extent to predict hemoglobin or WBC count. The ability to predict weight and glycosylated hemoglobin, however, was lost after adjusting for height. Finally, previous findings report the observed pattern of defects may be caused by parasitic helminth infection during childhood (Masterson et al., 2017). Therefore, we included a third analysis on a subsample that adjusted for presence of parasitic helminth infection during early childhood. Adjusting for parasitic helminth infection did not have a meaningful effect on the relationship between enamel defect extent and adolescent outcomes, although the subsample's limited power made it so that fewer associations

were detectable between enamel defects and adolescent outcomes (Table 2).

Extent of enamel defects and stunted growth were predictive of certain adolescent health outcomes. Enamel defects were predictive of anemia ($P = .02$), elevated WBC count ($P = .03$), and better metabolic health ($P = .047$). Stunted growth was predictive of anemia ($P = .03$) (Table 3). Each $1/3$ increase in defect extent predicted an 8% increase in prevalence of anemia, 12% increase in the prevalence of elevated WBC count, and 16% decrease in the prevalence of poor metabolic health. Compared to those with normal linear growth, stunted growth in adolescence predicted a 14% greater prevalence of anemia. Neither enamel defect extent nor stunted growth were predictive of overweight ($P = .18$ and $P = .26$, respectively), prediabetes or diabetes ($P = .07$ and $P = .09$, respectively), nor prehypertension/hypertension ($P = .63$ and $P = .89$, respectively) in adolescence (Table 3).

Extent of enamel defects and stunted growth appeared to perform similarly as classifiers of adolescent outcomes: anemia (AUC = 0.58, 95% CI: 0.51, 0.65 vs. AUC = 0.59, 95% CI: 0.52, 0.66, respectively; $P = .84$), prediabetes/type II diabetes (AUC = 0.61, 95% CI: 0.50, 0.72 vs. AUC = 0.58, 95% CI: 0.50, 0.67, respectively; $P = .69$), elevated WBC count (AUC = 0.61, 95% CI: 0.54, 0.69 vs. AUC = 0.58, 95% CI: 0.50, 0.65, respectively; $P = .51$), prehypertension/hypertension (AUC = 0.56, 95% CI: 0.45, 0.67 vs. AUC = 0.56, 95% CI: 0.44, 0.68, respectively; $P = .98$), and metabolic health (AUC = 0.60, 95% CI: 0.53, 0.67 vs. AUC = 0.60, 95% CI: 0.54, 0.66, respectively; $P = .94$). ROC analyses showed that addition of extent of enamel defects to stunted growth improved the AUC for all biomarkers in adolescence (0.1-0.7 improvement in AUC) (Figure 2).

4 | DISCUSSION

Defective enamel formation in infancy and childhood was predictive of adolescent shorter height, lower weight, lower hemoglobin, and higher WBC count. Such enamel defects may predict these adverse adolescent health outcomes as effectively as stunted growth in childhood. Our findings are consistent with bioarchaeological findings that have established an association between enamel defects and early mortality (Armélagos et al., 2009). They are also consistent with evidence from living human populations, which demonstrates that enamel defects predict subsequent outcomes, including stunted growth in late childhood and early adolescence (Rugg-Gunn et al., 1997; Santos & Coimbra, 1999) and celiac disease later in life (Pastore et al., 2008; Rashid et al., 2011).

The enamel defect pattern we observed, characterized by an orange peel texture on a large central depression, is

TABLE 2 Enamel defects and adolescent anthropometrics and biomarker linear regression results ($n = 336$)^a (difference in means and 95% CIs reported)

	Height (cm)	Weight (kg)	Hemoglobin ^b (g/dL)	Glycated Hemoglobin ^b (%A _{1c})	White Blood Cell count ^b (10 ⁹ /L)	Systolic blood pressure (mm Hg)	Diastolic blood pressure (mm Hg)
Extent of defects (per 1/3 of the tooth surface affected)	-1.35*** (-2.17, -0.53)	-0.98* (-1.70, -0.26)	-0.36*** (-0.59, -0.13)	-0.04* (-0.08, -0.000008)	0.74** (0.35, 1.14)	-0.41 (-1.56, 0.74)	0.19 (-0.88, 1.26)
Adjusted for SES	-1.27*** (-2.11, -0.44)	-0.92* (-1.64, -0.20)	-0.31* (-0.53, -0.08)	-0.03 (-0.08, 0.01)	0.69** (0.29, 1.09)	-0.10 (-1.27, 1.06)	0.37 (-0.73, 1.48)
Adjusted for adolescent HAZ	—	-0.33 (-0.85, 0.18)	-0.32*** (-0.54, -0.09)	-0.03 (-0.07, 0.01)	0.67** (0.27, 1.06)	-0.30 (-1.44, 0.84)	-0.11 (-0.96, 1.19)
Analysis on subsample ($n = 113$) with childhood parasitic helminth infection data available							
Extent of defects (per 1/3 of the tooth surface affected)	-0.79 (-2.19, 0.60)	0.39 (-1.10, 1.88)	-0.29 (-0.69, 0.11)	0.001 (-0.06, 0.07)	0.85* (0.05, 1.66)	1.08 (-1.23, 3.39)	1.51 (-0.66, 3.68)
Adjusted for parasitic helminth infection	-0.87 (-2.25, 0.52)	0.30 (-1.19, 1.79)	-0.28 (-0.69, 0.12)	-0.003 (-0.07, 0.06)	0.83* (0.02, 1.65)	1.12 (-1.26, 3.49)	1.52 (-0.71, 3.76)

^aAdjusted for covariates (sex and age) in adolescence.

^bReduced sample due to point-of-care device errors: hemoglobin $n = 335$, glycated hemoglobin $n = 319$, white blood cell count $n = 256$.

 $P < .10$ indicated in **bold font**. ***asterisk** indicates $P < .05$, ****double asterisk** indicates $P < .01$.

TABLE 3 Enamel defects, stunted growth and indicators of adolescent health risk, log-binomial regression results ($n = 336$)^a (prevalence ratios and 95% CIs reported)

	Overweight (BMI-for-age z-score > +1.0)	Anemic ^{a,b,d}	Prediabetes or diabetes ^d (%A _{1c} > 5.7)	Elevated WBC count ^{b,d}	Prehypertension or stage 1 hypertension ^b	Poor metabolic health ^{c,d}
Extent of defect (per 1/3 of the tooth surface affected)	0.83 (0.63, 1.09)	1.08* (1.01, 1.16)	0.73 (0.52, 1.02)	1.12* (1.01, 1.25)	0.90 (0.59, 1.37)	0.84* (0.71, 0.99)
Stunted growth (HAZ < -2.0)	0.73 (0.42, 1.26)	1.14* (1.01, 1.28)	0.51 (0.23, 1.12)	1.03 (0.87, 1.24)	0.95 (0.46, 1.97)	0.76 (0.53, 1.09)

^aAdjusted for covariates (sex and age) in adolescence.

^bSex and age specific thresholds for disease status.

^cAt least one of: overweight, prediabetes/type II diabetes, prehypertension/stage 1 hypertension.

^dReduced sample due to point-of-care device errors: hemoglobin $n = 335$, glycated hemoglobin/metabolic health $n = 319$, WBB count $n = 256$.

$P < .10$ indicated in **bold font**, *asterisk indicates $P < .05$.

unique and may represent a novel form of enamel hypoplasia since it doesn't fit the classical descriptions of hypoplastic grooves, pitting, or missing enamel from the tooth surface (Hillson, 2014). Alternatively, it may be related to the "plane form" hypoplasia observed in apes and other primates (Lukacs & Walimbe, 1998; Lukacs, 1999, 2001; Skinner & Newell, 2003) or have been affected by posteruptive erosion (Taji & Seow, 2010). Further description of this defect pattern is detailed elsewhere (Masterson et al., 2017). Investigation into potential early life causes of this defect pattern in this study sample suggested developmental origins are likely (Masterson et al., 2017).

Stressors that simultaneously disrupt the enamel formation process and adversely affect critical periods of development in early life explain why enamel defects may serve as a predictor of long-term health status (Barker & Osmond, 1986; Barker et al., 1989; Barker, 1994; Newnham & Ross, 2009; Petry, Desai, Ozanne, & Hales, 1997; Waterland & Michels, 2007). In this study sample, enamel defects were associated with stunted growth and parasitic infection during

early childhood (Masterson et al., 2017). In secondary analyses, however, the ability of enamel defect extent to predict adolescent outcomes was not explained by parasitic infection during early childhood. It is possible that the small study sample ($n = 113$) limited our ability to detect these associations, but it is also possible that another factor or multiple factors are responsible for explaining the enamel defect-adolescent outcome relationships. However, given the relationships of enamel defects with hemoglobin and with WBC count, it seems most plausible that the underlying mechanism is related to infection (Balarajan, Ramakrishnan, & Özalpin, 2011; Engle-Stone, Aaron, & Huang, 2017).

Results of the secondary analyses provide additional clues into how and why enamel defects are predictive of certain adolescent outcomes. First, though it is possible the relationships we observed are due to persistent exposures (common causes) or increased susceptibility throughout the life course, it does not appear that SES is such a factor. Next, adolescent body size, like childhood helminth infection, did not explain the ability of enamel defects to predict adverse

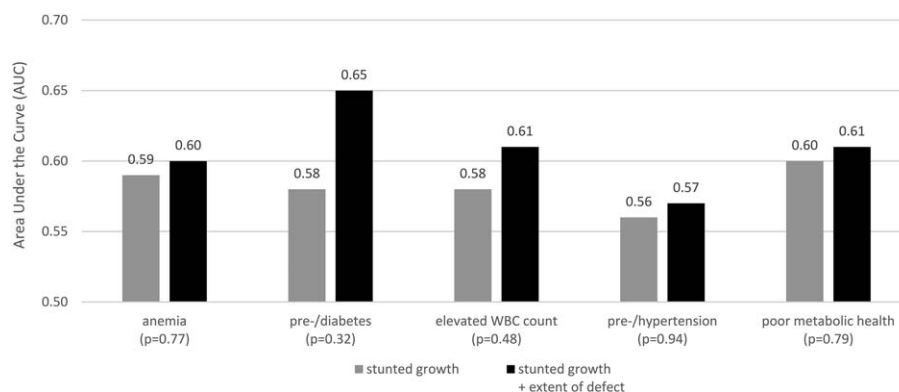


FIGURE 2 Accuracy of increasing subsets of predictive factors for classifying adolescent health outcomes (All ROC curves account for age and sex as precision variables)



adolescent health outcomes either. It seems plausible that the enamel defect-adolescent outcome associations we observed could be indicative of early life influences on subsequent health that were not evaluated in this analysis. However, it is a limitation of this study that SES and causes of enamel defects from early life were not measured repeatedly throughout the life course to enable more careful evaluation of the role of such factors.

Additional limitations of the current study include that adolescents who were missing both central maxillary incisors were excluded from analyses. If their lack of central incisors was due to enamel defects, the reported point estimates for enamel defect-adolescent outcomes may be underestimated due to the positive association between age and adverse adolescent health. It was not feasible for study participants to brush their teeth prior to taking the intraoral photographs, so dental plaque on the tooth surface may have made measurement of enamel defects less precise.

Enamel defects may have the potential to serve as a useful screening tool in pediatric clinics to alert clinicians to underlying disease risk that may not manifest until later in adulthood. An example of its utility in the existing literature is that enamel defects have been recognized as an early oral manifestation of celiac disease (El-Hodhod et al., 2012; Pastore et al., 2008; Rashid et al., 2011). Our results further support its potential utility for screening for general health risk, particularly because our results indicate that extent of enamel defects is associated with adverse health indicator measures in adolescence and also may predict different health outcomes than does stunted growth. In settings where childhood growth and health records are not available, enamel defects and stunted growth combined may be useful proxies for childhood stunting and for general health screening. However, measurement of enamel defects, especially by the particular patterns in which they present, must be improved and validated to be able to better investigate their etiology. This is important because enamel defects have the potential to serve as an important piece of the “toolkit” of biomarkers for studying the long-term consequences of childhood exposures. Future association studies of enamel defects and systemic conditions should carefully consider the etiologically-relevant exposure period for development of enamel defects when measuring systemic conditions. Future studies should also focus on potential underlying mechanisms to better understand how and why common causes during early life may play a role in connecting enamel defect patterns to systemic health conditions throughout the life course.

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AUTHOR CONTRIBUTIONS

EEM conceived of and designed the present study, collected and analyzed the data, interpreted findings, and drafted the present manuscript. ALF, DAE, LAM, DTAE, EC and PPH contributed to the conception and design of the study, interpretation of the findings, edited the manuscript for intellectual content and provided critical comments on the final version of the manuscript. All authors have critically revised and given their final approval of the submitted manuscript.

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