Original Research Article

Secular Change in the Timing of Dental Root Maturation in Portuguese Boys and Girls

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Objectives: In this study, we compare root formation in a modern sample of living Portuguese children ($n = 521$), between 6 and 18 years of age, with that of a similar sample of known sex and age Portuguese child skeletons ($n = 114$), who lived half a century earlier, to assess secular change in dental maturation.

Methods: The roots of seven developing permanent mandibular teeth were assessed for their maturation in both samples. The median age-of-attainment of root stages was calculated using logistic regression and compared between the samples. The potential influence of mortality bias in root development of the skeletal sample was tested.

Results: No mortality bias effect was detected. We find that the dentition of modern Portuguese boys and girls mature on average 1.22 years and 1.47 years earlier, respectively, compared to their counterparts born one half a century before. Our results also suggest that an earlier timing of attainment of root formation maturational stages was not accompanied by a change in the overall duration of root formation.

Conclusions: Although secular changes in human growth are frequently reported in the literature, a secular trend in dental maturation has not been consistently shown to date. Therefore, we demonstrate a clear and consistent acceleration in dental root maturation due to secular changes and show that the plasticity in dental development in response to environmental factors is greater than previously thought. Am. J. Hum. Biol. 22:791–800, 2010.

The purpose of this study is to compare timing and duration of tooth formation in a sample of modern children from Portugal with that of a similar sample of children who were born and lived half a century earlier, and assess a secular trend in dental maturation. Secular trend or change refers to the process of "children getting larger and growing to maturity more rapidly" (Eveleth and Tanner, 1990: 205). These changes are related to the material conditions of life which act upon human growth over generations. A common explanation for these secular changes is the improved quantity and quality of nutrition, the reduction of infectious disease burden brought by immunization and sanitation and increased access to health and medical care (Bogin, 1999; Eveleth and Tanner, 1990; Malina, 1990; Susanne, 1984; Tanner, 1989). Secular changes in body size (Cole, 2000; Olds and Harten, 2001; Susanne et al., 2001), body composition (Freedman et al., 1991; Olds and Harten, 2001; Thompson et al., 2002), tempo of growth (Hermanussen, 1997; Kalberg, 2002) and timing of sexual maturation (Jones et al., 2009; Padez, 2002; Susanne et al., 2001) have occurred during the last century and are well documented for most industrialized countries and several developing nations. It is an improvement in socioeconomic conditions, such as the European and North American economic recovery sparked by World War II, which causes the material conditions of life to change and, consequently, human growth (Bogin, 1999; Eveleth and Tanner, 1990; Malina, 1990; Susanne, 1984; Tanner, 1989). Similarly, a decline in overall living conditions can also result in a persistent decrease in body size or delayed maturation, in which case the trend is inverted and a negative secular change occurs (Bogin, 1999; Tobias, 1985).

Despite the current evidence, secular changes in skeletal and dental development have rarely been investigated and are less well known. Secular acceleration in skeletal maturation has only been clearly demonstrated for British (Himes, 1984) and South African children (Hawley et al., 2009), from hand-wrist radiographs. Other studies have shown that earlier reference standards for epiphyseal union are not appropriate for ageing modern human skeletons in a forensic context, suggesting a secular acceleration in skeletal maturation (Langley-Shirley and Jantz, 2010; Meijerman et al., 2007). Lin et al. (2006), Ranjitkar et al. (2006), and Ahmed and Warner (2007) have also examined changes over time in skeletal maturation, but a clear secular trend has not been detected in these studies.

Findings have been mixed with respect to secular changes in tooth eruption and emergence (Clements et al., 1953; Eskeli et al., 1999; Helm, 1969; Höuffding et al., 1984; Kerr, 1980; Leroy et al., 2003; Liversidge and Molleson, 2004; Niswander and Sujaku, 1960; Parner et al., 2001; Rönnerman, 1977; Rousset et al., 2003; Virtanen et al., 1994; Wedl et al., 2005) but the detection of a secular acceleration in the timing or in duration of tooth formation (Holtgrave et al., 1997; Monge et al., 2007; Nadler,

Contract grant sponsor: Fundação para a Ciência e Tecnologia; Contract grant number: SFRH/BPD/22142/2005; Contract grant sponsor: Public Health Service; Contract grant numbers: R01 DE016886, CDC 5R01 DD000350; Contract grant sponsor: The Foundation Fyssen.

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Received 5 May 2010; Revision received 13 June 2010; Accepted 28 June 2010

DOI 10.1002/ajhb.21084

Published online 18 August 2010 in Wiley Online Library (wileyonlinelibrary.com).

1998; Price, 2005; Simpson and Kunos, 1998) has been even more difficult to demonstrate. No study has been able to consistently and unequivocally show secular changes in dental maturation and those which attempt it reveal some limitations.

First and foremost, the analysis of secular trend in dental maturation has been hampered by the lack of comparative historic samples. According to Liversidge (2008), a secular trend would need a comparative reference sample that antedates World War II, and all large dental radiographic studies are later than this. Although secular changes were prominent in the 20th century, they became particularly pronounced after World War II, with the improvement in living standards and increased urbanization which were favorable to growth (Bogin, 1999). The time periods previously considered are usually insignificant for major societal changes to take place. In addition, those studies that reported a secular acceleration of tooth formation have been hampered by the use of inappropriate statistical techniques. In these studies, age-of-attainment of individual tooth stages has been calculated as the mean age at a stage, by averaging the age of children who are at a certain stage (Nadler, 1998; Price, 2005). This method is heavily influenced by sample size and age distribution, and it is particularly unsuitable for the most mature stage of formation (Liversidge, 2008; Smith, 1991). Only a few studies (Holtgrave et al., 1997; Monge et al., 2007; Simpson and Kunos, 1998) have used cumulative distribution methods to infer a secular acceleration in dental development. Unfortunately, two of these (Holtgrave et al., 1997; Monge et al., 2007) inferred a secular acceleration from comparisons with earlier published standards—the Moorrees et al. (1963) and Nolla's (1960) studies - and not from comparisons with earlier similar dental data.

Therefore, two important aspects need to be considered when analyzing possible secular changes in dental formation: (1) samples must be separated by a significant amount of time while maintaining comparability, and (2) appropriate statistical technique for estimating the mean age of attainment must be employed. In addition, it is critical to consider the age at a given dental stage (i.e. timing), and the time taken to complete enamel and dentine formation (i.e. duration) when interpreting possible secular changes. Here we compare dental maturation in a modern sample of living Portuguese children with that of a sample of known sex and age Portuguese child skeletons, who lived half a century earlier. These two samples provide a unique opportunity to assess secular changes because they reflect a fundamental improvement in the socioeconomic and living conditions of the Portuguese. The maturation of seven developing permanent mandibular teeth (excluding M3) was assessed in both samples. Because of the age range in the modern sample we were not able to study the whole maturation process and, consequently, we have only focused on the timing of root formation. The median age-of-attainment of root stages was calculated using logistic regression and secular acceleration in timing of root formation was assessed by comparing median age-of-attainment between the historic and modern samples. Secular changes in the duration of root formation were also examined by comparing differences in median age-of-attainment between consecutive stages in both samples. However, before we can compare dental development in a sample of living children with that in a sample of child skeletons, we need to address the issue of mortality bias (Saunders and Hoppa, 1993; Wood et al., 1992). Mortality bias means that child skeletons may reflect delayed growth due to their compromised health status, which eventually led to their premature death and may not be representative of their living counterparts. Therefore, before comparing dental maturation between the two samples of children, we evaluate the potential effects of mortality bias in the skeletal sample, by comparing dental maturation of children who died of natural causes with that of children who died of accidental causes.

MATERIALS AND METHODS

Samples

We utilized two samples of Portuguese children, which are over 50 years apart from each other. The earlier sample, described as the historic sample, is comprised of known sex and age skeletons from two museum skeletal collections; the Lisbon and Coimbra collections. This sample is comprised of 114 (60 girls and 54 boys) individuals born between 1887 and 1960 in Central and Southern Portugal, who died in Coimbra and Lisbon between 1903 and 1972. The majority of births (80%) occurred between 1910 and 1940, while most individuals (81%) died between 1920 and 1950. The individuals range in age between 6 and 18 years. Calendar ages were obtained by subtracting the date of birth from the date of death. These children are considered representative of the middle-to-low socioeconomic strata of the Lisbon and Coimbra population during the first half of the twentieth century, as inferred from their father's occupation stated in the birth record. These occupations include a large proportion of menial jobs. The Lisbon and Coimbra samples provide an extraordinary opportunity to assess the timing of tooth formation in earlier periods due to the fact that chronological age is known in these collections with great accuracy, from civil registration records. The skeletons in these collections have been obtained from abandoned burial plots at the local cemeteries in Lisbon and Coimbra, where temporary graves are cleared periodically for new internments. Once skeletonization is reached, the remains are exhumed and kept in small burial niches (ossuaries) for a rental fee. The remains are considered abandoned and, subsequently destroyed or reburied in the communal grave after several years of unpaid fees by the relatives. Prior to destruction or reburial, the remains are collected and curated by each Museum, under a special permit. For more details on the composition and history of these collections, see Rocha (1995) and Cardoso (2006).

The more recent sample, described as the modern sample, consists of patients attending the residency dental clinic at the Faculty of Dentistry of the University of Porto. This sample comprises 521 children (260 girls and 261 boys) born between 1985 and 1997, who were examined between 1998 and 2006. The age range is identical to the historic sample (6–18 years) and calendar ages were calculated by subtracting the date of birth from the date when the panoramic radiograph was taken. The radiographs were taken for diagnostic purposes and none of the children had undergone orthodontic treatment before taking the selected radiograph. Only children who were free of anomalies and pathologies were selected. The socioeconomic background of children in the modern sample can be described as middle-to-low, since the residency dental

TABLE 1. Age and sex distribution of the historic and modern samples

	Historic		Modern	
Age (years)	Girls	Boys	Girls	Boys
6.0–6.9	3	4	16	15
7.0–7.9	5	4	20	35
8.0–8.9	4	5	34	31
9.0–9.9	5	3	36	30
10.0–10.9	9	5	27	42
11.0–11.9	5	5	15	20
12.0–12.9	5	4	24	20
13.0–13.9	4	3	24	28
14.0–14.9	5	5	21	13
15.0–15.9	8	6	15	5
16.0–16.9	2	3	10	10
17.0–17.9	1	5	12	6
18.0–18.9	4	2	6	6
Total	60	54	260	261

Values are in number of individuals.

clinics provide lower cost services in comparison with the private clinics. The age and sex distribution of each sample is shown in Table 1.

The two samples represent an important environmental transition in Portugal, with its critical turning point roughly between 1960 and 1980. Although spanning several decades, children in the historic sample symbolize a population who was going through tremendous social and economic difficulties and where changes were occurring at an unbearable slow rate. Portugal remained a very isolated and underdeveloped society for most of the 20th century. The rise of a 50-year long dictatorship established a strong and repressive state which reinforced the closed economic and social nature of the country. Portuguese per capita income was one of the lowest in Europe and living conditions were very poor until quite recently. Children in the modern sample, on the other hand, represent a major change towards improved living conditions for the all population, but for children in particular. In 1974 a military coup overthrew the dictatorship and set the basis for a democratic pluralistic system in Portugal (Baiôa et al., 2003) that accomplished major improvements in economic and social welfare. Portugal experienced the first significant economic changes in 1960 with the country's union with the EFTA (European Free Trade Association) (Corkill, 2003). Per capita income doubled and tripled in just a few decades and initiated the exponential growth of the economy (Tortella, 1994). In parallel with the increase in per capita income is the twofold increase in public expenditures on health and in social security coverage (Lobo, 2000). In addition, the dictatorship years created an absent welfare state and it was not until the 1970s that public health and social welfare programs were established and later successfully implemented (Veiga et al., 2004). Health conditions in Portugal were long among the poorest in Western Europe and it was only between 1970 and 1990 that the greatest relative declines in both infant and post-neonatal mortality occurred (Barreto, 2000). Rapid changes are also expressed in dietary improvements since 1960–1980, namely increased per capita total daily caloric intakes and percentage of daily caloric intake from animal products, such as milk or meat (Cardoso, 2008).

Our analysis assumes that both samples do not differ genetically and any differences detected in dental root formation are likely to derive from environmental influences. Portugal has remained very genetically homogenous (Pereira et al. 2000), where foreign immigration is unlikely to have played an important role behind the changes in tooth formation. For example, Pereira et al. (2000) examined the diversity in mtDNA of the modern Portuguese population by comparing Northern, Central, and Southern Portugal and did not detect a statistically significant difference between the three regions. Similarly, a study using the Y-chromosome found with no statistically significant differences among the three regions (Beleza et al., 2006). In addition, any genetic contribution to the difference in dental root formation between the historic and modern samples is not possible to assess given that there is no known basis for the genetic determination of tooth formation differences between populations. This is supported by various recent studies, where it is shown that differences in tooth formation timing does not seem to depend predominantly on population differences (Braga et al., 2005; Liversidge and Speechly, 2001; Liversidge, 2003; Liversidge et al., 2006; Maber et al., 2006). This is further supported by the histological findings provided by Reid and Dean (2006) and Reid et al. (2008), who have found that population differences in crown formation timing are remarkably small, particularly for molars.

Data collection

The maturation of seven developing permanent teeth (excluding M3) in the mandible was assessed using Demirjian's eight stages (Demirjian et al., 1973). Stages were rated from radiographs in both the historic and modern samples, but the Lisbon collection data also includes stages rated from direct observations of loose teeth. There were some missing teeth in the historic sample and, hence, sample size for each tooth varies slightly in this sample. Because of the limitations associated with the age at which the children were radiographed in the modern sample, only the later stages of tooth formation, namely root formation, are included in this study. While the age of 6 was imposed as the lower limit of the samples' age range because of constraints of the modern sample, 18 years was set as the upper limit because it is the age at which the entire dentition (except M3) is fully formed in both samples. Data on Demirjian's staging was collected from the Lisbon sample (historic) by author H.C., from the Coimbra sample (historic) by Y.H. and from the Porto sample (modern) by P.J.

Assigning a categorical system to the continuous process of dental maturation includes problems of defining stages and subjectivity in identifying consecutive stages. Although the stages devised by Demirjian et al. (1973) probably comprise the most carefully defined system, based on radiographic pictures, diagrams and written criteria, it is essential to assess reliability of stage rating by conducting intra- and interobserver tests. Intra-individual repeatability and interindividual reproducibility were assessed using Cohen's Kappa and percent of agreement performed on double ratings of a subset of 28 orthopantomograms. Sign tests were used to assess whether the intra- or interobserver difference in scores was zero or equally likely to be positive (overscoring) or negative (underscoring). Intra- and interobserver agreement range from substantial to almost perfect agreement (Landis and Koch, 1977) (Table 2) and are consistent with reports in

TABLE 2. Cohen's Kappa and percentage of agreement (in parentheses) for intra-observer replicability and inter-observer reproducibility in double ratings of 28 orthopantomograms

		Reproducibility		
	Replicability	Observer 1	Observer 2	Observer 3
Observer 1	0.84 (88%)	–		
Observer 2	0.64 (73%)	0.64 (73%)	–	
Observer 3	0.87 (93%)	0.63 (85%)	0.75 (82%)	–

The difference between two scores did not exceed one stage for any tooth and sign tests showed that one observer was not systematically underscoring or overscoring relative to the other observer.

the literature (Dhanjal et al., 2006; Levesque and Demirjian, 1980; Leurs et al., 2005; Liversidge et al., 2006; Maber et al., 2006; Nykänen et al., 1998). Sign tests do not show any systematic errors in intra- or inter-observer assessment.

Assessing secular change

Dental maturation data was dichotomized into "stage not-attained" versus "stage attained" and the median age-of-attainment of root stages of individual teeth was calculated for each sample and sex separately, using a cumulative distribution function. At first, an exploratory analysis of the data was carried out and scatter-plots were visually inspected for outliers. Standardized residuals and Cook's distances were calculated for each observation, for detection of outliers and influential cases, respectively. Observations with standardized residuals greater than 3 in absolute value were excluded, as well as observations with a Cook's distance greater than one. Three outliers were detected in the historic sample and 15 in the modern sample. These markedly deviated observations represent spurious data which resulted from observation and transcription errors during data collection. The median age-of-attainment of a stage was calculated using logistic regression and is equivalent to the age when half the children (the median or the 50th percentile) reach or attain that stage (Liversidge, 2008). The median age-of-attainment has also been described as mean age-of-attainment (Liversidge et al., 2006; Liversidge, 2008), but is different from what has been called mean age at a stage, where the ages of children at a certain stage are averaged (Nadler, 1998). In addition to the median, the lower quartile (LQ, 25^{th} percentile), upper quartile (UQ, 75^{th} percentile) and the inter-quartile range (IQR) were also calculated to provide a measure of variability around the median. The median and quartiles were obtained from the logistic regression formulae and not directly from the sample distribution. The significance of differences in median age-of-attainment between samples was obtained by calculating an overall logistic regression model for each sex alone, where sample (historic versus modern) is treated as categorical covariate, and testing whether the coefficient for the variable "sample" is statistically different from zero (no statistically significant differences), using the Wald statistic. The use of logistic regression overcomes the difficulties of non-normality or heterocedasticity for the predictor variable (Pampel, 2000; Wright, 1995), as well as of an uneven age distribution and inadequate age range that hamper a number of studies on dental development (Liversidge and Molleson, 2004; Liversidge, 2008; Smith, 1991). In addition, the logistic regression approach is particularly appropriate for the last stage (H), because as it persists for an individual's whole life, timing cannot be estimated from other methods, such as mean age at that stage (Liversidge, 2008). The -2 Log Likelihood and the Hosmer and Lemeshow test were used to assess the quality of the logistic regression model adjusted to the data of each individual tooth stage. The significance of logistic regression coefficients was tested using the Wald statistic. Logistic regression models which fitted the data poorly and provided statistically insignificant coefficients for the constant and predictor variable (age) were not considered.

Differences in median age-of-attainment between consecutive stages (interval between stages) and between the earliest and latest stages observed for the root were calculated in both sexes, to assess changes in the duration of tooth (root) formation between the modern and historic samples. Wilcoxon matched pairs test was used to test the significance of differences in median age-of-attainment between stages in the modern and historic samples. Differences in median age-of-attainment between consecutive stages of male and female teeth were also compared using a Mann-Whitney test. This was carried out to assess sex differences in the duration of tooth formation.

Assessing mortality bias

The analysis of the effects of cause of death, to disentangle the influence of mortality bias, is rooted on the assertion that the health conditions which lead to a child's death are likely to influence his or her growth status: natural causes are likely to have an effect, accidental causes are not. Our assumption is that children who have died from an accident (e.g. drowning, fall, road accident) have a greater probability of most closely resembling their living counterparts, as their developmental status is not compromised by their health status which eventually determined their premature death. Since there is reliable information about cause of death in the historic sample, as obtained from the civil registration of death, we divided it into two distinct groups: one of children who died of natural causes ($n = 68$) and one of children who died of accidental violent deaths ($n = 13$). We then estimated dental age in both groups and compared it to chronological age in order to assess whether dental development in the group of children who died accidentally falls ahead that in the group of children who died of natural cases.

For each child in the historic sample, dental age was estimated for each individual tooth using the sex specific median age-of-attainment calculated from the modern sample and a mean age for each child was calculated from the ages obtained from each individual tooth. By doing this we make the general assumption that the child was average and that each group of children (natural versus accidental causes) represent the average of their group. Age estimation from mean age-of-attainment has been deemed appropriate for comparative purposes (Liversidge, 2008), such as in this case where the same approach is being applied to the two groups being compared. A measure of relative development was calculated by subtracting the chronological age from the estimated dental age and the sexes were pooled. Mean differences between dental and chronological age in each group were compared using a t-test to determine whether dental development is significantly delayed in the natural deaths group relative

TABLE 3. Median age-of-attainment in years (M) for each individual tooth stage in girls, and difference (Diff) in age-of-attainment between historic and modern samples

		Historic				Modern					
Tooth[a]	Stage	LQ	M	UQ	IQR	LQ	M	UQ	IQR	Diff	Sign
I1	G	7.78	8.55	9.31	1.53	6.49	6.70	6.91	0.42	1.85	0.003
	H	–	–	–	–	6.98	7.37	7.75	0.76	–	–
I2	G	8.12	8.60	9.08	0.97	7.07	7.43	7.78	0.71	1.17	0.002
	H	–	–	–	–	7.48	8.18	8.87	1.39	–	–
C	F	8.19	8.61	9.03	0.84	6.49	6.83	7.18	0.69	1.78	0.000
	G	10.46	10.83	11.19	0.73	9.41	9.99	10.57	1.16	0.84	0.015
	H	11.22	11.93	12.64	1.42	10.46	11.10	11.73	1.28	0.83	0.048
P1	E	–	–	–		6.63	6.91	7.20	0.57	–	–
	F	9.38	9.84	10.31	0.93	7.49	7.97	8.46	0.98	1.87	0.000
	G	10.51	11.04	11.57	1.06	9.92	10.45	10.97	1.06	0.59	0.095
	H	12.07	12.96	13.85	1.78	10.83	11.52	12.20	1.37	1.44	0.002
P2	E	8.63	9.17	9.71	1.08	6.84	7.40	7.96	1.12	1.77	0.000
	F	10.35	10.82	11.29	0.94	7.91	8.68	9.45	1.53	2.14	0.000
	G	11.84	12.82	13.80	1.96	10.52	11.20	11.87	1.35	1.62	0.001
	H	13.04	14.21	15.39	2.36	11.82	12.48	13.15	1.33	1.73	0.000
M1	G	7.27	8.08	8.89	1.62	6.32	6.65	6.98	0.66	1.43	0.001
	H	10.06	10.60	11.13	1.06	8.24	8.92	9.61	1.37	1.68	0.000
M2	E	–	–	–	–	7.64	8.27	8.91	1.27	–	–
	F	10.62	11.00	11.37	0.76	9.25	9.69	10.12	0.87	1.31	0.000
	G	11.90	12.71	13.52	1.62	10.41	11.06	11.70	1.30	1.65	0.000
	H	15.17	15.70	16.23	1.05	13.52	14.35	15.19	1.67	1.35	0.003

Sign refers to the *P*-value of differences using logistic regression. LQ, lower quartile (25th percentile); UQ, upper quartile (75th percentile); IQR, inter-quartile range.
[a]I1, central incisor; I2, lateral incisor; C, canine; PM1, first premolar; PM2, second premolar; M1, first molar; M2, second molar.

TABLE 4. Median age-of-attainment in years (M) for each individual tooth stage in boys, and difference (Diff) in age-of-attainment between historic and modern samples

		Historic				Modern					
Tooth[a]	Stage	LQ	M	UQ	IQR	LQ	M	UQ	IQR	Diff	Sign
I1	G	7.85	8.41	8.97	1.12	5.67	6.50	7.33	1.65	1.91	0.003
	H	8.51	9.51	10.51	2.00	7.63	8.12	8.61	0.98	1.39	0.003
I2	G	8.93	9.34	9.76	0.83	7.50	7.99	8.49	1.00	1.35	0.002
	H	9.97	10.24	10.50	0.53	8.51	8.97	9.43	0.92	1.27	0.002
C	F	8.49	9.34	10.19	1.70	7.12	7.79	8.46	1.34	1.55	0.001
	G	11.14	11.78	12.43	1.29	10.43	11.16	11.88	1.44	0.62	0.132
	H	12.63	13.74	14.85	2.22	11.71	12.38	13.04	1.33	1.36	0.006
P1	E	7.45	7.77	8.09	0.65	7.01	7.58	8.16	1.15	0.19	0.687
	F	9.97	10.46	10.94	0.96	8.13	8.58	9.03	0.90	1.88	0.000
	G	–	–	–	–	10.64	11.38	12.12	1.48	–	–
	H	11.73	12.55	13.37	1.64	11.66	12.33	13.00	1.34	0.22	0.671
P2	E	–	–	–	–	7.04	7.87	8.70	1.66	–	–
	F	10.42	10.89	11.35	0.93	8.36	9.14	9.93	1.58	1.75	0.000
	G	–	–	–	–	11.29	12.18	13.8	2.51	–	–
	H	13.19	14.41	15.62	2.42	12.43	13.22	14.01	1.58	1.19	0.028
M1	G	7.77	8.01	8.25	0.49	6.21	6.81	7.41	1.19	1.20	0.005
	H	11.22	11.57	11.93	0.70	8.90	9.59	10.29	1.39	1.98	0.000
M2	E	8.38	8.88	9.37	0.99	7.88	8.40	8.91	1.02	0.48	0.191
	F	–	–	–	–	9.58	10.20	10.82	1.24	–	–
	G	12.10	12.62	13.13	1.03	11.19	11.99	12.79	1.60	0.63	0.154
	H	15.79	16.20	16.60	0.82	13.70	14.44	15.18	1.48	1.76	0.002

Values are in years. Sign refers to the *P*-value of differences using logistic regression. LQ, lower quartile (25th percentile); UQ, upper quartile (75th percentile); IQR, inter-quartile range.
[a]I1, central incisor; I2, lateral incisor; C, canine; PM1, first premolar; PM2, second premolar; M1, first molar; M2, second molar.

to the accidental deaths group. Although the groups have a very different sample size, the *t*-test is considered a robust test, particularly when the variances are similar in the two groups (Zar, 1999), such as in this case. If there are no statistically significant differences in mean discrepancies between the two groups, then one group is not significantly delayed or advanced relative to the other in dental development and a premature death effect is not detected. Owing to the small sample size of the group of children who died of accidental violent deaths, a power analysis was also performed to assess the probability of making a type II error (Zar, 1999). Since we are concerned with whether the differences between groups can explain the difference between the historic and modern samples, we are only interested with a power analysis for a test which can detect a difference between groups of the magnitude of the difference between the historic and modern samples.

RESULTS

Secular change

In both sexes, all individual root stages show earlier median ages-of-attainment in the modern sample, showing that timing of formation is significantly advanced in the modern compared to the historic sample (Tables 3 and 4).

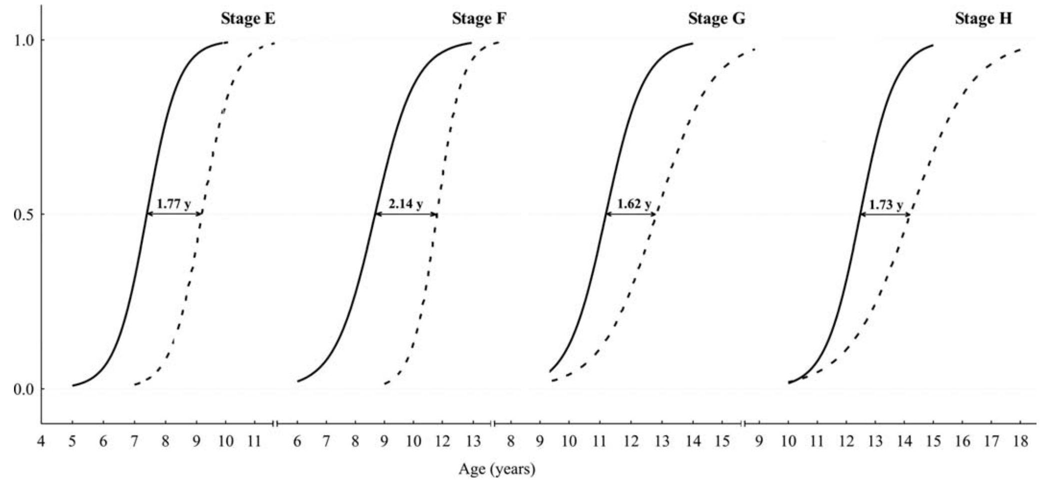

Fig. 1. Median secular advancement (in years) in formation timing of dental root stages E through H, of the second premolar in girls. The smoothed cumulative frequency distribution curves (logistic regression) for stages E through H, of the second premolar in girls, are depicted. The solid line corresponds to the historic data and the dashed line to the modern data.

TABLE 5. Differences between median age-of-attainment in years of one individual tooth stage and the next (O-N) and of the first and last stages observed (F-L), in girls

		Historic		Modern	
Tooth[a]	Stage	O-N	F-L	O-N	F-L
C	F	−2.22	−3.32	−3.16	−4.27
	G	−1.10	–	−1.11	–
	H	–	–	–	–
P1	E	–	–	b	b
	F	−1.20	−3.12	−2.48	−3.55
	G	−1.92	–	−1.07	–
	H	–	–	–	–
P2	E	−1.65	−5.04	−1.28	−5.08
	F	−2.00	–	−2.52	–
	G	−1.39	–	−1.28	–
	H	–	–	–	–
M1	G	−2.52	−2.52	−2.27	−2.27
	H	–	–	–	–
M2	E	–	–	b	b
	F	−1.71	−4.70	−1.37	−4.66
	G	−2.99	–	−3.29	–
	H	–	–	–	–
Mean		−1.87	−3.74	−1.98	−3.97

The incisors are not shown due to incomplete data.
[a]I1, central incisor; I2, lateral incisor; C, canine; PM1, first premolar; PM2, second premolar; M1, first molar; M2, second molar.
[b]Denotes missing data for historic sample, which has also been omitted in the modern sample for comparative purposes.

TABLE 6. Differences between median age-of-attainment in years of one individual tooth stage and the next (O-N) and of the first and last stages observed (F-L), in boys

		Historic		Modern	
Tooth[a]	Stage	O-N	F-L	O-N	F-L
C	F	−2.44	−4.40	−3.37	−4.59
	G	−1.96	–	−1.22	–
	H	–	–	–	–
P1	E	−2.69	−4.78	−1.00	−4.75
	F	–	–	b	–
	G	–	–	b	–
	H	–	–	–	–
P2	E	–	–	b	b
	F	–	−3.52	b	−4.08
	G	–	–	b	–
	H	–	–	–	–
M1	G	−3.56	−3.56	−2.78	−2.78
	H	–	–	–	–
M2	E	–	−7.32	b	−6.04
	F	–	–	b	–
	G	−3.69	–	−2.45	–
	H	–	–	–	–
Mean		−2.87	−4.72	−2.16	−4.45

The incisors are not shown due to incomplete data in girls.
[a]I1, central incisor; I2 lateral incisor; C, canine; PM1, first premolar; PM2, second premolar; M1, first molar; M2, second molar.
[b]Denotes missing data for historic sample, which has also been omitted in the modern sample for comparative purposes.

Advancement in median age-of-attainment in the modern sample relative to the historic sample ranged between 0.19 and 1.98 years (mean = 1.22 years) in boys and between 0.59 and 2.14 years in girls (mean = 1.47 years). However, not all historic-modern comparisons showed statistically significant differences ($P < 0.05$). The exceptions are: the canine (stage G), the first premolar (stages E and H), and the second molar (stages E and G) in boys; and the first premolar (stage G) in girls. Figure 1 illustrates secular acceleration in dental root maturation, by showing the mean advancement in formation timing of dental stages E through H of the second premolar in girls.

The age-of-attainment of root developmental stages was earlier in girls, except in the first incisor (stage G) in the modern and historic samples, and the first premolar (stage H), first molar (stage G) and second molar (stage G) in the historic sample (Tables 3 and 4). This indicates that girls begin to develop their teeth earlier than boys, and the mean sex difference in timing is about one half year, both in the historic and modern samples.

Tables 5 and 6 show that the mean age interval between consecutive root stages, and between the earliest and latest stages observed are similar in both the modern and historic samples. For example, in girls, the mean duration of formation of a single stage (F–L column) in the historic sample (1.87 years) is very similar to that of the modern sample (1.98 years). When comparing secular differences in duration of stage formation, only data which could be paired (modern and historic) were used to calculate the Wicoxon tests. This test did not show significant differences between the modern and historic samples in the duration of formation of individual stages, in either boys or girls.

In only a few teeth (second premolar in girls, and first premolar and second molar in boys) was it possible to determine the length of time required for the formation of tooth roots, obtained as the mean time interval between initial calcification (stage E) and the root completed stage (stage H). No significant differences were found between the modern and historic samples in duration of complete root formation. For example, the length of time required for the formation of the root of the first premolar in boys was 4.78 years in the historic sample and 4.75 years in the modern sample. Second premolar root formation time in girls was 5.04 in the historic sample and 5.08 in the modern sample.

There is also a tendency for a greater mean age interval between consecutive stages in boys (historic and modern), compared with that of girls. This suggests that girls advance more rapidly in root formation. The Mann-Whitney tests shows that sex differences in the duration of tooth formation are only significant in the historic sample (df = 17, $P = 0.0275$).

Mortality bias

Dental ages were estimated in the historic sample and the mean difference between dental and chronological age in the group of children who died of natural causes is −2.44 years (s.d. = 1.36), whereas the same difference is −2.66 years (s.d. = 1.25) for the group of children who died of accidental causes. A *t*-test failed to show statistically significant differences between the two groups ($P = 0.570$), thus suggesting that mortality bias has no noteworthy effect on root formation of the historic sample. The power analysis performed showed that the probability of making a Type II error when detecting a difference between the groups of 1.22 and 1.47 years (mean difference between the historic and modern samples for boys and girls) is 0.76 and 0.96, respectively. Therefore, the probability of accepting the null hypothesis of no differences between groups, when it is false, is between 0.24 and 0.04.

DISCUSSION

This study demonstrates a consistent secular acceleration in tooth formation timing, namely in the timing of root formation. We were able to show a statistically significant advancement in timing of dental maturation of Portuguese boys and girls over the last half a century. Results also suggest that an earlier timing of dental maturation was not accompanied by a change in the duration of root formation. Therefore, these results suggest that secular change is altering the age at which dentine starts to form and not how fast it is forming.

While other studies have suggested a secular acceleration in dental maturation (Holtgrave et al., 1997; Monge et al., 2007), this was accomplished by comparing a sample of modern children with earlier dental development standards. To the best of our knowledge, the only exception is the research carried out by Simpson and Kunos (1998), where dental development in two samples of American children, ~50 years a part, have been compared using suitable statistical techniques. In their study, a slight delay in root formation of the mandibular canine was noted in the earlier sample. Results shown here agree with the findings of Heuzé and Cardoso (2008), who showed that a Bayesian nongeographic-specific dental age estimation method, based on a diverse sample of modern children, consistently underestimated the age of a sample of children from the Lisbon collection by about one year. Our results are also consistent with the histological findings of Reid and Dean (2006) and Reid et al. (2008), who have shown that duration of crown formation has not changed over time. This leaves (1) a change in the timing of initiation or (2) a change in the rate or duration of dentinogenesis, to explain a secular acceleration in tooth maturation. A change in timing over time is precisely what we have found, together with an unchanged duration of root formation. In addition, results indicate that girls not only begin to develop their teeth earlier than boys, but also advance through root stages more rapidly (both historic and modern samples), comparing favorably with results reported by Demirjan and Levesque (1980).

Although mortality bias was an initial concern, analysis presented here shows that root formation in the group of children who died of natural causes is not significantly delayed relative to root formation in the group of children who died from accidents. This was confirmed by the power analysis performed in this study, which showed that there is a relatively low probability of assuming that the groups of children did not differed, when in fact they may have been different. There are only two ways of increasing the power of a test, and one is out of our reach (increasing sample size). We may, however, increase the power by changing the nature of the test (Sokal and Rolf, 1995), namely utilizing a nonparametric Mann-Whitney test. The significance of this test is roughly similar to that of the *t*-test ($P = 0.598$) obtained. If we assume that the death of children in the accidental deaths group cannot reflect the effects of their health status at the time of their death, their similarity with the children who died of natural causes supports the assertion that there is no significant effect of premature death on the developmental status of children in the historic sample. Although we cannot ignore that the Lisbon and Coimbra children are dead and died at a relatively earlier age than the modern children will eventually die, ultimately, the historic children died at a relatively earlier age because they had a worst environment. Therefore, the historic sample simply reflects a delayed growth as a consequence of the poor environment in which they lived and the comparison between the historic and modern sample is likely to reflect a fundamental change in the environment, which promoted better growth and a secular acceleration in development of the modern sample.

One of this study's limitations is the small size of the historic sample and its possible insufficient age range, which may account for earlier maturation in one or a few individual tooth stages. This may also help to explain why the earlier timing of dental development in the modern sample was not statistically significant for all individual tooth stages analyzed. However, it is unlikely that the small sample size can explain such a consistent pattern of dental delay in the historic sample, across the entire permanent dentition. Although the historic sample is relatively small, its importance lies in being representative of the modern sample at an early period, when radiographs or other sources of information about dental development of children are typically unavailable, at least in Portugal. The logistic regression approach used in this study accounts for the variation of development time of individual tooth stages and for variation which results from random fluctuations in the small historic sample. In addition, the sexes were treated separately so that what appears to be accelerated or delayed development cannot be accounted by sex differences in growth. The cross-sectional nature of the sample may also be deemed inappropriate to assess changes in duration of root formation, but results also show a consistent pattern across the whole dentition.

Although we have been able to demonstrate a secular acceleration towards earlier root formation in Portuguese children, what exactly it is that might underlie this secular trend in tooth development? The mechanism by which teeth mature earlier is unknown, but nutrition may play a significant role in such trend. In particular, nutrition may change the age at initiation of root formation. Although results are not conclusive, a few studies have examined the influence of nutrition on dental maturation. For example, Rai (2009) using third molars extracted from a sample of North Indian patients between the ages of 17 and 21, found that dental and chronological age did not differ in the group of well nourished individuals, but dental age underestimated chronological age in the group of malnourished subjects. In this study, the patients were categorized according to the nutritional risk index, which incorporates information about the subject's weight and plasma albumin level (Rai, 2009). A significant effect of malnutrition on dental development is strongly suggested by Murchinson et al. (1988) in an experimental study carried out with protein-deprived infant rhesus monkeys. Crown-root length in protein restricted monkeys was found to be significantly less than those of controls, but only for deciduous second molars. Hilgers et al. (2006) also found a significant effect of nutrition on dental development, but in conditions of overnutrition. BMI status was determined in a sample of children between the ages of 8 and 15, where a mean dental age acceleration of 1.53 years was found for obese individuals, compared to 0.68 years for normal children. Comparatively, Flores-Mir et al. (2005) and Cameriere et al. (2007) did not find a significant delay in tooth growth of stunted Peruvian children between the ages of 9.5 and 16.5.

Despite the conflicting results about the effects of nutrition on dental maturation of modern children, one of the primary explanations behind the secular trend phenomena is nutritional, involving an increase in calories, quality of diet, increased availability of certain foods and decrease in energy loss and expenditure, which accelerate somatic maturation timing (Bogin, 1999; Garn, 1987; Malina, 1990; Susanne, 1984; Tanner, 1989). The acceleration in dental root maturation timing of the Portuguese detected in this study, correlates well with a period of great political, social and economic changes initiated in Portugal in the 1960s and 1970s, which had critical consequences for the physical development of children, through access to better nutrition, especially more protein and caloric intake during infancy, but also through greater overall sanitary conditions and hygiene, lessening of disease, as well as delivery of social and medical care. While the historic sample reflects a period of less development during the 20th century, the children in the modern sample were all born after 1970 and represent a period of great changes towards modernization and improved environmental conditions. The improved environment for human growth allowed for greater growth in height and weight of Portuguese children (Cardoso, 2008), as well as for a decrease in age of menarche (Padez, 2003). These changes in body size and earlier maturation of the Portuguese ad to the widely documented secular trends that are taking place in the industrialized nations of the world (see Hauspie et al., 1996 and Bodzsar and Susanne, 1998, for a review). Temporal changes in height, weight and age at menarche have documented the great plasticity of the human growth process (Bogin, 1999), and now dental maturation.

Although environmental factors have been somewhat neglected as sources of variation in dental development, our results show that differences in timing of root stages of individual teeth between the modern and historic samples can be ascribed to a secular advancement in dental development of about 1–1.5 years. This secular trend effect has important consequences for a wide variety of research areas. For example, these results have an impact on medical clinical practice, by suggesting that earlier dental age standards may not be appropriate for orthodontic treatment of modern patients in the developed nations. In a forensic context, since children from developing countries mimic children from developed countries in earlier periods of time, dental age estimation methods based on children of the developed world may not perform well in undocumented refugee children and young illegal immigrants from the developing nations. Results also suggest that the variability of dental formation timing encountered in other Hominids and Hominins species should be more carefully interpreted since this variability could result from differences in environmental quality more than from genetic differences. Yet, future research is still needed to substantiate the findings in this study, particularly with respect to changes in the timing of enamel formation and to changes in the rate of tooth formation. Additionally, it is also interesting to explore further whether accelerated maturation is associated with advanced emergence.

CONCLUSION

The results in this study confirm previous work and show that the children in the modern sample are consistently advanced in timing of root maturation, compared to children in the historic sample, but they do not seem to differ in duration of root formation. The detection of a secular acceleration in dental maturation is particularly important because it provides an important additional insight into the link between human growth and the environment. In fact, this study shows that the plasticity in dental maturation is greater than previously thought. The acceleration in dental maturation detected here seems to reflect a greater access to better nutrition, lessening of disease, decrease in energy expenditure and improvement in overall living conditions experienced by modern Portuguese children, as a consequence of major political, economical, social and public health changes which occurred in Portugal at the late 20th century.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Barry Bogin for important insights and for his comments on an earlier version of this article; to Dr. Marina Andrade for statistical advice; to Dr. Helen Liversidge for important suggestions; to Dr. Ana Luísa Santos, whose diligence allowed us to obtain additional biographic information for the children in the Coimbra collection; to Dr. Américo Afonso, Dr. José António and Dr. Inês Caldas, for support during data collection at the Faculty of Dentistry in Porto; to Dr. Joan T. Richtsmeier for her editorial suggestions; to Dr. Janet Monge for re-prints; and to Carolan Wood for facilitating the access to important bibliographic sources. The two anonymous reviewers provided crucial comments which improved the paper significantly.

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