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AGENTS WITHIN A DEVELOPMENTAL COMPLEX ADAPTIVE SYSTEM: INTRAUTERINE MALE HORMONES AND DENTAL ARCH SIZE IN HUMANS

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ABSTRACT

Oral development is a complex adaptive system influenced by genetic, epigenetic and environmental factors. The dental arch develops from 6 weeks in utero until adult life, forming an accessible record to study general growth and development. Increased tooth size in female dizygotic opposite-sex (DZOS) twins compared with female dizygotic same-sex (DZSS) twins provides evidence for the masculinisation of females gestated with a male co-twin, possibly due to the intrauterine influence of male sex hormones: the "Twin Testosterone Transfer" (TTT) hypothesis. This study aimed to investigate the potential influence of intrauterine male hormones on dental arch size of female DZOS twins. Serial dental models of the primary and permanent dentitions of 69 female DZOS and DZSS twins were examined. Intercanine width, intermolar width, arch length and arch circumference were measured using a customised 2D image analysis system. Unpaired t-tests showed significant differences for mandibular intercanine width ($p = 0.03$; effect size = 0.6) and borderline differences for mandibular intermolar width and arch circumference ($p = 0.05$; effect size = 0.5). No significant differences were found in the permanent dentition. These findings provide support for the TTT hypothesis with some arch dimensions being larger in female DZOS twins. We have developed a model of assessing the effects of intrauterine male hormones on the epigenetic changes that last into postnatal life. Our evidence suggests that this is a moderate effect possibly interacting with numerous other environmental factors that may influence arch size.
Keywords: complex adaptive system, epigenetics, sex chromosomes, sexual dimorphism, twins.

1 INTRODUCTION

The development of the human dentition is a complex adaptive system influenced by genetic, epigenetic and environmental factors [1]. Sexual dimorphism in the human craniofacial complex has also been related to the growth promoting effects of the Y-chromosome [2,3] and sex hormones [4,5]. In the dentition, sexual dimorphism has been described in tooth size [6], arch dimensions [7,8] and underlying bone size [3], suggesting common influencing factors on dental and craniofacial development. However, the specific mechanisms by which sex chromosomes and prenatal sex hormones interact and influence craniofacial development require further investigation.

The first surge of intrauterine testosterone occurs around week 6 of human gestation and peaks around week 14. The SRY gene on the Y chromosome is responsible for initiating testicular differentiation in a male foetus [9,10]. A second surge occurs soon after birth, and the hormonal levels remain high during the first year of life. A third surge occurs during the adolescent growth spurt and is responsible for the development of male secondary sexual characteristics. Both the primary and permanent teeth, along with the supporting alveolar structures, develop during the first two surges and therefore provide a good model to study the effects of sex hormones on craniofacial development.

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Currently, foetal testosterone level is most accurately measured via amniocentesis [11], but the invasive nature of this procedure raises ethical issues and prevents its widespread application [10]. Alternatively, the Twin Testosterone Transfer (TTT) hypothesis, premised on hormonal transfer between foetuses from the same pregnancy [12–14], allows non-invasive and cost effective assessment of the influence of prenatal testosterone on human development. Previous twin studies supporting the TTT hypothesis have demonstrated increased masculine traits in human females from opposite-sex twin pairs as opposed to same-sex twin pairs. Examples include increased total brain volume [14], altered craniofacial growth and dental asymmetries [15], increased symptoms of Alcohol Use Disorder (AUD) [16], lower disordered eating attitudes [17] and increased tooth crown size [4,5]. These findings not only support the TTT hypothesis but also indicate that hormonal transfer may influence sensitivity of androgen receptors later in life (i.e. during pubertal hormone surges) [11].

2 AIMS

The aim of this study was to investigate the possible influence of intrauterine male hormones on dental arch dimensions (intercanine width, intermolar width, arch length and arch circumference) in both the primary and permanent dentitions of female dizygotic opposite-sex (DZOS) twins and female dizygotic same-sex (DZSS) twins. It was hypothesised that female DZOS twins would have larger dental arch dimensions than female DZSS twins, reflecting intrauterine male hormone diffusion.

3 MATERIALS AND METHODS

3.1 Materials

A convenience sample of 69 females from 33 DZOS and 36 DZSS twin pairs was selected for this study. Serial dental casts of the primary and permanent dentitions of the same individuals were obtained from the collection of records of twins enrolled in an ongoing study of dental and facial development at the School of Dentistry, University of Adelaide [18]. One co-twin from each pair of DZOS and DZSS twins was randomly selected by generating and assigning random numbers to avoid bias from inclusion of the same arch data from both co-twins. All participants were of European ancestry with no relevant medical or dental history. Twin zygosities were confirmed by analysing up to six highly variable genetic loci (*FES, vWA3l, F13A1, TH01, D21S11, FGA*) on six different chromosomes, using DNA obtained from buccal cells. The probability of dizygosity, given concordance for all systems, was <1% [19]. Dental impressions were taken when the twins were aged 4 to 7 years (mean age = 5.5 years) in the primary dentition and 12–17 years (mean age = 14.1 years) in the permanent dentition. The inclusion criteria included presence of all primary and permanent teeth, except second and third permanent molars. Participants with missing teeth, significant wear or partially erupted teeth were excluded.

Dental arches were examined using a 2D image analysis system, previously validated for tooth size and dental arch determination [20,21]. Each dental cast was clamped on a cast surveyor table and a tripod levelling device was used to level it accurately into a horizontal plane. The plane was defined by three reference points, including the central fossae of the second primary molars in the primary dentition, the first permanent molars in the permanent dentition and the contact area of the central incisors. An American Board of Forensic Odontology (ABFO) ruler No.2 (Lightening Powder Co Inc., Oregon, USA) was used as a reference scale (Fig. 1a–d). An occlusal photograph was then obtained for each dental cast for analysis.

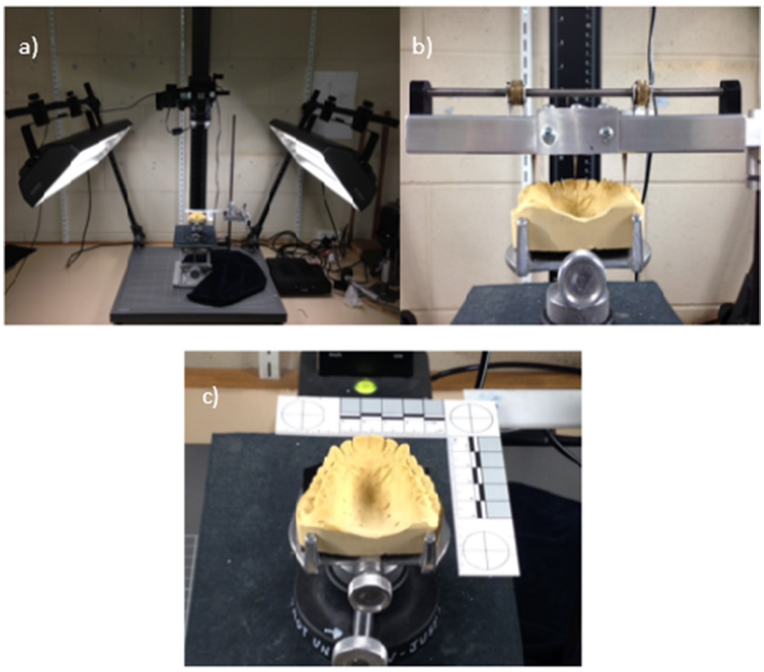

Figure 1: (a) 2D Image system (b) Tripod levelling device (c) Reference scale.

3.2 Measurement of arch dimensions

Based on previous studies [7,8,22], the four most repeatable landmarks (intercanine width, intermolar width, arch length and arch circumference) were chosen. Intercanine and intermolar widths were measured between the cuspal tips of canines and molars for both primary and permanent dentitions. Arch length was defined as a perpendicular distance between the contact area of the central incisors and the line passing through the most distal surface of the second primary molar in primary dentition, and the most mesial surface of the first permanent molar in permanent dentition. Arch circumference was measured as the line passing through the mesial and distal contact areas of each tooth from the first permanent molar/second primary molar of one side to the first permanent molar/second primary molar of the opposite side of the arch (Fig. 2a–d).

The images were analysed using ImageJ v. 1.48 (National Institute of Health, USA). Every image was first calibrated and landmarks digitised to identify the x, y coordinates which were then saved in an excel spreadsheet. A customised macro was used to calculate distances between landmarks corresponding to the parameters of interest.

Intra- and inter-operator repeatability tests were performed for all arch dimensions of primary and permanent dentitions in a randomly selected sub-group of 25% individuals. Mean differences between double determinations of intercanine width (0.15–0.27 mm), intermolar width (0.15–0.25 mm), arch length (0.02–0.10 mm) and arch circumference (0.15–0.25 mm) were non-significant, indicating that systematic errors were unlikely to introduce any bias to the analysis. In addition, the Dahlberg statistic was calculated and the error variance (Se^2) was expressed as a percentage of the observed variance for each variable to determine the extent to which variability due to experimental error affected the observed variance. Error percentages for all arch dimensions were less than 2%. Overall, random errors of method were small for all variables and unlikely to bias the results of the study.

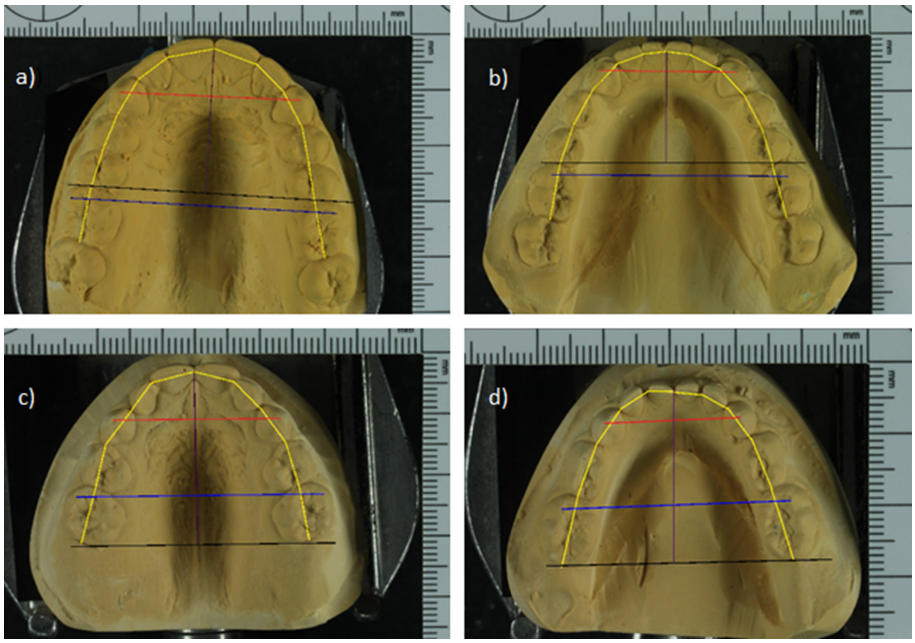

Figure 2: Landmarks on study models for arch dimensions: (a) permanent maxillary arch, (b) permanent mandibular arch, (c) primary maxillary arch and d) primary mandibular arch. Red - Intercanine width. Blue - Intermolar width. Purple - Arch length. Yellow – Arch circumference. Black – Reference line.

Analysis of histograms showed that all variables were approximately normally distributed and parameter descriptive statistics including mean and standard deviation (SD) were calculated. Unpaired t-tests were conducted to determine whether there were significant differences in arch dimensions among the two zygosity groups. The analysis was performed using SPSS version 20 (IBM 2011, USA). The effect size was also calculated to assist in interpreting the intensity of the male hormone effect (i.e. small, medium or large), given that p values alone are not adequate to fully understand the results [23]. Adjustments for multiple t-tests were not made to avoid Type II (β) error in our sample.

4 RESULTS

In the primary dentition, there were significant differences in the mandibular intercanine width ($p = 0.03$; effect size = 0.6) and borderline significance in mandibular intermolar width ($p = 0.05$; effect size = 0.5) and arch circumference ($p = 0.05$; effect size = 0.5) (Table 1). No significant differences were noted in any of the four measurements in the maxillary arch ($p>0.05$) (Table 1). In the permanent dentition, there were no significant differences in the arch dimensions between the two zygosity groups ($p>0.05$) (Table 2).

5 DISCUSSION

This study is the first to test the TTT hypothesis by comparing dental arch dimensions of both human primary and permanent dentitions in female twins. DZOS twin pairs provide a unique model to investigate the influence of prenatal sex hormones on dental development, since female DZOS twins

Table 1: Comparison of dental arch dimensions in the primary dentition of DZOS and DZSS female twins.

	DZOS females			DZSS females			Effect size
	n	mean	SD	n	mean	SD	
Maxillary arch							
Intercanine width	33	27.5	1.89	36	27.5	2.07	0.0
Intermolar width	33	40.5	1.92	36	40.7	2.13	0.1
Arch length	33	27.3	2.00	36	27.1	2.03	0.1
Arch circumference	33	71.5	3.59	36	71.3	4.03	0.0
Mandibular arch							
Intercanine width	33	22.9*	1.36	36	22.0*	1.87	0.6
Intermolar width	33	35.5†	1.76	36	34.6†	1.99	0.5
Arch length	27	24.4	1.53	34	23.8	1.56	0.4
Arch circumference	33	66.8†	2.93	36	65.4†	3.09	0.5

*Mean values in DZOS females were significantly greater than DZSS females at $p<0.05$
†Mean values in DZOS females were greater than DZSS females at borderline significance at $p = 0.05$

Table 2: Comparison of dental arch dimensions in the permanent dentition of DZOS and DZSS female twins.

	DZOS females			DZSS females			Effect size
	n	mean	SD	n	mean	SD	
Maxillary arch							
Intercanine width	29	33.6	2.26	36	27.5	2.07	0.0
Intermolar width	32	50.6	3.05	24	50.8	3.03	0.2
Arch length	32	25.0	2.49	24	25.4	3.02	0.1
Arch circumference	29	93.3	4.68	23	93.7	6.32	0.1
Mandibular arch							
Intercanine width	32	25.6	1.65	23	26.1	1.53	0.3
Intermolar width	33	43.1	2.66	24	43.5	2.67	0.2
Arch length	33	21.4	1.96	24	21.5	2.55	0.0
Arch circumference	31	84.2	4.27	23	85.0	5.08	0.2

No significant differences were found in the permanent arch dimensions between DZOS and DZSS females ($p>0.05$).

develop under different concentrations of sex hormones compared to female DZSS twins [12]. By studying arch size independently, it is possible to consider whether they vary together and are affected by the same factors or whether they vary independently and are influenced by different factors.

Some mandibular arch dimensions of the primary dentition were larger in DZOS female twins than DZSS female twins, providing support for the TTT hypothesis. It appears that the effect of intrauterine male hormones produced by the male counterpart on the female DZOS twin lasts until the age of 5–7 years, with limited effects on arch dimensions later in the permanent dentition. In the permanent dentition, postnatal environmental factors, such as tooth wear from diet and tooth grinding, play a major role in influencing the size and shape of the dental arch [24]. Comparison of data for the primary and permanent dentition in our study imply the occurrence of a priming effect (possibly an epigenetic change) in DZOS females in utero. In addition, our findings indicate greater effects of testosterone on the growth and development of the mandible than the maxilla, which is consistent with the notion that mandible displays greater plasticity to growth and adaptation than the cranium [25].

A proposed mechanism to explain the increased arch size in DZOS females is that testosterone binds to the androgen receptor in the cytoplasm of the cells, resulting in ligand-receptor activation and dimerisation. The activated complex then localises to the nucleus, where it bonds to DNA and affects transcription [26]. Once primed, the effect of testosterone can continue even at low levels. Immunohistochemical studies have also shown the presence of oestrogen, progesterone and testosterone receptors in human foetal cartilaginous tissue [27], indicating a possible direct effect of sex hormones on prenatal dentoalveolar skeletal development. Thus, part of the action of intrauterine testosterone on the dental arches of female DZOS twins may be on the supporting dentoalveolar bone.

Our findings of dental arch dimensions conforming to the TTT hypothesis may have broader applications in craniofacial biology research and clinical practice, given that dental arch development underpins craniofacial morphometry [24]. Interestingly, our findings are consistent with reports of accelerated craniofacial and statural growth in boys with delayed puberty [28,29]. So far, little attention has been given to the dental arch development in children undergoing testosterone treatment, or in children with sex hormone imbalance, but this is an evolving field of research involving multi-disciplinary management.

6 CONCLUSIONS

This is the first study to provide evidence of the effect of intrauterine testosterone on dental arch dimensions of female DZOS twins. In addition to earlier findings of the influence of testosterone on tooth size, this study highlights intrauterine testosterone as an important developmental factor contributing to sexual dimorphism in the human dental arches. Further investigations into the organisational effects of testosterone on other cranial structures will provide additional insights into how genetic, epigenetic and environmental factors influence craniofacial growth and development [30].

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