Check for updates

The Laryngoscope
© 2024 The Authors. *The Laryngoscope* published by Wiley Periodicals LLC on behalf of The American Laryngological, Rhinological and Otological Society, Inc.

Micro-CT Imaging of Tracheal Development in Down Syndrome and Non-Down Syndrome Fetuses

M. Matthijs Fockens, MD; Yousif Dawood, MD; Mika J. Zwart, BS; Daniël Docter, MD; Jaco Hagoort, BEng; Frederik G. Dikkers, MD, PhD; Bernadette S. de Bakker, MD, PhD

Objectives: Down syndrome (DS) is associated with airway abnormalities including a narrowed trachea. It is uncertain whether this narrowed trachea in DS is a consequence of deviant fetal development or an acquired disorder following endotracheal intubation after birth. This study aimed to compare the tracheal morphology in DS and non-DS fetuses using microfocus computed tomography (micro-CT).

Methods: Twenty fetal samples were obtained from the Dutch Fetal Biobank and divided into groups based on gestational age. Micro-CT images were processed to analyze tracheal length, volume, and cross-sectional area (CSA).

Results: Mean tracheal length and tracheal volume were similar in DS and non-DS fetuses for all gestational age groups. Mean, minimum, and maximal tracheal CSA were statistically significantly increased in the single DS fetus in the group of 21–24 weeks of gestation, but not in other gestational age groups. In 90% of all studied fetuses, the minimum tracheal CSA was located in the middle third of the trachea.

Conclusion: Tracheal development in DS fetuses was similar to non-DS fetuses between 13 and 21 weeks of gestation. This suggests that the narrowed tracheal diameter in DS children may occur later in fetal development or results from postnatal intubation trauma. The narrowest part of the trachea is in majority of DS and non-DS fetuses the middle third.

Key Words: Down syndrome, fetal development, micro-CT, trachea.

Level of Evidence: Level 3

***Laryngoscope*, 00:1–7, 2024**

INTRODUCTION

Down syndrome (DS) is the most prevalent chromosomal disorder in humans. It is associated with multiple disorders of the airway.[1] DS children are vulnerable to airway obstruction at the level of the trachea from congenital and acquired disorders.[2,3] A smaller airway diameter at the proximal part of the trachea is considered to be a characteristic feature of DS.[4] It is not known whether this narrowed trachea is a congenital anomaly (i.e., already present at birth), whether it is related to delayed growth of the trachea after birth, or whether it is an acquired disorder attributed to endotracheal intubation.[5,6] Intubation with a tube size adjusted to the expected narrowed diameter is an important measure to minimize the risk of subglottic and tracheal stenosis.

The etiology of tracheal disorders in DS children has largely remained unknown.[3] Investigating embryonic and fetal development of the trachea can provide valuable insights into the origins of tracheal disorders. In the last decade, microfocus computed tomography (micro-CT) has significantly contributed to a better understanding of embryonic and fetal human development.[7,8] It has emerged as a powerful technique that provides high-resolution visualization of the anatomy from 6 to 24 weeks of gestation. Like other X-ray-based techniques, micro-CT imaging requires density differences for tissue differentiation. To study soft tissue, an addition of a contrast agent is required. Commonly, this is achieved using iodine as a contrast agent and then referred to as diffusible iodine-based contrast-enhanced computed tomography (diceCT).[9] Micro-CT also enables three-dimensional (3D) reconstruction of anatomical structures, which allows morphometric analysis for research and educational purposes. Furthermore, micro-CT has clinical value as it can contribute to perinatal fetal autopsy in a noninvasive manner as it outperforms high-field MRI in

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

From the Department of Otorhinolaryngology Head and Neck Surgery (M.M.F., F.G.D.), Amsterdam UMC location University of Amsterdam, Amsterdam, The Netherlands; Department of Obstetrics and Gynaecology (Y.D., D.D., B.S.D.B.), Amsterdam UMC location University of Amsterdam, Amsterdam, The Netherlands; Amsterdam Reproduction and Development Research Institute (Y.D., D.D., B.S.D.B.), Amsterdam, The Netherlands; Department of Medical Biology (M.J.Z., J.H.), Amsterdam UMC location University of Amsterdam, Amsterdam, The Netherlands; Department of Pediatric Surgery (D.D.), Amsterdam UMC location University of Amsterdam – Emma Children's Hospital, Amsterdam, The Netherlands; Amsterdam Gastroenterology Endocrinology Metabolism (D.D.), Amsterdam, The Netherlands; and the Department of Pediatric Surgery (B.S.D.B.), Erasmus University Medical Center – Sophia Children's Hospital, Rotterdam, The Netherlands.

Additional supporting information may be found in the online version of this article.

Editor's Note: This Manuscript was accepted for publication on April 12, 2024.

Presented at the 104th Annual Meeting of the American Broncho-Esophagological Association, Chicago, IL, USA; May 15–19, 2024.

The authors have no funding, financial relationships, or conflicts of interest to disclose.

Send correspondence to M. Matthijs Fockens, Department of Otorhinolaryngology Head and Neck Surgery, Amsterdam UMC location University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands. Email: m.m.fockens@amsterdamumc.nl

DOI: 10.1002/lary.31468

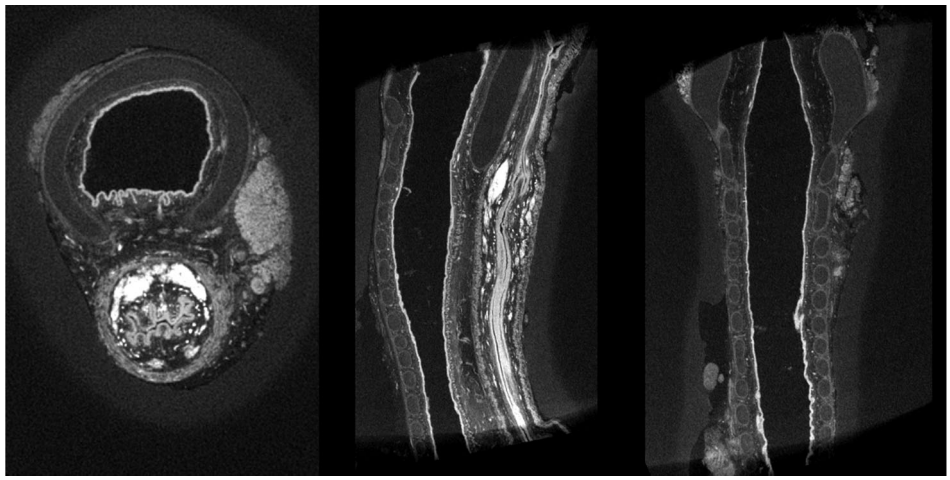

Fig. 1. Micro-computed tomography images of the upper part of the trachea of sample number 83 (axial, sagittal, and coronal plane).

postmortem whole-body fetal imaging.[10] It can therefore be considered the imaging study of choice upon early pregnancy loss investigation, albeit not standard practice in most centers at this moment.[11]

The aim of our study was to compare the airway morphology of the trachea in DS and non-DS fetuses with micro-CT imaging. Identification of developmental differences emerging during early fetal development could potentially offer insights into prenatal tracheal disorders associated with DS and enhance our understanding of fetal airway development in general.

MATERIALS AND METHODS

Fetal samples were obtained from the Dutch Fetal Biobank, a nationwide program coordinated from Amsterdam UMC in Amsterdam, the Netherlands.[12] After elective pregnancy termination or immature birth, parents are asked to donate their fetus for research purposes. By November 2023, the Biobank consists of more than 350 human fetuses and is continuously expanding (see www.3dhumandevelopment.com). For each DS fetus, at least one non-DS fetus was selected that matched gestational age. Fetal samples were divided in groups based on gestational age: 13–16 weeks, 16–18 weeks, 18–21 weeks, and 21–24 weeks. Ethical approval was acquired through the accredited Medical Research Ethics Committee of Amsterdam UMC (METC2016_285, #B2017369). All practices are in accordance with the Dutch law and legislation.

Donated samples are submerged in 4% paraformaldehyde (PFA) within 6 h of decease for 2–7 days for fixation, depending on sample size. Samples were either fixed as a whole-body fetus or tissue was dissected prior to fixation, depending on study requirements and comorbidities of the fetus. When adequately fixed, the samples are stored in 0.2% PFA until they are requested and approved for research. To differentiate between soft tissues, a contrast agent was added to improve contrast. Here, a 3.75% buffered Lugol's solution (B-Lugol) was utilized.[13] Lugol is an iodine-based contrast agent that provides good soft tissue contrast for most types of tissue and is safe to use.[14]

Samples were stained by submersion in this solution for 1–2 weeks depending on sample size. After adequate soft tissue contrast was reached, the samples were rinsed in phosphate-buffered saline (PBS) to remove excess B-Lugol. Next, the samples were mounted in 1% agarose to prevent movement during scanning and to protect the samples during transport. Agarose provides adequate sample fixation without compromising image quality. Scans were performed on a Phoenix V|tome|x M300 (Waygate Technologies GmbH, Wunstorf, Germany) at Wageningen University & Research (The Netherlands), or on a Phoenix Nanotom M (Waygate Technologies GmbH) or UniTOM XL (TESCAN GmbH, Dortmund, Germany) at Catholic University Leuven (Belgium). Due to varying sizes of the samples, a single acquisition protocol would not suffice as this would negatively impact the image quality of the smallest and largest samples. Therefore, the protocol was optimized per sample by an experienced micro-CT operator. Acquisition settings per sample can be found in Supplement 1.

Micro-CT images were viewed and processed with Amira software (version 2022.1; Thermo Fischer Scientific, Waltham, MA, USA) (see Fig. 1). Image processing started with segmentation of the tracheal lumen from the inferior border of the cricoid ring until the top of the carina. Subsequently, tracheal lumen segmentation was processed into a 3D model for quantitative morphometric analysis (see Fig. 2). Morphometric analysis of segmentations was performed with 3D Slicer (version 5.2.2), an open source software application for biomedical sciences (www.slicer.org).[15] The total volume of the segmented tracheal lumen was calculated. Tracheal length was measured using a centerline in the center point of the lumen to correct for the physiological curved course of the trachea. This centerline also corrected for potential bowing of the dissected DS samples caused during specimen processing and mounting. The cross-sectional area (CSA) of the tracheal lumen was measured over the entire tracheal length in a plane perpendicular to the centerline. Subsequently, the minimum CSA was measured, as this represents the narrowest part of the airway lumen. Furthermore, the position of the narrowest CSA in the trachea was measured from the inferior border of the cricoid and specified as a percentage of the total tracheal length (where 0% represents the cranial end, and 100% the caudal end). The mean CSA and maximum CSA of the trachea were also calculated. For centerline generation and cross-sectional measurements, the Vascular Modeling Toolkit (VMTK) extension (http://www.vmtk.org) for 3D Slicer was used.

Statistical analyses were performed with IBM SPSS software (version 28.0; IBM Corp, Armonk, NY, USA). The Shapiro–Wilk test was used to test the normality of the distribution of continuous variables. All continuous variables were normally distributed and presented as mean ± standard deviation. To compare the DS and non-DS groups, the unpaired t-test was

15314995, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/lary.31468 by Erasmus University Rotterdam Universiteitsbibliotheek, Wiley Online Library on [03/06/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

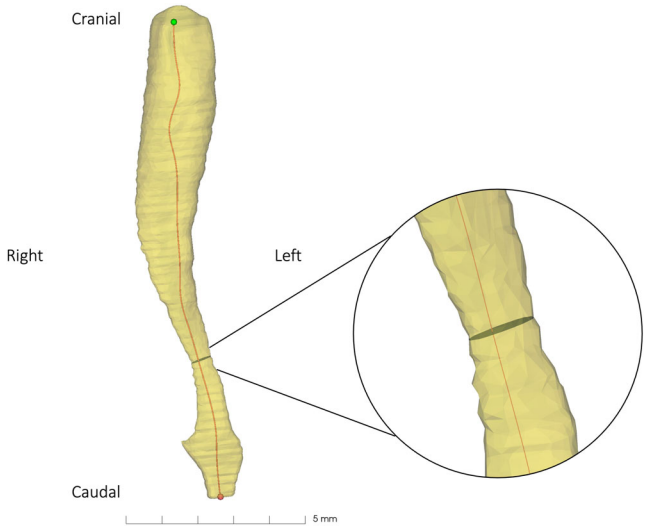

Fig. 2. Anterior view of a three-dimensional segmentation model of the tracheal lumen of a Down syndrome fetus (sample number 217, gestational age 16 + 5 weeks). Tracheal volume was calculated from the complete segmentation model (13.4 mm^3; yellow). The centerline was measured for tracheal length (15.8 mm; red line). Cross-sectional area (CSA) was measured perpendicular to the centerline over the entire length of the centerline. Here, the minimum CSA is displayed (0.1 mm^2; grey slice) and is located at 63.2% of the length of the tracheal lumen. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

performed. A p value of <0.05 was considered statistically significant.

RESULTS

The study population consisted of 6 DS fetuses and 14 non-DS fetuses (see Table I). Gestational age of DS fetuses ranged from 13 + 3 to 21 + 2 weeks (94–149 days; mean 119 ± 19 days). All DS fetuses were donated after a planned termination of pregnancy because of the prenatal DS diagnosis made with ultrasound in combination with noninvasive prenatal blood test and in some cases also invasive prenatal testing. In DS fetuses, the larynx, trachea, and lungs were dissected in continuity to optimize micro-CT image acquisition. Gestational age of non-DS fetuses ranged from 13 + 4 to 23 + 3 weeks (95–164 days; mean 128 ± 22 days). They were scanned as whole fetal samples. In this control group, pregnancy was terminated because of parental request on social reasons ($n = 9$), Klinefelter syndrome ($n = 1$), Marfan syndrome ($n = 1$), and Alpers syndrome ($n = 1$). Furthermore, the control group consisted of two fetuses that were donated after immature birth. The groups of fetuses with a gestational age of 13–16 weeks and 16–18 weeks each included two DS fetuses and three non-DS fetuses. The groups of fetuses with a gestational age of 18–21 weeks and 21–24 weeks each consisted of one DS fetus and four non-DS fetuses.

Results of morphometric analyses are presented in Table II and Figure 3. Mean tracheal length and tracheal volume were not significantly different in DS and non-DS fetuses for all gestational age groups. Mean, minimum, and maximal tracheal CSA did not differ between DS and non-DS fetuses in all groups, except in the group of 21–24 weeks. In this group, the mean, minimum, and maximum CSA were all significantly higher in the DS fetus compared with the four non-DS fetuses (4.7 vs. 3.3 ± 0.1 mm^2 [$p = 0.001$], 3.5 vs. 2.5 ± 0.3 mm^2 [$p = 0.035$] and 6.4 vs. 4.3 ± 0.3 mm^2 [$p = 0.007$], respectively). Particularly, morphometric analysis of the tracheal lumen of the fetus with Marfan syndrome was not different from DS fetuses and other control fetuses of the same gestational age.

The location of the minimum tracheal CSA did not differ between the DS and non-DS fetuses for all gestational ages. In 90% of all studied fetuses, the minimum tracheal CSA was located in the middle third of the trachea. In one non-DS fetus, the location of the minimum tracheal CSA was in the most proximal third of the trachea (sample number 112, gestational age 16 + 2 weeks; narrowest CSA located at 6.2%). In one DS fetus, the location of the minimum tracheal CSA was in the most distal third of the trachea (sample number 83, gestational age 15 + 2 weeks; narrowest CSA located at 76.4%).

DISCUSSION

Fetal development of the tracheal airway was investigated with micro-CT in DS and non-DS fetuses between 13 and 24 weeks of gestation. Morphometric analysis of 3D segmentation models showed that tracheal length and tracheal volume were similar in DS and non-DS fetuses. In fetuses of 21–24 weeks of gestation, tracheal CSA measurements were significantly increased in the DS fetus in comparison with the non-DS fetuses. Tracheal CSA measurements did not differ in other age groups. This study indicates that narrowing of the tracheal airway in DS patients does not occur during early fetal development.

Morphometric results of tracheal length and tracheal CSA reported in this study are in line with normative data on fetal tracheal development.[16–18] In a study on fetuses aged 14–25 weeks of gestation, the tracheal volume was notably increased compared with our results, whereas the tracheal length and tracheal CSA were generally equal to our results.[18] We believe the authors of this study have overestimated the tracheal volume, as this was calculated by multiplying the proximal tracheal CSA with the tracheal length. This calculation was based on the assumption that the trachea is uniform in shape throughout its length. In our study, we have demonstrated that all fetuses have a narrowed part of the trachea and that the shape of the tracheal lumen is not consistently uniform throughout its length. In our study, the location of the narrowest part of the trachea (minimum tracheal CSA) was in the middle third of the trachea in the majority of fetuses (90%). This observation corresponds with a previous study that demonstrated that the proximal end of the trachea is wider than the distal end until 28 weeks of gestation.[19] It is important to acknowledge that the mature or "normal" trachea, shaped through the processes of embryonic, fetal, and postnatal development, can exhibit a wide range of measurements.[20,21] An autopsy study in adults showed that

15314995, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/lary.31468 by Erasmus University Rotterdam Universiteitsbibliotheek, Wiley Online Library on [03/06/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE I.
Overview of Fetal Samples Used to Examine the Trachea.

Gestational Age (Weeks + Days)	Sample Number	TOP/ IB	Indication for TOP	Sex	CRL (cm)	Weight (g)	Clinical Aspects
Down syndrome fetuses							
13 + 3	103	TOP	Down syndrome	M	8	35	Hydrops fetalis, hygroma colli
15 + 2	83	TOP	Down syndrome	M	11	77	Hydrops fetalis, ventricular septal defect
16 + 4	187	TOP	Down syndrome	M	12.8	132	Hygroma colli
16 + 5	217	TOP	Down syndrome	F	13	132	
18 + 6	138	TOP	Down syndrome	M	14.7	214	
21 + 2	70	TOP	Down syndrome	F	21	430	Tetralogy of Fallot, atrioventricular septal defect, right sided aortic arch
Non-Down syndrome fetuses							
13 + 4	157	TOP	Klinefelter syndrome	M	9.4	52	
13 + 4	196	IB		M	8	39	
14 + 5	78	TOP	Marfan syndrome	F	11	53	
16 + 2	29	TOP	Parental request	—	—	—	
16 + 2	112	TOP	Alpers syndrome	F	11	85	
17 + 0	27	TOP	Parental request	—	13.5	—	
18 + 2	119	TOP	Parental request	F	15	201	
19 + 0	37	TOP	Parental request	F	15.5	232	
19 + 1	56	TOP	Parental request	M	16	271	
19 + 6	176	IB		M	16	292	
21 + 1	24	TOP	Parental request	M	18	—	Unilateral cleft lip, alveolus and palate
21 + 5	9	TOP	Parental request	—	—	—	Dilated intestines
22 + 3	23	TOP	Parental request	F	19.5	—	
23 + 3	12	TOP	Parental request	—	—	—	

Em dash (—) represents missing data.
CRL = crown rump length; IB = immature birth; TOP = termination of pregnancy.

airway diameters at the level of the fourth tracheal ring almost doubled between subjects, ranging from 14.0 to 24.6 mm in the coronal plane and 13.5 to 24.4 mm in the midsagittal plane.[22] Given the significant association between the height of children and the diameter of the proximal trachea, it is noteworthy that height serves as a more reliable predictor of endotracheal tube size than age.[23]

Our study demonstrates that the tracheal CSA in DS fetuses is similar or larger than the tracheal CSA in non-DS fetuses. This finding is contradictory to an earlier study in DS children. Shott demonstrated that the airway diameter of the subglottic larynx and proximal trachea was decreased in DS children aged 18 months to 8 years old.[4] Subsequently, her advice was to intubate DS children with an endotracheal tube two sizes smaller than the size appropriate for age. Although this advice should not necessarily be rejected, it is important to acknowledge some limitations of Shott's study. First, it is debatable whether the recommendation to use a smaller tube size is feasible for DS neonates and infants. The endotracheal tubes for children of this age are already very small (inner diameter ranging from 2.5 to 4.0 mm for premature to 18-month old children), leaving little room for down staging.[24] Second, the assessment of MRI scans did not specify whether the included DS children had previously undergone one or more endotracheal intubations. This information is crucial to distinguish whether the decreased airway diameter is a feature characteristic of DS or a result of previous intubation. Although the results of Shott's study contradict our findings, it is important to acknowledge that fetal research should be compared with research in living children with careful consideration. There is a gap in knowledge, as development of the trachea in the period between 24 weeks of gestation and birth has not been investigated in DS fetuses. We hypothesize that the discrepancy could be attributed to a delay in tracheal growth during later stages of fetal development (>24 weeks) or in early life. If the latter is the case, a narrowed trachea should not be considered a congenital feature.

The most important strength of this study is that it is the first report on development of the fetal trachea in DS. This was achieved with the valuable collection of the Dutch Fetal Biobank.[12] Additionally, the integration of micro-CT images and segmentation tools facilitated the generation of high-resolution images and 3D models. These tools allowed for accurate measurements of length,

15314995, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/lary.31468 by Erasmus University Rotterdam Universiteitsbibliotheek, Wiley Online Library on [03/06/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE II.
Quantitative Morphometric Analysis of Three-Dimensional Segmentations of the Trachea in 20 Fetuses.

	All Samples	Down Syndrome Fetuses	Control Fetuses	*p*
Tracheal length (mm)	15.5 ± 3.9	15.4 ± 4.1	15.5 ± 4	*0.929*
13–16 weeks GA	10.7 ± 2.2	11.6 ± 2.8	10.4 ± 2.1	*0.618*
16–18 weeks GA	13.8 ± 1.4	15.1 ± 1	12.9 ± 0.8	*0.059*
18–21 weeks GA	17 ± 1.1	16.7	17.1 ± 1.2	*0.770*
21–24 weeks GA	20.2 ± 1.7	22	19.8 ± 1.6	*0.312*
Tracheal volume (mm^3)	30.5 ± 24.9	27.2 ± 29.7	32 ± 23.7	*0.704*
13–16 weeks GA	8.4 ± 5	11.3 ± 7.2	6.5 ± 3.3	*0.371*
16–18 weeks GA	16.1 ± 6.8	14.1 ± 1	17.4 ± 9.3	*0.663*
18–21 weeks GA	28.8 ± 7.2	25.9	29.6 ± 8.1	*0.710*
21–24 weeks GA	68.7 ± 11.5	86.3	64.3 ± 6.8	*0.063*
Mean tracheal CSA (mm^2)	1.8 ± 1.2	1.7 ± 1.5	1.9 ± 1.1	*0.797*
13–16 weeks GA	0.8 ± 0.4	1.1 ± 0.5	0.6 ± 0.2	*0.201*
16–18 weeks GA	1.1 ± 0.5	0.9 ± 0.8	1.3 ± 0.7	*0.444*
18–21 weeks GA	1.7 ± 0.4	1.6	1.7 ± 0.4	*0.861*
21–24 weeks GA	3.6 ± 0.6	4.7	3.3 ± 0.1	***0.001***
Minimum tracheal CSA (mm^2)	1.2 ± 1	1.1 ± 1.2	1.2 ± 0.9	*0.795*
13–16 weeks GA	0.5 ± 0.3	0.8 ± 0.4	0.4 ± 0.2	*0.139*
16–18 weeks GA	0.7 ± 0.6	0.3 ± 0.3	0.9 ± 0.7	*0.347*
18–21 weeks GA	0.9 ± 0.2	0.8	0.9 ± 0.2	*0.743*
21–24 weeks GA	2.7 ± 0.5	3.5	2.5 ± 0.3	***0.035***
Maximum tracheal CSA (mm^2)	2.6 ± 1.5	2.7 ± 1.9	2.5 ± 1.4	*0.852*
13–16 weeks GA	1.2 ± 0.6	1.7 ± 0.9	0.9 ± 0.2	*0.219*
16–18 weeks GA	1.8 ± 0.6	1.8 ± 0.4	1.8 ± 0.8	*0.946*
18–21 weeks GA	2.5 ± 0.6	2.7	2.5 ± 0.7	*0.794*
21–24 weeks GA	4.8 ± 1	6.4	4.3 ± 0.3	***0.007***
Location of minimum tracheal CSA (%)	51 ± 13.3	54.1 ± 13.1	49.7 ± 13.7	*0.518*
13–16 weeks GA	57.4 ± 11.8	61.3 ± 21.5	54.8 ± 4.6	*0.623*
16–18 weeks GA	42.9 ± 21.5	56.2 ± 10	34 ± 24.1	*0.321*
18–21 weeks GA	56.2 ± 6.3	46.6	58.6 ± 3.9	*0.073*
21–24 weeks GA	47.6 ± 4.1	42.8	48.8 ± 3.5	*0.224*

CSA = cross-sectional area; GA = gestational age.

volume, and area, minimizing the potential for observer bias that is often present with older measurement techniques.[17] Our study also has some limitations. First, it is important to recognize that the sample size is relatively small. The single DS fetus aged 21 + 2 weeks of gestation that showed a significantly increased CSA can be considered an outlier in terms of crown rump length (CRL) or weight, but missing data in the non-DS group prevents us from drawing this conclusion. Maternal factors like gestational diabetes could have influenced early fetal growth, but this information is not registered in the Dutch Fetal Biobank. As the Biobank consists of only one DS fetus of 21–24 weeks of gestation, we were unable to enlarge this sample size and further investigate the statistically significant result. This is explained by the early moment of prenatal DS diagnosis and subsequent termination of pregnancy, usually around 12–18 weeks of gestation. We did not encounter any tracheal anomalies in either DS or non-DS fetuses, which can be attributed to the rarity of these anomalies.[25] Second, the lower airways were dissected in the DS fetuses, whereas whole-body fetal imaging was performed in the non-DS fetuses. Our experience with micro-CT scanning technique improved over time (primarily by optimization of tissue staining and fixation). By the time the control samples were scanned, we had learned that tissue dissection was not necessary for this study. Although DS and non-DS fetuses underwent different tissue processing, we reason that this did not influence study results as the robust construction of the airway is not compromised by our staining and fixation techniques. Third, it is important to recognize the heterogeneity of the non-DS group. The group contains fetuses whose pregnancy was terminated because of parental request, in addition to fetuses with specific syndromes. Marfan syndrome has been associated with tracheomegaly, but morphometric analysis of tracheal lumen of this fetus was not different from DS fetuses and other control fetuses of the same gestational age.[26] Tracheal anomalies have not been described as being part of Alpers syndrome and Klinefelter syndrome.

15314995, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/lary.31468 by Erasmus University Rotterdam Universiteitsbibliotheek, Wiley Online Library on [03/06/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

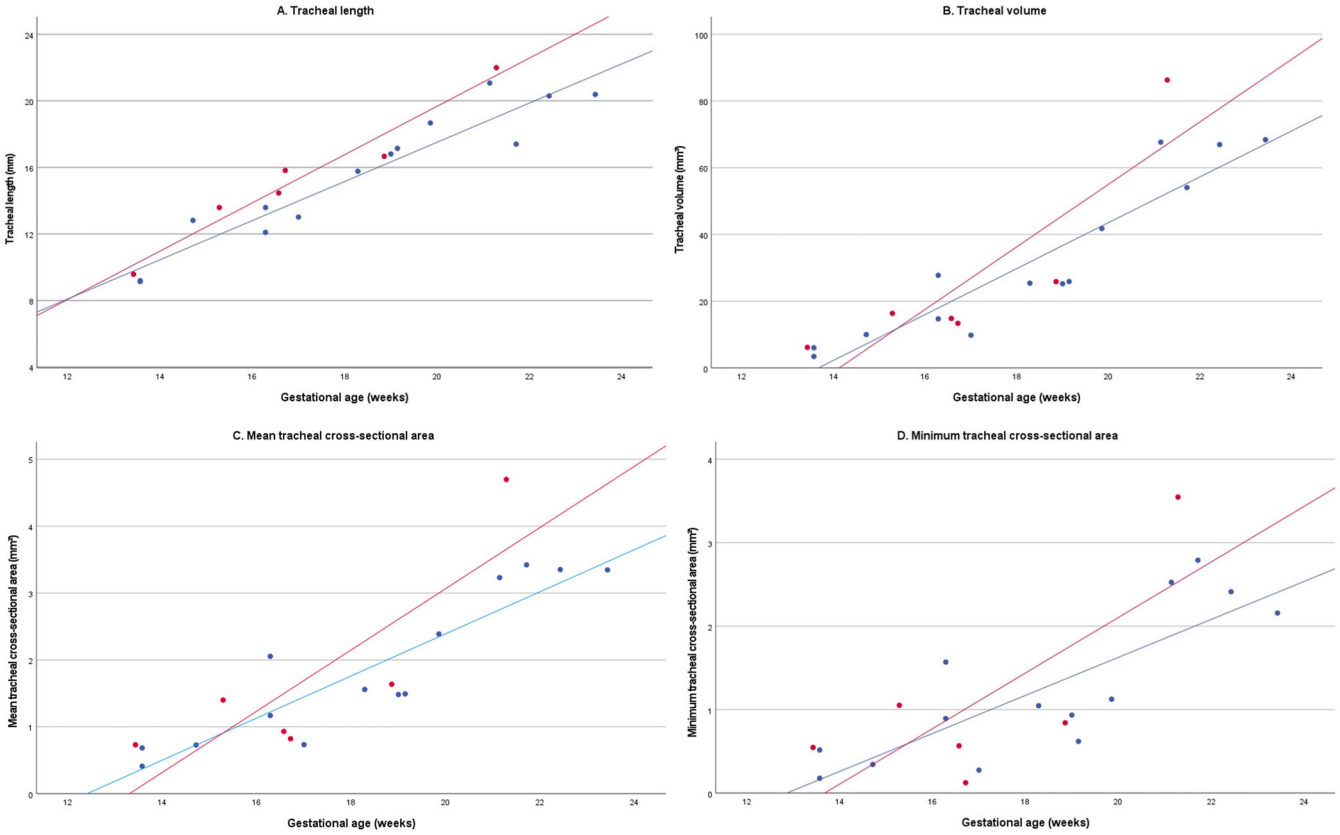

Fig. 3. Scatter plots of tracheal length (A), tracheal volume (B), tracheal cross-sectional area (C), and minimum tracheal cross-sectional area (D) in relation to the gestational age for both the Down syndrome (DS) fetuses (red) and non-DS fetuses (blue). [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

CONCLUSION

This study demonstrates that tracheal development in DS fetuses is similar to non-DS fetuses between the gestational age of 13 and 21 weeks. Although a narrowed diameter of the tracheal lumen is considered a feature of DS children, examination of fetal DS samples did not provide confirmation. We hypothesize that the narrowed tracheal diameter in DS children cannot be explained by a deviant development in early fetal period, but may occur later in fetal development or result from intubation trauma after birth. Future research should be performed with a larger cohort and encompass the unexplored timeframe spanning from 24-week gestational age to birth. It is crucial to emphasize that the latter recommendation may encounter hurdles in implementation and is difficult to perform due to the sensitive nature of these cases.

ACKNOWLEDGMENTS

The authors thank Dr. Thomas O'Mahoney for his valuable contribution.

BIBLIOGRAPHY

1. Chin CJ, Khami MM, Husein M. A general review of the otolaryngologic manifestations of Down syndrome. *Int J Pediatr Otorhinolaryngol*. 2014; 78(6):899-904.
2. Hamilton J, Yaneza MMC, Clement WA, Kubba H. The prevalence of airway problems in children with Down's syndrome. *Int J Pediatr Otorhinolaryngol*. 2016;81:1-4.
3. Fockens MM, Hölscher M, Limpens J, Dikkers FG. Tracheal anomalies associated with Down syndrome: a systematic review. *Pediatr Pulmonol*. 2021;121(4):382-822.
4. Shott SR. Down syndrome: analysis of airway size and a guide for appropriate intubation. *Laryngoscope*. 2000;110(4):585-592.
5. Boseley ME, Link DT, Shott SR, Fitton CM, Myer CM, Cotton RT. Laryngotracheoplasty for subglottic stenosis in down syndrome children: the Cincinnati experience. *Int J Pediatr Otorhinolaryngol*. 2001;57(1): 11-15.
6. Arianpour K, Forman SN, Karabon P, Thottam PJ. Pediatric acquired subglottic stenosis: associated costs and comorbidities of 7,981 hospitalizations. *Int J Pediatr Otorhinolaryngol*. 2019;117:51-56.
7. Dawood Y, de Bakker BS. Micro-CT of early human development. *Radiology*. 2020;297(1):32.
8. Dawood Y, Buijtendijk MFJ, Shah H, et al. Imaging fetal anatomy. *Semin Cell Dev Biol*. 2022;131:78-92.
9. Gignac PM, Kley NJ, Clarke JA, et al. Diffusible iodine-based contrast-enhanced computed tomography (diceCT): an emerging tool for rapid, high-resolution, 3-D imaging of metazoan soft tissues. *J Anat*. 2016; 228(6):889-909.
10. Hutchinson JC, Kang X, Shelmerdine SC, et al. Postmortem microfocus computed tomography for early gestation fetuses: a validation study against conventional autopsy. *Am J Obstet Gynecol*. 2018;218(4):445.e1-445.e12.
11. Dawood Y, Honhoff C, Post A-S, et al. Comparison of postmortem whole-body contrast-enhanced microfocus computed tomography and high-field magnetic resonance imaging of human fetuses. *Ultrasound Obstet Gynecol*. 2022;60(1):109-117.
12. Dawood Y, Buijtendijk MFJ, Bohly D, et al. Human embryonic and fetal biobanking: establishing the Dutch Fetal Biobank and a framework for standardization. *Dev Cell*. 2023;58(24):2826-2835.
13. Dawood Y, Hagoort J, Siadari BA, et al. Reducing soft-tissue shrinkage artefacts caused by staining with Lugol's solution. *Sci Rep*. 2021;11(1): 19781.
14. Docter D, Dawood Y, Jacobs K, et al. Microfocus computed tomography for fetal postmortem imaging: an overview. *Pediatr Radiol*. 2023;53(4): 632-639.

15314995, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/lary.31468 by Erasmus University Rotterdam Universiteitsbibliotheek, Wiley Online Library on [03/06/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

15. Fedorov A, Beichel R, Kalpathy-Cramer J, et al. 3D Slicer as an image computing platform for the Quantitative Imaging Network. *Magn Reson Imaging*. 2012;30(9):1323-1341.
16. Fayoux P, Marciniak B, Devisme L, Storme L. Prenatal and early postnatal morphogenesis and growth of human laryngotracheal structures. *J Anat*. 2008;213(2):86-92.
17. Szpinda M, Daroszewski M, Woźniak A, Szpinda A, Mila-Kierzenkowska C. Tracheal dimensions in human fetuses: an anatomical, digital and statistical study. *Surg Radiol Anat*. 2011;34(4):317-323.
18. Szpinda M, Daroszewski M, Szpinda A, et al. New quantitative patterns of the growing trachea in human fetuses. *Med Sci Monit*. 2012;18(6):PH63-PH70.
19. Adamiec E, Dzieciołowska-Baran E, Czerwiński F, Miklaszewska D, Teul I. Prenatal development of the human trachea. *Folia Morphol*. 2002;61(2):123-125.
20. Munguía-Canales DA, Ruiz-Flores J, Vargas-Mendoza GK, Morales-Gómez J, Méndez-Ramírez I, Murata C. Tracheal dimensions in the Mexican population. *Cir Cir*. 2011;79(6):505-510.
21. Kamel KS, Lau G, Stringer MD. In vivo and in vitro morphometry of the human trachea. *Clin Anat*. 2009;22(5):571-579.
22. Randestad KE, Lindholm CE, Fabian P. Dimensions of the cricoid cartilage and the trachea. *Laryngoscope*. 2000;110(11):1957-1961.
23. Coordes A, Rademacher G, Knopke S, et al. Selection and placement of oral ventilation tubes based on tracheal morphometry. *Laryngoscope*. 2011;121(6):1225-1230.
24. Tweedie DJ, Skilbeck CJ, Cochrane LA, Cooke J, Wyatt ME. Choosing a paediatric tracheostomy tube: an update on current practice. *J Laryngol Otol*. 2007;122(2):161-169.
25. Schweiger C, Cohen AP, Rutter MJ. Tracheal and bronchial stenoses and other obstructive conditions. *J Thorac Dis*. 2016;8(11):3369-3378.
26. Dutau H, Cavailles A, Fernandez-Navamuel I, Breen DP. Tracheal compression in a patient with Marfan's syndrome-associated tracheomegaly treated by an XXL stent: the largest diameter airway stent ever placed in a previously undescribed airway condition. *Respiration*. 2009;77(1):97-101.

15314995, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/lary.31468 by Erasmus University Rotterdam Universiteitsbibliotheek, Wiley Online Library on [03/06/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License