# Quantitative Analysis of Digital Videokymography: A Preliminary Study on Age- and Gender-Related Difference of Vocal Fold Vibration in Normal Speakers

\*Akihito Yamauchi, †Hisayuki Yokonishi, \*Hiroshi Imagawa, ‡Ken-Ichi Sakakibara, \*Takaharu Nito, §Niro Tayama, and \*Tatsuya Yamasoba, \*†§Tokyo and ‡Ishikari-Gun, Hokkaido, Japan

**Summary: Introduction.** Kymography is an effective method for assessing temporal patterns of vocal fold vibrations. Because kymographic data for a number of normal speakers based on high-speed digital imaging (HSDI) were limited in the literature, this prospective study was conducted to provide normative kymographic HSDI data and clarify gender- and age-related normal variations.

**Methods.** Vocally healthy adults were divided into young ( $\leq$ 35 years) and elderly groups ( $\geq$ 65 years). Kymograms were recomposed from HSDI data at the midglottal level, and kymographic parameters were analyzed quantitatively. Then gender- and age-related differences were evaluated.

**Results.** A total of 26 young subjects (9 men and 17 women, mean age: 27 years) and 20 elderly subjects (8 men and 12 women, mean age: 73 years) were investigated. Obtained data generally matched the values in the literature. Slight asymmetry was seen in all groups, with the elderly subjects having more evident asymmetry than the young subjects. Most of the kymographic parameters showed a negative correlation with fundamental frequency ( $F_0$ ), whereas the open quotient displayed a positive correlation with  $F_0$ . There were significant intergroup differences in  $F_0$ , amplitude and lateral peak at a speaking  $F_0$ .

**Conclusions.** The present quantitative findings generally matched the qualitative kymographic data reported in the literature. When judging whether a vibratory pattern is normal or pathological, both gender and age should be taken into account, because gender- and age-related variations of symmetry,  $F_0$ , and phase were frequently observed in the present study. **Key Words:** Voice–Normal–Kymography–Kymogram–High-speed digital imaging–Digital videokymography.

## INTRODUCTION

Vibration of the vocal folds is an essential part of voice production, and a method of accurately visualizing vocal fold vibration is essential for the detection, diagnosis, and treatment of various laryngeal disorders. For this purpose, voice specialists have clinically used videostroboscopy and high-speed digital imaging (HSDI) in recent years.<sup>1–4</sup>

Videostroboscopy is usually the method of first choice because of its low cost, rapidity, and utility. However, production of videostroboscopic illusory images requires synchronizable stable acoustic phonation, so this method is not applicable to brief, unstable, subharmonic, or aperiodic phonations that frequently occur in patients with vocal disorders. Furthermore, images obtained by videostroboscopy are averaged and recomposed, resulting in decreased reliability of assessment. On the other hand, HSDI can be used to observe vocal fold vibrations in real time with an extremely high frame rate. HSDI can be

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used in persons with severe hoarseness or short phonation, allowing much wider clinical application than videostroboscopy. In addition, direct observation of intracycle vibratory behavior is possible, so HSDI allows more accurate and reliable assessment than videostroboscopy.<sup>1–4</sup>

Moreover, various methods for analysis of HSDI data are now available, resulting in superior qualitative and quantitative evaluation compared with videostroboscopy. These approaches include the glottal width waveform,<sup>5</sup> glottal area waveform,<sup>6</sup> phonovibrography,<sup>7</sup> laryngotopography,<sup>8–11</sup> and digital kymography (DKG).<sup>12</sup> Among these techniques, DKG analysis is considered to be the best choice for evaluating the temporal characteristics of HSDI data.

A kymogram displays vocal fold movements along a single horizontal line (transverse to the glottis) over the selected time period in a single image. When the appropriate scan line is selected, a kymogram gives a good overview of the vocal fold movements and information such as periodicity, which is hard to interpret through simple frame-by-frame analysis of HSDI data. Kymography dates back to 1971, when Gall et al<sup>13</sup> used a cine-camera to pioneer this technique (photokymography). Through recent advances, this technique has since developed into videokymography (VKG)<sup>14</sup> and (DKG).<sup>12</sup> Strobovideokymography, which is based on a videostroboscopic technique, is also available,<sup>15</sup> but it is less widely used than VKG and DKG, presumably because of providing less reliable assessment than that obtained with the high frame rates of these other methods.

A major drawback of DKG is the limited availability of normative data, which are essential for making accurate distinctions between normal and pathological vocal fold vibrations. Although there have already been several normative

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From the \*Department of Otolaryngology, The University of Tokyo Hospital, Tokyo, Japan; †Department of Otolaryngology, The Tokyo Metropolitan Police Hospital, Tokyo, Japan; ‡Department of Communication Disorders, The Health Sciences University of Hokkaido, Ishikari-Gun, Hokkaido, Japan; and the §Department of Otolaryngology and Tracheo-esophagology, The National Center for Global Health and Medicine, Tokyo, Japan.

Address correspondence and reprint requests to Akihito Yamauchi, Department of Otolaryngology, The University of Tokyo Hospital, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. E-mail: drachilles23@yahoo.co.jp

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kymographic studies, only a few parameters were assessed.<sup>16–22</sup> In addition, age-related differences of vocal fold vibrations in normal speakers have not been investigated in a kymographic study, although knowledge of normal variations is essential to allow clinical judgment to be modified according to the gender and age of patients.

Furthermore, there is a paucity of information about correlations between kymographic parameters and acoustic/aerodynamic parameters. Mehta et al<sup>23–25</sup> did investigate the relationships between HSDI-derived parameters (from DKG and glottal area waveform) and acoustic/spectral/cepstral measures recently, and found some significant correlations between HSDI parameters and acoustic measures (between jitter and the standard deviation of left-right phase asymmetry/amplitude asymmetry, and between shimmer and the standard deviation of open quotient),<sup>23</sup> and between HSDI parameters and a cepstrum-based acoustic measure (between cepstral peak magnitude and F<sub>0</sub> deviation/speed quotient/lateral phase difference),<sup>24</sup> but not between HSDI parameters and spectral tilt measures.<sup>25</sup> For the better interpretation of vocal fold vibrations observed by DKG, however, further data accumulation on this matter is definitely needed.

Accordingly, the aims of the present study were to obtain normative quantitative data for multiple DKG parameters based on HSDI, to clarify gender- and age-related normal variations, and to elucidate the relationship between DKG parameters and aerodynamic/acoustic parameters.

# MATERIALS AND METHODS

#### Subjects

Vocally healthy volunteers with no vocal symptoms and no history of laryngeal disease were recruited to participate in the present study, and were divided into a young group (aged 21 to 35 years) and an elderly group (aged  $\geq 65$  years). All subjects were required to sign a consent form that was approved by our institutional review board. A total of 46 persons (29 women and 17 men) were enrolled in the present study, including 26 subjects (9 men and 17 women) in the young group and 20 subjects (8 men and 12 women) in the elderly group.

Demographic data included the age, gender, and the chronic medical condition (CMC) score. The CMC score is an objective measure of the burden of chronic medical conditions, which was proposed by Mau et al based on the Medical Outcome Study of Stewart et al<sup>26,27</sup> The CMC score is calculated as follows: six points for coronary artery disease, chronic heart failure, or depression; three points for chronic obstructive pulmonary disease; and two points for diabetes, back problems, or arthritis. A high CMC score indicates an unfavorable health condition.

# High-speed digital imaging

A high-speed digital camera (FASTCAM-1024PCI; Photoron, Tokyo, Japan) was connected to a rigid endoscope (#4450.501, Richard Wolf, Vernon Hills, IL, USA) via an attachment lens (f = 35 mm, Nagashima Medical Inc., Tokyo, Japan). Recording was performed under illumination with a 300-W xenon light source at a frame rate of 4500 fps and a spatial resolution of 512x400 pixels, with an 8-bit grayscale and a recording duration of 1.86 s. High-speed digital images of sustained phonation of the vowel /i/ with a comfortable intensity were recorded at a low frequency, a speaking fundamental frequency, and a high frequency.

# **Digital kymography**

From the recorded HSDI data, a segment with good focus, brightness, and contrast was selected by visual inspection. Subsequently, the images were rotated until the glottal axis and the kymographic line were perpendicular to each other, and a kymogram was constructed at the middle of the membranous glottis. Then DKG parameters related with vibratory characteristics listed in "size parameter," "time parameter," and "size and time parameter" paragraphs were calculated. Creation of kymograms and parametric measurement were performed with a custom MATLAB software (Mathworks Inc., Natick, MA, USA) programed by two of the coauthors (H.Y and H.I.) at our institution. Each parameter was averaged over three consecutive cycles. At 4500 fps, the frame size of kymogram corresponded to 0.089 seconds (400 frames). Figure 1 shows an example of kymographic analysis (a kymogram obtained from a 27-year-old man phonating /i/ at his speaking  $F_0$ ).

#### Normalization

Size parameters were basically normalized by the vocal fold length (VFL) (pixel). VFL was calculated from an image obtained during phonation by measuring the distance between the anterior commissure and the vocal process. When the anatomical landmarks were not visible because of tilting of the epiglottis or an overhanging arytenoid, the points for measurement were extrapolated from the shape of the vocal fold edge during phonation.

Furthermore, because the HSDI studies in the literature usually used the vocal fold width (VFW) as a benchmark of size normalization, selected parameters were also normalized by VFW mean, which was calculated as (left VFW + right VFW)/2 (pixel).

Time parameters were normalized by glottal cycle, and size and time parameters were normalized by both glottal cycle and VFL.

In the present study, " $N_L$ -" meant a parameter normalized by VFL; " $N_W$ -" meant a parameter normalized by VFW mean; " $N_G$ -" meant a parameter normalized by glottal cycle; and " $N_{GL}$ -" meant a parameter normalized by both glottal cycle and VFL.

#### Size parameter

Vocal fold width. VFW mean was normalized by VFL ( $N_L$ -VFW mean), which was calculated as VFW mean  $\times$  100/VFL (%).  $N_L$ -VFW mean indicates a lateral-to-longitudinal ratio of the vocal fold.

Amplitude. Amplitude was the peak-to-peak lateral displacement of the vocal fold (Figure 1). Amplitude mean was calculated as (left amplitude + right amplitude)/2 (pixel), and amplitude difference was calculated as the absolute value of



**FIGURE 1.** An example of kymographic analysis is shown. A digital kymogram of a 27-year-old male phonating /i/ at a speaking  $F_0$  is displayed. Parameters concerning amplitude, mucosal wave, glottal width, glottal cycle, glottal closure, phase, and asymmetry were evaluated: VFL was 193 pixels, N<sub>L</sub>-VFW mean was 32.6 (%); N<sub>L</sub>-amplitude mean was 6.7 (%) and N<sub>L</sub>-amplitude difference was 1.0 (%); N<sub>L</sub>-mucosal wave magnitude mean was 6.7 (%) and N<sub>L</sub>-axis shift was 0.52 (%); N<sub>GL</sub>-lateral peak index mean was 5.8 (‱) and N<sub>GL</sub>-lateral peak index difference was 0.13 (‱); N<sub>GL</sub>-IGW was 8.2 (%), N<sub>GL</sub>-IGW difference was 0.30 (%) and the IGWA index was 3.6 (%); kymographic F<sub>0</sub> was 161 (Hz); open quotient was 0.61; N<sub>G</sub>-lateral phase difference was 7.1 (%), speed quotient mean was 1.16 and speed index mean was 0.059; N<sub>G</sub>-mucosal wave persistence mean was 60.7 (%) and N<sub>G</sub>-mucosal wave persistence difference was 14.3 (%).

(left amplitude – right amplitude) (pixel). These parameters were then normalized by VFL (N<sub>L</sub>-amplitude mean or difference) or VFW mean (N<sub>W</sub>-amplitude mean or difference): N<sub>L</sub>-amplitude mean or difference = amplitude mean or difference  $\times$  100/VFL (%); and N<sub>W</sub>-amplitude mean or difference = amplitude mean or difference  $\times$  100/VFW mean (%).

Left-right amplitude asymmetry  $(AA)^{19,20}$  was also calculated using the following formula:  $AA = (left amplitude - right amplitude) \times 100/(left amplitude + right amplitude) (%).$ 

Axis shift. Axis shift is the mediolateral distance traveled by the vocal folds during closed phase.<sup>14</sup> In the present study, a left-to-right axis shift was defined as positive and vice versa. Axis shift was normalized by VFL (N<sub>L</sub>-axis shift = axis shift  $\times$  100/VFL) (%), and by the sum of left and right amplitude (AS = axis shift  $\times$  100/(left amplitude + right amplitude)) (%).<sup>19,20</sup>

Mucosal wave magnitude. The lateral traveling distance of mucosal wave was parameterized as mucosal wave magnitude, a size parameter of mucosal wave (Figure 1). Left and right mean and difference of mucosal wave magnitude were normalized by VFL and VFW mean:  $N_L$ - or  $N_W$ -mucosal wave magnitude mean = (left mucosal wave magnitude + right mucosal wave magnitude)  $\times$  100/(2  $\times$  VFL or VFW mean) (%); and  $N_L$ - or  $N_W$ -mucosal wave magnitude – right mucosal wave magnitude |  $\times$  100/(2  $\times$  VFL or VFW mean) (%); (2  $\times$  VFL or VFW mean) (%).

### **Time parameter**

Kymographic  $F_0$ . Kymographic  $F_o$  (Hz) was calculated as 1/ glottal cycle.

Lateral phase difference. Lateral phase difference was the left-right difference of the time at maximum lateral vocal fold displacement.<sup>19</sup> The absolute value of lateral phase difference normalized by glottal cycle (N<sub>G</sub>-lateral phase difference) was calculated as |the time frame of maximum left vocal fold displacement – the time frame of maximum left vocal fold displacement| × 100/glottal cycle (%). Left-right phase asymmetry (PA)<sup>19,20</sup> was also calculated: PA = (the time frame of maximum left vocal fold difference) of maximum left vocal fold difference of maximum left vocal fold deflection – the time frame of maximum left vocal fold difference).

**Open quotient.** Open quotient were calculated as open phase  $\times$  100/glottal cycle (%).

**Speed quotient, speed index.** Both speed quotient and speed index were measured. Speed quotient was calculated as opening phase/closing phase, and speed index was calculated as (opening phase – closing phase)/(opening phase + closing phase). Because different speed quotients and speed indexes could be obtained from left and right vocal folds, speed quotient mean, calculated as (left speed quotient + right speed quotient)/ 2, and speed index mean, calculated as (left speed index + right speed index)/2, were introduced in the present study.

Mucosal wave persistence. The duration for which the mucosal wave remained visible was termed mucosal wave persistence, a novel time parameter for mucosal wave. The average value of left and right mucosal wave persistence normalized by glottal cycle (N<sub>G</sub>-mucosal wave persistence mean), and the absolute value of left and right mucosal wave persistence difference normalized by glottal cycle (N<sub>G</sub>-mucosal wave persistence difference) were evaluated in the present study: N<sub>G</sub>-mucosal wave persistence + right mucosal wave persistence  $\times$  100/

 $(2 \times \text{glottal cycle})$  (%); and N<sub>G</sub>-mucosal wave persistence difference = |left mucosal wave persistence - right mucosal wave persistence|  $\times 100/(2 \times \text{glottal cycle})$  (%).

### Size and time parameter

Lateral peak index. The lateral peak is the junctional point at which the opening of the vocal fold finishes and the closing starts, and the shape of lateral peak is evaluated qualitatively (sharp or rounded).<sup>14</sup> The sharpness of the lateral peak reflects a patent vertical phase difference, and the rounded lateral peak is usually an unfavorable sign that indicates the stiffened vocal fold.<sup>14</sup> In the present study, "lateral peak index" was newly introduced as a quantitative parameter of lateral peak shape. In Figure 1, the angle A is expressed as left amplitude/left opening phase, whereas the angle B is expressed as (left amplitude - axis shift)/left closing phase. (Angle A + angle B), a supplementary angle of angle C, was termed as lateral peak index, where small lateral peak index signifies rounded lateral peak, and large lateral peak index signifies sharp lateral peak. The left-right mean and difference of lateral peak index normalized by VFL and glottal cycle was evaluated in the present study: NGL-lateral peak index mean = (left lateral peak index + right lateral peak index)  $\times$  100/  $(2 \times VFL \times glottal cycle)$  (‱); and N<sub>GL</sub>-lateral peak index difference = |left lateral peak index - right lateral peak index  $\times 100/(2 \times \text{VFL} \times \text{glottal cycle})$  (%00).

Integral glottal width. As a parameter to reflect the averaged glottal width in one glottal cycle, integral glottal width (IGW) was newly introduced. IGW was calculated as the integral of glottal width over one glottal cycle, which was measured by the *MAT-LAB* program. In the present study, IGW normalized by VFL and glottal cycle (N<sub>GL</sub>-IGW) was evaluated (%): N<sub>GL</sub>-IGW = IGW × 100/(VFL × glottal cycle) (%). Additionally, the left-right difference of IGW was evaluated by two parameters, N<sub>GL</sub>-LGW difference and asymmetry index (AI).<sup>15,28</sup> IGW was divided into left- and right-half portions. When there was no glottal closure, the midpoint of the minimum glottal width was chosen for the end point of the dividing line. N<sub>GL</sub>-IGW difference was calculated as |left-half IGW – right-half IGW| × 100/(VFL × glottal cycle) (%), and AI was calculated as (left IGW – right IGW) × 100/(left IGW + right IGW) (%).<sup>15,28</sup>

#### Aerodynamic studies and acoustic analysis

Vocal function and voice quality were evaluated by measuring aerodynamic and acoustic parameters. Aerodynamic parameters included the maximum phonation time (MPT), mean flow rate (MFR) and laryngeal resistance measured by the Nagashima PE-77E Phonatory Function Analyzer (Nagashima Medical Inc., Tokyo, Japan). Acoustic parameters such as the fundamental frequency (AA-F<sub>0</sub>, F<sub>0</sub> from acoustic analysis), amplitude perturbation quotient (APQ), period perturbation quotient (PPQ) and harmonics-to-noise ratio (HNR) were measured using a custom *MATLAB* software program (Mathworks Inc., Natick, MA, USA) made by one of the coauthors (H.I.) at our institution.

Aerodynamic and acoustic studies were performed approximately 30 minutes before HSDI recording because

simultaneous recording was not available at our institution. Both evaluations were done under as similar conditions as possible to allow comparison between the DKG parameters and the aerodynamic/acoustic parameters.

### **Statistical analysis**

To investigate differences of DKG parameters in relation to frequency, age or gender, one-factor analysis of variance (AN-OVA) was performed. If a significant difference was identified, *post-hoc* analysis (Scheffe *F* test) was done subsequently. Associations between DKG parameters and aerodynamic/acoustic parameters were assessed by Spearman rank correlation analysis. In all analyses, P < 0.05 was regarded as indicating statistical significance.

### RESULTS

# Demographic profile and acoustic/aerodynamic parameters

Table 1 lists the demographic data, aerodynamic parameters, and acoustic parameters of all subjects along with normal values for Japanese subjects (except for HNR or CMC score

#### TABLE 1.

Demographic Data and Results of Aerodynamic/Acoustic Measures are Summarized

Women			
Parameter (Unit)	Normal Value <sup>28,29</sup>	Young (n = 17)	Elderly (n = 12)
Age (y) CMC score MPT (s) MFR (mL/s) AA-F <sub>0</sub> (Hz) APQ (%) PPQ (%) HNR (dB)	$\begin{array}{c} {\sf N/A}\\ {\sf N/A}\\ 20.3\pm 6.7\\ 102.0\pm 36.0\\ 251.5\pm 24.4\\ 2.07\pm 0.68\\ 0.47\pm 0.32\\ {\sf N/A} \end{array}$	$26.2 \pm 3.2 \\ 0.0 \pm 0.0 \\ 23.7 \pm 7.0 \\ 127.9 \pm 39.2 \\ 236.3 \pm 23.2 \\ 2.68 \pm 1.36 \\ 0.28 \pm 0.19 \\ 23.8 \pm 3.9 \\ \end{array}$	$71.8 \pm 5.3 \\ 0.67 \pm 1.61 \\ 17.1 \pm 4.8 \\ 126.5 \pm 30.6 \\ 204.5 \pm 45.5 \\ 3.29 \pm 1.71 \\ 0.39 \pm 0.60 \\ 21.7 \pm 3.7 \\ \end{array}$
Men			
Parameter (Unit)	Normal Value <sup>28,29</sup>	Young (n = 9)	Elderly (n = 8)
Age (y) CMC score MPT (s) MFR (mL/s) AA-F <sub>0</sub> (Hz) APQ (%) PPQ (%) HNR (dB)	N/A N/A 29.7 $\pm$ 9.3 120.0 $\pm$ 41.0 132.0 $\pm$ 19.7 2.19 $\pm$ 0.72 0.31 $\pm$ 0.14 N/A	$28.8 \pm 3.1 \\ 0.0 \pm 0.0 \\ 30.5 \pm 10.9 \\ 131.8 \pm 41.5 \\ 119.1 \pm 17.0 \\ 1.80 \pm 0.91 \\ 0.16 \pm 0.07 \\ 23.5 \pm 4.7$	$74.4 \pm 4.3 \\ 0.0 \pm 0.0 \\ 21.0 \pm 8.5 \\ 150.6 \pm 40.0 \\ 138.6 \pm 24.4 \\ 3.08 \pm 1.20 \\ 0.19 \pm 0.11 \\ 21.2 \pm 3.4$

Abbreviations: CMC, chronic medical condition; MPT, maximum phonation time; MFR, mean flow rate; AA-F<sub>0</sub>, fundamental frequency measured by acoustic analysis; APQ, amplitude perturbation quotient; PPQ, period perturbation quotient; HNR, harmonics-to-noise ratio; SD, standard deviation; N/A, not applicable.

Notes: Normal values are quoted from the Japanese literature.<sup>29,29</sup> Values signify "mean  $\pm$  SD".

1	1	3

Displayed					
Size Parameter (U)	L	SFo	Н	ANOVA	Post-hoc Analysis
VFL (pixel)	141.7 ± 33.1	175.4 ± 40.3	179.8 ± 35.8	<0.001***	L-SF <sub>0</sub> **, L-H**
VFW mean (pixel)	53.7 ± 16	54.5 ± 17.5	56.4 ± 16.9	0.718	_
N <sub>L</sub> -VFW (%)	39.2 ± 11.7	31.8 ± 9.2	31.4 ± 6.8	<0.001***	L-SF <sub>0</sub> **, L-H**
N <sub>L</sub> -amp. mean (%)	10.7 ± 3.1	7.9 ± 2.8	6.2 ± 2.1	<0.001***	L-SF <sub>0</sub> **, SF <sub>0</sub> -H**, L-H**
N <sub>L</sub> -amp. diff. (%)	$4.5 \pm 3.6$	2.3 ± 1.9	1.9 ± 1.6	<0.001***	L-SF <sub>0</sub> **, L-H**
N <sub>w</sub> -amp. mean (%)	30.6 ± 16.5	26.2 ± 10.0	$20.6 \pm 7.4$	<0.001***	L-H**
N <sub>w</sub> -amp. diff. (%)	12.5 ± 12.2	7.9 ± 7.2	$6.0 \pm 5.2$	0.002**	L-SF <sub>0</sub> *, L-H**
AA (%) <sup>19,20</sup>	-17.6 ± 47.1	-10.8 ± 33.1	-17.1 ± 25.8	0.610	_
N <sub>L</sub> -MWM mean (%)	$23.0 \pm 9.7$	17.7 ± 8.4	$11.2 \pm 4.9$	<0.001***	L-SF <sub>0</sub> **, SF <sub>0</sub> -H**, L-H**
N <sub>L</sub> -MWM diff. (%)	$5.4 \pm 5.0$	4.2 ± 2.3	2.9 ± 2.3	0.008**	L-H**
N <sub>W</sub> -MWM mean (%)	61.5 ± 24.0	58.0 ± 24.9	35.9 ± 13.4	<0.001***	L-SF <sub>0</sub> **, SF <sub>0</sub> -H**
N <sub>W</sub> -MWM diff. (%)	14.5 ± 12.0	14.3 ± 15.1	9.8 ± 7.7	0.109	_
N <sub>L</sub> -axis shift (%)	2.6 ± 1.9	1.7 ± 1.6	1.1 ± 1.0	<0.001***	L-SF <sub>0</sub> *, L-H**
AS (%) <sup>19,20</sup>	4.8 ± 17.2	5.3 ± 15.0	6.6 ± 11.3	0.843	_

<b>Overall Data of Size</b>	Kymographic Parameters at all	Frequencies, ANOVAs an	d Post-Hoc Analyses as to	Frequencies are
Displayed				

*Abbreviations:* ANOVA, analysis of variance; SD, standard deviation; U, unit; L, low frequency; SF<sub>0</sub>, speaking fundamental frequency; H, high frequency; VFL, vocal fold length; VFW, vocal fold width; N<sub>L</sub>, normalized (by VFL); Amp., amplitude; Diff., difference; N<sub>W</sub>, normalized (by VFW); AA, amplitude asymmetry<sup>19,20</sup>; MWM, mucosal wave magnitude; AS, axis shift.<sup>19,20</sup>

*Notes:* Values for frequency columns show "mean ± SD", values for ANOVA column show *P* value, and the column of *post-hoc* analysis lists a pair with significant difference.

\*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001.

TABLE 2

which was not found in the literature).<sup>29,30</sup> The CMC scores of all subjects was  $0.2 \pm 0.9$ .

#### **DKG data at all frequencies**

Tables 2 and 3 display DKG data obtained at each frequency. Kymographic  $F_0s$  for a low frequency, speaking fundamental

frequency, and high frequency were significantly different among themselves, which indicated that the phonation tasks were sufficiently performed in terms of pitch. Principally, size parameters, except for AA, N<sub>w</sub>-mucosal wave magnitude difference and AS, showed peak values at a low frequency, and were negatively correlated with frequency (Table 2).

# TABLE 3. Overall Data of Time/Size and Time Kymographic Parameters at all Frequencies, ANOVAs and *Post-hoc* Analyses as to Frequencies are Displayed

Parameter	L	SF <sub>0</sub>	Н	ANOVA	Post-hoc Analysis
Time parameter (U)					
Kymographic F <sub>0</sub> (Hz)	157.8 ± 39.2	210.2 ± 58.6	331.0 ± 102.8	<0.001***	L-SF <sub>0</sub> **, SF <sub>0</sub> -H**, L-H**
N <sub>G</sub> -LPD (%)	$9.2 \pm 6.4$	8.6 ± 7.3	9.0 ± 7.9	0.909	
PA (%) <sup>19,20</sup>	$-3.3 \pm 10.8$	-3.3 ± 10.8	$-4.6 \pm 11$	0.792	_
Open quotient	0.52 ± 0.12	0.55 ± 0.15	0.66 ± 0.19	<0.001***	L-H**, SF <sub>0</sub> -H**
Speed quotient mean	0.77 ± 0.26	0.95 ± 0.54	0.87 ± 0.51	0.152	_
Speed index mean	$-0.19 \pm 0.17$	-0.12 ± 0.18	$-0.15 \pm 0.2$	0.145	_
N <sub>G</sub> -MWP mean (%)	45.2 ± 17	53.3 ± 17.7	50.0 ± 17.7	0.086	_
N <sub>G</sub> -MWP diff. (%)	12.5 ± 9	13.4 ± 10.1	12.0 ± 11.5	0.825	_
Size and time parameter (	(U)				
N <sub>GL</sub> -LPI mean (‱)	11.3 ± 4.9	14.0 ± 8.9	23.0 ± 14.3	<0.001***	L-H**, SF <sub>0</sub> -H**
N <sub>GL</sub> -LPI diff. (‱)	2.8 ± 2.9	$3.6 \pm 8.3$	$3.9 \pm 3.5$	0.626	_
N <sub>GL</sub> -IGW mean (%)	10.8 ± 3.9	8.3 ± 3.4	7.6 ± 3.3	<0.001***	L-SF <sub>0</sub> **, L-H**
N <sub>GL</sub> -IGW diff. (%)	2.9 ± 2.3	1.9 ± 1.6	1.7 ± 1.7	0.005**	L-SF <sub>0</sub> *, SF <sub>0</sub> -H**
AI (%) <sup>15,27</sup>	31.4 ± 29.5	23.7 ± 19.4	22.0 ± 17.9	0.117	-

Abbreviations: ANOVA, analysis of variance; SD, standard deviation; U, unit; L, low frequency; SF<sub>0</sub>, speaking fundamental frequency; H, high frequency; F<sub>0</sub>, fundamental frequency; N<sub>G</sub>, normalized (by glottal cycle); LPD, lateral phase difference; PA, phase asymmetry<sup>19,20</sup>; MWP, mucosal wave persistence; Diff., difference; N<sub>GL</sub>, normalized (by vocal fold length and glottal cycle); LPI, lateral peak index; IGW, integral glottal width; Al, asymmetry index<sup>15,27</sup>; ‱, 0.01%. *Notes:* Values for frequency columns show "mean ± SD", values for ANOVA column show *P* value, and the column of *post-hoc* analysis lists a pair with significant difference.

\*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001.

Both  $N_L$ - and  $N_W$ -size parameters similarly responded to frequency, although benchmarks of normalization (VFL and VFW mean) behaved differently in response to  $F_0$ .VFL revealed a positive correlation with  $F_0$  whereas VFW mean revealed no significant correlation with  $F_0$ .

In time parameters, only open quotient showed a significant correlation with frequency. The peak open quotient was found at a high frequency, and this parameter revealed a positive correlation with  $F_0$  (Table 3). The other time parameters showed no significant correlations with frequency.

In size and time parameters,  $N_{GL}$ -LPI mean showed a significant positive correlation with frequency, whereas  $N_{GL}$ -IGW mean and difference revealed significant negative correlations with frequency (Table 3).  $N_{GL}$ -LPI difference and AI demonstrated no significant correlation with frequency.

# Gender- and age-related differences of DKG parameters

Tables 4 and 5 display DKG data of all subgroups at a speaking F<sub>0</sub>. In size parameters, only N<sub>W</sub>-amplitude mean revealed a significant intergroup difference (ANOVA, P = 0.012) with a significant difference between young female and elderly male (P < 0.05). By gender, the amplitude mean was larger in males than in females: N<sub>L</sub>-amplitude mean was 9.0 ± 3.1% in males and 7.3 ± 2.5% in females (P = 0.042) and N<sub>W</sub>-amplitude mean was 30.7 ± 12.4% in males and 23.4 ± 7.0% in females (P = 0.015). By age, N<sub>W</sub>-amplitude mean was larger in the elderly group (29.9 ± 12.1%) than in the young group (23.3 ± 6.7%) (P = 0.025); N<sub>W</sub>-amplitude difference was larger in the elderly group (10.7 ± 8.4%) than in the young group (5.7 ± 5.4%) (P = 0.020); and N<sub>W</sub>-mucosal wave mean was larger in the elderly group (66.7 ± 26.5%)

than in the young group  $(51.4 \pm 21.8\%)$  (P = 0.037). The other size parameters revealed no significant intergroup difference.

In time parameters, kymographic  $F_0$  revealed a significant intergroup difference, as expected. Speed index mean was negatively larger in the elderly group (-0.19 ± 0.18) than in the young group (-0.067 ± 0.166) (P = 0.024). Although the other time parameters showed no significant intergroup differences, AS, N<sub>G</sub>-lateral phase difference and PA tended to be larger in the elderly group than the young group.

In size and time parameters, N<sub>GL</sub>-lateral peak mean revealed a significant intergroup difference (ANOVA, P < 0.001): young females showed the largest value of all (the sharpest lateral peak), with significant differences between young female and elderly female (P < 0.05), young female and young male (P < 0.01), and young female and elderly male (P < 0.05). Although the other size and time parameters revealed no significant intergroup difference, N<sub>GL</sub>-IGW difference and AI tended to be larger in the elderly group than the young group.

#### Correlations

Table 6 lists the correlations between DKG parameters and aerodynamic/acoustic parameters. A strong correlation  $(0.7|r| \le 1.0)$  was found between AA-F<sub>0</sub> and kymographic F<sub>0</sub> (r = 0.742). For aerodynamic parameters, moderate correlations  $(0.4 < |r| \le 0.7)$  were found between MFR and N<sub>GL</sub>-IGW difference (r = -0.411), MFR and AI (r = -0.496), and laryngeal resistance and N<sub>GL</sub>-lateral peak index (r = 0.474). For acoustic parameters, moderate correlations were noted between AA-F<sub>0</sub> and N<sub>L</sub>-amplitude mean (r = -0.431), AA-F<sub>0</sub> and open quotient (r = -0.582), AA-F<sub>0</sub> and N<sub>GL</sub>-IGW (r = -0.409), AA-F<sub>0</sub> and N<sub>GL</sub>-lateral peak index mean (r = 0.496), speed

TABLE 4.

Kymographic Size Parameters, ANOVAs and Post-hoc Analyses as to Subgroup at a Speaking Fundamental Frequency are Displayed

YF	EF	YM	EM	ANOVA	Post-hoc Analysis
157.5 ± 37.5	190.3 ± 46	167.2 ± 32.3	200.1 ± 27.7	0.033	_
53.3 ± 14.6	53.1 ± 15.8	55.7 ± 21.7	57.8 ± 22.8	0.93	_
34.3 ± 7	29.2 ± 10	33.6 ± 11.1	28.5 ± 9.9	0.32	_
7 ± 2.8	7.6 ± 2.1	9.1 ± 2.8	8.9 ± 3.5	0.226	_
1.7 ± 1.3	2.7 ± 1.9	2.2 ± 1.4	$4.4 \pm 4.4$	0.077	_
20.6 ± 5.8	27.2 ± 7	28 ± 5.9	33.8 ± 17.1	0.012	YF-EM
6.3 ± 5.3	9.2 ± 7.2	$4.6 \pm 5.8$	12.9 ± 10.1	0.074	_
-13.3 ± 37	$-8 \pm 32.5$	$-5.3 \pm 24.2$	-16.1 ± 38.6	0.897	_
17.2 ± 8.4	18.2 ± 10.3	$16.6 \pm 6.5$	19.1 ± 8.2	0.928	_
$3.6 \pm 4.4$	4.2 ± 3.2	$6.2 \pm 3.4$	$3.3 \pm 2.9$	0.339	_
50.2 ± 21.2	64.3 ± 26.5	53.5 ± 24.1	70.3 ± 28	0.199	_
12.8 ± 21.6	14.7 ± 9.1	20.2 ± 11.6	10.5 ± 6.3	0.568	_
1.7 ± 1.9	1.5 ± 1	1.5 ± 1.4	2.3 ± 1.8	0.741	_
6.7 ± 5.7	6.4 ± 12	4.7 ± 4.5	4.7 ± 4.5	0.449	_
	$\begin{array}{r} \mbox{YF} \\ 157.5 \pm 37.5 \\ 53.3 \pm 14.6 \\ 34.3 \pm 7 \\ 7 \pm 2.8 \\ 1.7 \pm 1.3 \\ 20.6 \pm 5.8 \\ 6.3 \pm 5.3 \\ -13.3 \pm 37 \\ 17.2 \pm 8.4 \\ 3.6 \pm 4.4 \\ 50.2 \pm 21.2 \\ 12.8 \pm 21.6 \\ 1.7 \pm 1.9 \\ 6.7 \pm 5.7 \end{array}$	$\begin{array}{c cccc} YF & EF \\ \hline 157.5 \pm 37.5 & 190.3 \pm 46 \\ 53.3 \pm 14.6 & 53.1 \pm 15.8 \\ 34.3 \pm 7 & 29.2 \pm 10 \\ 7 \pm 2.8 & 7.6 \pm 2.1 \\ 1.7 \pm 1.3 & 2.7 \pm 1.9 \\ 20.6 \pm 5.8 & 27.2 \pm 7 \\ 6.3 \pm 5.3 & 9.2 \pm 7.2 \\ -13.3 \pm 37 & -8 \pm 32.5 \\ 17.2 \pm 8.4 & 18.2 \pm 10.3 \\ 3.6 \pm 4.4 & 4.2 \pm 3.2 \\ 50.2 \pm 21.2 & 64.3 \pm 26.5 \\ 12.8 \pm 21.6 & 14.7 \pm 9.1 \\ 1.7 \pm 1.9 & 1.5 \pm 1 \\ 6.7 \pm 5.7 & 6.4 \pm 12 \\ \end{array}$	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $

*Abbreviations:* SD, standard deviation; ANOVA, analysis of variance; YF, young female; EF, elderly female; YM, young male; EM, elderly male; VFW, vocal fold width; N<sub>L</sub>, normalized (by VFL); Amp., amplitude; Diff., difference; N<sub>W</sub>, normalized (by VFW); AA, amplitude asymmetry<sup>19,20</sup>; MWM, mucosal wave magnitude; AS, axis shift.<sup>19,20</sup>

Notes: Values for frequency columns show "mean ± SD", values for ANOVA column show P value, and the column of post-hoc analysis lists a pair with significant difference.

equency are Display	eu					
rameter	YF	EF	YM	EM	ANOVA	PHA
me parameter (U)						
K- F <sub>o</sub> (Hz)	256.1 ± 31.5	209.3 ± 47	147.5 ± 55.5	184.7 ± 43.2	<0.001***	YF-YM**
						YF-EM**
						EF-YM*
N <sub>G</sub> -LPD (%)	8.3 ± 8.8	$9.8 \pm 6.3$	$6.2 \pm 5.4$	10.0 ± 7.3	0.66	_
PA (%) <sup>19,20</sup>	$-4.5 \pm 11.3$	-1.5 ± 12	0.43 ± 8.51	-7.6 ± 10	0.421	_
N <sub>G</sub> -MWPM (%)	60.1 ± 19.5	53.1 ± 17.7	46.5 ± 12.2	47.0 ± 16.1	0.184	_
N <sub>G</sub> -MWPD (%)	15.5 ± 13.4	12.5 ± 7.5	15.3 ± 8.4	7.9 ± 5.3	0.325	_
Open quotient	0.53 ± 0.17	0.58 ± 0.14	$0.56 \pm 0.14$	0.54 ± 0.12	0.811	_
Speed quotient	$1.1 \pm 0.76$	0.86 ± 0.38	$1.02 \pm 0.34$	$0.69 \pm 0.16$	0.298	_
Speed index	-0.087 ± 0.18	$-0.16 \pm 0.21$	$-0.029 \pm 0.137$	$-0.23 \pm 0.13$	0.087	_
ze and time parame	ter (U)					
N <sub>GL</sub> -LPI-M (‱)	20.5 ± 11.4	12.3 ± 4.1	7.1 ± 2.8	11.2 ± 2.8	<0.001***	YF-EF*
						YF-YM**
						YF-EM*
N <sub>GL</sub> -LPI diff. (‱)	6.3 ± 13.4	2.9 ± 3	1.3 ± 1.7	2.1 ± 2.1	0.437	_
N <sub>GL</sub> -IGW-M (%)	$6.8 \pm 3.2$	8.7 ± 3.1	$9.6 \pm 2.3$	$9.3 \pm 4.6$	0.141	_

Kymographic Time/Size and Time Parameters, ANOVAs and Post-hoc Analyses as to Subgroup at a Speaking Fundamental
Frequency are Displayed

*Abbreviations:* ANOVA, analysis of variance; SD, standard deviation; U, unit; YF, young female; EF, elderly female; YM, young male; EM, elderly male; PHA, post-hoc analysis; K-F<sub>0</sub>, kymographic fundamental frequency; N<sub>G</sub>, normalized (by glottal cycle); LPD, lateral phase difference; PA, phase asymmetry<sup>19,20</sup>; MWPM, mucosal wave persistence mean; MWPD, mucosal wave persistence difference; ‰, 0.01%.

 $1.4 \pm 1.7$ 

 $15.4 \pm 18.7$ 

 $2.1 \pm 1.3$ 

 $26.2 \pm 16.1$ 

*Notes:* Values for frequency columns show "mean ± SD", values for ANOVA column show *P* value, and the column of *post-hoc* analysis lists a pair with significant difference.

\*P < 0.05; \*\*P < 0.01; \*\*\*P < 0.001.

N<sub>GL</sub>-IGW diff. (%)

AI (%)<sup>15,27</sup>

quotient mean and HNR (r = 0.410), and speed index mean and HNR (r = 0.478).

 $1.7 \pm 1.6$ 

 $24.3 \pm 22.7$ 

### DISCUSSION

TABLE 5.

Pa Ti

Si

Although DKG and VKG share many features, there are certain differences with which phonosurgeons should be acquainted. Although DKG involves construction of kymograms from HSDI data obtained with a high-speed digital camera, VKG involves direct acquisition of kymographic images with a special video camera that not only registers standard images of the whole glottis but also performs high-speed scanning of the larvnx along a single horizontal line. Although the amount of data obtained is relatively small, VKG achieves better spatial and temporal resolution than DKG.<sup>4</sup> VKG has several other advantages over DKG, including a lower cost and more rapid data storage and processing.<sup>4</sup> On the other hand, DKG has some advantages over VKG. Kymograms can be created at any level of the glottal plane<sup>4,11,31</sup>; the glottal axis can be adjusted before a kymogram is constructed<sup>11,19,23–25</sup>; and other HSDI analyses can be done concomitantly using the same HSDI data, which allows parametric comparison across different HSDI analysis methods.<sup>9,10,23–25,31</sup> DKG was selected in the present study because it was one of the serial HSDI researches on normophonic subjects.<sup>11,12,32,33</sup> Advantages of DKG included normalization by VFL which facilitates parametric comparison with videostroboscopic studies that frequently use size normalization by VFL.<sup>34–36</sup> Normalization by VFL also allows parameterization of more various vibratory characteristics (eg, amplitude, mucosal wave, or IGW). It is important to select the appropriate technique by considering these merits and demerits.

0.578

0.524

 $2.4 \pm 1.9$ 

 $28.2 \pm 17.9$ 

Most of the previous normative DKG studies adopted a subjective rating system, and quantitative parameters, especially for the mucosal wave, are limited. Quantification of mucosal wave can facilitate detection, diagnosis, and treatment of laryngeal pathologies with altered mucosal wave such as vocal fold cyst, scar, or carcinoma, for instance. The present study therefore introduced quantitative parameters of amplitude (N<sub>L</sub>-/N<sub>w</sub>-amplitude mean or difference, and AA) and mucosal wave (N<sub>L</sub>-/N<sub>w</sub>-mucosal wave magnitude mean or difference, and N<sub>G</sub>-mucosal wave persistence mean or difference), and overall, the obtained data for amplitude and mucosal wave at a speaking F<sub>0</sub> generally agreed with the reported norms in the literature. N<sub>w</sub>-amplitude mean was  $26.2 \pm 10\%$  in the present study, which was generally matched but slightly smaller than the reported norm (1/3 to 1/2 of theVFW).<sup>3,37</sup> Minor variation in the intensity or pitch across studies may play a role here because amplitude is reported to show a positive correlation with intensity, and a negative correlation with pitch<sup>5,32</sup>: a negative correlation between N<sub>L</sub>and N<sub>w</sub>-amplitude mean and F<sub>0</sub> found in the present study (Tables 2 and 6) supports this previous findings. AA in the present study  $(-10.8 \pm 33.1\%)$  accorded with the reported value in the literature.<sup>19,20,32</sup> However,  $N_L$ -/ $N_W$ -amplitude difference and AA revealed found only weak correlations with aerodynamic parameters in the present study, and thus

TABLE 6.

Parameter	MPT	MFR	LR	AA-F <sub>0</sub>	APQ	PPQ	HNR
N <sub>L</sub> -amplitude mean	0.146	0.035	-0.095	-0.431**	-0.137	-0.102	0.036
N <sub>L</sub> -amplitude difference	0.341*	-0.387**	0.215	-0.075	-0.004	0.016	0.069
N <sub>w</sub> -amplitude mean	-0.076	0.081	-0.097	-0.363*	-0.066	-0.117	-0.116
N <sub>w</sub> -amplitude difference	0.143	-0.264	0.168	-0.061	0.051	0.027	-0.046
AA <sup>19,20</sup>	-0.056	0.166	-0.305*	-0.04	0.091	0.139	-0.237
N <sub>L</sub> -MWM Mean	-0.121	0.163	-0.084	-0.165	0.075	0.215	-0.142
N <sub>L</sub> -MWM difference	-0.081	0.314*	-0.308*	-0.203	-0.219	-0.115	0.171
N <sub>w</sub> -MWM mean	-0.288	0.201	-0.127	-0.19	0.136	0.194	-0.26
N <sub>w</sub> -MWM difference	-0.133	0.264	-0.307*	-0.083	-0.191	-0.113	0.139
N <sub>L</sub> -axis shift	0.224	-0.380**	0.25	-0.107	-0.128	0.015	0.076
AS <sup>19,20</sup>	0.037	-0.137	0.261	0.057	-0.128	-0.129	0.227
Kymographic $F_0$	-0.069	-0.143	0.302*	0.742***	0.044	0.138	0.195
N <sub>G</sub> -lateral phase difference	-0.130	-0.156	0.001	-0.05	-0.091	0.125	-0.05
PA <sup>19,20</sup>	0.022	-0.023	-0.159	0.001	0.112	0.214	-0.17
Open quotient	-0.055	0.198	-0.321*	-0.582***	-0.21	-0.191	0.024
Speed quotient mean	0.031	-0.038	0.111	0.099	-0.245	-0.128	0.41**
Speed index mean	0.109	0.001	0.026	-0.044	-0.310*	-0.192	0.478***
N <sub>G</sub> -MWP mean	-0.144	0.087	0.026	0.205	0.115	0.271	-0.018
N <sub>G</sub> -MWP difference	-0.172	0.195	0.008	0.104	0.066	0.201	-0.168
N <sub>GL</sub> -IGW	-0.08	0.12	-0.239	-0.409**	-0.07	0.001	-0.136
N <sub>GL</sub> -IGW difference	0.22	-0.411**	0.225	-0.008	-0.028	0.07	-0.005
AI <sup>15,27</sup>	0.259	-0.496***	0.313*	0.123	-0.032	0.033	0.106
N <sub>GL</sub> -LPI mean	0.128	-0.249	0.474***	0.496***	-0.038	0.077	0.251
N <sub>GL</sub> -LPI difference	0.16	-0.327*	0.396**	0.184	-0.059	0	0.229

*Abbreviations:* MPT, maximum phonation time; MFR, mean flow rate; LR, laryngeal resistance; AA-F<sub>0</sub>, fundamental frequency of acoustic analysis; APQ, amplitude perturbation quotient; PPQ, period perturbation quotient; HNR, harmonics-to-noise ratio; N<sub>L</sub>, normalized (by vocal fold length); N<sub>W</sub>, normalized (by vocal fold width); AA, amplitude asymmetry<sup>19,20</sup>; MWM, mucosal wave magnitude; AS, axis shift<sup>19,20</sup>; F<sub>0</sub>, fundamental frequency; N<sub>G</sub>, normalized (by glottal cycle); PA, phase asymmetry<sup>19,20</sup>; MWP, mucosal wave persistence; N<sub>GL</sub>, normalized (by vocal fold length and glottal cycle); IGW, integral glottal width; AI, asymmetry index<sup>15,27</sup>; LPI, lateral peak index.

\**P* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001.

these parameters may not be primarily related to the vocal function or voice quality in normophonic subjects. N<sub>W</sub>-mucosal wave magnitude mean in the present study (58 ± 24.9%) coincided with the reported value in the literature (1/2 and over of the VFW).<sup>3,37</sup> Although N<sub>W</sub>-mucosal wave magnitude mean showed a negative correlation with F<sub>0</sub>, N<sub>G</sub>-mucosal wave persistence mean had no correlation with F<sub>0</sub> and thus was considered to be more robust in terms of frequency than N<sub>G</sub>-mucosal wave magnitude mean and N<sub>G</sub>-mucosal wave persistence mean are considered to reflect different characteristics of mucosal wave, it is advisable to evaluate both parameters in the assessment of laryngeal pathologies with possible alteration of mucosal wave.

In this study, the open quotient was found to be  $0.55 \pm 0.15$  at a speaking F<sub>0</sub> at the midpoint of the membranous portion, which generally matched the values in some previous reports, <sup>23,28,31,38,39</sup> but was rather smaller than those in other reports (Table 7).<sup>5,20</sup> These differences may stem from differences in the data acquisition technique (videostroboscopy<sup>23,39</sup> or HSDI<sup>28,31,38</sup>), the method of analysis (DKG,<sup>31,38</sup> glottal width waveform,<sup>5</sup> or glottal area waveform<sup>39</sup>), monitored longitudinal level,<sup>12,38</sup> or variation in the examination conditions. According to previous reports, open quotient increases as  $F_0$  increases and decreases as the intensity increases.<sup>5,39</sup> In the present study, the same pitch-associated behavior was observed in open quotient (Table 3). At the same time, however, the open quotient revealed a negative correlation with AA-F<sub>0</sub> at a speaking F<sub>0</sub> (Table 6), which appears contradictory to some previous findings. Female subjects with a high speaking F<sub>0</sub> showed higher open quotient than male subjects with a low speaking F<sub>0</sub>.<sup>31,39</sup> More research is needed to clarify the relationship between open quotient and F<sub>0</sub> across different gender or age groups.

The speed quotient  $(0.95 \pm 0.54)$  at a speaking F<sub>0</sub> in the present study was in accord with some of the previous reports,<sup>20,38</sup> but smaller than the report by Woo<sup>39</sup> and larger than the report by Timcke et al (Table 7).<sup>5</sup> Minor variations of intensity at the time of evaluation may play a role in such variation, because the speed quotient has been reported to increase as the intensity increases.<sup>5,39</sup> The minor variation in monitored longitudinal level can lead to altered speed quotient, as well.<sup>12,38</sup> Speed quotient mean was smaller in the elderly subjects than young subjects. This age-associated decrease in the speed quotient mean may originate from structural and functional alteration of the vocal fold resulting from geriatric change, leading to the decreased speed of vocal folds returning to the midline. Closing phase characteristics have been linked to acoustic quality (eg the

#### TABLE 7.

Kymographic Parameters at a Speaking Fundamental Frequency of the Present Study Were Compared With Those of the
Literature. Values Signify "Mean ± SD", and Values With    Signify the Absolute Value

Parameter	Present Study	Literature
N <sub>W</sub> -amplitude mean	26.2 ± 10	One third to one half of VFW $^{3,37}$
AA <sup>10,20</sup>	$-10.8 \pm 33.1$	6.5 ± 4.7 <sup>1</sup> ; range, –9 to –1 <sup>20</sup> ; Female  10  ± 10, male  18  ± 18 <sup>31</sup>
N <sub>w</sub> -MWM mean	58 ± 24.9	One half of VFW <sup>3</sup> ; at least one half of VFW <sup>37</sup>
AS <sup>19,20</sup>	5.3 ± 15	$10.4 \pm 8.3^{19}$
PA <sup>19,20</sup>	-3.3 ± 10.8	6.3 ± 4.3 <sup>19</sup> ; range, –4 to –1 <sup>20</sup>
AI <sup>15,27</sup>	31.4 ± 29.5	$2.4 \pm 2.5^{27}$
Open quotient	0.55 ± 0.15	Range, 0.64–0.88 <sup>5</sup> ; range, 0.62–0.76 <sup>20</sup>
		$0.5 \pm 0.13^{23}$ ; 0.46 $\pm 0.05^{28}$
		Female 0.63 ± 0.13, male 0.47 ± 0.12 <sup>31</sup>
		Female 0.66 ± 0.14, male 0.56 ± 0.1 <sup>38</sup>
		Female 0.64, male 0.66 <sup>39</sup>
Speed quotient mean	$0.95 \pm 0.54$	Range, 0.50–0.78 <sup>5</sup> ; range, 0.91–1.15 <sup>20</sup>
		Female 0.85 ± 0.21, male 0.88 ± 0.28 <sup>38</sup>
		Female 1.29, male 1.16 <sup>39</sup>

Abbreviations: N<sub>W</sub>, normalized (by vocal fold width); AA, amplitude asymmetry<sup>19,20</sup>; MWM, mucosal wave magnitude; AS, axis shift<sup>19,20</sup>; PA, phase asymmetry<sup>19,20</sup>; AI, asymmetry index<sup>15,27</sup>; VFW, vocal fold width.

spectral tilt): a larger ratio of closing phase to glottal cycle (smaller speed quotient/index mean) leads to steeper rolloff of spectral tilt, decreased maximum area declination rate, and decreased sound pressure level.<sup>25,40</sup> In the present study, the speed quotient mean and speed index mean were moderately correlated with HNR. Perhaps among acoustic parameters measured in the present study, HNR, an acoustic parameter that includes pitch perturbation, amplitude perturbation, and turbulent noise, might be most sensitive to the alteration in closing phase.

PA  $(-3.3 \pm 10.8\%)$  and AS  $(5.3 \pm 15\%)$  in the present study generally matched the value in the literature (Table 7).<sup>19,20</sup> These values were rather small in number, and no significant correlations were found between these two parameters and aerodynamic/acoustic parameters in the present study. Lateral phase difference and axis shift may therefore be considered to play only a secondary role in the characteristics of normal voice.

Previously, the shape of lateral peak was evaluated qualitatively (sharp or rounded).<sup>14,41</sup> Because its shape is dependent on the frame rate and pixel size in the lateral direction, lateral peak in the present study was normalized by VFL and glottal cycle. N<sub>GL</sub>-lateral peak index mean was largest in young females, meaning that the lateral peak in this subpopulation was sharpest. A positive correlation between N<sub>GL</sub>-lateral peak mean and F<sub>0</sub> stands to reason: the elevated vocal fold tension at a high frequency should lead to the quickened glottal opening and closure, constituting a sharp lateral peak, and vice versa. Although the obtained data regarding lateral peak seems reasonable in general, further refinement of the analytical technique is definitely needed in the future: because the degree of lateral peak was approximated by the angle created by tangents at the lateral peak in the present study, the obtained values may not truly reflect the actual lateral peak.

NGL-IGW and NGL-IGW difference are other new parameters that were introduced in the present study. NGL-IGW is considered as a comprehensive parameter in which both size and temporal aspects of glottal width are reflected. As shown in Table 3, N<sub>GL</sub>-IGW showed a negative correlation with F<sub>0</sub> just as N<sub>L</sub>amplitude, although NGL-IGW could also have presented a positive correlation with F<sub>0</sub> from a temporal standpoint just as open quotient. The contribution of size aspect to N<sub>GL</sub>-IGW may therefore be greater than that of temporal aspect. AI in the present study  $(31.4 \pm 29.5\%)$  was considerably higher than the value reported by Kim et al.<sup>28</sup> This discrepancy may result from a difference in the used technique (videostroboscopy<sup>28</sup>) or HSDI) or in demographics of recruited subjects such as age or gender. AI demonstrated a negative correlation with MFR in the present study (Table 6), which was an unexpected result. Because AI is an indicator of asymmetry, AI was expected to have a positive (rather than negative) correlation with MFR because high MFR is an unfavorable condition. The clinical use of these novel parameters and their relevance to aerodynamic/acoustic parameters will need to be validated by further studies on clinical cases with various laryngeal disorders.

Generally, the elderly group demonstrated larger asymmetry parameters (N<sub>W</sub>-amplitude difference, AS, N<sub>GL</sub>-IGW difference, N<sub>G</sub>-lateral phase difference and PA) than the young group. Changes related to aging (eg, asymmetry of the laryngeal frame and atrophic degeneration of the lamina propria or laryngeal musculature) can result in the left-right differences of the closing force, volume, tension, and mucoelasticity, leading to more prominent asymmetry.<sup>42–46</sup> In the present study, the CMC score, an indicator of physiological aging, was measured because the aging of voice is reported to be more influenced by physiological age than chronological aging *per se.*<sup>47,48</sup> The CMC score of recruited subjects in the present study  $(0.2 \pm 0.9)$  was significantly smaller than that of patients with presbylarynx, the voice disorder resulting from pathological vocal aging, in the literature  $(1.3 \pm 2.4)$ ,<sup>49</sup> which serves as a rationale for regarding the subjects recruited in the present study as physiologically normal.

In consideration of the recent increase in clinical use of HSDI, understanding the normal range of vocal fold vibrations and their variations is more important than before for otolaryngologists who are engaged in evaluating and treating vocal disorders. The present findings not only add to normative data for DKG, but also reveal normal variations of DKG parameters associated with gender and age. Knowledge of the age-related variations demonstrated in the present study is especially important considering the worldwide trend for aging of society.<sup>49,50</sup> In addition, quantitative evaluation of some DKG parameters that have mainly been assessed in a qualitative manner so far (eg, amplitude, mucosal wave, and lateral peak) was introduced in this study. Some novel parameters were developed, as well (eg, mucosal wave persistence, lateral peak index, and IGW). It is hoped that the findings of the present study will encourage the routine clinical application of DKG analysis.

The present study had several limitations. First, the age range and number of subjects were limited: few male subjects were relatively few, and middle-aged subjects were not examined. Still, because the aerodynamic/acoustic data of recruited subjects in the present study generally matched those of the previous reports on Japanese population,<sup>29,30</sup> HSDI data in the present study are estimated to represent the larger Japanese population. Future studies on a larger number of subjects with wider age range will definitely be needed, however. Second, the analytical method is still preliminary, and depends on manual measurement to a great extent, making analysis timeconsuming and vulnerable to intra- or interrater bias. Further improvement of the analytical method with more automation and high precision should be explored in the future study to shorten the analysis time and to lower the influence of bias. Third, there may be inevitable individual variations in the pitch range and intensity across phonation tasks, because the phonation tasks were mainly based on self-selected pitch and self-controlled intensity. Although significant differences of kymographic  $F_0$  among a low frequency, a speaking  $F_0$  and a high frequency indicate that the phonation tasks were sufficiently performed in terms of pitch in the present study, the introduction of stricter pitch and intensity control, perhaps with a stimulus tone or sound level meter, should be warranted in the future study. Fourth, simultaneous recording of HSDI and aerodynamic or acoustic parameters was not available at our center, presumably resulting in the relatively low correlations obtained in this study, although the strong correlation between AA-F<sub>o</sub> and kymographic F<sub>0</sub> (r = 0.742) should stand as a rationale for comparing DKG parameters and aerodynamic/acoustic parameters in the present study (Table 6). Establishment of a system for simultaneous recording of HSDI and aerodynamic/ acoustic parameters should result in stronger interparametric correlations, leading to better understanding of their relationships.

# CONCLUSION

The present study provided quantitative normative data for DKG parameters using HSDI, which were generally in agreement with the results of previous qualitative studies. Some novel parameters were also introduced, to better describe vibratory characteristics. Furthermore, age- and gender-related differences were analyzed, revealing differences of  $F_0$ , normalized amplitude and lateral peak, with elderly subjects showing more prominent asymmetry than young subjects. When deciding whether a vocal fold pattern is normal or pathological, the gender- and age-related differences observed in the present study should be taken into account.

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