

## Tongue thickness and its clinical significance

Keiko FUJIMOTO, Tsuyoshi HONDA, Hideki SUITO, Kan NAGAO,

Tetsuo ICHIKAWA

キーワード : tongue thickness, oral function, elderly, assessment

**Abstract :** **Background:** The tongue occupies most of the space in the oral cavity, and it plays an important role in oral functions such as mastication, swallowing and articulation. The tongue continues to move from the time of intake to swallowing, particularly during masticating. A method for evaluating tongue thickness at rest by ultrasonography has been proposed; however, the association between tongue thickness and various oral functions remains unclear.

**Objective:** The purpose of this study was to investigate the relation between tongue thickness and oral functions, and to clarify the clinical significance of tongue thickness measurements in oral function maintenance.

**Materials and Methods:** Elderly outpatients were serially screened for enrollment and a total of 106 subjects (men, 54; women, 52; mean age,  $75.2 \pm 6.5$  years) were selected. Age, body mass index, and functional teeth number including implant and pontic of fixed partial denture number were recorded as the basic attributes. Tongue thickness, tongue pressure, tongue thrust pressure and tongue motor function were measured as tongue assessments. Cheek pressure, oral moisture and occlusal force were measured as other oral functions.

**Results:** Subjects with thick tongue tended to have higher BMI, stronger muscle strengths and lower diadochokinesis. Diadochokinesis of /ka/, cheek pressure and functional teeth number were extracted as the independent factors affecting tongue thickness.

**Conclusion:** Tongue thickness does not necessarily reflect oral functions in healthy elderly people except for a negative association between tongue thickness and oral diadochokinesis of /ka/. Multiple assessments of tongue would be required to evaluate oral function, and the assessment of tongue thickness might have a different clinical meaning.

### Introduction

The tongue occupies most of the space in the oral cavity and it plays an important role in oral functions such as mastication, swallowing and articulation. The tongue continues to move from the time of intake to swallowing, particularly during masticating.

Assessments of tongue include functional and morphological assessments. The functional ones are tongue movement, tongue pressure, tongue motor function and tongue-related electromyograms (EMGs)<sup>1)</sup>. Tongue movement has been measured using videofluoroscopic examination of swallowing (VF)<sup>2, 3)</sup> and ultrasonic diagnostic equipment<sup>4)</sup>. Tongue pressure has been measured using a strain

gage-type pressure sensor<sup>5)</sup>, pressure sheet sensor<sup>6)</sup> and the balloon-type tongue pressure sensor<sup>7, 8)</sup>. Tongue motor function has been measured the maximum repetition number during the monosyllable utterance on tongue (oral diadochokinesis)<sup>9)</sup>. An assessment of tongue morphology using ultrasonography, that is tongue thickness, has been reported by Tamura et al. T, who reported that the tongue thickness at rest was significantly related to nutritional condition<sup>10)</sup>, body mass index (BMI) and period of care needed<sup>11)</sup>. However, the relationship between tongue thickness and various oral functions is not clear.

The purpose of this study was to investigate the

relationship between tongue thickness and oral functions, which can be provided easily at chair side; and to clarify the clinical significance of tongue thickness measurements in the management of oral function.

## Materials and methods

### Subjects

We enrolled 106 elderly outpatients with regular maintenance from January 2017 to August 2017 in the dental division of Tokushima University Hospital. 100 of 106 subjects were used in a previous research with the different research question<sup>12</sup>. The exclusion criteria of the participants were those with a history of an ingesting/swallowing disorder and with maxillofacial defect.

This study was approved by the clinical research institutional review board of the Ethics Committee of the Tokushima University Hospital (Approval No. 2225). Consent was obtained with a document from all participants after providing sufficient explanations.

### Basic attribute

Age, height and weight were gathered through interviews and then BMI was calculated. Functional teeth number including implant and pontic of fixed partial denture number was counted during the oral examination.

### Tongue assessment

#### 1) Tongue thickness

Tongue thickness was defined as the distance from the mylohyoid muscle surface to the tongue dorsum surface when an ultrasound probe (frequency, 4.0–8.0 MHz; contact face size, 9 × 25 mm) of ultrasonography (Vscan with Dual Probe, GE, Tokyo, Japan) was positioned to the middle of bilateral second mandibular premolars perpendicular to the Frankfurt plane<sup>10,11</sup> (Figure 1). Measurements were performed in the image when the tongue was in a stable position after swallowing saliva. Measurements were repeated three times, and the mean value was used as a representative value.

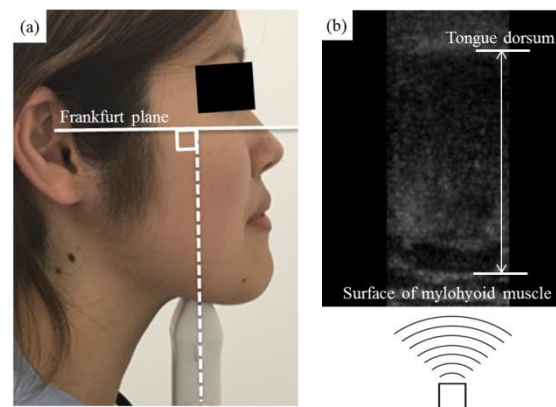
#### 2) Tongue pressure and tongue thrust pressure

The maximum tongue pressure and tongue thrust pressure were measured using a commercially-available tongue pressure meter (JMS tongue pressure measurement device, JMS, Hiroshima, Japan). As for the maximum tongue pressure, a balloon-type tongue pressure probe was placed between the tongue and palate, and the participants

were asked to press it as much as possible for 7 seconds. As for tongue thrust pressure, the probe was held lightly against the upper and lower right first premolars and the participants were asked to close their lips and press the balloon with the tongue apex as much as possible for 7 seconds. Measurements were repeated three times and the mean value was used as a representative value.

#### 3) Tongue motor function

Oral diadochokinesis was measured as an indicator of tongue motor function<sup>9</sup>. Participants were asked to utter repeatedly either of the syllables, /ka/ or /ta/, as quickly as possible for 5 seconds. The utterance frequency (number per second) of /ka/ or /ta/ was measured using an automatic counter (Kenkokun Handy, Takei Scientific Instruments Co., Niigata, Japan) as assessments of tongue apex and dorsum, respectively. Measurements were taken three times for each subject and the maximum value was recorded as a representative value.



**Figure 1** Tongue thickness using ultrasonography. (a) Probe position. (b) Sonogram. Tongue thickness is a distance from the surface of mylohyoid muscle to the tongue dorsum.

### Cheek pressure

Cheek pressure was measured using the tongue pressure meter based on a previous study<sup>13</sup>. Participants were asked to bite at a habitual occlusal position and the balloon-type probe was held between the right buccal mucosa and the buccal surface of the upper and lower first molars. Participants were asked to press it by the buccal mucosa as much as possible for 7 seconds. Measurements were repeated three times and the mean value was used as a representative value.

**Table 1** Categorization of tongue thickness for the regression analysis

Tongue thickness (mm)	
Thin group (0)	< 49.4
Thick group (1)	≥ 49.4

**Table 2** Differences in measurement factors between the thin and thick groups of tongue thickness

	Tongue thickness		p-value
	Thin group (n=53) Median (interquartile range)	Thick group (n=53) Median (interquartile range)	
Age	75.00 (69.00-81.00)	75.00 (69.00-80.00)	0.955
BMI, kg/m <sup>2</sup>	22.06 (20.23-23.64)	23.44 (20.62-25.03)	0.061
Tongue pressure, kPa	27.90 (21.67-33.02)	29.07 (24.20-33.65)	0.410
Tongue thrust pressure, kPa	25.07 (17.17-29.75)	26.00 (20.93-31.65)	0.221
/ta/, times	6.40 (5.60-7.00)	6.20 (5.40-6.60)	0.101
/ka/, times	6.00 (5.40-6.50)	5.60 (2.80-6.20)	0.021*
Cheek pressure, kPa	15.73 (13.22-18.02)	17.03 (14.52-21.08)	0.032*
Occlusal force, N	203.10 (122.50-355.15)	278.70 (166.25-432.65)	0.083
Oral moisture	29.00 (27.80-29.80)	28.40 (27.15-29.35)	0.146
Functional teeth number	9.00 (2.50-20.50)	15.00 (8.00-21.50)	0.114

\* Significance (p < 0.05)

### Oral moisture

Oral moisture was measured using an oral moisture checker (Mucus, Life Co., Saitama, Japan). Participants were asked to stick out their tongue and the sensor head was positioned vertically against the portion of the tongue dorsum to approximately 10 mm apart from the tongue apex with contact pressure of approximately 200 gf during 2 seconds. Measurements were repeated three times and the median was recorded as the representative value.

### Occlusal force

A pressure-indicating film (Dental Prescale 50H, GC, Tokyo, Japan) and Occluzer 709 (GC, Tokyo, Japan) were used to measure the total sum of the occlusal force in maximum clenching at the intercuspal position for 3 seconds. Measurements were performed three times and the maximum value was used as representative values.

### Statistical analysis

Before regression analysis, the participants were divided into two groups: “thin group (0)” and “thick group (1)” according to tongue thickness as shown in Table 1.

Mann–Whitney U test was used to analyze the differences between the thin and thick groups. Next, a univariate logistic regression analysis using the forced entry method with the tongue thickness as the dependent variables was performed to clarify the association with each factor. A multivariate logistic regression analysis was also performed to assess the interactions between variables. The backward elimination method with the likelihood ratio test was used to select the variables.

Before conducting these tests, Shapiro-Wilk test was used to confirm whether the data had a normal distribution. The SPSS 24.0 for MAC (IBM Co., Tokyo, Japan) was used for all statistical analysis and the level of statistical significance was set at a p value of 0.05.

**Table 3** Association of tongue thickness with measurement factors

	Tongue thickness			
	Odds ratio	95% CI		P-value
		Lower limit	Upper limit	
Age	1.000	0.943	1.061	0.988
BMI, kg/m <sup>2</sup>	1.138	1.005	1.289	0.042*
Tongue pressure, kPa	1.020	0.974	1.068	0.410
Tongue thrust pressure, kPa	1.032	0.983	1.083	0.203
/ta/, times	0.673	0.444	1.020	0.062
/ka/, times	0.613	0.413	0.908	0.015*
Cheek pressure, kPa	1.104	1.000	1.220	0.050*
Occlusal force, N	1.001	0.999	1.003	0.203
Oral moisture	0.934	0.798	1.092	0.391
Functional teeth number	1.035	0.993	1.079	0.101

\* Significance (p < 0.05)

**Table 4** Extracted factors affecting tongue thickness in the multivariate logistical regression analysis

	Odds ratio	95% CI		P-value
		Lower limit	Upper limit	
	/ka/, times	0.481	0.306	0.757
Cheek pressure, kPa	1.145	1.026	1.278	0.016*
Functional teeth number	1.056	1.008	1.106	0.022*

\* Significance (p < 0.05)

## Results

### Characteristics of participants

The baseline characteristics of the 106 elderly participants showing the means and standard deviations were as follows: age  $75.2 \pm 6.5$  years, BMI  $2.5 \pm 3.3$  kg/m<sup>2</sup>, functional teeth number  $13.4 \pm 9.5$ , tongue thickness  $49.7 \pm 6.4$  mm, tongue pressure  $27.7 \pm 8.4$  kPa, tongue thrust pressure  $24.7 \pm 8.1$  kPa, oral diadochokinesis of /ta/  $6.2 \pm 1.0$  times, oral diadochokinesis of /ka/  $5.7 \pm 1.1$  times, cheek pressure  $6.3 \pm 4.1$  kPa, occlusal force  $280.2 \pm 210.9$  N, and oral moisture  $28.3 \pm 2.5$ .

Because Shapiro-Wilk test showed that tongue thickness did not conform to normal distribution, Mann-Whitney U test was performed to compare the groups of tongue thickness in the measurement factors. Significant differences were found between the diadochokinesis of /ka/ (hereinafter called /ka/) and cheek pressure (Table 2). Subjects with thick

tongue tended to have higher BMI, stronger muscle strengths and lower diadochokinesis.

### Comparison of tongue thickness and various factors

Tables 3 and 4 show the results for two types of logistic regression analysis.

A univariate logistic regression analysis revealed that BMI, /ka/ and cheek pressure were extracted as significant factors affecting tongue thickness. A multivariate logistic regression analysis revealed that /ka/, cheek pressure and functional teeth number were extracted as significant independent factors affecting tongue thickness.

## Discussion

In the elderly, eating and conversation are a great pleasure and worth living for. Maintenance and improvement of oral functions such as mastication,

swallowing and articulation in the old state have been reported to have a significant effect on improvement of the quality of life<sup>14, 15, 16</sup>). The tongue plays a primary role in mastication, swallowing and conversation; and the decline of tongue function causes various problems such as difficulty of chewing, articulation and swallowing, and pulmonary aspiration. It is important to detect it at an earlier state and to take appropriate measures including rehabilitation and exercise before it becomes a serious problem. Although this study focused on the tongue, aspects of tongue movement are complicated. It is difficult to perform quantitative assessments based on direct observation and therefore, various indirect assessment methods have been developed.

Both functional and morphological assessments of the tongue are available. Functional assessments include the observations of tongue movements using VF<sup>2, 3</sup>) and ultrasonic diagnostic equipment<sup>4, 17</sup>); tongue exercise ability using articulatory behavior such as oral diadochokinesis<sup>9</sup>); tongue muscle strength such as maximum tongue pressure<sup>7,8</sup>); tongue-related EMGs<sup>1</sup>); tongue-palate contact pattern; and tongue contact pressure<sup>5,6</sup>). Morphological assessments include the assessment of the tongue shape of the sagittal plane using a cephalogram<sup>18</sup>), examination of the structure and three-dimensional shape of tongue using magnetic resonance imaging<sup>19</sup>), and observations of the sagittal and coronal planes using VF and ultrasonic testing<sup>10, 11, 20, 21</sup>).

Tongue thickness is one of morphological assessments of the tongue. Tamura et al.<sup>10</sup>) suggested that malnutrition might induce sarcopenia of the tongue as well as the skeletal muscle considering that tongue thickness was affected by the mid-arm muscle area and age. Moreover, Okayama et al.<sup>11</sup>) reported that tongue thickness of the elderly with care needs was associated to BMI and the period of care needed, and tongue thickness would help assess nutritional condition and oral functions. Furthermore, some reports have suggested that tongue thickness of amyotrophic lateral sclerosis (ALS) patients was associated to BMI and maximum tongue pressure, and that tongue thickness could help to evaluate swallowing function of ALS patients<sup>22, 23</sup>).

In this study, we investigated the relationship between tongue thickness with oral functions in 106 elderly participants, who were 65 years old, and examined the significance of tongue thickness in the assessment of oral functions. Statistical analysis revealed a bias in the data distribution of tongue thickness, and the explanatory variable which was

executed as a categorical variable divided into two groups: the thin and thick groups according to the median value.

Previous reports have suggested that a thick tongue showed high tongue pressure, and might be related to nutritional benefit<sup>10, 11</sup>). In this study, the values of /ka/ and cheek pressure were significantly different in the tongue thickness groups. Moreover, BMI, /ka/ and cheek pressure were extracted as the interactive variables in the univariate logistic regression analysis; and /ka/, cheek pressure and functional teeth number were extracted in the multivariate logistic regression analysis. The thicker the tongue was, the more both BMI and tongue pressure tended to be high, although the differences were not significant.

In this study, tongue thickness tended to be greater as the value of oral muscle strength was higher, but no significant difference was observed except for cheek pressure. Although both cheek pressure and functional teeth number were determined to be significant factors, their respective odds ratios were found to be 1.145 and 1.056, respectively, therefore they had only a weak association with tongue thickness. In contrast to previous studies of elderly people with care needs and of ALS patients<sup>11, 22, 23</sup>), the subjects in this study were mostly healthy elderly receiving outpatient dental care for maintenance who had relatively high tongue functions. Most subjects were also believed to have adequate nutrition intake. Therefore, the results in this study would be different from previous studies.

It is reported that fiber atrophy and fattening of the extrinsic and intrinsic tongue muscles were commonly found in healthy elderly of 65 years older<sup>24</sup>), and the positions of the larynx and hyoid bone after 60 years old were lower than those of young people<sup>25</sup>). Such age-related changes might cause a low tongue position in healthy elderly. Oral diadochokinesis of /ka/ is used to assess the tongue motor function by raising tongue dorsum to the soft palate<sup>26, 27</sup>). In case of the low tongue position, it is speculated that the movement distance of tongue for the utterance of /ka/ becomes long. The relationship between the tongue thickness and low tongue position is not clear, but a low tongue position could be a factor in a lower frequency of /ka/. Moreover, when tongue thickness increases, tongue volume and mass increase and then the movement speed will decrease. Therefore, a greater tongue thickness was associated with a lower frequency of /ka/.

In this study, we assessed tongue function by using tongue thickness. However, tongue function is so

influenced by tongue position, volume and mass. The present study suggested that tongue thickness did not necessarily reflect the movement function of the tongue or other oral functions. Therefore, tongue function should not be assessed solely by tongue thickness but to be done by using multiple examinations.

The tongue is mainly muscle tissue. Every muscle of the whole body during everyday activities usually works not with maximum power, but with plenty of power. It is known as reserved capacity, which is important for the maintenance of bodily functions for health and longevity, but that of elderly people is lower than that of younger people<sup>28, 29</sup>. It is reported that the elderly has a high risk for dysphagia because the reserve capacity of swallowing also declines with aging<sup>30</sup>. It may be therefore possible that the reserve capacity declined even if the subjects had no problem on mastication and swallowing in the present study. The function of tongue dorsum which is assessed by the oral diadochokinesis of /ka/, is mainly to retain the bolus in the oral cavity and to push it into the pharynx at the time of mastication to swallowing. The decline might result in the laryngeal penetration of the bolus during swallowing and decline of swallowing pressure which finally causes pulmonary aspiration. Considering the report that tongue thickness decreases in elderly people with care needs<sup>11</sup>, the continuous measurements of tongue thickness might have a different clinical significance as assessments of oral functions of the elderly.

It is possible to identify associated factors, but very difficult to determine the cause and effect association, while this study is based on a cross-sectional design. Therefore, future longitudinal and intervention studies are necessary to clarify those associations.

### Conclusion

This study showed that tongue thickness does not necessarily reflect oral functions in healthy elderly people except for a negative association between tongue thickness and oral diadochokinesis of /ka/. It is speculated that multiple assessments of tongue would be required to evaluate oral function and the assessment of tongue thickness might have a different clinical meaning.

### Acknowledgement

We gratefully acknowledge the work of past and present members of our laboratory. The authors also thank Dr. Omar Marianito Maningo Rodis (Tokushima University) for proofreading this

manuscript. Special thanks also go to the hospital staffs that provided their help and sincere encouragement.

### References

- 1) Sasaki M, Arakawa T, Nakayama A and Yamaguchi M: Method of tongue movement estimation based on suprahyoid muscle coordination. *Trans Jpn Soc Med Biol Eng.*50 (1) , 31–37(2012) (in Japanese)
- 2) Matsuo K and Palmer JB: Kinematic linkage of the tongue, jaw, and hyoid during eating and speech. *Arch Oral Biol.* 55(4), 325-331(2010)
- 3) Okada A, Honma M, Nomura S and Yamada Y: Oral behavior from food intake until terminal swallow *Physiol Behav.* 90, 172-179(2007)
- 4) Steffi Galén, Paul-G and Jost-Brinkmann: B-mode and M-mode Ultrasonography of Tongue Movements during Swallowing. *Journal of Orofacial Orthopedics* 71(2), 125-135(2010)
- 5) Ono T, Hori K and Nokubi T: Pattern of tongue pressure on hard palate during swallowing. *Dysphagia.* 19(4), 259-264(2004)
- 6) Hori K, Ono T, Tamine K, Kondo J, Hamanaka S, Maeda Y, Dong J and Hatsuda M: Newly developed sensor sheet for measuring tongue pressure during swallowing. *J Prosthodont Res* 53(1), 28-32(2009)
- 7) Hayashi R, Tsuga K, Hosokawa R, Yoshida M, Sato Y and Akagawa Y: A novel handy probe for tongue pressure measurement. *Int J Prosthodont.* 15(4), 385-388(2002)
- 8) Utanohara Y, Hayashi R, Yoshikawa M, Yoshida M, Tsuga K and Akagawa Y: Standard values of maximum tongue pressure taken using newly developed disposable tongue pressure measurement device. *Dysphagia.* 23(3), 286-290(2008)
- 9) Yamada A, Kanazawa M, Komagamine Y and Minakuchi S: Association between tongue and lip functions and masticatory performance in young dentate adults. *J Oral Rehabil.* 42, 833-839(2015)
- 10) Tamura F, Kikutani T, Tohara T, Yoshida M and Yaegaki K: Tongue Thickness Relates to Nutritional Status in the Elderly. *Dysphagia.* 27(4), 556–561(2012)
- 11) Okayama H, Tamura F, Tohara T and Kikutani T: A Study on the Tongue Thickness of the

- Elderly with Care Needs. *Journal of Japanese society for disability and oral health* 31, 723-729(2010)
- 12) Fujimoto K, Honda T, Suito H, Hongama S, Nagao K and Ichikawa T: Do assessments of oral function predict satisfaction with mastication in the elderly? (in submitted to Plos One)
  - 13) Tsuga K, Maruyama M, Yoshikawa M, Yoshida M and Akagawa Y: Manometric evaluation of oral function with a hand-held balloon probe. *J Oral Rehabil.* 38, 680-685(2011)
  - 14) Yoshida Y, Hatanaka Y, Ogawa Y, Imaki M, Takabatake S and Shinsyo F: Cross-Sectional Study of Chewing Activity and Competence of Daily Living in Elderly Residents. *Seikatsu Eisei* 46, 13-21 (2002)
  - 15) Hiura H and Arai Y: Self-Related Health and Chewing Activity in Japanese Elderly Residents. *Japanese Journal of Gerodontology* 12, 50-54 (1997)
  - 16) Morisaki N, Miura H, Moriya S and Hara S: Relationship between the swallowing function and the health-related QOL among community-dwelling dependent elderly persons. *Japanese journal of geriatrics* 51(3), 259-263(2014) (in Japanese)
  - 17) Koshino H, Hirai T, Ishijima T and Ikeda Y: Tongue motor skills and masticatory performance in adult dentates, elderly dentates, and complete denture wearers. *J Prosthet Dent.* 77, 147-152(1997)
  - 18) Ralph L. Shelton and James F. Bosma: Maintenance of the pharyngeal airway. *J. Appl. Physiol.* 17(2), 209-214(1962)
  - 19) Fujita S, Dang Jianwu, Suzuki N and Honda K: A Computational Tongue Model and its Clinical Application. *Oral Science International.* 4(2), 97-109(2007)
  - 20) Ren YF, Isberg A, Henningsson G and Larson O: Tongue posture in cleft palate patients with a pharyngeal flap. *Scand J Plast Reconstr Surg Hand Surg.* 26(3), 307-312(1992)
  - 21) Taniguchi H, Matsuo K, Okazaki H, Yoda M, Inokuchi H, Gonzalez-Fernandez M, Inoue M and Palmer JB: Fluoroscopic evaluation of tongue and jaw movements during mastication in healthy humans. *Dysphagia.* 28(3), 419-27(2013)
  - 22) Nakamori M, Hosomi N, Takaki S, Oda M, Hiraoka A, Yoshikawa M, Matsushima H, Ochi K, Tsuga K, Maruyama H, Izumi Y and Matsumoto M: Tongue thickness evaluation using ultrasonography can predict swallowing function in amyotrophic lateral sclerosis patients. 127(2), 1669-1674(2016)
  - 23) Umemoto G, Furuya H, Arahata H, Sugahara M, Sakai M and Tsuboi Y: Relationship between tongue thickness and tongue pressure in neuromuscular disorders. 4(4), 142-145(2016)
  - 24) Susan G. Butler, Catherine Rees Lintzenich, Xiaoyan Leng, Andrew Stuart, Xin Feng, MD, J. Jeffrey Carr and Stephen B. Kritchevsky: Tongue Adiposity and Strength in Healthy Older Adults. *Laryngoscope.* 122(7), 1600-1604(2012)
  - 25) Kaneko I: A cinefluorographic study of hyoid bone movement during deglutition. *Nihon Jibiinkoka Gakkai Kaiho.* 95(7), 974-987(1992) (in Japanese)
  - 26) Ito K, Yoshihara A, Takano N, Ishigami K, Seida Y, Inoue M, Kitahara M and Miyazaki H: A comparison of methods for the measurement of oral diadochokinesis. *Jpn J Gerodontology.* 24(1), 48-54(2009) (in Japanese)
  - 27) Ziegler W: Task-related factors in oral motor control: speech and oral diadochokinesis in dysarthria and apraxia of speech. *Brain Lang.* 80, 556-575(2002)
  - 28) Covinsky KE, Palmer RM, Fortinsky RH, Counsell SR, Stewart AL, Kresevic D, Burant CJ and Landefeld CS: Loss of independence in activities of daily living in older adults hospitalized with medical illnesses: increased vulnerability with age. *J Am Geriatr Soc.* 51(4), 451-458(2003)
  - 29) Gill TM, Allore HG, Gahbauer EA and Murphy TE: Change in disability after hospitalization or restricted activity in older persons. *JAMA.* 304(17):1919-1928(2010)
  - 30) Nicosia MA, Hind JA, Roecker EB, Carnes M, Doyle J, Dengel GA and Robbins J: Age effects on the temporal evolution of isometric and swallowing pressure. *J Gerontol A Biol Sci Med Sci.* 55(11), 634-640(2000)