

# Changes in surface tension of saliva in Down syndrome

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**Abstract. – OBJECTIVE:** Surface tension in saliva might contribute to the maintenance of upper airway patency. The present study aimed to determine whether salivary surface tension is altered in patients with Down syndrome who are predisposed to upper airway collapse.

**PATIENTS AND METHODS:** We used the pull-off force technique to measure surface tension in samples (100  $\mu$ L) of saliva collected from twenty-three male patients with Down syndrome and twenty-three healthy males (controls).  $p < 0.05$  was considered to indicate significance.

**RESULTS:** Salivary surface tension was significantly lower in the patients than in the controls ( $57.3 \pm 4.9$  vs.  $60.3 \pm 4.7$  mN/m;  $p = 0.039$ ). Age and surface tension positively correlated in the patients ( $p = 0.001$ ).

**CONCLUSIONS:** The lower surface tension of saliva in patients with Down syndrome might compensate for an anatomical predisposition towards upper airway collapsibility and other risk factors. The function of surface tension in saliva might be altered due to aging in such patients.

## Key Words:

Surface tension, Down syndrome, Upper airway patency.

ing disorder<sup>10-12</sup>, which is associated with upper airway obstruction, daytime hyper-somnolence<sup>13</sup>, and cardiovascular diseases.

The surface tension forces of human saliva are similar to those of the fluid that lines the upper airway<sup>14,15</sup> and helps to maintain upper airway patency<sup>16-18</sup>. We recently found that decreased salivary surface tension associated with increasing salivary phosphatidylcholine concentrations in patients with obstructive sleep apnea hypopnea might correlate with a decrease in the hypopnea index but not with total event rates (apnea hypopnea index) or the apnea index<sup>19</sup>.

Although craniofacial malformations are regarded as a primary cause of upper airway obstruction in patients with Down syndrome, whether salivary function, including surface tension, could contribute to the pathogenesis of upper airway obstruction in these patients remains unclear. Individuals with Down syndrome breathe through the mouth and have a reduced rate of saliva secretion. While upper airway patency is influenced by many factors, significantly altered surface tension forces in saliva are associated with reduced secretory rates<sup>14,20</sup> and breathing routes<sup>21</sup>. We investigated whether salivary surface tension differs between patients with Down syndrome and controls.

## Introduction

Down syndrome is the most common genetic cause of developmental disability and mental retardation, with an incidence of 1 per 660 live births<sup>1</sup>. Down syndrome is characterized by midfacial and mandibular hypoplasia<sup>2-4</sup>, enlarged tongue<sup>5</sup>, adenoid and tonsillar hypertrophy, and obesity. Salivary characteristics, including pH, buffer capacity<sup>6</sup>, and salivary flow rate<sup>7-9</sup> might also be associated with Down syndrome. Furthermore, 30-60% of individuals with Down syndrome have some degree of sleep-related breath-

## Patients and Methods

### Participants

Twenty-three men with Down syndrome (age,  $31.2 \pm 12.7$  y; body mass index [BMI],  $22.4 \pm 4.1$  kg/m<sup>2</sup>) and 23 age-matched healthy men without sleep apnea (age,  $35.5 \pm 12.7$  y; BMI,  $23.0 \pm 4.8$  kg/m<sup>2</sup>) were recruited for the present study. The patients either received routine dental care or resided in facilities neighboring the Department of Special Care Dentistry at Nagasaki Univer-

sity Hospital, Nagasaki Oral Health Center. A detailed clinical history was obtained to exclude individuals with severe congenital heart disease, currently under medication, insulin management, or currently or had previously smoked. The Human Investigation Committee at Nagasaki University Graduate School of Biomedical Science approved the experimental protocol and written informed consent was obtained from all healthy individuals and the parents or legal guardians of the patients.

## Measurements

### **Surface Tension Measurement and Instrument Calibration**

The surface tension of saliva in samples (100  $\mu\text{L}$ ) was determined using the pull-off force technique as described<sup>14,19</sup>. We used a setup based on a surface force apparatus with two cylindrical ( $R$  D 2 cm) polished silica discs mounted in a crossed-cylinder configuration. The lower disc was mounted at the end of a double-cantilevered spring with a spring constant ( $k$ ) of  $\sim 3 \times 10^4 \text{ Nm}^{-1}$  and connected to a shaft. The upper disc was mounted on a stainless steel holder, and the surface separation was changed with a motor-driven micrometer connected to the shaft with the attached spring. With the surfaces a few millimeters apart, a droplet ( $\sim 0.2 \mu\text{L}$ ) was placed in the center of the lower disc, which was then brought up until the droplet also contacted the upper disc, forming an annulus at the center. The micrometer was then slowly reversed until the discs jumped apart and the droplet snapped. The distance of the jump was determined by reading the micrometer. The setup was calibrated with water (from a Milli-Q unit), ethylene glycol (Sigma-Aldrich Corp., St. Louis, MO, USA), and oleic acid  $\geq 99\%$  (GC) (Sigma-Aldrich Corp., St. Louis, MO, USA). A calibration curve for the surface tension instrument was created by plotting the known values of  $\gamma$  for a series of standards (oleic acid, ethylene glycol, and water: 32.5, 47.7, and 71.2 mN/m, respectively) vs. the measured jump distance. The primary output of the surface tension measurement device is the gap created between the two silica surfaces (jump distance), and it is proportional to the force required to separate the two surfaces contacting by a given liquid. Salivary surface tension was then calculated by determining jump distances for all samples.

### **Protocol**

The participants were instructed not to consume any food or fluids for one hour before saliva collection on the day of the study. Two salivary samples ( $\sim 100 \mu\text{L}$  each) were collected from beneath the tongues of seated participants using 20  $\mu\text{L}$  continuously adjustable air displacement pipettes (Pipetman<sup>®</sup>; Gilson Inc., Madison, WI, USA) before the overnight sleep study. All samples were stored frozen ( $-20^\circ\text{C}$ ) for up to three weeks, before being shipped on dry ice to a laboratory that measured surface tension (Teijin Pharma Limited, Tokyo, Japan) within one month of collection. The device was calibrated with liquids of known surface tension and compared with control reference samples that were sent with each batch to ensure sample integrity during storage and transportation. The samples were defrosted at room temperature for 30 minutes and then salivary protein was removed by centrifugation for five minutes at  $15,000 \times g$  and  $20^\circ\text{C}$ . Surface tension was then measured in supernatants (10  $\mu\text{L}$ ).

## Analysis

### **Salivary Surface Tension**

Surface tension in saliva was determined from calibration curves based on reference jump-distance values obtained from standard solutions.

### **Statistical Analysis**

All data were statistically analyzed using Prism version 5.0 (GraphPad Software Inc., La Jolla, CA, USA). Between-group values were assessed using unpaired  $t$ -tests. Pearson's correlation coefficient analysis and simple regression were used to assess the relations between the surface tension of saliva and age. Statistical significance was assumed for values with  $p < 0.05$ . Data are presented as means  $\pm$  standard deviation (SD) unless otherwise noted.

### **The Ethical Approval**

The experimental protocol was approved by the Human Investigation Committee of the Nagasaki University Graduate School of Biomedical Sciences (0844-6), and written informed consent was obtained from all patients.

## Results

### Salivary Surface Tension

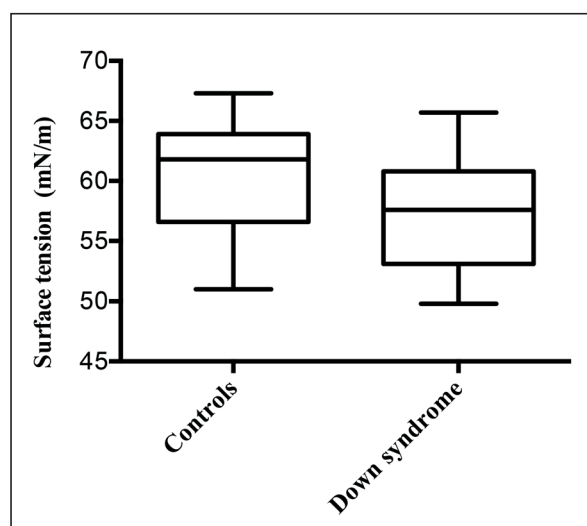
Salivary surface tension was significantly lower in the patients than in the controls ( $57.3 \pm 4.9$  vs.  $60.3 \pm 4.7$  mN/m,  $p = 0.039$ ; Figure 1). Age significantly correlated with surface tension in the patients ( $r^2 = 0.40$ ,  $p = 0.0011$ ; Figure 2), but not in the controls.

## Discussion

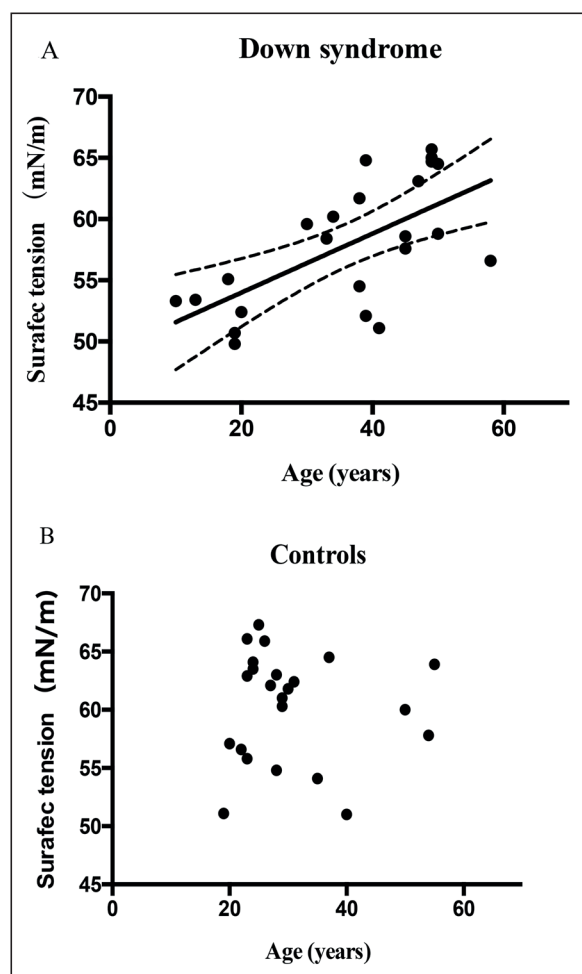
This is the first study to show that salivary surface tension is significantly lower in male patients with Down syndrome than in healthy, age- and sex-matched controls. Furthermore, surface tension might increase with age.

### Factors Contributing to Physiological Role of Surface Tension ( $\Gamma$ ) Forces of Saliva

The salivary surface tension from our controls ( $60.3 \pm 4.7$  mN/m) was consistent with values determined during sleep ( $61.9 \pm 4.1$  mN/m)<sup>22</sup> and during anesthesia ( $60.9 \pm 4.1$  mN/m)<sup>23</sup>. Greater surface tension of the fluid lining the upper airway can predispose the airway to collapse, but applying a surfactant significantly reduces this propensity<sup>22,24</sup>. The surface tension of the



**Figure 1.** Salivary surface tension in twenty-three patients with Down syndrome and twenty-three controls. Values were significantly lower in patients than controls ( $57.3 \pm 4.9$  vs.  $60.3 \pm 4.7$  mN/m,  $p = 0.039$ ). Values are shown as means  $\pm$  SD.



**Figure 2.** Correlations between surface tension and age in twenty-three controls and twenty-three patients with Down syndrome. Age and salivary surface tension significantly correlate ( $p = 0.001$ ) in patients (A), but not in controls (B).

upper airway fluid is similar to that of saliva in non-apneic control persons<sup>14,15</sup>. This is consistent with the notion that swallowing moves saliva to the back of the throat and coats the upper airway such that saliva is the primary constituent of the fluid lining the upper airway<sup>14</sup>. We recently found that increased phosphatidylcholine concentrations in saliva reduce surface tension and improve airway patency in patients with obstructive sleep apnea<sup>19</sup>. Furthermore, another study of the surface tension of airway aspirates withdrawn during neonatal resuscitation<sup>25</sup> has indicated that surface tension correlates significantly with phosphatidylcholine concentrations, which in turn correlate with gestational age. The main physiological role of phosphatidylcholine is

lipid metabolism. Based on current knowledge, we speculate that the reduced surface tension in patients with Down syndrome is associated with altered phosphatidylcholine concentrations. If so, reduced surface tension might be a compensatory mechanism involved in opening a collapsed upper airway during sleep. Bohm et al<sup>26</sup> described lower phosphatidylcholine values in the extracellular milieu of obese patients. These authors speculated that phosphatidylcholine is reduced due to increased endogenous phosphatidylcholine synthesis and/or reduced phosphatidylcholine release in adipocytes. Taken together, we suggest that compensatory mechanisms such as changes in salivary surface tension and the upper airway mucosa cannot be further activated due to low or absent phosphatidylcholine release at the extracellular level. That is, when patients have other risk factors such as obesity and diabetes, surface tension might increase because of reduced phosphatidylcholine levels. Changes in lipid metabolism and/or surfactant protein might regulate the physiological function of surface tension in patients with Down syndrome. The association between sleep-disordered breathing and saliva in patients with Down syndrome who has increased risk factors for reduced upper airway patency requires further investigation.

### **Features of Surface Tension in Down Syndrome**

We found significantly lower salivary surface tension in patients with Down syndrome compared with controls. The lower surface tension might compensate for increased upper airway collapsibility induced by predisposing anatomical features. Lower surface tension means partially depressed functional ability, probably due to specific features of Down syndrome such as breathing through the mouth. We also found that surface tension increases with age. We speculate that if a compensatory mechanism is established to maintain upper airway patency in younger patients with Down syndrome, it might be depressed in elderly patients. Breathing through the mouth and dry mouth are common features of patients with Down syndrome and breathing through the mouth is closely associated with oral mucosal wetness and salivary surface tension forces according to Verma et al<sup>21</sup>. They indicated that breathing through the mouth increases the surface tension of the fluid lining the upper airway in healthy individuals while awake. Therefore, breathing through the mouth might

interfere with physiological compensatory mechanisms associated with surface tension in Down syndrome. In addition to breathing routes, Yarat et al<sup>27</sup> demonstrated significantly lower salivary flow rates in patients with Down syndrome compared with healthy controls (0.11 vs. 0.67 mL/min; respectively). Sato et al<sup>28</sup> described that alleviating oral mucosal dryness in patients with Down syndrome relieves sleep apnea symptoms. The numbers of salivary reservoirs in the oral cavity available for wetting the mucosal surfaces and maintaining the surface tension of the fluid lining the airway might be reduced in Down syndrome due to both breathing through the mouth and lower salivary rates.

### **Methodological Limitations**

Several limitations are associated with the present study. The sample size was relatively small because of challenges associated with collecting saliva samples from the patients. Only male patients with Down syndrome were included to avoid hormonal influences on upper airway dilator muscle activity<sup>29-31</sup>. Samples of saliva were stored frozen and sent in batches to a remote location for measurement. Although we did not study the effect of storage duration on surface tension, our preliminary findings (data not shown) suggested that variability related to this is small and within repeated measurement error. However, all samples from the patients and controls were similarly processed. Therefore, the likelihood of error due to sample storage causing bias between the groups is low. Furthermore, we believe that our findings are reliable because our control values for salivary surface tension were consistent with reported values.

### **Clinical Implications**

The prevalence of sleep-disordered breathing associated with the upper airways of children with Down syndrome is 30-50%<sup>10-12</sup> and tends to increase with age. In contrast, the prevalence in otherwise healthy children is only 2%. Trois et al<sup>32</sup> recently suggested that persons with Down syndrome aged > 40 years had periodic breathing for 24% of the night and a median life expectancy of 49 years. Such early mortality might be associated with untreated obstructive sleep apnea symptoms, such as obstructive apnea, nocturnal hypoxemia, hypoventilation, and sleep fragmentation. Although the impact of sleep-disordered breathing in patients with Down syndrome is likely to be high, continuous positive airway

pressure and oral appliances might not be the most effective treatment modalities for such patients. Alternative therapeutic strategies to treat obstructive sleep apnea, such as lowering surface tension and improving oral mucosal fluid properties using pharmacological agents<sup>15,17,33,34</sup>, might help those unable to comply with traditional means of treatment. As such, salivary surface tension might be an important factor in controlling upper airway patency and might be involved in a compensatory pathway against oral dryness in persons with Down syndrome. Furthermore, salivary surface tension might be a predictive alternative screen for sleep disorders breathing. The role of salivary surface tension in sleep disorder breathing should be investigated.

### Conclusions

The salivary surface tension of patients with Down syndrome is lower than that of healthy controls and might serve as a compensatory response to an anatomical predisposition towards upper airway collapsibility. However, our data also indicate that such a compensatory mechanism might be depressed with age in male patients with Down syndrome. Further investigations should investigate the effects of aging on surface tension on salivary function in a larger patient cohort and in female patients.

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### Conflict of Interest

The Authors declare that they have no conflict of interests.

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