Original Article

# Reference equations of the impulse oscillatory in healthy Thai adults

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**Background:** The ethnicity is significantly under-reported and this may limit the applicability of current impulse oscillometry (IOS) equations to heterogeneous patient populations. Establishing predictive equations for the IOS in the Thai adult population is still required. Therefore, this study aimed to establish reference equations for the IOS in the Thai adult population.

**Methods:** This retrospective cross-sectional study of IOS parameters in healthy adults aged greater than 20 years old with normal spirometry and who had no chronic respiratory diseases. Pre-bronchodilator (BD) IOS was performed in all subjects. Reference equations were calculated separately for men and women using multivariable linear regression analysis.

**Results:** A total of 127 subjects (87 men and 40 women) with a mean age of 48.7±17.2 (range, 22–92) years were included. The resistance at 5 Hz (R5), resistance at 20 Hz (R20), and area under reactance curve between 5 Hz and resonant frequency (AX) were significantly higher in women compared to men. The reference equations of the IOS parameters were established for men and women. Age, height, and bodyweight were shown to be the influential predictor as they contributed to the most of IOS indices except for the R5–R20 in men equations. Bodyweight was shown to be the influential predictor as it contributed to the most IOS indices except for the X5 in women's equations.

**Conclusions:** We provided the reference equations for the IOS indices in Thai adults. IOS indices including R5, R20, and AX were significantly higher in women compared to men.

**Keywords:** Impulse oscillometry (IOS); predictive value; reference equation; Thai; adult

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## Introduction

Impulse oscillometry (IOS) is a simple, non-invasive method that requires only tidal breathing for measuring both airway resistance and airway reactance (1,2). This technique has been developed by Michaelson *et al.* (3) since 1975. Previous study suggested that it is more sensitive than spirometry for detecting small airway dysfunction (4). Nowadays, IOS has been used for assessing various

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respiratory diseases especially for the diagnosis of chronic obstructive pulmonary disease (COPD) (5-11) and assessment of asthma control (12-16). The most common IOS parameters reported are resistance at 5 Hz (R5), resistance at 20 Hz (R20), difference of resistance (R5–R20), resonant frequency (Fres), reactance at 5 Hz (X5), and area under reactance curve between 5 Hz and resonant frequency (AX) (2,17).

Following other lung function tests including spirometry, diffusing lung capacity for carbon monoxide (DLCO), and total lung capacity (TLC), choosing optimal reference values is crucial for the interpretation of IOS results. Previous studies have demonstrated that IOS indices are associated with sex, age, height, and bodyweight (18-22). Previous study also suggested that there are differences in lung volumes between ethnic groups (23). The IOS prediction equations should be separately provided for men and women (24). Moreover, the predictive values of IOS can be different depending on the types of device that is used (25). This may have an impact on the predictive values and normal ranges of IOS parameters. The lack of appropriate reference equations and normal ranges of IOS have hindered the application of IOS in clinical practice. The previous study suggested that ethnicity is significantly under-reported and this may limit the applicability of current equations to heterogeneous patient populations (24). Establishing predictive equations for the IOS in the Thai adult population is still required. Therefore, this study aimed to establish reference equations for the IOS in the Thai adult population. We present the following article in accordance with the STROBE reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-21-1989/rc).

## Methods

### *Study procedures*

This retrospective cross-sectional study of IOS parameters in healthy Thai adults aged greater than 20 years old in the previous studies were included (11,16). The data of healthy controls in the Coronavirus Disease 2019 (COVID-19) study and wildland firefighter study were also included. All subjects were recruited from the same area in Chiang Mai, Thailand. This study was conducted at the Lung health center, Division of Pulmonary, Critical Care, and Allergy, Department of Internal Medicine, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Research Ethics Committee, Faculty of Medicine, Chiang Mai University (study code: MED-2564-08533, date of approval: 12 November 2021) and filed under Clinical Trials Registry (study ID: TCTR20211014002, date of approval: 14 October 2021). Individual consent for this retrospective analysis was waived.

### *Subjects*

One hundred and fifty-four subjects were screened for eligibility for enrolment in the study. Subjects unable to perform spirometry and IOS were excluded. IOS or spirometry not met standard recommended by King *et al.* (26) according to the European Respiratory Society (ERS) standard and ERS/American Thoracic Society (ATS) 2019 standard (27), respectively were also excluded. Subjects had a history of cigarette smoking greater than 5 pack-years, had a history of asthma, other chronic lung disease or current acute respiratory tract infection, and obesity [body mass index (BMI) >30 kg/m$^2$] were also excluded. The healthy control subjects were subjects with normal spirometry [forced vital capacity (FVC), forced expiratory volume in the first second ($FEV_1$), and the ratio of $FEV_1$/FVC greater than the statistically defined fifth percentile of normal (lower limit of normal; LLN)] (27), and who had no known other chronic systemic diseases (e.g., cardiovascular diseases, kidney diseases, liver diseases, neurological diseases, musculoskeletal diseases, autoimmune diseases, and malignancy). Baseline characteristics including age, height, bodyweight, BMI, underlying disease, and history of smoking were recorded. Pulmonary function test data including IOS and spirometry results were also recorded.

### *IOS*

Pre-bronchodilator (BD) IOS was performed in all subjects before spirometry for ensuring that there was no effect of forced expiration on the respiratory tract smooth muscle (28). The IOS was measured using IOS (Master Screen IOS, Viasys GmbH, Hoechberg, Germany). The parameters in IOS that represent the resistance of the airways include the R5, R20, and R5–R20 values. The parameters in IOS that represent the reactance of the airways include the X5, Fres, and AX. All subjects were asked to perform tidal breathing for 30–45 seconds via a mouthpiece that was connected to a loudspeaker which

 

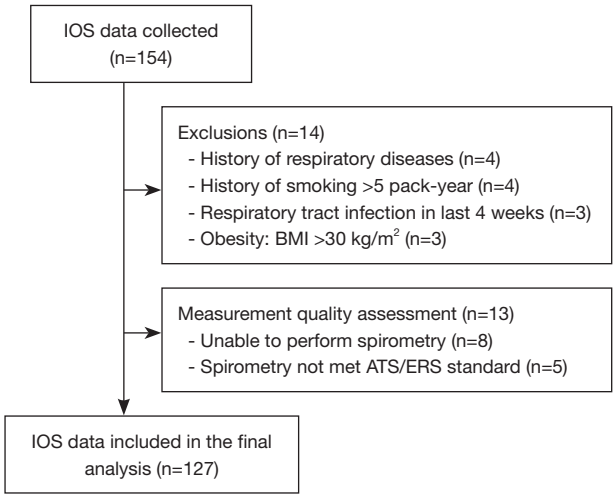


**Figure 1** Study flow chart. IOS, impulse oscillometry; BMI, body mass index; ATS, American Thoracic Society; ERS, European Respiratory Society.

generates pressure oscillations composed of multiple frequencies. Subjects were asked to sit on a chair in an upright position, wore a nose clip, and were directed to firmly support their cheeks with both hands. A minimum of three trials was performed following the standard recommended by ERS standard (26). The average values from three IOS measurements were recorded. The coherence of each measurement was ≥0.8 at 5 Hz and ≥0.9 at 20 Hz. Unacceptable data were excluded from the analysis.

### *Spirometry*

Spirometry was assessed in all subjects using a spirometer (Vmax Encore 22, Care Fusion, Hoechberg, Germany). Pre-BD spirometry was performed according to the standards of ATS/ERS 2019 (27). Spirometry parameters including FVC, $FEV_1$, ratio of $FEV_1$/FVC, and forced expiratory flow at 25–75% of FVC ($FEF_{25-75\%}$) were recorded. The predicted values and z-score of FVC, $FEV_1$, ratio of $FEV_1$/FVC, and $FEF_{25-75\%}$ were calculated from the Global Lung Initiative (GLI) 2012 (Southeast Asian sub-group) reference equations (29).

### *Study size calculation*

The study size of the study was calculated based on data from the previous study (19) using G*Power Version 3.1.9.2 which included two factors and $R^2$ of R5–R20 was 0.2249. Therefore, at least 74 subjects (37 for each gender) needed to be included in this study (power =0.8 with statistical significance <0.05).

### *Statistical analysis*

Results for continuous data were expressed as mean ± standard deviation (SD) or median, interquartile range (IQR) as appropriate. Results for categorical data were expressed as frequencies and percentages. Independent sample *t*-tests and the Mann-Whitney U test were used to compare differences between the sex groups for parametric and non-parametric data, respectively. Fisher's exact test was used to compare the categorical data between groups. Reference equations were calculated separately for men and women using multivariable linear regression analysis. Scatter plots were drawn to observe the linear relationship between IOS indices and predictor variables [Figure S1 (male), Figure S2 (female)]. Normal P-P plots and residual plots were drawn to examine the normality and equal variance of the residuals. As R5–R20 and AX were non-normal distribution, thus R5–R20 and AX were calculated as log10 transformation (logR5–R20 and logAX) in the equations. Predictor variables including age, height, and bodyweight were selected using the stepwise method, in which predictors would enter the model if the P value <0.10. The fitness of the model was assessed by the coefficient of determination ($R^2$). The upper limit of normal (ULN) and LLN of IOS parameters was calculated as followed = predictive value + 1.645 × root mean square error (RMSE) and predictive value – 1.645 × RMSE, respectively. All statistical analyses were performed using STATA version 16 (StataCorp, College Station, TX, USA).

## Results

A total of 154 subjects were recruited. However, fourteen subjects were excluded due to various reasons including having a history of chronic respiratory diseases (n=4), having a history of smoking ≥5 pack-years (n=4), having respiratory tract infection in the last four weeks (n=3), and obesity (n=3). Eight subjects were unable to perform spirometry. Therefore, they were excluded. After the measurement of the quality of spirometry and IOS, five subjects were excluded due to the spirometry not meeting ATS/ERS 2019 standard. More data are shown in *Figure 1*. The distribution of age, height, and bodyweight according to sex is shown in *Figure 2*.

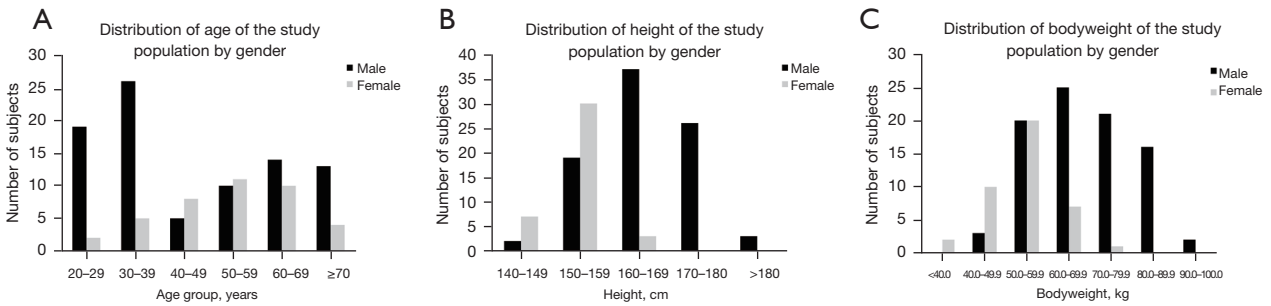


**Figure 2** Distribution of characteristics including age, height, and bodyweight according to the sex of the 127 subjects. (A) Age. (B) Height. (C) Bodyweight.

**Table 1** Baseline characteristics of study population (n=127)

| Clinical characteristics | Men (n=87) | Women (n=40) |
|---|---|---|
| Age, years (range) | 46.7±18.2 (22–92) | 52.6±12.9 (25–74) |
| Height, cm (range) | 165.6±7.6 (148–184) | 152.9±4.1 (145–162) |
| Bodyweight, kg (range) | 68.4±11.7 (43.0–99.0) | 53.5±7.5 (39.0–70.0) |
| BMI, kg/m$^2$ (range) | 24.8±2.9 (19.1–30.0) | 22.9±2.9 (17.1–29.5) |
| Smoking status | | |
| Non-smoker | 63 (72.4) | 40 (100.0) |
| Current-smoker | 4 (4.6) | 0 (0.0) |
| Ex-smoker | 20 (23.0) | 0 (0.0) |
| Smoking pack-year | 1.3±1.8 | 0.0±0.0 |

Data are mean ± SD unless otherwise stated. BMI, body mass index; SD, standard deviation.

The baseline characteristics of the study population are presented in the *Table 1*. The mean age of men and women was 46.7±18.2 and 52.6±12.9 years, respectively. All of the females were non-smokers and 72.4% of male subjects were non-smokers. More data are shown in *Table 1*.

The spirometric and IOS data of subjects are shown in *Table 2*. There was no significant difference between the percent predicted and z-score of FVC, $FEV_1$, the ratio of $FEV_1$/FVC, and $FEF_{25–75\%}$ between men and women. The R5, R20, and AX were significantly higher in women compared to men. The R5–R20 tends to be higher in women compared to men but not a significant difference. The X5 was more negative in women compared to men but not a significant difference. However, there was no significant difference between the Fres between men and women. More data are shown in *Table 2*.

The spirometric and IOS data of smokers and non-smokers in male subjects are shown in *Table 3*. The percent predicted and z-score of FVC, $FEV_1$, the ratio of $FEV_1$/FVC, and $FEF_{25–75\%}$ between smokers and non-smokers were not significantly different. All IOS indices were also comparable between smokers and non-smokers. More data are shown in *Table 3*.

The reference equations for the IOS parameters for men and women are presented in *Table 4*. Age, height, and bodyweight were shown to be the influential predictor as they contributed to the most of IOS indices except for the R5–R20 in men equations. Bodyweight was shown to be the influential predictor as it contributed to the most of IOS indices except for the X5 in women's equations. Age was shown to be the influential predictor for IOS indices in men but not for women except for the Fres equation. More data are shown in *Table 4*.

## Discussion

Our study provided the reference equations for the IOS parameters in Thai healthy adults for both men and women. IOS indices including R5, R20, and AX were significantly higher in women compared to men. Compared to the previously published IOS predictive values in the Chinese and Australian populations that used the same IOS device (19,20), we found that the R5 and R20 in Thai adults were higher. We also found that the X5 was more negative in Thai adults compared to the previous findings (19,20).

Sex-specific IOS reference equations of the commonly used IOS indices are created for Thai adults. Our results showed that age, height, and bodyweight were shown to be the influential predictors as they contributed to the most of IOS indices in men equations which were supported by the previous studies indicating that anthropometric

**Table 2** Spirometric and IOS data of subjects (n=127)

| Parameters | Men (n=87) | Women (n=40) | P value |
|---|---|---|---|
| Spirometry | | | |
| % predicted FVC | 100.0±14.1 | 102.6±10.1 | 0.299 |
| z-score of FVC | 0.107 (–0.595, 0.805) | 0.148 (–0.251, 0.639) | 0.575 |
| % predicted $FEV_1$ | 99.0±12.8 | 101.7±12.4 | 0.264 |
| z-score of $FEV_1$ | 0.081 (–0.716, 0.616) | 0.142 (–0.517, 0.634) | 0.400 |
| $FEV_1$/FVC, % | 82.3±5.8 | 82.9±5.0 | 0.589 |
| z-score of $FEV_1$/FVC | –0.208 (–0.684, 0.370) | –0.094 (–0.553, 0.372) | 0.684 |
| % predicted $FEF_{25–75\%}$ | 103.0±24.1 | 108.9±32.7 | 0.264 |
| z-score of $FEF_{25–75\%}$ | 0.065 (–0.609, 0.568) | 0.335 (–0.827, 0.842) | 0.447 |
| IOS | | | |
| R5, $cmH_2O$/L/s | 3.29±0.99 | 4.17±1.11 | <0.001 |
| R20, $cmH_2O$/L/s | 2.79±0.79 | 3.57±0.87 | <0.001 |
| R5–R20, $cmH_2O$/L/s, median (IQR) | 0.38 (0.20, 0.71) | 0.52 (0.33, 0.78) | 0.108 |
| X5, $cmH_2O$/L/s | –0.90±0.44 | –1.08±0.57 | 0.061 |
| Fres, Hz | 12.38±3.71 | 12.37±3.82 | 0.997 |
| AX, $cmH_2O$/L, median (IQR) | 2.15 (1.30, 4.08) | 3.98 (2.03, 5.41) | 0.024 |

Data are mean ± SD unless otherwise stated. IOS, impulse oscillometry; FVC, forced vital capacity; $FEV_1$, forced expiratory volume in the first second; $FEF_{25–75\%}$, forced expiratory flow at 25–75% of FVC; R5, resistance at 5 Hz; R20, resistance at 20 Hz; R5–R20, heterogeneity of resistance between R5 and R20; IQR, interquartile range; X5, reactance at 5 Hz; Fres, resonant frequency; AX, the area under reactance curve between 5 Hz and resonant frequency; SD, standard deviation.

parameters including height and weight are influential predictors for contributing to the IOS reference equation in men (21,22). For women, our study showed that height and bodyweight were shown to be the influential predictors for the contribution of the IOS predictive value which was supported by the previous studies indicating that height and weight were predictors that affect the IOS reference equation in women (21,22,30). We found a negative correlation between height and airway resistance indices including R5 and R20 which were supported by the previous studies (18,19,21,22,30). The systematic review suggested that the relation between airway resistance and height may be due to the relation between height and lung volume (24). We also found a positive correlation between bodyweight and airway resistance and reactance indices which were supported by the previous studies (19,21,22,30). The previous finding also suggested that the increase in airway resistance and decrease in X5 with increasing weight supported that weight plays a role in the determination of impedance (24). Pellegrino *et al.* (31) hypothesized that these findings may reflect obesity-related pulmonary inhomogeneity, possibly as a consequence of mechanical airway compression and microatelectasis.

Our study showed a significant increase in airway resistance including R5 and R20 in men compared to women which were comparable to those of previous studies, which showed that the R5 and R20 were significantly higher in men (20,30). Previous studies postulated that higher airway resistance in women in comparison to men likely results from smaller average lung volumes or possibly smaller airway diameters in women (32,33). We also found a higher airway reactance (more negative X5) in females compared to males which was similar to the previous studies (20,21). Previous studies explained that one plausible explanation for this finding could be a higher BMI in women compared to men (21). However, the BMI of women in our study was lower compared to men. Therefore, the more negative X5 in females may be due to the shorter height which was suggested in the previous findings (19).

A previous study suggested that ethnicity is significantly

 

**Table 3** Spirometric and IOS data of smokers and non-smokers in male subjects (n=87)

| Parameters | Smokers (n=24) | Non-smokers (n=63) | P value |
|---|---|---|---|
| Spirometry | | | |
| % predicted FVC | 96.1±11.3 | 101.6±14.8 | 0.104 |
| z-score of FVC | –0.020 (–0.883, 0.259) | 0.135 (–0.521, 0.884) | 0.171 |
| % predicted $FEV_1$ | 97.4±10.0 | 99.6±13.7 | 0.476 |
| z-score of $FEV_1$ | –0.095 (0.779, 0.486) | 0.081 (–0.716, 0.748) | 0.314 |
| $FEV_1$/FVC, % | 83.4±5.1 | 81.9±6.0 | 0.280 |
| z-score of $FEV_1$/FVC | 0.030 (–0.612, 0.694) | –0.208 (–0.708, 0.175) | 0.301 |
| % predicted $FEF_{25–75\%}$ | 105.2±25.4 | 102.2±23.7 | 0.616 |
| z-score of $FEF_{25–75\%}$ | –0.109 (–0.565, 0.672) | 0.088 (–0.649, 0.559) | 0.613 |
| IOS | | | |
| R5, $cmH_2O$/L/s | 3.47±1.04 | 3.22±0.97 | 0.287 |
| R20, $cmH_2O$/L/s | 2.85±0.92 | 2.76±0.75 | 0.624 |
| R5–R20, $cmH_2O$/L/s, median (IQR) | 0.43 (0.30, 0.89) | 0.34 (0.17, 0.70) | 0.145 |
| X5, $cmH_2O$/L/s | –0.99±0.48 | –0.87±0.42 | 0.259 |
| Fres, Hz | 12.74±3.58 | 12.23±3.77 | 0.575 |
| AX, $cmH_2O$/L, median (IQR) | 2.15 (1.41, 6.16) | 2.12 (1.22, 3.87) | 0.314 |

Data are mean ± SD unless otherwise stated. IOS, impulse oscillometry; FVC, forced vital capacity; $FEV_1$, forced expiratory volume in the first second; $FEF_{25–75\%}$, forced expiratory flow at 25–75% of FVC; R5, resistance at 5 Hz; R20, resistance at 20 Hz; R5–R20, heterogeneity of resistance between R5 and R20; X5, reactance at 5 Hz; Fres, resonant frequency; AX, the area under reactance curve between 5 Hz and resonant frequency; SD, standard deviation.

under-reported and this may limit the applicability of current equations to heterogeneous patient populations (24). Despite the increasing use of IOS in clinical practice, population-specific reference equations are lacking (21). Some IOS indices in our Thai prediction value were higher when compared with the Chinese and Australian predictive values (19,20). For example, the R5 were higher about 22.5–32.1% and 11.9–15.4% for men and women, respectively when compared to previous findings (19,20). The R20 were also higher about 11.9–15.4% and 19.5–20.6% for men and women, respectively when compared to the Chinese and Australian predictive values (19,20). Moreover, the X5 in our prediction value were more negative when compared with the Chinese and Australian predictive values (ranging from -6.2% to -6.9% and -5.6% to -14.1% for men and women, respectively) (19,20). The differences in airway resistance and reactance between our study and the previous studies may be due to the average height of our study were shorter compared to the previous studies (165.6 and 152.9 cm *vs.* 170.0 to 175.9 cm and 159.0 to 164.6 cm for men and women, respectively (19,20). Moreover, the differences in airway impedance between our study and the previous one may be due to the average BMI of our study were lower compared to the previous study in Australian population (24.8 and 22.9 kg/$m^2$ *vs.* 27.2 and 28.5 kg/$m^2$ for men and women, respectively (20). Additionally, the diversity of ethnic origins may have resulted in differences in airway resistance and reactance (24). The utility of IOS is fast gaining acceptability for accurate clinical diagnosis, and its use has increased significantly among Thai clinicians in recent years because of the ease to perform compared to spirometry. Thus, developing IOS reference equations for the Thai population is important. To the best of our knowledge, our study is the first study to present the IOS reference equation in the Thai adult population aged 22–92 years.

### *Strength and limitation of this study*

The strength of our study is its value as the first study that

 

**Table 4** Sex-specific reference equations of the IOS indices

| Parameters | Equations | RMSE | Adjusted $R^2$ |
|---|---|---|---|
| Men | | | |
| R5, $cmH_2O/L/s$ | 18.7428 – 0.0163 (age) – 0.1106 (height) + 0.0531 (weight) | 0.8194 | 0.3431 |
| R20, $cmH_2O/L/s$ | 13.6060 – 0.0169 (age) – 0.0743 (height) + 0.0332 (weight) | 0.6818 | 0.2933 |
| logR5–R20, $cmH_2O/L/s$ | 1.0414 – 0.0087 (height) | 0.4131 | 0.0249 |
| X5, $cmH_2O/L/s$ | –6.5532 + 0.0045 (age) + 0.0349 (height) – 0.0051 (weight) | 0.3940 | 0.2182 |
| Fres, Hz | 49.1797 + 0.0296 (age) – 0.2560 (height) + 0.0615 (weight) | 3.3240 | 0.2233 |
| logAX, $cmH_2O/L$ | 4.3201 – 0.0255 (height) + 0.0039 (weight) | 0.3413 | 0.1676 |
| Women | | | |
| R5, $cmH_2O/L/s$ | 12.6580 – 0.0838 (height) + 0.0809 (weight) | 0.9868 | 0.2489 |
| R20, $cmH_2O/L/s$ | 15.7253 – 0.0977 (height) + 0.0519 (weight) | 0.7834 | 0.2333 |
| logR5–R20, $cmH_2O/L/s$ | –4.2198 + 0.0052 (age) + 0.0166 (height) + 0.0202 (weight) | 0.3170 | 0.2046 |
| X5, $cmH_2O/L/s$ | –6.1651 + 0.0333 (height) | 0.5648 | 0.0560 |
| Fres, Hz | –4.2714 + 0.1079 (age) + 0.2048 (weight) | 3.4200 | 0.2415 |
| logAX, $cmH_2O/L$ | –0.7026 + 0.0064 (age) + 0.0162 (weight) | 0.3573 | 0.1296 |

Models were built using multivariable linear regression analysis taking age (in years), weight (in kg), and height (in centimeters) as main explanatory variables. IOS, impulse oscillometry; RMSE, root mean square error; R5, resistance at 5 Hz; R20, resistance at 20 Hz; R5–R20, heterogeneity of resistance between R5 and R20; X5, reactance at 5 Hz; Fres, resonant frequency; AX, the area under reactance curve between 5 Hz and resonant frequency.

provides the IOS reference equation in the Thai adult population. However, this study has some limitations. Firstly, there was no data from childhood and adolescent subjects aged less than 22 years due to limitations of available data. Thus, the Thai IOS reference equation from childhood and adolescence should be presented in future studies. Secondly, this is a single-center study that small samples in Northern Thailand were included. Thus, the multicenter study that included more sample size in all parts of Thailand is needed to confirm these findings. However, we had calculated sample size and could recruit subjects more than calculated one. Thirdly, the data about air pollutants and biofuel exposure were not mentioned in our study. This could have an impact on IOS parameters. However, annual report of the particulate matter with a diameter of 2.5 micrometres or less (PM2.5) in our city was 30.6±4.2 microgram/$m^3$ (ranged from 24.0 to 36.0 microgram/$m^3$) over the past decade (34) whereas the gaseous pollutants were within acceptable level by the World Health Organization (WHO). Thus, the detail about air pollutants and biofuel exposure should be focused in future studies. Fourthly, 24 (27.6%) of male subjects with smoking history of was 1.3±1.8 pack-year were included in this study. However, all included subjects were classified as non-smoker according to the U.S. Centers for Disease Control which defines a never-smoker as someone who has smoked <100 cigarettes or 5 pack-year per lifetime (35). Although, the smoking history of these light smokers was classified as non-smokers, IOS possibly might detect very early airway changes in them. Therefore, only absolute non-smokers should be recruited in future studies.

## Conclusions

Our study provided the reference equations for the IOS parameters in Thai adults for both men and women. IOS indices including R5, R20, and AX were significantly higher in women compared to men.

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## Footnote

*Reporting Checklist:* The authors have completed the STROBE reporting checklist. Available at https://jtd.amegroups.com/article/view/10.21037/jtd-21-1989/rc

*Data Sharing Statement:* Available at https://jtd.amegroups.com/article/view/10.21037/jtd-21-1989/dss

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at https://jtd.amegroups.com/article/view/10.21037/jtd-21-1989/coif). The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Research Ethics Committee, Faculty of Medicine, Chiang Mai University (study code: MED-2564-08533, date of approval: 12 November 2021) and filed under Clinical Trials Registry (study ID: TCTR20211014002, date of approval: 14 October 2021). Individual consent for this retrospective analysis was waived.



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