The measurement of cough response to bronchoconstriction induced by methacholine inhalation in healthy subjects: An examination using the Astograph method

Title Page

TITLE:

The Measurement of Cough Response to Bronchoconstriction Induced by Methacholine Inhalation in Healthy Subjects: An Examination Using the Astograph Method

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Authors' contributions:

JH designed the study, and wrote the initial draft of the manuscript. MF and KK contributed to analysis and assisted in the preparation of the manuscript. JH, TS, KY, HK, SW, TY and SN were involved in the recruitment of healthy subjects. NO and MA were involved in the data interpretation. TS, HK and YI were involved in the study design and data interpretation. All authors have approved the final version of this manuscript for submission.

ABSTRACT:

Background:

We demonstrated that heightened cough response to bronchoconstriction is a fundamental feature of cough variant asthma (CVA). To evaluate this physiological feature of CVA in daily clinical practice, it is necessary to clarify the cough response to bronchoconstriction in healthy subjects. We evaluated cough response to methacholine (MCh)-induced bronchoconstriction in healthy subjects. A forced oscillometry technique was used to measure airway resistance changes with Mch.

Methods:

Healthy never-smokers (21 men, 20 women; mean 22.3 ± 3.7 years) participated. None had a >3-week cough history, clinically significant respiratory or cardiovascular disorders, or disorders that might put subjects at risk or influence the study results or the subjects' ability to participate. Twofold increasing concentrations of Mch chloride diluted in phosphate-buffered saline (0.039 to 160 mg/mL) were inhaled from nebulizers at 1-minute intervals during subjects' tidal breathing after the baseline respiratory resistance (Rrs) was recorded. Mch inhalation continued until Rrs reached twice the baseline value and FEV_1 decreased to <90% of baseline value. Spirometry was measured before Mch inhalation and immediately after Rrs had increased twofold.

Coughs were counted during and for 30 minutes after Mch inhalation. The cough reflex sensitivity to capsaicin was also examined.

Results:

The number of coughs was 11.1 ± 14.3 (median, 7.0; range, 0 to 71; reference range, 0 to 39.7). There was no significant difference in the cough response between the sexes. The reproducibility of the cough response to bronchoconstriction was sufficient. No correlation existed between the bronchoconstriction-induced cough response and capsaicin cough-reflex sensitivity.

Conclusions:

Using the Astograph method, cough response to bronchoconstriction could be measured easily, safely and highly reproducibly in healthy subjects.

Clinical Trial Registration

The Kanazawa University Hospital's Medical Ethics Committee approved this study (registration number 2015-039, UMIN 000020804).

Running Head:

Bronchoconstriction and cough in Healthy Subjects

Keywords:

Bronchoconstriction

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Cough variant asthma

Methacholine chloride

Bronchial provocation Tests

Abbreviations:

AC = Atopic cough

ACE = angiotensin converting enzyme

CVA = cough variant asthma

FEV_1 = forced expiratory volume in 1 second

Mch = Methacholine

Rrs = respiratory resistance

SBS = sinobronchial syndrome

INTRODUCTION:

Chronic cough has been defined as “cough lasting longer than 8 weeks as the only symptom and whose cause is not apparent by physical examination and routine testing such as CXR and spirometry”.(1) A persistent cough often interferes with daily living and sleep. In Japan, cough variant asthma (CVA) and atopic cough (AC) are major causes of chronic non-productive cough (2). There are at least two possible mechanisms of chronic non-productive cough: increased cough reflex sensitivity (such as AC (3), gastroesophageal reflux disease (4) or angiotensin converting enzyme [ACE]-inhibitor induced cough (5)) and bronchoconstriction-triggered cough (such as CVA (6)). Our series of studies have clearly demonstrated that the cough receptor sensitivity was never involved in the pathology of cough at all in pure CVA patients, diagnosed on the grounds that cough was completely or almost resolved by only bronchodilator therapy (7, 8). Recently, we reported that the heightened cough response to bronchoconstriction is a fundamental physiological feature of CVA using partial and full flow-volume curves.(6, 9) To evaluate this physiological feature of CVA in daily clinical practice in the future, it is necessary to clarify the cough response to bronchoconstriction in healthy subjects at the present time.

The aim of this study was to evaluate the cough response to

bronchoconstriction induced by Mch using the Astograph method in healthy subjects.

METHODS:

Subjects

Forty-one healthy, never-smoking subjects (21 males and 20 females; mean age 22.3 ± 3.7 years) participated in this single-arm and non-randomized study. None of them had a history of cough lasting for more than three weeks, bronchial asthma, any clinically significant respiratory disorders, ischemic heart disease, cardiovascular disease, or a disorder that might put them at risk or influence the study results or their ability to participate. As far as possible to exclude the subjects who had experienced non-infectious cough from this study, we excluded the subjects having history of cough lasting more than 3 weeks.

Informed consent was obtained from all subjects. This study was performed at Kanazawa University Hospital between February 2016 and July 2016 and was approved by the Medical Ethics Committee of Kanazawa University Hospital (registration number 2015-039, UMIN 000020804).

Methacholine inhalation protocol

Methacholine inhalation was performed by an Astograph (Jupiter 21; CHEST; Tokyo, Japan), according to the method of Takishima et al (10). Briefly, respiratory

resistance (Rrs. $cmH_2O/L/sec$) was measured by the forced oscillation method (3 Hz) during continuous inhalation of Mch in stepwise incremental concentrations, until the Rrs reached twice the baseline value (10, 11). Mch chloride (Wako Pure Chemical Industries, Ltd; Osaka, Japan) was diluted in phosphate-buffered saline solution with 2-fold increasing concentrations, from 0.0195 to 160 mg/mL. The PBS and Mch solution was inhaled for 1 minute. Each subject wore a nose clip and was examined during quiet breathing in a sitting position.

Assessment of cough response to bronchoconstriction induced by Mch

Spirometry was measured, using a computed spirometer (CHESTAC-9800; CHEST; Tokyo, Japan), before Mch inhalation and immediately after the Rrs had increased twofold. At that time, if FEV_1 did not decrease to less than 90% of the baseline value, inhalation of Mch was restarted at the same concentration. An observer counted coughs, and cough counts were collected for the interval beginning <1 minute before and for 30 minutes (total 30+α min) following inhalation of Mch, at which the Rrs and FEV_1 were archived. Throat clearing was easily identified and was disregarded. Because our preliminary data in cough response to bronchoconstriction using partial and full flow-volume curves showed that healthy subjects did not have any cough in 30 or

more minutes after the inhalation of Mch, we adopted 30 minutes as time for count of coughs.

After completion of the measurement of the cough response, salbutamol was inhaled via the Astograph until the Rrs recovered to the baseline value.

To evaluate reproducibility of the cough response measurements, the cough response was measured twice over a 7-day interval in 24 subjects (14 males, 10 females), in order to avoid the tachyphylaxis to inhaled methacholine.

Assessment of cough-reflex sensitivity to inhaled capsaicin

Cough-reflex sensitivity was assessed by the capsaicin provocation test(12) in 19 healthy subjects. Capsaicin (30.5 mg) was dissolved in Tween 80 (1 mL) and ethanol (1 mL) and then dissolved in physiological saline (8 mL) to make a stock solution of 10 mmol/L, which was stored at -20°C. This solution was diluted with physiological saline to make solutions starting at a concentration of 0.49 μmol/L; then, the concentration was doubled sequentially up to 1 mmol/L. Each subject inhaled a control solution of physiological saline followed by progressively increasing concentrations of the capsaicin solution. Solutions were inhaled for 15 seconds every 60 seconds by tidal mouth-breathing subjects who wore a nose clip from a Bennett Twin nebulizer

(3012-60cc, Puritan-Bennett Co., Carlsbad, California, USA). Increasing concentrations were inhaled until five or more coughs were elicited. The number of cough was counted for total 60 seconds, i.e., 15 seconds of inhalation plus 45 seconds of observation in each concentration of capsaicin solutions. The nebulizer output was 0.21 mL/min. A blinded medical technician in our pulmonary function laboratory counted the number of capsaicin-induced coughs. The cough threshold was defined as the lowest concentration of capsaicin that elicited five or more coughs. Our previous study showed that Mch induced bronchoconstriction had no effect on cough reflex sensitivity to capsaicin in healthy subjects.(13), patients with asthma or chronic bronchitis (12). Therefore, the assessment of first time cough response to bronchoconstriction and that of cough-reflex sensitivity to inhaled capsaicin were examined on the same day, in the order of above description.

Data analysis

Data, excluding the capsaicin cough threshold and maximum concentration of inhaled Mch, were presented as the mean ± standard deviation (range). The capsaicin cough threshold and maximum concentration of inhaled Mch were expressed as the geometric mean with geometric standard error of the mean (GSEM). Statistical

differences between the groups and within groups were analyzed using the Mann-Whitney U test and Wilcoxon signed-rank test, respectively. The reproducibility of the cough response between the first and second measurement was analyzed using a Bland-Altman analysis and intra-class correlation coefficient. Results with *P* values of <0.05 were considered to be statistically significant.

RESULTS:

The characteristics of the subjects are shown in Table 1. The capsaicin cough threshold and maximum concentration of inhaled Mch are shown in Table 1. Figure 1A displays the number of coughs provoked after inhalation of Mch (11.1 ± 14.3/30+α min; median, 7; range, 0-71; reference range, 0-39.7). The number of coughs was 11.3 ± 16.3/30+α min (median, 7; range, 0-71) and 11.0 ± 12.0/30+α min (median, 8; range, 0-43) in males and females, respectively (Figure 1B). The cough response to bronchoconstriction was not significantly different between males and females ($p = 0.86$). Figure 2A shows the plot of the cough number in 24 healthy subjects. There was a strong linear relationship between cough response to bronchoconstriction on two separate days (Spearman's rank correlation coefficient; $r = 0.927$, $p = 0.0005$). The mean and standard deviation (SD) of differences between the 24 pairs of repeated measurements (Figure 2B) was -0.83 and 7.79, respectively (95% confidence interval,-4.12 to 2.46). There was no correlation between the differences and the size of the cough number (Spearman's rank correlation coefficient; $r = 0.567$, $p = 0.694$). The intra-class correlation coefficients between cough responses to bronchoconstriction on two separate days were as follows: the value of single measures was 0.900 and the one of average measures was 0.947. From these results, we concluded that there was good

reproducibility in the cough response to bronchoconstriction by the method of this study when the measurements were performed at more than a 7-day interval. The number of coughs was 11.7 ± 13.8/15+α min (median, 10.0; range, 0-61; reference range, 0-39.3). Figure 3A shows the time course of coughs during and after Mch inhalation. The majority of coughs appeared within 15 min, and coughs were not provoked 30 or more minutes after Mch inhalation. A very small count of cough (0.44 ± 0.75; median, 0; range, 0-2) was provoked before the endpoint of Mch inhalation. There was a strong linear relationship between the cough number counted for 15+α minutes and 30+α minutes intervals (Spearman's rank correlation coefficient; $r = 0.995$, $p < 0.0001$) (Figure 3B). FEV_1 was measured before Mch inhalation and immediately after the Rrs had doubled post-Mch inhalation. The parameters before and after inhalation of Mch are shown in Table 1. The change in FEV_1 was -18.2 ± 7.1% (median, -15.7%; range, -10.0 to -32.0%). The values of the SpO_2 and heart rate were significantly altered by inhalation of Mch ($p = 0.0006$ and < 0.0001, respectively). There was no correlation between cough response and change in FEV_1 (Spearman's rank correlation coefficient; $r = 0.037$, $p = 0.863$) and between cough response to bronchoconstriction and maximum concentration of inhaled Mch (Spearman's rank correlation coefficient; $r = -0.114$, $p = 0.914$) (Figure 4A and 4B, respectively). There was no correlation between cough

response to bronchoconstriction and cough threshold to inhaled capsaicin (Spearman's rank correlation coefficient; $r = 0.114$, $p = 0.960$) (Figure 5). Only a few subjects reported mild dyspnea and chest tightness besides the cough during and after Mch inhalation. All subjects were asymptomatic when leaving our hospital, and no subjects reported respiratory symptoms in the days after the test.

DISCUSSION:

Cough is a common symptom that worsens the QOL.(14) A large-scale, Japanese cohort study revealed that the prevalence of cough was 10.2% and the prevalence of chronic cough was >2%.(14) A productive cough is primarily due to sputum hypersecretion.(2) On the other hand, a non-productive cough is evoked by two possible mechanisms: 1) increased cough receptor sensitivity, e.g., AC (3), gastroesophageal reflux disease (GERD) (4) and ACE-inhibitor induced cough (15); and 2) heightened cough response to bronchoconstriction, e.g., CVA.(6) In a Japanese cohort study, AC, CVA and sinobronchial syndrome (SBS) were three major causes of chronic cough.(2) Patients with CVA but not AC can develop a non-reversible airflow limitation,(16) and the response to antitussive drugs except corticosteroids is completely different between AC and CVA. We need to determine the differential diagnosis of the causative disease producing the persistent cough through medical-history taking, physical and clinical examinations, and diagnostic therapy. Generally, the diagnosis of the causative disease was dependent on therapeutic diagnostic procedures to some extent. There were several problems with therapeutic diagnosis, e.g. spontaneous relief of cough leading to a false-positive result, resistance to the therapy leading to a false negative result,(17) and a limitation of the diagnostic ability in the case of multiple

causative diseases. Thus, we believe that a pathophysiologic diagnostic procedure should be established in the future.(17)

We previously reported that CVA had a heightened cough response to Mch-induced bronchoconstriction,(6) and conversely patients with bronchial asthma had an impaired cough response to Mch-induced bronchoconstriction (9) compared with healthy subjects. Recently, we have also shown that in contrast to CVA, AC had a normal cough response to Mch-induced bronchoconstriction.(18) In these studies, the inhalation of Mch was performed by a standardized method recommended by the Japanese Society of Allergology,(19) and a repeated flow-volume curve following each 2-minute inhalation of increasing concentrations of Mch was needed to validate the degree of bronchoconstriction. Therefore, expiration with maximum effort is performed by the examinee after each concentration of Mch, and this method requires an examiner's technique and would be time-consuming. The Astograph is usually used to estimate non-specific airway hyperresponsiveness. By using this method, it is possible to achieve the continuous 1-minute inhalation of each increasing dose of Mch and to measure the continuous changes of Rrs. This method requires no effort by the subject to measure the cough response. In the current study, a strong linear relationship between the cough numbers counted for intervals of 15+α minutes and 30+α minutes was

observed. Therefore, we expect that the Astograph method would be useful for evaluating the cough response to Mch-induced bronchoconstriction without imposing restrictions on the daily clinical practice. In the future study, we need to examine the cough response to bronchoconstriction in CVA patients using this method.

Niimi et al reported that the decrease in FEV_1 was -19.3 ± 6.9% (median, -19.7%; range, -8.0 to -31.4%) after Mch inhalation continued until Rrs reached twice the baseline value using the Astograph method in patients with asthma.(11) Our study subjects showed that the decrease in FEV_1 was -18.2 ± 7.1% (median, -15.7%; range, -10.0 to -32.0%) after Mch inhalation. The decrease in FEV_1 was used as an index of the airway narrowing in our study of healthy subjects; therefore, if FEV_1 did not decrease to less than 90% of baseline value when the Rrs reached twice the baseline value, inhalation of Mch was restarted at the same concentration. Because in our previous two studies, using a standardized method, mean bronchoconstriction equivalent to a 6.30 ± 3.03% and 7.60 ± 10.6% fall in FEV_1 in healthy subjects was used as mild bronchoconstriction (6, 9), Mch inhalation was continued until %decrease in FEV_1 was 10% as round number in this study. Thousands of Mch challenge tests have been performed by laboratories with no serious side effects.(20) In our study, only a few subjects reported mild dyspnea and chest tightness besides the cough during and after

Mch inhalation, and all subjects were asymptomatic when leaving our hospital; furthermore, no subjects reported respiratory symptoms in the days after the test. Using the Astograph method, we could evaluate the cough response to bronchoconstriction safely.

In our previous two studies using a standardized method,(6, 9) mild bronchoconstriction equivalent to a 6.30 ± 3.03% and 7.60 ± 10.6% fall in FEV_1 provoked few coughs (median, 0/32 min; range, 0-13 and median, 0.5/32 min; range, 0-15), and more severe bronchoconstriction equivalent to a 22.5 ± 10.4% and 22.6 ± 15.0% fall in FEV_1 provoked more coughs (median, 20/32 min; range, 0-54 and median, 22.5/32 min; range, 0-85) in healthy subjects. In this study using the Astograph method, the cough number induced by bronchoconstriction equivalent to 18.2 ± 7.1% fall in FEV_1 provoked coughs (median, 7; range, 0-71/30+α min). Both of the cough number and the fall in FEV_1 in this study were slightly weaker than those in severe bronchoconstriction of our previous two studies and we thought that cough number in this study was considered acceptable compared to our previous data. Though, we could not examine the cough response to saline using the identical method, Mch inhalation protocol using Astograph method was unlikely to provoke extreme high number of coughs. From results of our 3 studies, it is likely that the extent of bronchoconstriction

has a significant influence on the cough response, regardless of the inhalation procedure of Mch and the change in FEV_1 seems appropriate to be an index of bronchoconstriction for clinical cough response to Mch-induced bronchoconstriction in the presence of many different Mch dosing methods and many different dosing protocols. There is also the possibility that mucus secretion induced by Mch could result in cough as a mechanical stimuli, but the healthy subjects who take Mch challenge test don't present productive cough.

There was no correlation between cough response and change in FEV_1 among healthy subjects in this study. On the other hand, our previous two studies have shown that the stronger the bronchoconstriction was provoked, the more likely cough was evoked in the same healthy individuals, patients with CVA or bronchial asthma (6, 9). From these results, we expect that cough response to bronchoconstriction varies among individuals and that the extent of bronchoconstriction itself decides the cough response in the same individual. There was no direct correlation of cough response to bronchoconstriction with maximum concentration of inhaled Mch in healthy subjects. This result may support our idea that bronchial hypersensitivity is not principal feature in CVA (21).

Several investigators have reported on the bronchoconstriction induced cough

response in humans. Chausow et al. have reported that histamine induced bronchoconstriction equivalent to a 10.3 ± 7.3% (range, 6.0-26.2%) fall in FEV_1 provoked total 2.6 ± 4.1 coughs (range, 0-10) in healthy subjects. This degree of cough response to bronchoconstriction was similar to that in mild bronchoconstriction of our previous two studies (6, 9). Mch challenge has not been examined in the healthy subjects. In patients with asthma, histamine or Mch induced bronchoconstriction equivalent to a 35.8 ± 7.8% (range, 23-47.7%) or 33.3 ± 7.0% (range, 25.3-46.4%) fall in FEV_1 provoked total 5.6 ± 8.0 (range, 0-21) or 0.9 ± 2.3 coughs (range, 0-6). In patients with chronic cough, histamine or Mch induced bronchoconstriction equivalent to a 22.5 ± 11.7% (range, 7.4-49.3%) or 18.9 ± 12.4% (3.8-39.6%) fall in FEV_1 provoked total 29.8 ± 21 (range, 7-93) or 22.7 ± 20.6 coughs (range, 0-86). Milder degree of bronchoconstriction have caused more coughs in the patients with chronic cough than coughs induced by more severe bronchoconstriction in the patients with asthma (22). Inhalation of neurokinin A induced bronchoconstriction equivalent to a mean 48% (SEM 12%) fall in sGaw has provoked no coughs in asthmatic subjects, but has not caused bronchoconstriction in the healthy subjects (23). Griffin et al. have examined the relationship between leukotriene D4 or histamine induced bronchoconstriction equivalent to 20 to 30% fall in V30 (maximum expiratory airflow

late at 30% of the vital capacity above residual volume) and cough response in patients with asthma. Cough response were present only after histamine challenge, but the exact cough number was not mentioned (24). Using the identical method to this study, Matsumoto and Niimi, et al. have shown that patients with CVA coughed more frequently during methacholine-induced bronchoconstriction, equivalent to Rrs reached twice the baseline value, than did patients with classic asthma (25). From these previous reports of another groups, however, the degree of bronchoconstriction was unequal within the study or among studies, protocol of bronchial challenge was varied and the data about cough response to bronchoconstriction in healthy subjects was scarce, we thought that 1) in patients with bronchial asthma, coughs are hardly provoked by bronchoconstriction equivalent to over 20% fall in FEV_1, 2) in part of patient with chronic cough, bronchoconstriction-triggered cough response was more enhanced than that in asthmatic patient, 3) the inhalation procedure of Mch had little influence to bronchoconstriction induced cough response.

There was good reproducibility in the cough response to bronchoconstriction using the Astograph method at an interval of more than 7 days in healthy subjects. Cough-reflex sensitivity to inhaled capsaicin is heightened in females.(26) In contrast, cough response to bronchoconstriction was not influenced by gender. This study is

limited by its use of a small-sized cohort, as well as an age bias among recruited study subjects. We could not determine whether or not an age difference was present, as with cough-reflex sensitivity.(26, 27) It may be necessary to plan a future study to investigate these points.

We have also obtained the following data from basal study using naïve guinea pig (28). 1) Mch-induced bronchoconstriction provoked cough. There was a significant positive correlation between the increase in the index of bronchoconstriction and the number of coughs. 2) Procaterol completely abolished both the bronchoconstriction and coughs in naïve guinea pigs. 3) Capsaicin desensitization had no effect on the Mch-induced bronchoconstriction or coughs. 4) Moguisteine, that has been shown to inhibit the excitatory response of RARs to tussive stimuli in guinea pigs, dose-dependently inhibited the cough induced by Mch without affecting the Mch induced bronchoconstriction. From these results, we have concluded that cough evoked by capsaicin and Mch relies on different afferent pathways. Mch-induced cough appears to be bronchoconstriction-triggered via RARs, not C-fibers.

This study showed that there is no significant correlation between cough response to bronchoconstriction and cough-receptor sensitivity to inhaled capsaicin in the same healthy subjects. Our previous studies have shown that inhaled capsaicin did

not decrease FEV_1 at threshold dose in healthy subjects (12) andthat Mch induced bronchoconstriction had no effect on cough reflex sensitivity to capsaicin in healthy subjects, patients with asthma or chronic bronchitis (12, 13). These results may support the idea that two cough reflexes are mediated by separate afferent pathways to the cough center.

In conclusion, using the Astograph method, cough response to bronchoconstriction could be measured easily, safely and highly reproducibly in healthy subjects. This method could be performed in a relatively short time. The median bronchoconstriction-induced cough number was 7 (range, 0-71; reference range, 0-39.7/30+α min) when FEV_1 decreased by about 18%. The cough response was not influenced by gender. We could not determine whether or not an age difference was present. A future prospective study is needed for confirming the cough response in patients with chronic cough to create a criterion for diagnosing CVA using this method.

DECLARATION OF INTERESTS

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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FIGURE LEGENDS:

Figure 1

Mch-induced cough number in all subjects. After inhalation of Mch at Rrs increased twice the baseline, 11.1 ± 14.3/30+α min (median, 7; range, 0-71) coughs were provoked (A). The comparison of the cough response to bronchoconstriction between males and females (B).

Figure 2

The plot of the cough number in 24 healthy subjects. The interval between the first and second measurements was more than 7 days in each subject (A). Bland-Altman analysis. Average (X-axis) of and differences (Y-axis) in values for the cough number measured on two separate days are plotted (B). The mean and SD of differences between the 24 pairs of repeated measurements was -0.83 and 7.79, respectively. 95% confidence interval was -4.12 to 2.46).

Figure 3

The time course of coughs during and after Mch inhalation (A). Relationship between the cough number counted for 15+α and 30+α minute intervals (B).

Figure 4

Relationship between cough response to bronchoconstriction and change in FEV_1 (A). Relationship between cough response to bronchoconstriction and maximum concentration of inhaled Mch (B).

Figure 5

Relationship between cough response to bronchoconstriction and capsaicin cough threshold.

TABLES:

Table 1

Characteristics of the subjects -

Gender (Male/Female)	21/20	-
Age (years)	22.3 ± 3.1	-
Height (cm)	163.7 ± 8.1	-
Body weight (kg)	55.3 ± 8.8	-
	pre~~before~~ Mch inhalation	post~~after~~ Mch inhalation
FVC (L~~%pred~~)	4.12 ± 1.02 ~~(98.8 ± 12.9)~~	3.83 ± 1.00
FVC (%pred)	98.8 ± 12.9	91.8 ± 12.9
FEV_1 (L~~%pred~~)	3.66 ± 0.85 ~~(98.9 ± 12.3)~~	3.02 ± 0.79
FEV_1 (%pred)	98.9 ± 12.3	81.3 ± 12.8
Change in FEV_1 between pre- and post-Mch	-	81.8± 7.1

inhalation (%)		
FEV_1/FVC ratio (%)	89.5 ± 6.6	79.0 ± 7.2
SpO_2	97.4 ± 1.57	96.6 ± 1.27*
Heart Rate	75.0 ± 11.3	83.6 ± 14.9*
C5 (µmol/l)	20.1 (1.35)	-
maximum concentration of inhaled Mch (mg/ml)	20.5 (1.21)	-

Table legends

Data, excluding the capsaicin cough threshold and maximum concentration of inhaled Mch, were presented as the mean ± standard deviation (range). The capsaicin cough threshold and maximum concentration of inhaled Mch were expressed as the geometric mean with geometric standard error of the mean (GSEM).

Figure 1A

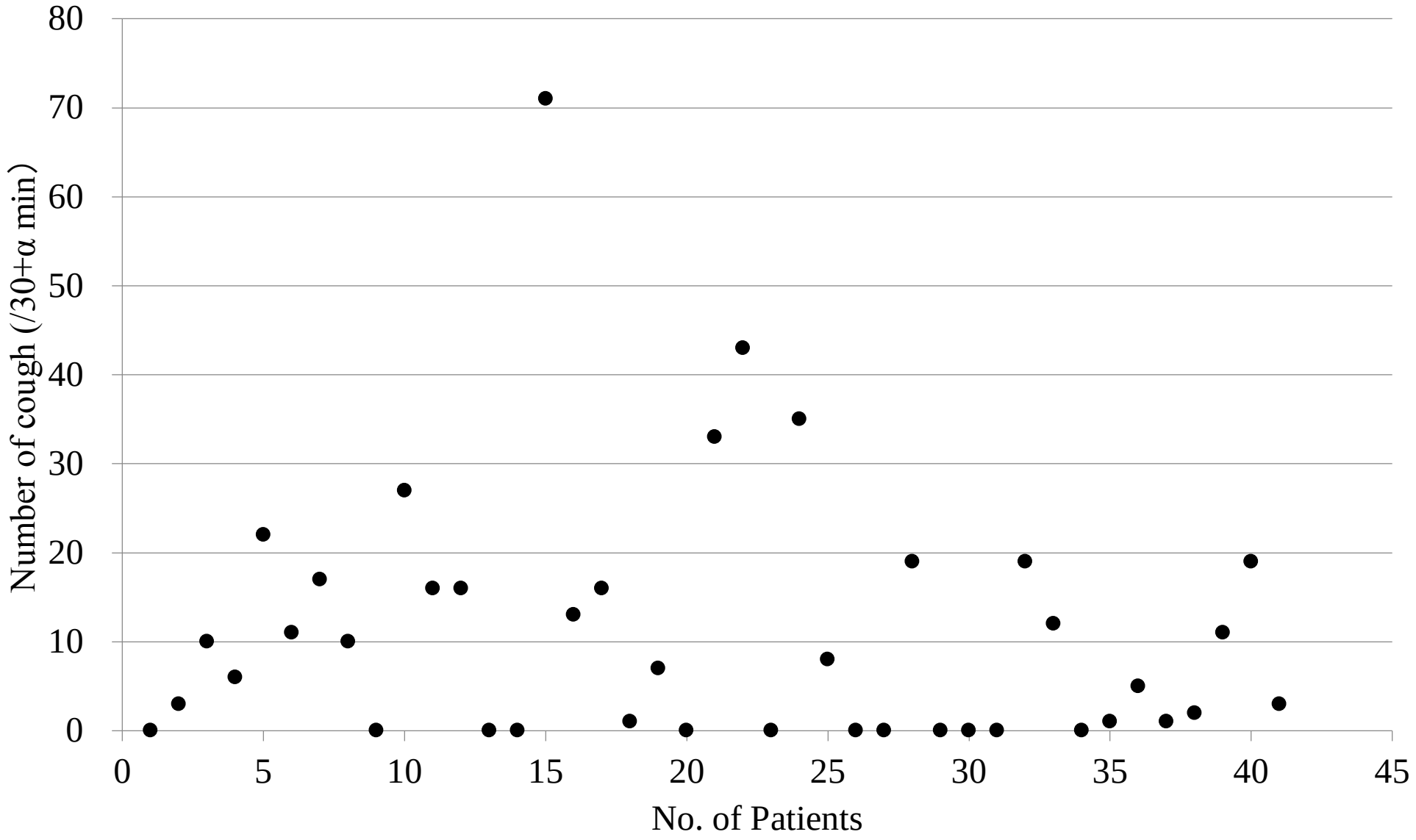

80
70
60
50
40
30
20
10
0
Number of cough (/30+α min)
0
5
10
15
20
25
30
35
40
45
No. of Patients

Figure 1B

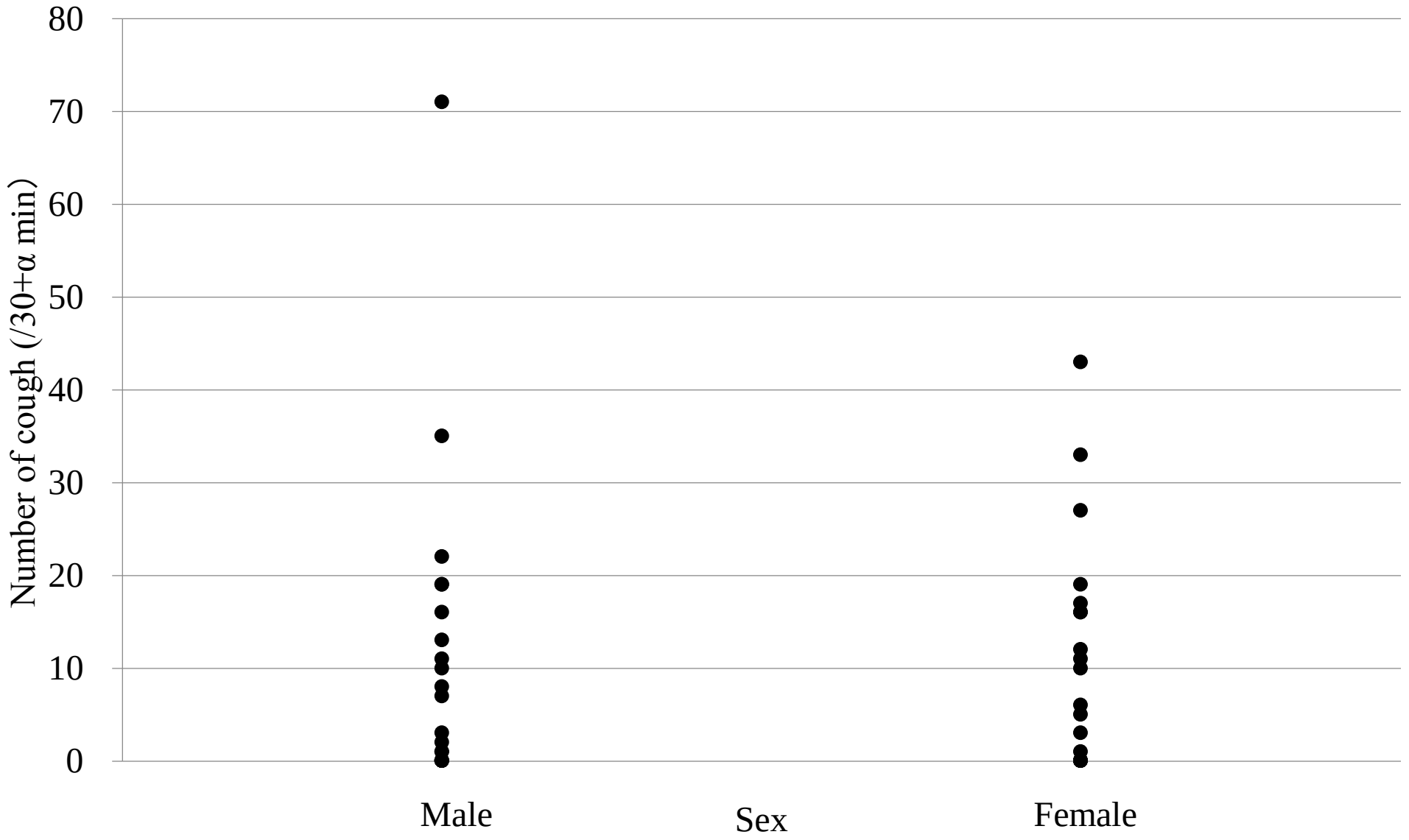


Figure 2A

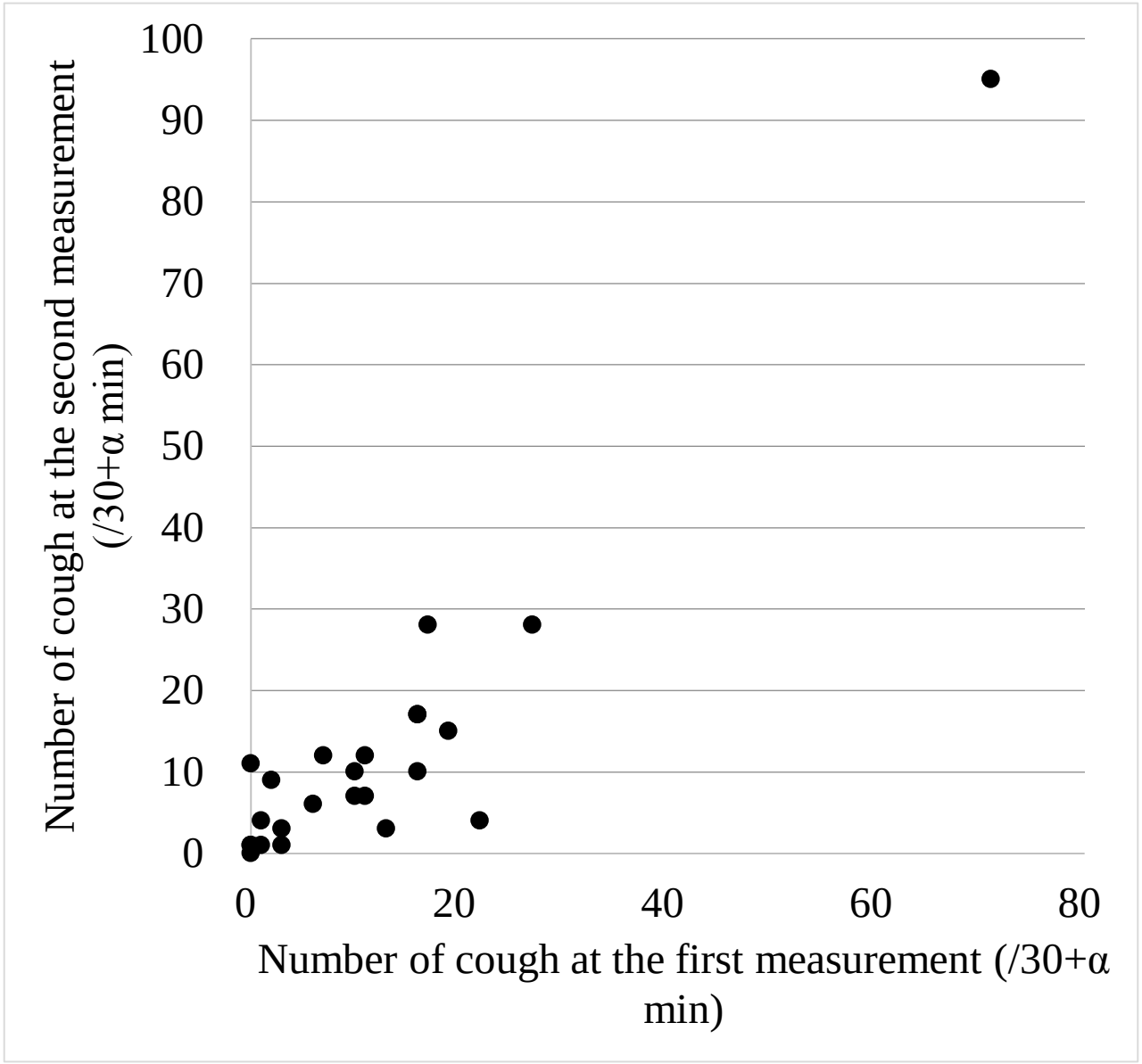

Number of cough at the second measurement (/30+α min)
100
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
Number of cough at the first measurement (/30+α min)

Figure 2B

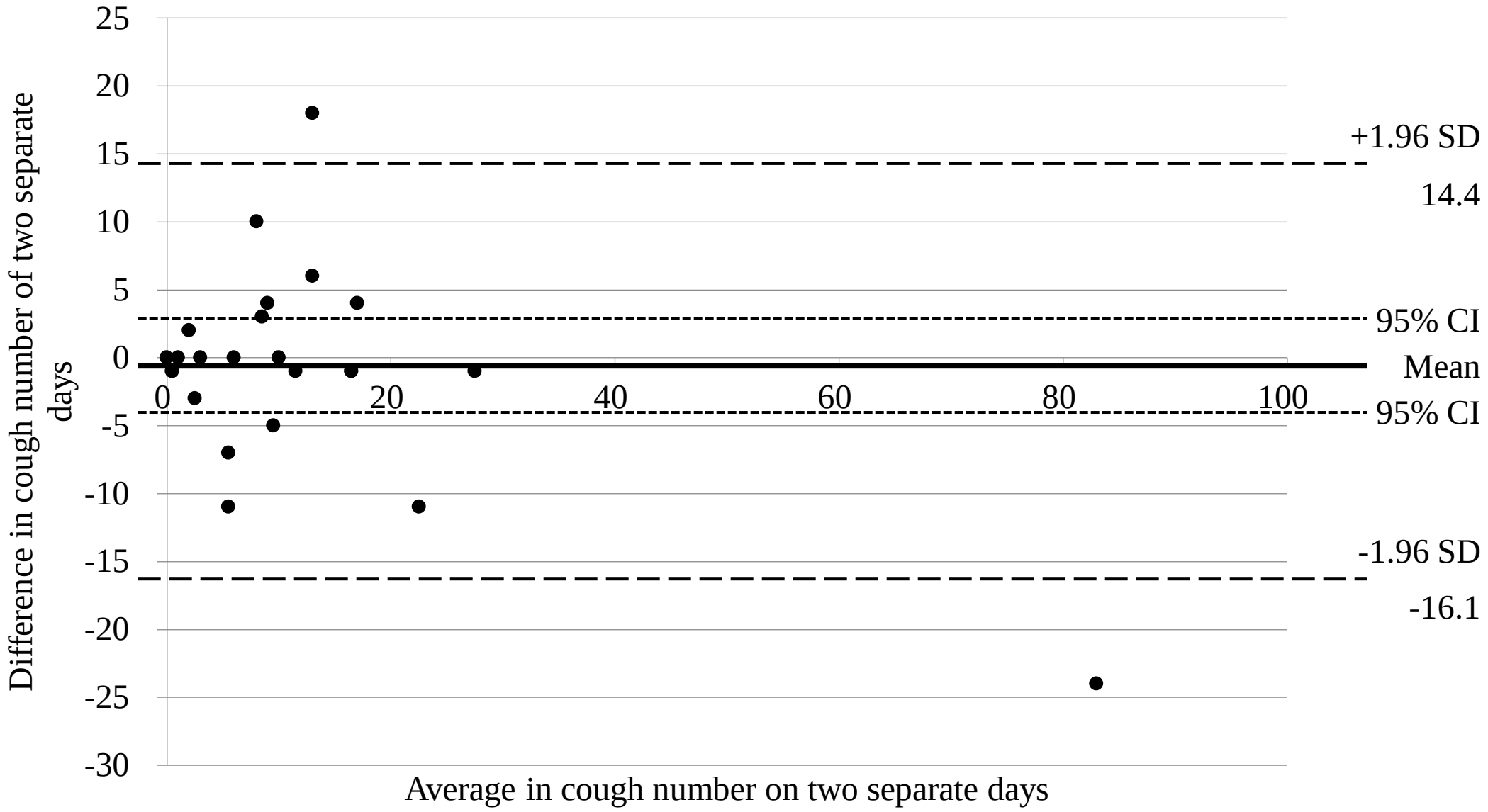

25
20
15
10
5
0
-5
-10
-15
-20
-25
-30
0
20
40
60
80
100
Difference in cough number of two separate days
Average in cough number on two separate days
+1.96 SD
14.4
95% CI
Mean
95% CI
-1.96 SD
-16.1

Figure 3A

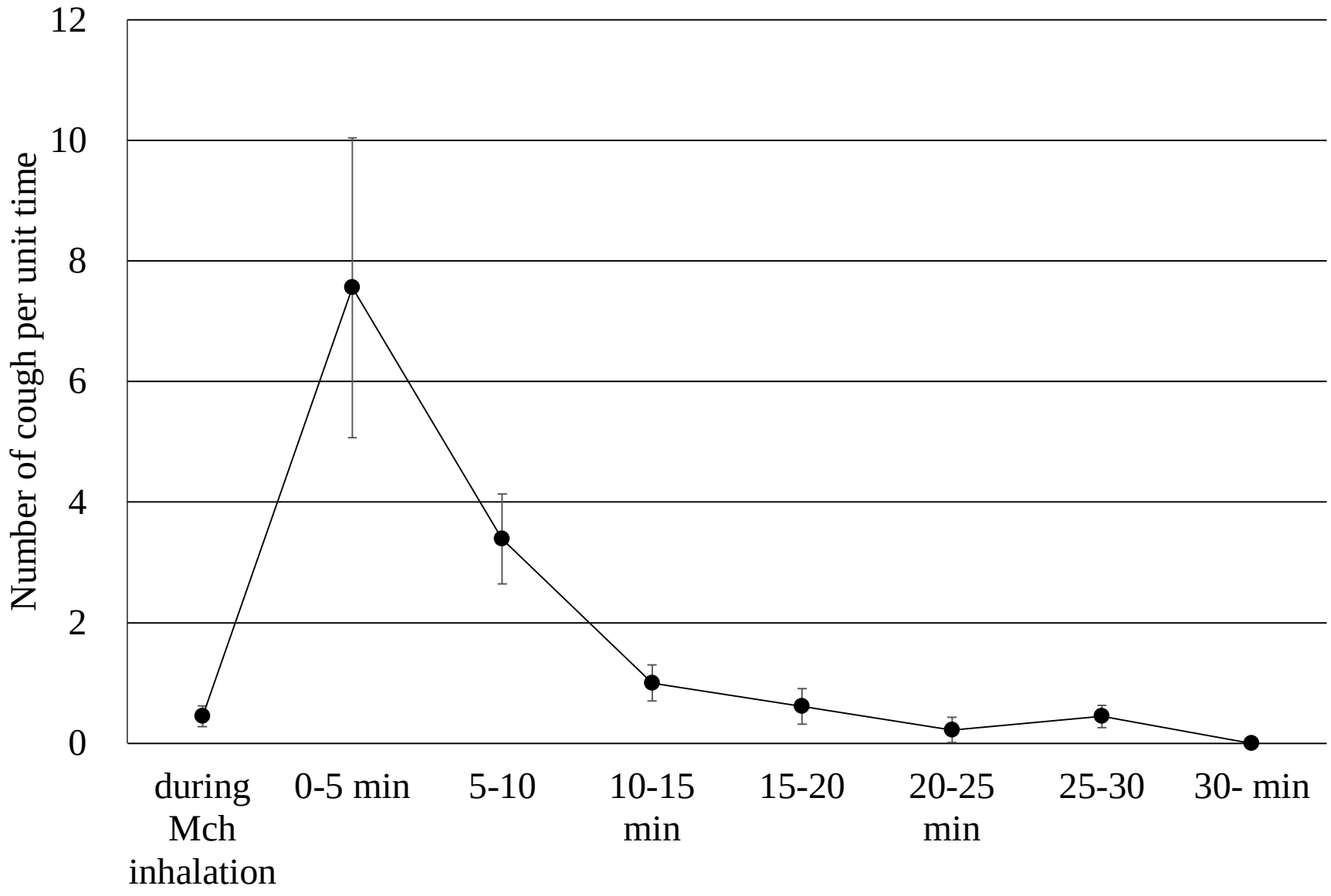

12
10
8
6
4
2
0
Number of cough per unit time
during Mch inhalation
0-5 min
5-10
10-15 min
15-20
20-25 min
25-30
30- min

Figure 3B

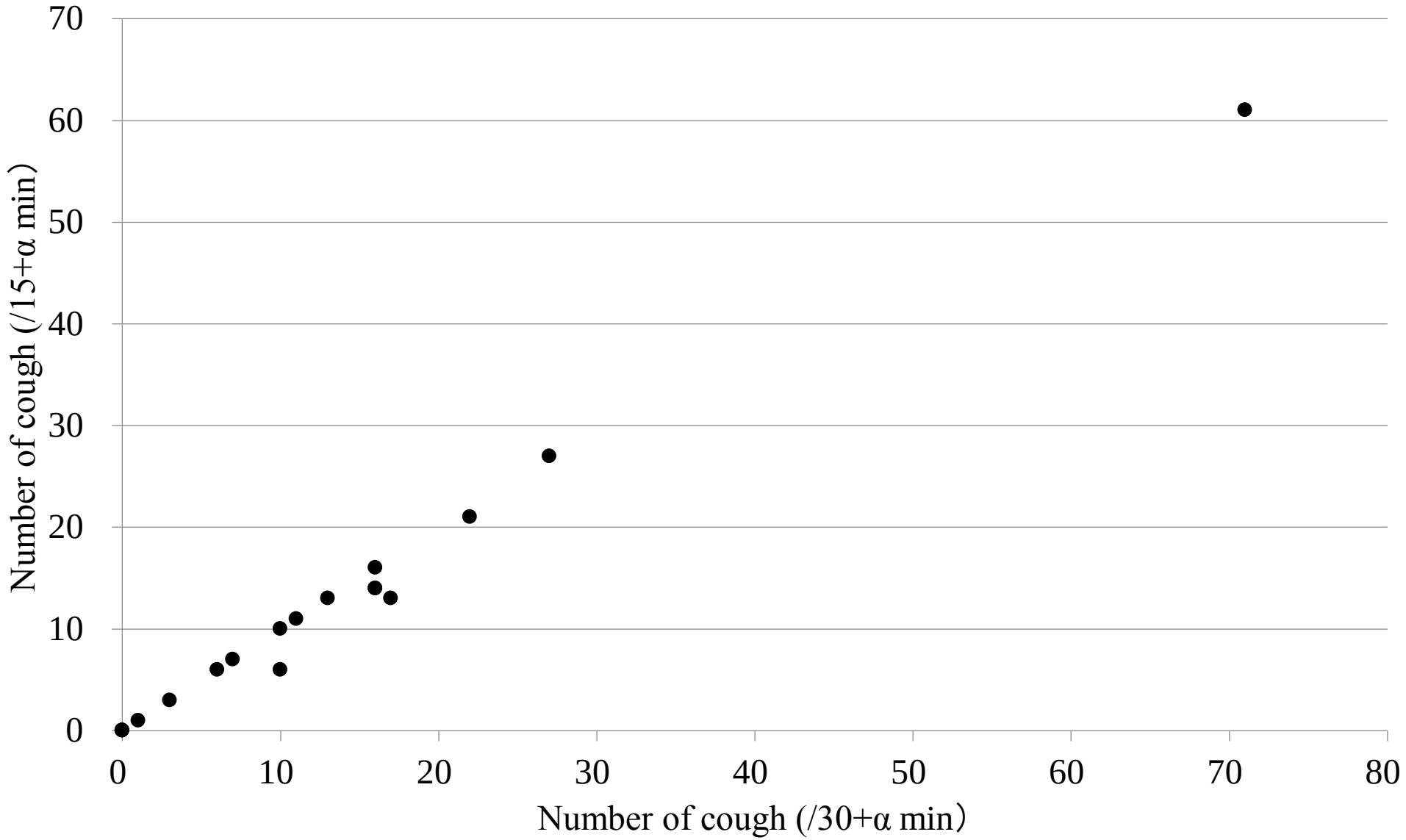

70
60
50
40
30
20
10
0
Number of cough (/15+α min)
0
10
20
30
40
50
60
70
80
Number of cough (/30+α min)

Figure 4A

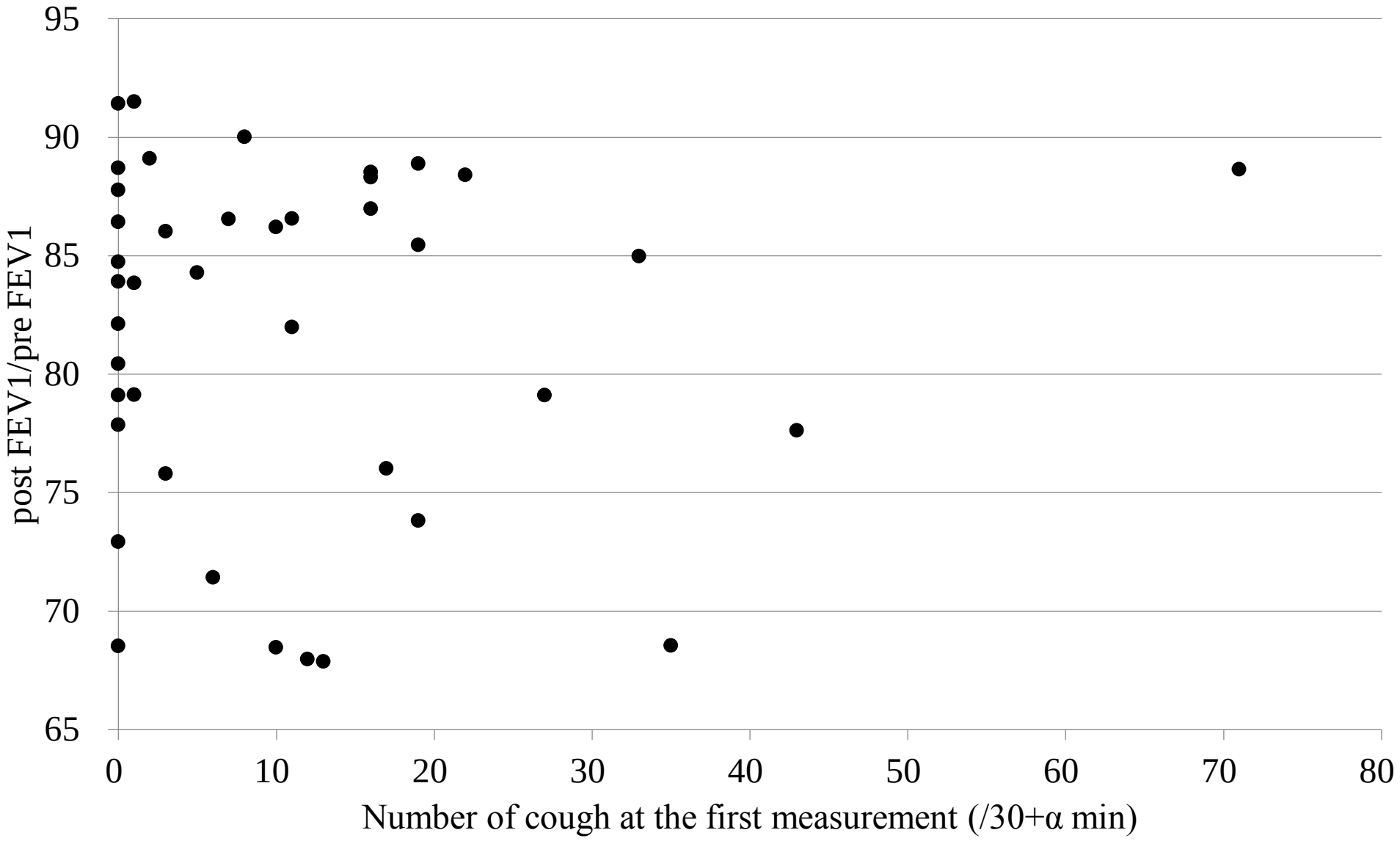

95
90
85
80
75
70
65
post FEV1/pre FEV1
0
10
20
30
40
50
60
70
80
Number of cough at the first measurement (/30+α min)

Figure 4B

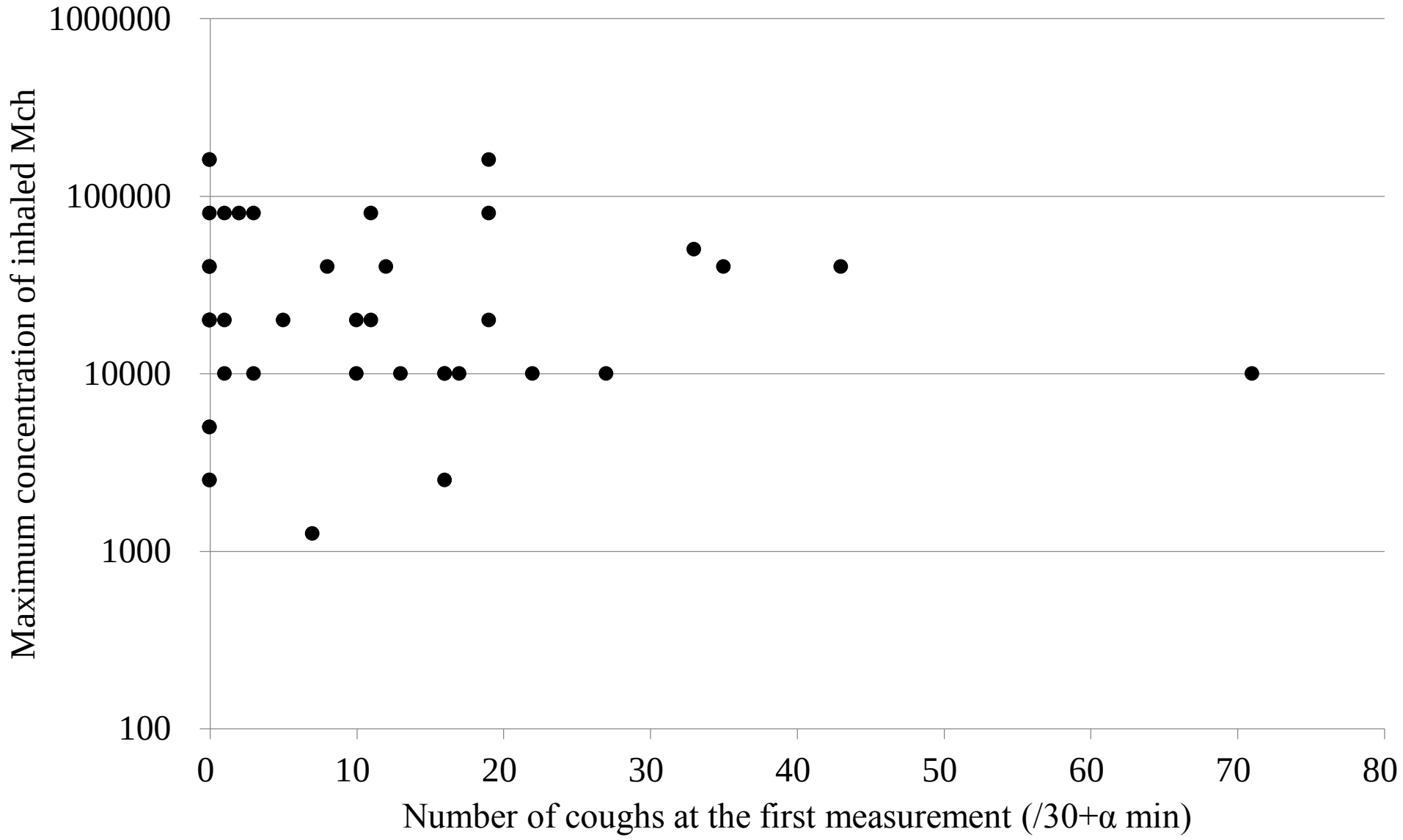
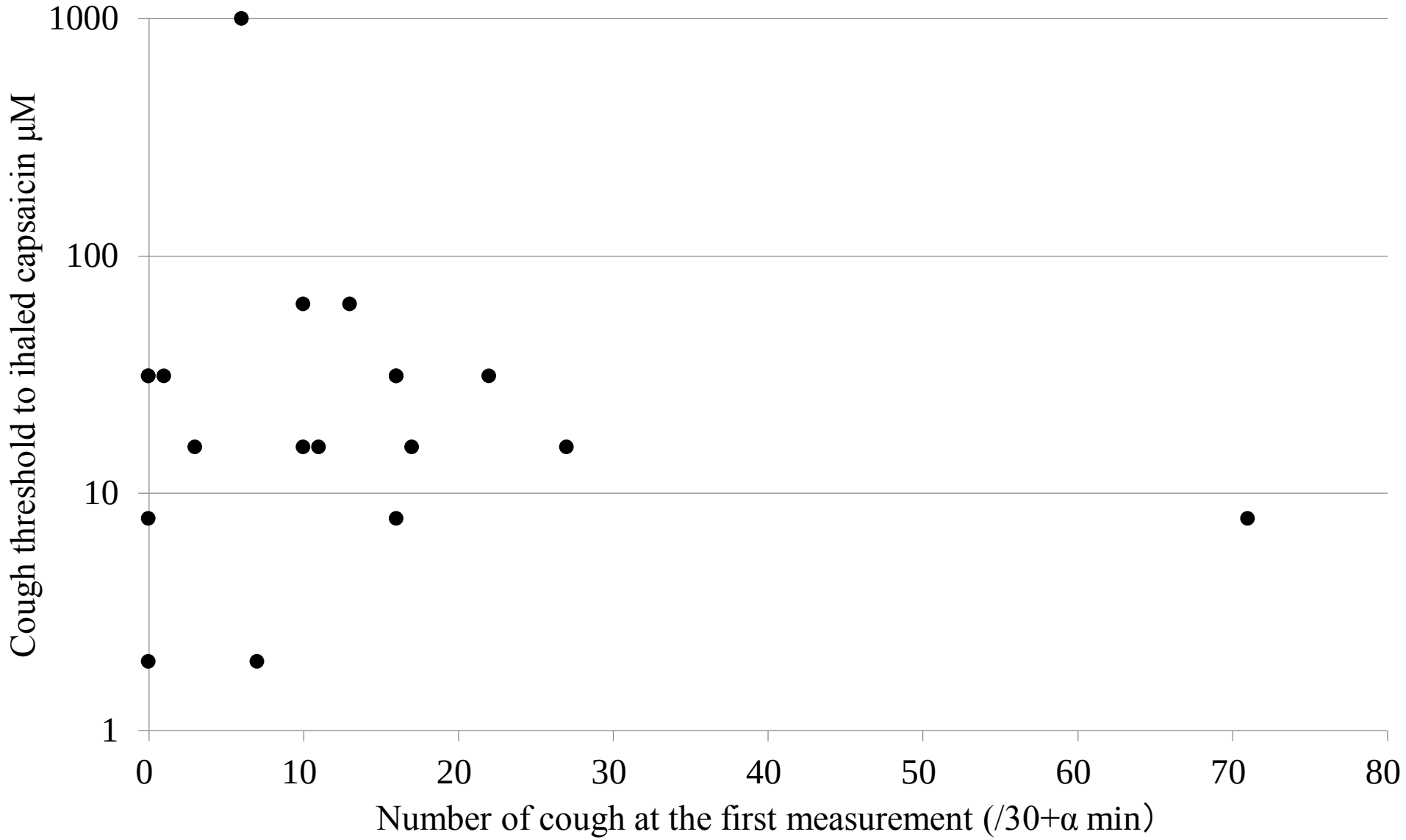

1000000
100000
10000
1000
100
Maximum concentration of inhaled Mch
0
10
20
30
40
50
60
70
80
Number of coughs at the first measurement (/30+α min)

Figure 5


1000
100
10
1
Cough threshold to ihaled capsaicin μM
0
10
20
30
40
50
60
70
80
Number of cough at the first measurement (/30+α min）