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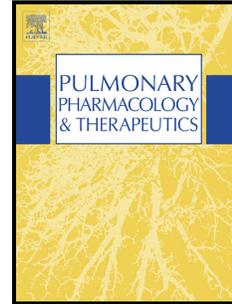
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- 1 SHR; sensory hyperreactivity
- 2 TRP channel; transient receptor potential ion channel
- 3 TRPV1; transient receptor potential channel vanilloid subunit 1
- 4 TRPM8; transient receptor potential melastin subunit 8
- 5
- 6
- 7
- 8
- 9

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1 **Abstract**

2 Down-regulation of cough sensitivity in humans is rarely discussed in terms other than  
3 pharmacological treatment of cough or hypersensitive cough reflex. Chronic cough and  
4 increased cough sensitivity could be due to a number of airway and other diseases. When such  
5 conditions are excluded, there still remains a group of patients with no evident medical  
6 explanation for persistent coughing; such patients are often described as having “chronic  
7 idiopathic cough”.

8           The aim of this study was to use a standardized eucapnic dry air provocation  
9 among patients with chronic idiopathic cough in order to study physiological parameters and  
10 measure their possible influence on capsaicin cough sensitivity. Fourteen female patients with  
11 chronic idiopathic cough and ten healthy controls underwent a capsaicin inhalation  
12 provocation on two occasions. In all patients, irritating environmental factors were known to  
13 induce cough and airway symptoms. One of the two capsaicin provocations was preceded by  
14 a eucapnic dry air provocation. Number of coughs, spirometry, respiratory rate, pulse rate,  
15 end-tidal CO<sub>2</sub>, and oxygen saturation by pulse oximetry (PSaO<sub>2</sub>) were registered and  
16 compared. The patients showed increased capsaicin sensitivity compared with the control  
17 subjects. This sensitivity was decreased when the capsaicin test was preceded by a eucapnic  
18 dry air provocation. Before the dry air provocation and after the capsaicin provocations, end-  
19 tidal CO<sub>2</sub> was decreased among the patients in comparison with the controls. After dry air  
20 provocation, spirometry values remained unchanged.

21 The results suggest that in patients with chronic idiopathic cough, physiological down-  
22 regulation of the cough sensitivity is possible with a eucapnic dry air provocation.

23

24 **Key words:** capsaicin, chemical sensitivity, dry air, cough, hyperventilation, sensory  
25 hyperreactivity

## 1 **1. Introduction**

2 Causes of down-regulation of cough in humans are only rarely discussed in non-  
3 pharmaceutical terms [1,2]. Several disorders and conditions are followed by cough and  
4 cough reflex hypersensitivity, sometimes to such an extent that the quality of life is affected  
5 more than by the original illness [3]. On the other hand, an impaired cough reflex may be an  
6 expression of various central nervous disorders, such as stroke, coma, and Parkinson's  
7 disease, but little is known about the underlying mechanisms of these down-regulations [4-6].  
8 Furthermore, it has been reported that several conditions or situations, like acute hypoxia,  
9 hypercapnoea, sleep, and anaesthesia, may down-regulate the cough reflex, though most  
10 studies have thus far involved animals [2,7-10]. There seem to be no definitive studies on the  
11 effect of exercise on cough though there are anecdotal impressions that cough are inhibited  
12 during exercise [2].

13  
14 Coughing is a common clinical problem. When it persists for more than 2 months, it is  
15 regarded as chronic, though the definition of "chronic cough" varies in the literature [11]. The  
16 pathophysiology behind coughing is fairly well understood in a number of conditions, but it is  
17 not always possible to attribute a persistent cough to an airway disorder or any other medical  
18 disorder. In some cases, chronic cough is related to reflux and indigestion [12], and some  
19 authors claim a postnasal drip syndrome as a common reason for enduring cough [13]. Still,  
20 there remains a group of patients with no obvious explanation for their coughing; these  
21 patients are often described as presenting with chronic idiopathic cough (CIC) [14,15].  
22 Patients with CIC are commonly referred to as middle-aged women with an increased cough  
23 reflex sensitivity and there is an association with organ specific autoimmune disease in some  
24 patients with CIC [16].

25

1 Previous studies have described patients with upper and lower airway symptoms induced by  
2 chemicals and scents [17-24]. Inducing factors might include perfume, flowers, cleaning  
3 agents, car exhaust fumes, and cigarette smoke. Steroids,  $\beta_2$ -agonists, and other  
4 pharmacological treatment for asthma or allergy have weak effects or none at all. Common  
5 symptoms are heavy breathing, difficulty getting air, cough, hoarseness, phlegm, nasal  
6 blockage, rhinorrhea, and eye irritation. These symptoms are often misinterpreted as asthma  
7 and/or allergy, but some patients also report symptoms such as sweating, headache, dizziness  
8 and fatigue. These patients have been suggested to be suffering from airway sensory  
9 hyperreactivity (SHR), since they have increased cough sensitivity to inhaled capsaicin,  
10 which is known to reflect the sensory nerve reactivity of the airways and of the transient  
11 receptor potential vanilloid subunit 1 (TRPV1) [17,25]. Cough is a salient symptom in this  
12 group of patients, mainly middle-aged females, and many of them could also be diagnosed  
13 with chronic idiopathic cough, though SHR is not included in the classic CIC panorama  
14 [22,23].

15  
16 SHR is a frequent problem that affects more than 6% of the adult population in Sweden [21].  
17 Findings of changed levels of nerve growth factor in nasal secretions after capsaicin  
18 inhalation indicate a neurochemical imbalance in the airways [26].

19  
20 The TRPV1 receptor is activated not only by capsaicin but also by different noxious stimuli,  
21 as well as heat, lipids, and protons [27-29]. An increased expression of TRPV1 is found in  
22 several conditions characterized by general hypersensitivity, such as thermal hyperalgesia,  
23 vulvodinia, rectal hypersensitivity, oesophagitis, and chronic cough [30-34]. TRPV1 is often  
24 regarded as an integrator of the thermosensitive members of the group I of the TRP family  
25 [28,35]. Ambient temperatures are sensed by TRPV3 and TRPV4, which are also

1 mechanosensitive and activated by changes in osmotic cell swelling [36-38].

2

3 The possibility that hyperpnoea and exercise could down-regulate cough sensitivity may  
4 indicate involvement of TRPV4 in the airways as an effect of osmotic changes. In  
5 consequence with the known up-regulation of the TRPV1 in SHR, an increased expression of  
6 TRPV4 could be followed by an augmented down-regulation of the cough reflex compared to  
7 healthy subjects.

8

9 The aim of this study was to use a standardized dry air provocation among patients with SHR  
10 and chronic idiopathic cough in order to study different physiological parameters and to  
11 measure the possible influence of eucapnic dry air ventilation on capsaicin cough sensitivity  
12 in these patients and in healthy subjects.

13

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## 1 **2. Materials and methods**

### 2 *2.1. Subjects*

3 The study group included 14 consecutively recruited non-smoking patients, all women, aged  
4 between 43 and 64 years with a mean age of 56 (95% confidence interval [CI] 52–60). They  
5 had been referred to an asthma outdoor clinic because of cough and other symptoms  
6 suggesting asthma or allergy, and were screened using a questionnaire on airway symptoms in  
7 response to chemicals and scents. They all had a history of at least 1 year (mean 11, 95% CI  
8 7–15) of upper and lower airway symptoms induced by irritating environmental stimuli like  
9 perfume, flowers, cleaning agents, car exhaust fumes, and cigarette smoke. All had daily or  
10 weekly problems with cough, meeting the criteria of chronic cough [11]. All had previous  
11 positive reactions to the capsaicin inhalation test, as described by Johansson et al. and fulfilled  
12 the criteria for SHR [25]. Within the last three years, all patients had tested negative to the  
13 skin prick test with a standard panel of 10 allergen sources common to Sweden, and  
14 methacholine tests administered according to international guidelines were negative [39,40].  
15 None of the participants showed bronchial reversibility or had a history of cardiac disease,  
16 gastroesophageal reflux, or post-nasal drip.

17  
18 The control group consisted of ten subjectively healthy non-smoking women aged between 46  
19 and 63 years with a mean age of 53 (95% CI 48–59). They were likewise screened using  
20 questions on airway symptoms and symptoms in response to chemicals and scents. None of  
21 the controls had a history of symptoms in response to scents, chemicals, cold air, allergy, or  
22 asthma. No further medical examination was performed.

23  
24 Informed consent was obtained from all subjects at the start of the investigation. The study  
25 was approved by the Regional Ethical Review Board of Gothenburg, Sweden.

1

2 *2.2. Protocol*

3 Each participant visited the clinic twice, and was randomized to either a standardized  
4 eucapnic voluntary hyperventilation (EVH) test followed by a capsaicin test, or a capsaicin  
5 test only. Patients were asked to take no medication for a minimum of 12 hours before any  
6 test. Long-acting  $\beta_2$ -agonists were withheld for at least 72 hours. Provocations were not  
7 carried out in subjects who had experienced respiratory infections during the previous month.

8

9 Cough registration was performed manually during the provocations, by the same investigator  
10 in all cases. Cough was defined as the characteristic sound that follows a forced expiratory  
11 effort against a closed glottis, and distinguished from other sounds such as clearing the throat  
12 [41,42].

13

14 *2.3. Eucapnic voluntary hyperventilation test*

15 Using a mouthpiece (with a nose clip on), the participants hyperventilated a mixture of  
16 compressed, dry gas including 20.1% O<sub>2</sub> and 4.98% CO<sub>2</sub>-balanced N<sub>2</sub> for 6 minutes. The test  
17 was conducted in a seated position with an effort mimicking hard physical exercise. Prior to  
18 the test, subjects were coached to maintain the target balloon of 5.4 L half full with air for the  
19 duration of the test. Before the EVH test and 0, 5, and 10 minutes after, respiratory rate, pulse  
20 rate, end-tidal CO<sub>2</sub>, and oxygen saturation (PSaO<sub>2</sub>) were registered in all participants by  
21 capnography and pulse oximetry (OSCARoxy Multigas Monitor and Pulse Oximeter  
22 SCO123.05.EN; Datex-Ohmeda Division Instrumentarium Corporation, Helsinki, Finland).

23 Also before the EVH test, each participant performed a forced expiratory volume test in 1  
24 second (FEV<sub>1</sub>) using a spirometer (Vitalograph, Buckingham, UK). On both occasions, FEV<sub>1</sub>  
25 values showed less than 5% difference between each test. The highest value of FEV<sub>1</sub> was

1 multiplied by 30 to obtain the target value of 85% MVV (maximum voluntary ventilation)  
2 flow in the Aiolos Asthma Test for EVH (Aiolos Medicinsk Teknik, Karlstad, Sweden),  
3 administered in accordance with international guidelines [39,40].

4

5 The total number of induced coughs was counted for the 6 minutes duration of the EVH test  
6 and during 10 minutes after the dry air provocation.

7

8 Post-challenge FEV<sub>1</sub> testing was conducted 0, 5, and 10 minutes after the test, and followed  
9 immediately by the capsaicin provocation test.

10

#### 11 *2.4. Capsaicin provocation*

12 Three concentrations of capsaicin, 0.4, 2.0, and 10.0  $\mu\text{mol/L}$ , were prepared from a stock  
13 solution of capsaicin (1 mmol/L in ethanol (99.5%); Sigma-Aldrich Sweden AB, Stockholm,  
14 Sweden) in accordance with a previous study [23]. Capsaicin was nebulized (Pari Boy 36,  
15 type 37.0130; Paul Ritzau Pari-Werk GmbH, Starnberg, Germany) and inhaled through a  
16 mouthpiece (Pari Inhalierboy, No. 36.75) by tidal volume breathing; a nose clip was not used.  
17 Saline (1 mL) and the three increasing concentrations of capsaicin (0.4, 2.0, and 10.0  $\mu\text{mol/L}$ )  
18 in a 1 mL solution were inhaled to completion, or for a maximum of 6 minutes, in order to  
19 induce coughing; this was followed by a 4-minute rest period. The total number of induced  
20 coughs was counted for 10 minutes from the onset of each inhaled capsaicin concentration  
21 [23]. The total duration of each capsaicin inhalation provocation was approximately 35  
22 minutes. Out of consideration for the patients, provocation was stopped if one concentration  
23 of capsaicin provoked more than 50 coughs. Forced expiratory volume in 1 second was  
24 measured before and after each provocation, and the higher of two values was recorded.

25

1 2.5. *Statistical analysis*

2 The Mann-Whitney *U*-test was used for non-paired data and the Wilcoxon signed-rank test  
3 was used for paired data. Data are presented as means with 95% CIs. Results were considered  
4 significant if  $p < 0.05$ . All data were analyzed using StatView 5.0.1.0 (SAS Institute, Inc.,  
5 Cary, NC, USA) and SPSS 13.0 (SPSS, Inc., Chicago, IL, USA).

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### 1 3. Results

#### 2 3.1. Eucapnic voluntary hyperventilation test

3 All participants performed the dry air provocation according to the protocol. The mean target  
4 ventilation (85% of MVV) among the patients was 66 L/min (95% CI 61–72) and did not  
5 differ from that among the controls, 66 L/min (95% CI 63–70). Recordings of FEV<sub>1</sub>,  
6 respiratory rate, pulse rate, end-tidal CO<sub>2</sub>, and PSaO<sub>2</sub> are reported in Table 1. There were no  
7 significant changes in either FEV<sub>1</sub> or PSaO<sub>2</sub> in either of the groups. Pulse rate was  
8 significantly increased in patients ( $p<0.05$ ), but not in controls, directly after the EVH test as  
9 compared with before. Both before and directly after the EVH test, respiratory rate and pulse  
10 rate did not differ between the groups. Compared with the measurements taken before the  
11 EVH test, the patients, but not the controls, still had a significantly higher respiratory rate  
12 after 5 ( $p<0.01$ ) and 10 minutes ( $p<0.05$ ). With regard to end-tidal CO<sub>2</sub>, the patients had  
13 significantly lower values compared to the controls before the EVH test ( $p<0.05$ ) (Table 1).

14  
15 In the patient group, the mean number of coughs during the 6 minutes EVH test was 18 (95%  
16 CI 5–32), while among the controls, one subject coughed three times and another one time  
17 ( $p<0.01$ ). During the 10 minutes after the dry air provocation, before the onset of the  
18 capsaicin provocation, the mean number of coughs among the patients was 29 (95% CI 19–  
19 39), compared with 12 among the controls (95% CI 0–27) ( $p<0.01$ ).

#### 21 3.2. Capsaicin provocations

22 Results of the provocations with the three concentrations of capsaicin (0.4, 2.0, and 10.0  
23  $\mu\text{mol/L}$ ) are presented in Figures 1 (patients) and 2 (controls). On 10.0  $\mu\text{mol/L}$  capsaicin  
24 seven of the 14 patients coughed more than 50 times in the tests *without* a preceding dry air  
25 provocation, and two coughed more than 50 times *with* preceding dry air; the capsaicin

1 provocation was stopped in all these cases. In accordance with dose-response relationships  
2 seen in several earlier studies using the same capsaicin provocation model [17,20,24,43], the  
3 missing data for those patients whose provocations were halted due to excess coughing were  
4 filled in by doubling the number of coughs evoked by 2.0  $\mu\text{mol/L}$  capsaicin to represent the  
5 cough response to 10  $\mu\text{mol/L}$  capsaicin. All individuals in the control group completed the  
6 capsaicin tests in accordance with the protocol on both occasions.

7

8 Recordings of FEV<sub>1</sub>, respiratory rate, pulse rate, end-tidal CO<sub>2</sub>, and PSaO<sub>2</sub> after capsaicin  
9 challenge with a preceding dry air provocation are reported in Table 1. There were no  
10 significant changes in FEV<sub>1</sub>, pulse rate, or PSaO<sub>2</sub> in either of the groups on either of the two  
11 test occasions.

12

13 The patients had significantly higher respiratory rate ( $p<0.05$ ) before the capsaicin  
14 provocation with preceding EVH compared to the capsaicin provocation without EVH [mean  
15 value 20 (95% CI 17–23) and 16 (95% CI 14–19) respectively]. Also among the control  
16 subjects respiratory rate was higher ( $p<0.01$ ) when the capsaicin provocation was preceded by  
17 EVH [mean value 16 (95% CI 14–18) and 13 (95% CI 12–14) respectively]. Otherwise  
18 respiratory rate did not differ significantly between the two capsaicin provocations in either of  
19 the groups.

20

21 The patient group coughed significantly more than the control group with all three  
22 concentrations of capsaicin, both with ( $p<0.01$ ) and without ( $p<0.01$ ) the preceding dry air  
23 challenge. Patients also differed significantly from the controls in terms of higher respiratory  
24 rate ( $p<0.01$ ) and lower end-tidal CO<sub>2</sub>, after both the capsaicin provocations ( $p<0.05$ ).

25

1 On the concentration of 0.4  $\mu\text{mol/L}$  capsaicin, the patients' mean number of coughs was 14  
2 (95% CI 5–23) without preceding EVH, and 10 (95% CI 4–17) when combined with dry air  
3 challenge (*ns*). The corresponding values for the concentration of 2.0  $\mu\text{mol/L}$  capsaicin were  
4 43 (95% CI 31–55) and 28 (95% CI 18–38) respectively ( $p < 0.05$ ), while those for 10  $\mu\text{mol/L}$   
5 capsaicin were 87 (95% CI 65–109) and 57 (95% CI 46–69) respectively ( $p < 0.05$ ) (Figure  
6 1).

7  
8 The mean number of coughs in the control group was 0 without or with preceding EVH test  
9 on the concentration of 0.4  $\mu\text{mol/L}$  capsaicin. The corresponding values for the concentration  
10 of 2.0  $\mu\text{mol/L}$  capsaicin were 9 (95% CI 5–14) and 9 (95% CI 4–15) respectively (*ns*), and  
11 those for 10  $\mu\text{mol/L}$  capsaicin were 22 (95% CI 12–32) and 27 (95% CI 15–39) respectively  
12 (*ns*) (Figure 2).

13

#### 1 4. Discussion

2 The main finding of this study was that after eucapnic dry air provocation, capsaicin cough  
3 sensitivity was decreased in a group of patients with chronic idiopathic cough and airway  
4 sensory hyperreactivity, while no such changes were seen in the control group. Furthermore,  
5 the patients had a general tendency to lower end-tidal CO<sub>2</sub> compared to the controls, and there  
6 was a significant difference before the dry air provocations. The patients also had higher  
7 respiratory rate and lower end-tidal CO<sub>2</sub> after capsaicin provocations.

8  
9 The tidal breathing method of capsaicin challenge used in the present study was developed for  
10 patients with SHR; it has been modified over time, and has shown good reproducibility  
11 [17,22,23,25]. Another common test for cough sensitivity with capsaicin inhalation involves a  
12 “single-breath” technique using increasing concentrations of capsaicin until the individual  
13 coughs, for example, two or five times (C2 or C5); this has also shown good reproducibility  
14 [44,45]. In the present study, the patients but not the control subjects displayed a down-  
15 regulation of the capsaicin cough sensitivity. A more sensitive method such as the single-  
16 breath technique may have also shown a change among the controls, and this protocol will be  
17 used in future studies with larger groups of patients and controls in accordance with ERS  
18 guidelines [42].

19  
20 The present findings suggest that hyperreactive cough reflexes from capsaicin are probably  
21 also linked to increased reactivity of other, osmo-sensitive, TRP channels, and there may be  
22 an interplay between the different TRP channels and their respective stimuli. Earlier findings  
23 that SHR patients have increased cough response to capsaicin after exercise in cold air [46]  
24 may indicate an up-regulation of the cold-sensing TRPM8; these results have now been  
25 proven not to be transferable to the more pure osmotic stimulation of an EVH test. In the light

1 of earlier suggestions of a down-regulated cough reflex after hyperpnoea and exercise [2], we  
2 hypothesize that this mechanism is dependent on TRPV4 activation. In SHR patients, who are  
3 already known to have an augmented TRPV1 response, a corresponding up-regulation of  
4 TRPV4 receptors could be followed by decreased cough sensitivity after evaporation in  
5 comparison to normal subjects.

6  
7 Addition of 5% CO<sub>2</sub> has earlier been shown to reduce the incidence and severity of airway  
8 related problems including cough during anaesthesia [7]. In animals, the reflex responses to  
9 airway irritation are known to interact with the background chemical ventilatory drive [10]. In  
10 humans, too, there are indications that changes in the CO<sub>2</sub> ventilatory drive can modify reflex  
11 responses to airway irritation since in anesthetized patients; an increase in CO<sub>2</sub> ventilatory  
12 drive and hypercapnoea decreased the degree of reflex responses to irritating stimuli [47,48].  
13 The findings, in accordance with previous animal studies may be a parallel to the results in  
14 the present study.

15  
16 The eucapnic hyperventilation in this study did down-regulate the earlier known increased  
17 cough sensitivity to capsaicin in combination with a more “normalized” end-tidal CO<sub>2</sub> in  
18 comparison to the controls. It is worth asking whether this kind of down-regulation of cough  
19 sensitivity is only present in patients with idiopathic cough problems and a tendency to  
20 hyperventilation, or whether it could be a question of dose, both regarding dry air provocation  
21 load and regarding capsaicin concentration. The cause of and mechanisms behind the  
22 demonstrated down-regulation of cough sensitivity remain unknown, and extended studies are  
23 needed.

24

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5 Giovanni Fontana, Department of Critical Care, University of Florence, Italy for valuable  
6 advices and fruitful discussions. We are grateful to Inger Winberg for excellent help with the  
7 recordings.

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1 TABLE 1.  
 2 Data on FEV<sub>1</sub>, respiratory rate, pulse rate, end-tidal CO<sub>2</sub>, and PSaO<sub>2</sub> in 14 patients with  
 3 chronic idiopathic cough and 10 healthy control subjects before and after dry air provocation  
 4 and after the following capsaicin provocation. Data are presented as means with 95%  
 5 confidence intervals (CIs).

<b>Patient group</b>	FEV <sub>1</sub> , % predicted	Respiratory rate	Pulse rate	End-tidal CO <sub>2</sub> , kPa	PSaO <sub>2</sub> , %
Before dry air provocation	103 (94-112)	15 (13-17)	75 (70-80)	5.0 (4.7-5.3)	98 (97-98)
After dry air provocation	101 (92-111)	20 (17-23)	80 (75-86)	4.4 (4.1-4.8)	98 (97-98)
After capsaicin provocation	101 (92-110)	18 (17-20)	71 (65-77)	4.3 (3.9-4.8)	97 (96-98)
<b>Control group</b>					
Before dry air provocation	98 (89-107)	14 (12-17)	74 (64-80)	5.4 (5.1-5.7)	98 (97-99)
After dry air provocation	97 (87-107)	19 (16-22)	75 (67-83)	4.8 (4.4-5.2)	98 (97-99)
After capsaicin provocation	97 (87-108)	16 (14-18)	69 (61-77)	4.9 (4.6-5.3)	98 (97-99)

6

7

1

2 **Figure Legends**

3

4 **Figure 1**

5 Mean number of coughs ( $\pm$  95% confidence intervals, CIs) after inhalation of 0.4, 2.0 and  
6 10.0  $\mu\text{mol/L}$  capsaicin in 14 patients with airway sensory hyperreactivity with or without a  
7 preceding eucapnic dry air provocation.

8

9 **Figure 2**

10 Mean number of coughs ( $\pm$  95% confidence intervals, CIs) after inhalation of 0.4, 2.0, and  
11 10.0  $\mu\text{mol/L}$  capsaicin in 10 control subjects with or without a preceding eucapnic dry air  
12 provocation.

13

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2

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ACCEPTED MANUSCRIPT

Fig 1

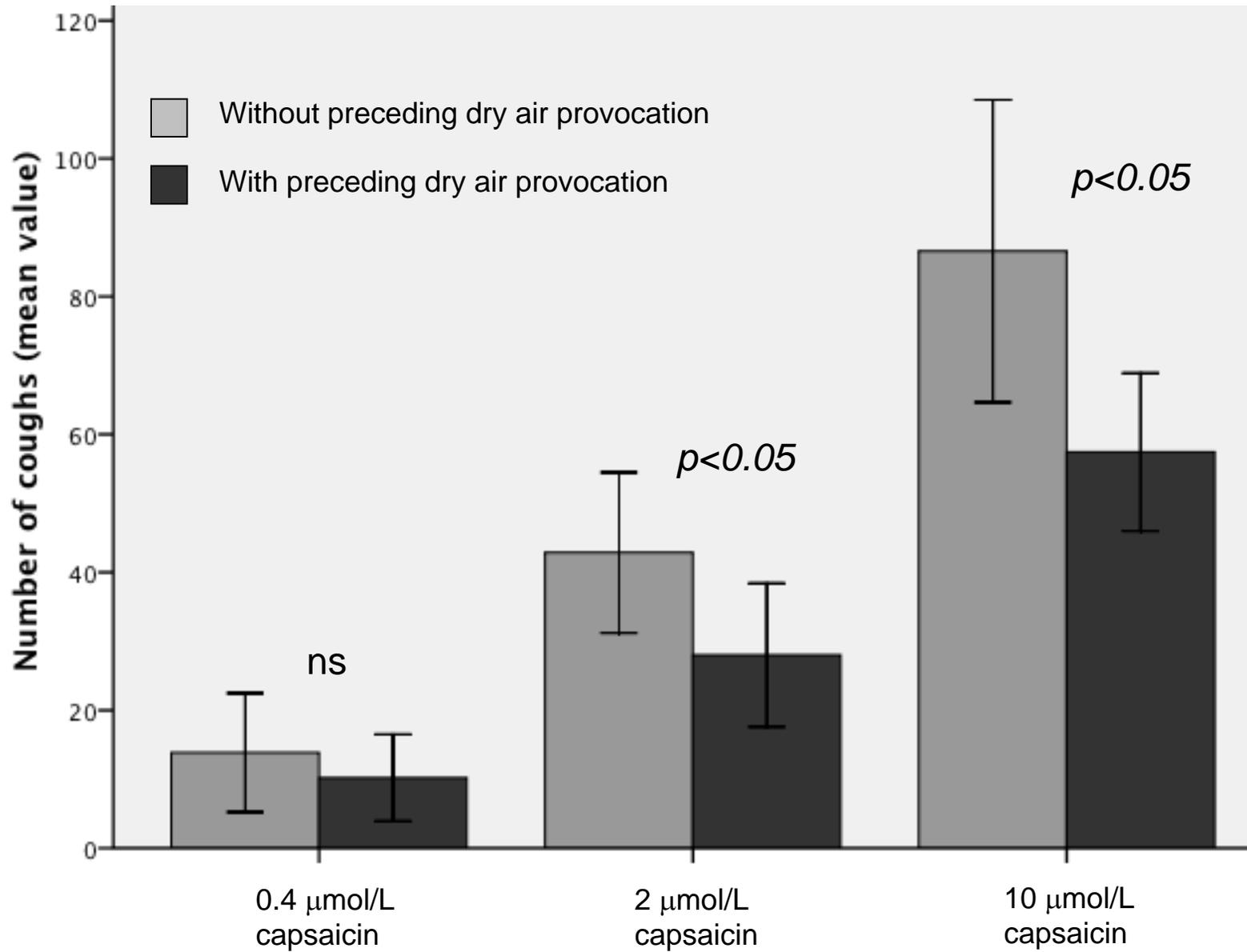


Fig 2

