TNF and IL-1β exposure increases airway narrowing but does not alter the bronchodilatory response to deep inspiration in airway segments

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Summary at a glance

It is unclear whether the attenuated bronchodilatory response to deep inspiration (DI) in asthma is related to an inflammatory environment. Using whole bronchial segments *in vitro*, we show that culture with pro-inflammatory cytokines, TNF and IL-1β, increases airway narrowing but does not affect the bronchodilatory response to DI.

Abstract

Background and objective: Whilst chronic inflammation of the airway wall and the failure of deep inspiration (DI) to produce bronchodilation are both common to asthma, whether pro-inflammatory cytokines modulate the airway smooth muscle (ASM) response to strain during DI is unknown. The primary aim of the study was to determine how an inflammatory environment (simulated by the use of pro-inflammatory cytokines) alters the bronchodilatory response to DI.

Methods: We used whole porcine bronchial segments *in vitro* that were cultured in medium containing tumour necrosis factor (TNF) and interleukin-1β (IL-1β) for 2 days. A custom-built servo-controlled syringe pump and pressure transducer was used to measure airway narrowing and to simulate tidal breathing with intermittent DI manoeuvres.

Results: Culture with TNF and IL-1β increased airway narrowing to acetylcholine but did not affect the bronchodilatory response to DI.

Conclusions: The failure of DI to produce bronchodilation in patients with asthma may not necessarily involve a direct effect of pro-inflammatory cytokines on airway tissue. A relationship between inflammation and airway hyper-responsiveness is supported, however, regulated by separate disease processes than those which attenuate or abolish the bronchodilatory response to DI in patients with asthma.

Keywords: asthma, bronchoconstriction, inflammation, cytokines and strain

Short title: TNF and IL-1β does not alter DI

Introduction

Airway hyper-responsiveness (AHR) is a primary characteristic of asthma[1] and is considered a major contributor to airflow limitation[2, 3]. The cause(s) of AHR remain unclear but may involve abnormalities in airway smooth muscle (ASM) structure/function following chronic inflammation of the airway wall present in patients with asthma[4]. Increased levels of pro-inflammatory cytokines, such as tumour necrosis factor (TNF) and interleukin-1β (IL-1β), which are released from numerous cell types, including mast cells[5], have been detected in the sputum of patients with symptomatic asthma[5-11]. Changes in ASM force production in the presence of TNF[7-10] and/or IL-1β[7, 11] could, theoretically, contribute to AHR.

More recently, the dynamic mechanical environment of the lung has been identified as an important regulator of airway responsiveness and may be susceptible to inflammatory disease processes, contributing to AHR[12]. In normal healthy individuals *in vivo*, deep inspiration (DI) produces a transient (i.e. for ~1 to 2min) reversal of bronchoconstriction (i.e. bronchodilation) to a number of different inhaled ASM contractile agonists[13-16]. However, the bronchodilatory response to DI is attenuated or abolished in patients in asthma[14-16]. The underlying mechanism by which DI produces bronchodilation is thought to involve a strain-induced decrease in ASM force[17-21], due to perturbed cross-bridge binding[19, 20] and/or de-polymerisation of the contractile apparatus[22]. Whilst chronic inflammation of the airway wall and the failure of DI to produce bronchodilation are both common to asthma, whether pro-inflammatory cytokines modulate the ASM response to strain and therefore, the bronchodilatory response to DI, is unknown.

The primary aim of the study was to determine how an inflammatory environment (simulated by the use of pro-inflammatory cytokines) alters the bronchodilatory response to DI, in a manner similar to how airway narrowing is expected to increase and contribute to AHR. We hypothesised that culturing airway segments in medium containing TNF and IL-1β for 2 days would decrease the bronchodilatory response to DI.

Methods

Animal handling and bronchial segment preparation

All animal experiments conformed to institutional ethics and animal care unit regulations (Animal Ethics Committee, University of Western Australia, Crawley, WA, Australia). Bronchial segments, ~25mm long, were dissected from male White Landrace pigs (n=18, ~35kg)[21, 23, 24]. The mode generation was 18 at the distal and 12 at the proximal end (where the generation of the trachea=0), with an internal diameter of ~2mm at the distal and ~3mm at the proximal ends.

Bronchial segment culture

Prior to culturing, cannulated bronchial segments were washed in bovine serum albumin containing 1% penicillin/streptomycin, 0.5% gentamycin and 1% Amphotericin B under sterile conditions. Bronchial segments were cultured in Dulbecco's modified Eagle medium (DMEM) containing 1% penicillin/streptomycin, 0.5% gentamycin, 1% L-glutamine and 2% foetal bovine serum (FBS) at 37°C with 5% CO_2, 5% humidity. The culture medium was replaced every 24hr to remove the effect of metabolites produced by the tissue. In the TNF+IL-1β treatment group, the culture medium always contained human TNF (100ng/mL) and human IL-1β (20ng/mL). The use of TNF and IL-1β was based on a systematic literature search, which identified these pro-inflammatory cytokines as playing a potentially important role in asthma pathophysiology.

Organ bath and syringe pump

The bronchial segment was mounted horizontally in an organ bath, connected to a liquid filled servo-controlled syringe pump and pressure transducer, used to measure lumen

volume and to apply fixed-transmural pressure (P_{tm}) oscillations (i.e. tidal breathing and DI manoeuvres)[21, 25, 26] (Figure 1).

Protocol 1: Contractile response of bronchial segments before and after culture

Airways were studied in the organ bath fresh (i.e. within ~1hr of being dissected and prior to culture) and again on day 2 of culture without pro-inflammatory cytokines (n=6). Small fixed-P_{tm} oscillations, simulating tidal breathing ($\Delta 5cmH_2O$ at 0.25Hz), were also briefly applied in the relaxed state to calculate the specific compliance of the airway wall. Following oscillation, airway narrowing dose-response curves (DRC) were constructed to ACh (10^{-7} to $3x10^{-3}$M) under static conditions ($5cmH_2O$ P_{tm}).

Protocol 2: Effect of TNF and IL-1β on bronchodilation to DI airway narrowing

Airways were studied on day 2 of culture in the control (left or right lower lobe) and TNF+IL-1β treatment group (alternate lower lobe, n=6 per group). DRCs were constructed to ACh (10^{-7} to $3x10^{-3}$M) under both static ($5cmH_2O$ P_{tm}) and oscillatory conditions in a randomised order, separated by 45min. The oscillatory protocol comprised tidal breathing ($\Delta 5cmH_2O$ at 0.25Hz) and intermittent DI manoeuvres ($\Delta 25cmH_2O$, a 2s inflation, a 2s hold at the peak of inflation and a 2s deflation) applied once contraction at each dose of ACh had plateaued[21]. To assess whether any changes in airway narrowing observed with TNF and IL-1β were as a result of increased ASM force, in a separate group of airways, DRCs were constructed to ACh under isovolumic conditions to measure active lumen pressure (proportional to ASM tension).

Morphometry

After experimentation, bronchial segments were fixed in 4% formaldehyde solution and processed into paraffin blocks. Transverse sections (5μm) were stained with haematoxylin and eosin. The internal lumen perimeter (P_i) and area (A_i), the area enclosed by the outer ASM perimeter (A_{mo}) and area of the ASM layer[27] were measured using ImageJ (version 1.45j, National Institutes of Health, MD, U.S.A.). The area of the ASM layer was expressed as √area of the ASM layer/P_i.

Analysis and statistics

The volume of the relaxed airway lumen was measured by the volume that could be withdrawn until closure at 5cmH_2O P_{tm}[28]. Airway narrowing to ACh was expressed as %lumen volume. Active lumen pressure to ACh was measured from the change in P_{tm}. Dynamic measurements were made at the troughs of the pressure cycle. Maximum response (E_{max}) and sensitivity (PD_2=–$\log_{10}$, dose producing 50% E_{max}) to ACh was calculated from variable slope sigmoidal curves fitted to individual airways. Specific compliance was calculated from volume strain (Δvolume/lumen volume)/ΔP_{tm}[24, 28, 29].

The ASM strain during breathing manoeuvres (ΔP_{tm}) was calculated from the trough to peak change in the outer ASM perimeter (P_{mo}) during DI as a proportion of the P_{mo} immediately prior to DI. The P_{mo} of the bronchial segment in the organ bath was calculated as previously described[21, 25, 26] and assumes inner wall area is constant at all P_{tm}, that P_{mo} is circular and that the lumen is cylindrical. The bronchodilatory response to DI was defined as %reversal of airway narrowing to ACh measured immediately after DI[24, 28, 29]. Bronchodilation to DI was not calculated at doses ($\leq 3x10^{-6}$M), which produced minimal airway narrowing.

Differences between groups were analysed using paired t-tests and 2-way ANOVA. Data analysis and statistical tests were performed using Statistica (version 8.0; StatSoft, Tulsa, OK, U.S.A.) and GraphPad Prism (version 5.0d; GraphPad Software, La Jolla, CA, U.S.A.). Data are presented as means±SEM, where n=number of animals.

Results

Protocol 1: Contractile response of bronchial segments before and after culture

After 2 days in culture without pro-inflammatory cytokines, there was a non-significant decrease in E_{max} ($p=0.11$) but no difference in PD_2 (Figure 2). There was no difference in specific compliance after culture (fresh before culture $0.010\pm0.0024 cmH_2O^{-1}$; after $0.017\pm0.0048 cmH_2O^{-1}$). Histological examination revealed denuding of the airway epithelium after culture.

Protocol 2: Effect of TNF and IL-1β on bronchodilation to DI airway narrowing

Following 2 days of culture in medium containing TNF+IL-1β, there was no difference in lumen volume, P_i, area of the ASM layer or specific compliance of the airway wall in the relaxed state, compared to the control group (Table 1). Under static conditions, airway narrowing (Figure 3A) and active lumen pressure (Figure 3B) E_{max} was greater in the TNF+IL-1β treatment group, compared with the control group. There was no difference in PD_2 between groups.

Airways stiffened strongly in response to ACh (Figure 4A). Further, when expressed as the change in specific compliance from the relaxed state, airway wall stiffening to ACh was greater in the TNF+IL-1β treatment group, compared with the control group (Figure 4B).

The primary aim of the study was to determine how an inflammatory environment (simulated by the use of pro-inflammatory cytokines) alters the bronchodilatory response to DI. Scatter plots of bronchodilation measured immediately after DI against

the ASM strain produced by DI had linear lines-of-best-fit fitted to individual airways (Table 2). The average linear lines-of-best-fit are shown in Figure 5A. Bronchodilation was positively correlated with ASM strain for all airways in both groups (i.e. greater bronchodilation with increasing ASM strain during DI). However, there was no difference in the average intercept or slope of the lines fitted between bronchodilation and ASM strain between groups. Bronchodilation to DI fell substantially with increasing dose of ACh (Figure 5B). There was also no difference in the bronchodilatory response to DI between groups at comparable doses of ACh. This result was the same whether bronchodilation was measured immediately after DI or at the peak of DI.

Discussion

In addition to AHR, a reduced bronchodilatory response to DI is now believed to play an important role in asthma pathophysiology. A reduced bronchodilatory response to DI has recently been shown to correlate with the perception of dyspnoea in subjects with asthma[29], although the mechanism for the dysfunction is unknown. The present study determined how pro-inflammatory cytokines, TNF and IL-1β, alters the bronchodilatory response to DI in bronchial segments. Results show that whilst culture with TNF and IL-1β increases airway narrowing, it does not affect the bronchodilatory response to DI.

Before discussing the significance of our findings, there are aspects of the methodology that require discussion. Airway function was modelled using whole bronchial segments, which retain the normal morphology of the airway wall, tissue-to-tissue interactions and physiological loading conditions. Culturing relatively large segments of tissue for an extended period is challenging due to a potential restriction in the supply of nutrients or the removal of metabolites. There was epithelial loss after two days in culture, which likely occurred as a result of restricted luminal perfusion of nutrients. The impaired epithelial barrier did not increase airway narrowing, since in the present study contractile agonists were delivered to the serosal surface[30]. There was, instead, a non-significant decrease in maximum response to ACh after 2 days of culture, consistent with studies using ASM strips/rings[9, 11]. Although the presence of FBS is necessary to provide appropriate mitogen/survival signals, it may have contributed to the decrease in airway responsiveness[31]. Importantly, FBS was present in both the control and TNF+IL-1β treatment groups. Finally, TNF and IL-1β were used in combination to increase our chances of observing an effect on the bronchodilatory response to DI and/or airway

narrowing. The drawback of this approach is that we are therefore unable to identify the separate effects of these cytokine and it is also possible that they may operate in opposition.

Previous studies examining airway responsiveness *in vitro* following pro-inflammatory cytokines have done so under static conditions (i.e. in the absence of oscillatory ASM strain associated with breathing manoeuvres)[7-11]. Given that the dynamic mechanical environment is now considered an important regulator of airway caliber[12], we questioned whether pro-inflammatory cytokines would impact the normal response to DI. Our results showed that 2 days of culture with TNF and IL-1β did not affect ASM response to strain during DI, which suggests that inflammation may not directly alter bronchodilation to DI. It remains unclear how other pro-inflammatory cytokines, such as interleukins 2, 4, 5 and 6 (IL-2, IL-4, IL-5 and IL-6) or granulocyte macrophage colony stimulating factor (GM-CSF)[5, 6] impact bronchodilation to DI.

One limitation of our study was that due to concerns regarding tissue viability, it was not possible to extend the cytokine exposure beyond 48hrs and while this was sufficient to modify ASM force and narrowing, this does not necessarily mean the response to DI will be similarly affected. There is however evidence that the response to mechanical strain elicited by DI can be modified by inflammation within a much shorter period of exposure than that used in the present study. The response to DI in human subjects is disrupted 4hrs after ozone exposure[32], which the authors concluded was most likely due to an induced inflammatory event, previously shown to occur 3hrs after ozone exposure[33]. *In vitro*, force induced re-lengthening of isolated ASM, analogous to

bronchodilation produced by DI, was enhanced by corticosteroid treatment after only 2hrs[34]. While there is likely to be a direct effect of corticosteroids on ASM contraction[34], length oscillation of ASM increases inflammatory gene expression (including IL-1β)[35], supporting possible interaction between mechanical perturbation of the ASM, the inflammatory cascade and response to corticosteroids.

Evidence for an association between airway inflammation and response to DI in human subjects is nonetheless mixed. One study reported an inverse association between bronchodilation to DI and mast cells and $CD4^+$ lymphocytes in the airways of subjects with mild asthma, but not COPD[36], while another study showed no such relationship between any inflammatory cell in mild asthma[37]. There is evidence that the impaired response to DI in asthma can be therapeutically targeted with anti-inflammatories. Bronchodilation response to DI was improved in subjects with asthma following a two-week course of oral corticosteroids[38]. Putting our own findings in context, if the bronchodilatory response to DI is disrupted by an inflammatory cascade, our data suggests that the underlying mechanism may not be related to an alteration in the airway wall response to mechanical strain. The response to DI *in vivo* is a balance between hysteresis of the airway wall and hysteresis of the surrounding lung parenchyma[39], the latter of which reduces bronchodilation. It is therefore possible that increased parenchymal inflammation, rather than airway inflammation, contributes to a reduced bronchodilatory response to DI in subjects with asthma by increasing parenchymal hysteresis[40].

Our laboratory[21, 41] and others[42] have previously shown that ASM strain is the critical determinant of bronchodilation to DI. By use of fixed-P_{tm} DI, the amplitude of DI and the stiffness of the airway wall determine the magnitude of ASM strain. Under high levels of ASM activation where the airway wall stiffens, the magnitude of ASM strain becomes negligible and the bronchodilatory response to DI is attenuated. In both groups, bronchodilation to fixed P_{tm} DI fell substantially with increasing dose of ACh but there was no difference in the lines fitted between bronchodilation and ASM strain. The critical level of ASM strain required to produce bronchodilation was ~3%, which was not affected by culture with TNF and IL-1β. In the diseased state, a reduction in the magnitude of ASM strain produced by DI below 3% (e.g., as a result of excessive airway wall stiffening) could abolish the bronchodilatory response to DI, without necessarily modulating the relationship between ASM strain and bronchodilation. The reduced response to DI in patients with asthma may, therefore, be due to airway wall stiffening[43] possibly as a consequence of airway remodelling, and not a direct result of exposure to pro-inflammatory cytokines.

A new finding is the apparent change in the ASM response to strain during DI following 2 days of culture, compared with fresh bronchial segments in our previous study[21] (Figure 6). Increasing ASM strain produced less bronchodilation following culture (i.e. the slope of the lines fitted between bronchodilation and ASM strain was steeper in fresh bronchial segments, compared to the control group in the present study). Since we observed denudation of the airway epithelium with culture, this raises the possibility that the release of epithelial-derived mediators contributes to the bronchodilatory response to DI. Brown and Mitzner[44] showed in dogs *in vivo,*

bronchodilation to DI is abolished by inhibiting nitric oxide (NO) synthesis. However, in pilot studies, blocking NO synthesis (L-NOARG), or manual denudation of the epithelium (data not shown) did not attenuate the bronchodilatory response to DI. An alternative possibility is that there is a phenotypic change to the ASM in culture whereby the response to mechanical strain is modified, as has been proposed to occur in asthma[45]

We confirm the findings of numerous studies *in vitro*[7-11], where culture with TNF and/or IL-1β increased maximum isometric ASM force under static conditions (i.e. without breathing stresses and strains), which in the present study, was reflected by an increase in maximum active lumen pressure to ACh. The ASM force hyper-contractility following culture with TNF and IL-1β (~40% increase in maximum active lumen pressure to ACh) we found in whole bronchial segments was somewhat greater than that reported in isolated ASM strips, where there was an ~8 to ~30% increase in maximum isometric force to ACh[7, 8, 10]. Differences in pro-inflammatory cytokine-induced hyper-contractility between studies are likely explained by relative concentrations: TNF (100ng/mL) and IL-1β (20ng/mL) in the present study, compared to 10 to 100ng/mL and/or 10 to 25ng/mL, respectively, in previous studies. There are some contrary findings in the literature, such as the study by Wills-Karp and colleagues[46], who report no effect of TNF and/or IL-1β on contractile response.

We found no difference in the area of the ASM layer, suggesting that the increase in ASM force is due to an increase in ASM contractility. The ASM hyper-contractility could occur as a result of a TNF-induced increase in intra-cellular Ca^{2+} release from the

sarcoplasmic reticulum[47-49], Ca^{2+}-sensitization through the G-protein coupled receptor RhoA/Rho kinase pathway[50] and/or an IL-1β-induced up-regulation of TNF mRNA expression[11].

The underlying assumption of studies demonstrating enhanced isometric force is that this will also increase ASM shortening in the presence of normal mural mechanical afterloads. We now extend previous findings to show that the increase in ASM force due to TNF and IL-1β is sufficient to increase maximum airway narrowing by ~23% lumen volume, which was made possible using our bronchial segment model. To our knowledge, no previous studies *in vitro*, have shown an increase in airway narrowing, as opposed to isometric ASM force, following culture with pro-inflammatory cytokines. In contrast to the increase in maximum response to ACh produced by TNF and IL-1β, we found no difference in sensitivity, which is also consistent with studies using ASM strips and/or rings[8-10].

With greater cytokine-mediated ASM force production, airway stiffness is also expected to increase if there are more bound cross-bridges. An increase in airway wall stiffness will intuitively decrease the magnitude of ASM strain during DI at the same dose of ACh. While there was no difference in airway wall stiffness between groups at comparable doses of ACh, the change in specific compliance (reduction) from the relaxed state was greater in the TNF+IL-1β treatment group. Despite enhanced airway stiffening with TNF+IL-1β exposure, the bronchodilatory response to DI was unaltered at any dose of ACh.

In conclusion, culture with TNF and IL-1β increases airway narrowing but does not affect bronchodilation to DI. The failure of DI to produce bronchodilation in patients with asthma may not necessarily involve a direct effect of pro-inflammatory cytokines on airway tissue. A relationship between inflammation and airway hyper-responsiveness is supported, however, regulated by separate disease processes than those which attenuate or abolish the bronchodilatory response to DI in patients with asthma.

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Conflict of Interest

The authors declare no conflict of interests.

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Tables

Table 1. Lumen volume, internal perimeter (Pi), area of the ASM layer and specific compliance of the airway wall in the control and TNF+IL-1β treatment groups.

	Lumen Volume (μL)	**P_i (mm)**	**Area of the ASM layer ($\sqrt{mm^2}/mm$)**	**Specific Compliance (cmH_2O^{-1})**
Control Group	171.3±9.5	9.13±0.65	0.0835±0.0062	0.0129±0.0011
TNF+IL-1β Treatment Group	190.0±24.6	9.70±0.28	0.0786±0.0027	0.0185±0.0034

There was no difference in lumen volume, P_i, area/thickness of the ASM layer or specific compliance of the airway wall in the relaxed state between groups. n=6 per group. Mean±SEM.

Table 2. Slope, intercept and Pearson's correlation coefficients for scatter plots of bronchodilation against ASM strain.

Airway		**1 Left**	**2 Right**	**3 Left**	**4 Right**	**5 Left**	**6 Right**
Control	Slope	258.9	609.6	184.8	284.8	228.0	142.8
Group	Intercept	0.024	0.056	0.036	0.032	0.035	0.011
	r	0.98	0.98	0.97	0.96	0.91	0.97
	p value	0.0006	0.0007	0.0015	0.0033	0.0127	0.0011
Airway		**1 Right**	**2 Left**	**3 Right**	**4 Left**	**5 Right**	**6 Left**
TNF+IL-1β	Slope	128.9	188.9	239.3	262.6	266.5	489.4
Treatment	Intercept	0.018	0.074	0.014	0.029	0.015	0.025
Group	r	0.99	0.97	0.99	0.95	0.87	0.91
	p value	0.0002	0.0012	0.0001	0.0074	0.0243	0.0110

Bronchodilation is the %reversal in airway narrowing following DI and ASM strain is the change in P_{mo} produced by DI in the control (airway 1 to 6, left or right lower lobe) and TNF+IL-1β treatment groups (alternate lower lobe).

Figures

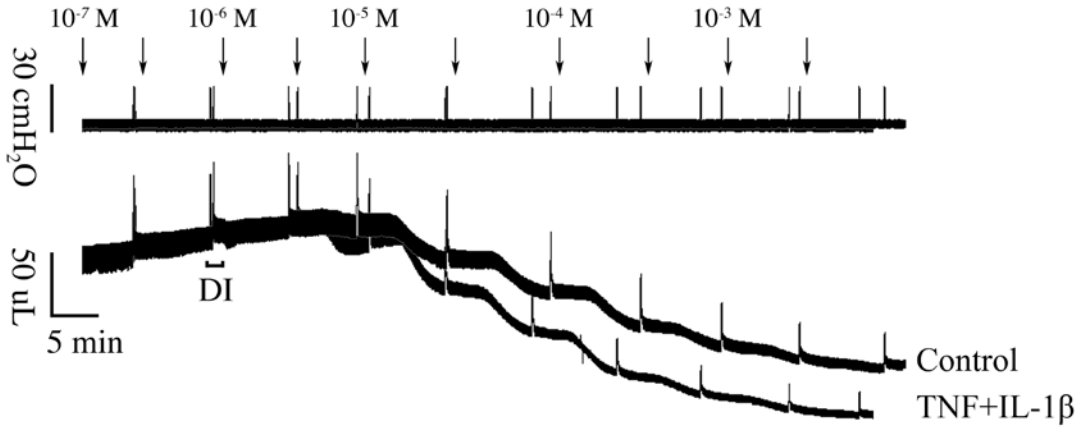


Figure 1. Example traces of transmural pressure (P_{tm}, above) and lumen volume (below) to cumulative doses of acetylcholine (ACh, 10^{-7} to $3x10^{-3}$M, *arrows*, text labels shown only for whole log doses). Fixed-P_{tm} oscillations were applied to control airways and airways treated with TNF (100ng/mL)/IL-1β (20ng/mL). Traces from the control and TNF+IL-1β treatment groups have been temporally shifted to better distinguish the curves. At the time scale shown, tidal oscillations are not visible but appear as a thick line, the thickness of which indicates the magnitude of the P_{tm} and volume oscillations. Individual DI are visible prior to the subsequent dose of ACh. In response to ACh, lumen volume decreased in a dose-dependent manner. Stiffening of the airway wall produced by ACh appears as a reduced volume oscillation in response to P_{tm} oscillation.

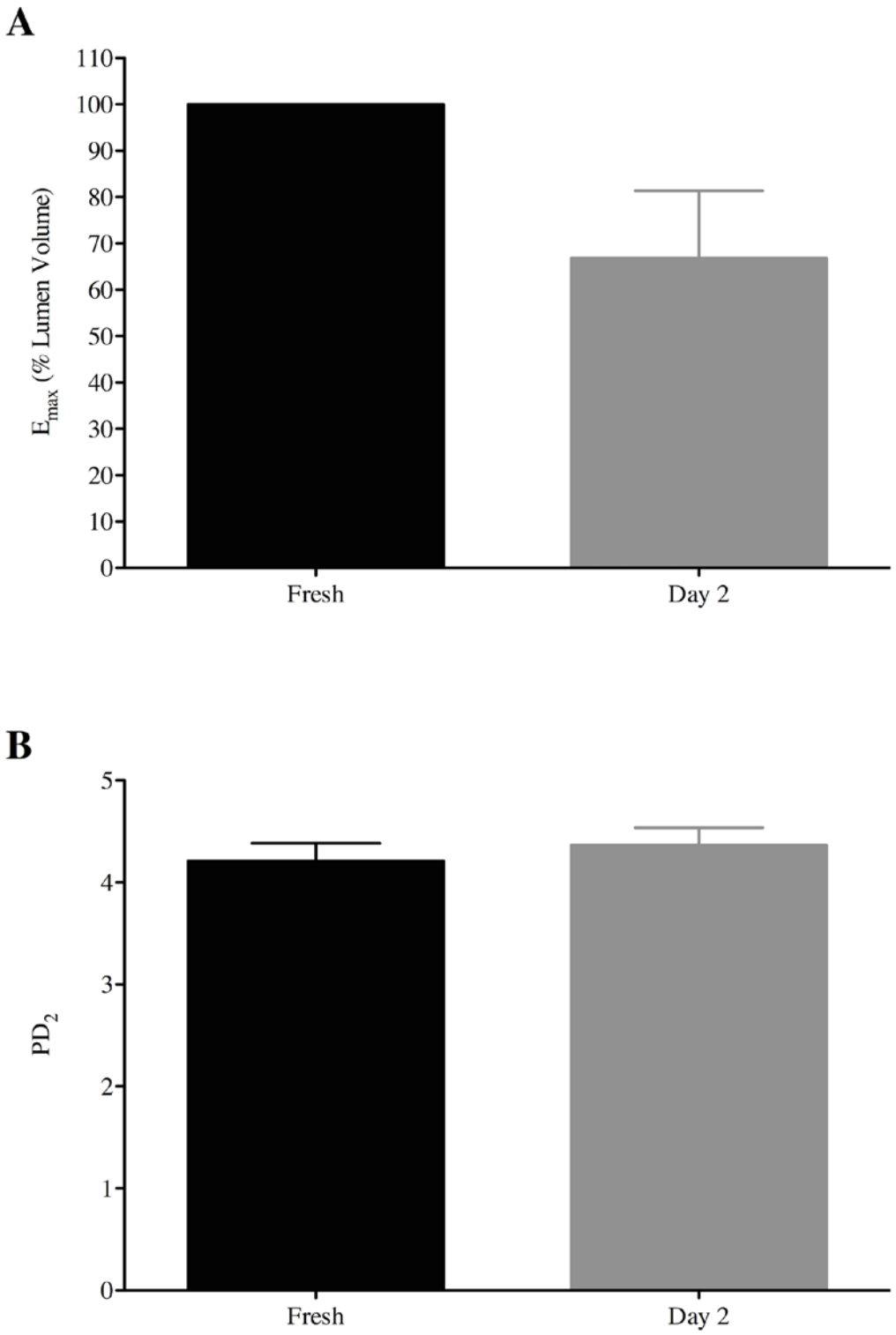
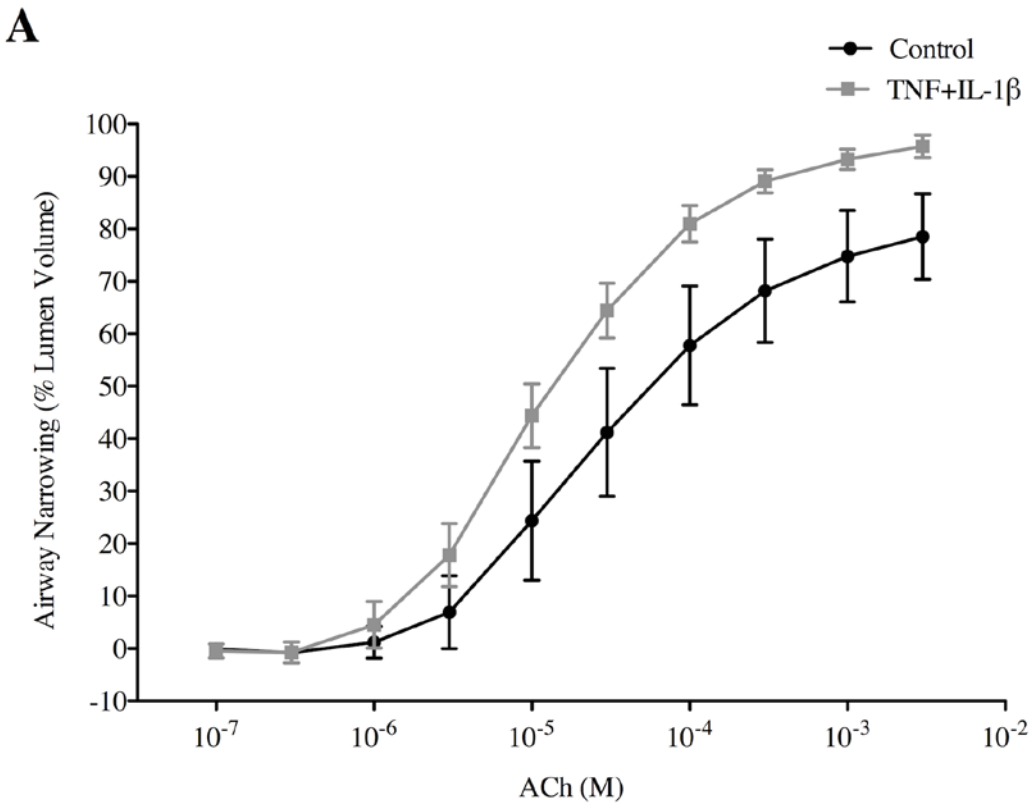


Figure 2.The effect of culture on maximum response (E_{max}, %lumen volume, A) and sensitivity (PD_2, B) to ACh under static conditions. There was a non-significant

reduction in E_{max} following 2 days of culture (66.8±14.5% lumen volume), compared with the fresh airway before culture (100.0±0.0% lumen volume i.e. airway closure in all airways, p=0.11). There was no difference in PD_2 following 2 days of culture (4.37±0.17), compared with the fresh airway before culture (4.21±0.17). n=6. Mean±SEM.


A
Control
TNF+IL-1β
Airway Narrowing (% Lumen Volume)
100
90
80
70
60
50
40
30
20
10
0
-10
10-7
10-6
10-5
10-4
10-3
10-2
ACh (M)

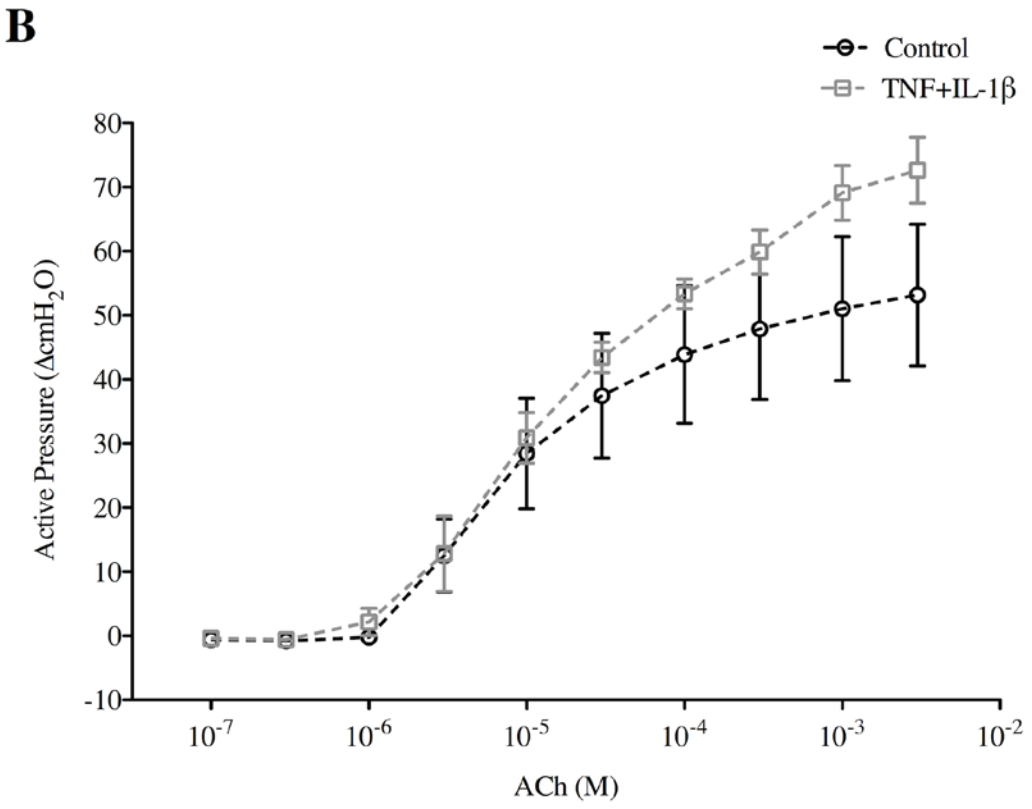

B
Control
TNF+IL-1β
Active Pressure (ΔcmH2O)
80
70
60
50
40
30
20
10
0
-10
10-7
10-6
10-5
10-4
10-3
10-2
ACh (M)

Figure 3. Cumulative DRC to ACh (10^{-7} to $3x10^{-3}$M) for airway narrowing (%Lumen Volume, A) and active lumen pressure (ΔcmH_2O, B) in the control and TNF+IL-1β treatment groups. Culture with TNF and IL-1β increased both airway narrowing (control group; 77.7±7.8% lumen volume, TNF+IL-1β treatment group; 95.3±2.1% lumen volume, $p<0.05$) and active lumen pressure (control group; 51.4±11.1cmH_2O, TNF+IL-1β treatment group; 72.2±6.0cmH_2O, $p<0.05$). There was no difference in PD_2 between groups for airway narrowing (control group; 5.54±0.22, TNF+IL-1β treatment group; 4.94±0.12) or active lumen pressure (control group; 4.95±0.26, TNF+IL-1β treatment group; 4.96±0.26). n=6 per group. Mean±SEM.

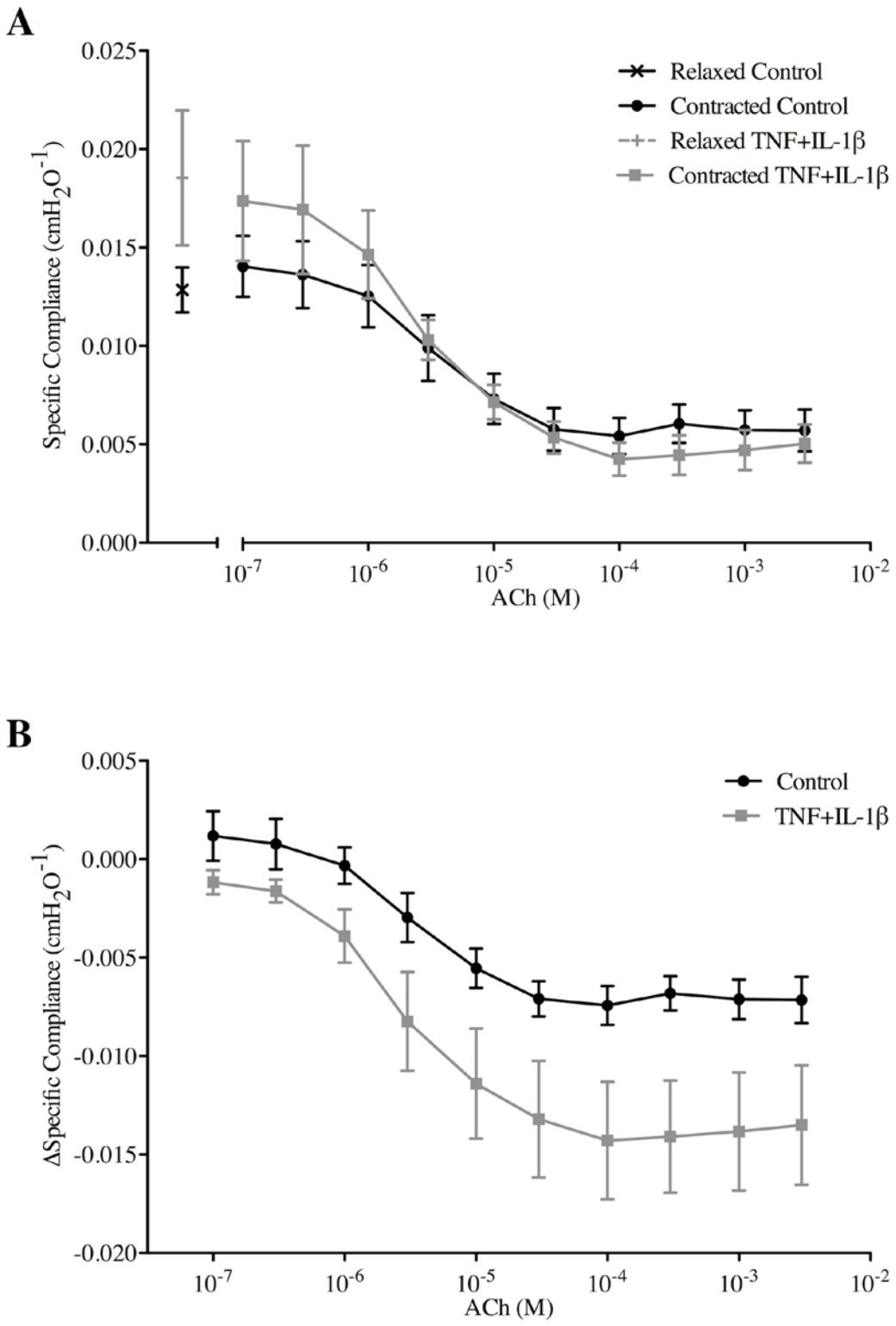


Figure 4. Specific compliance of the airway wall (cmH_2O^{-1}, A) in the relaxed state and in airways narrowed to ACh (10^{-7} to $3x10^{-3}$M), as well as the change in specific

compliance from the relaxed state (ΔcmH_2O^{-1}, B) in the control and TNF+IL-1β treatment groups. Airways stiffened strongly in response to ACh (control group; $0.0129\pm0.0011 cmH_2O^{-1}$ in the relaxed state, to $0.0057\pm0.0011 cmH_2O^{-1}$, $p<0.001$, TNF+IL-1β treatment group; $0.0185\pm0.0034 cmH_2O^{-1}$ in the relaxed state, to $0.0050\pm0.0010 cmH_2O^{-1}$, $p<0.001$). There was no difference in airway specific compliance between groups. The change in specific compliance from the relaxed state produced by ACh was greater in the TNF+IL-1β treatment group ($p<0.05$). n=6 per group. Mean±SEM.

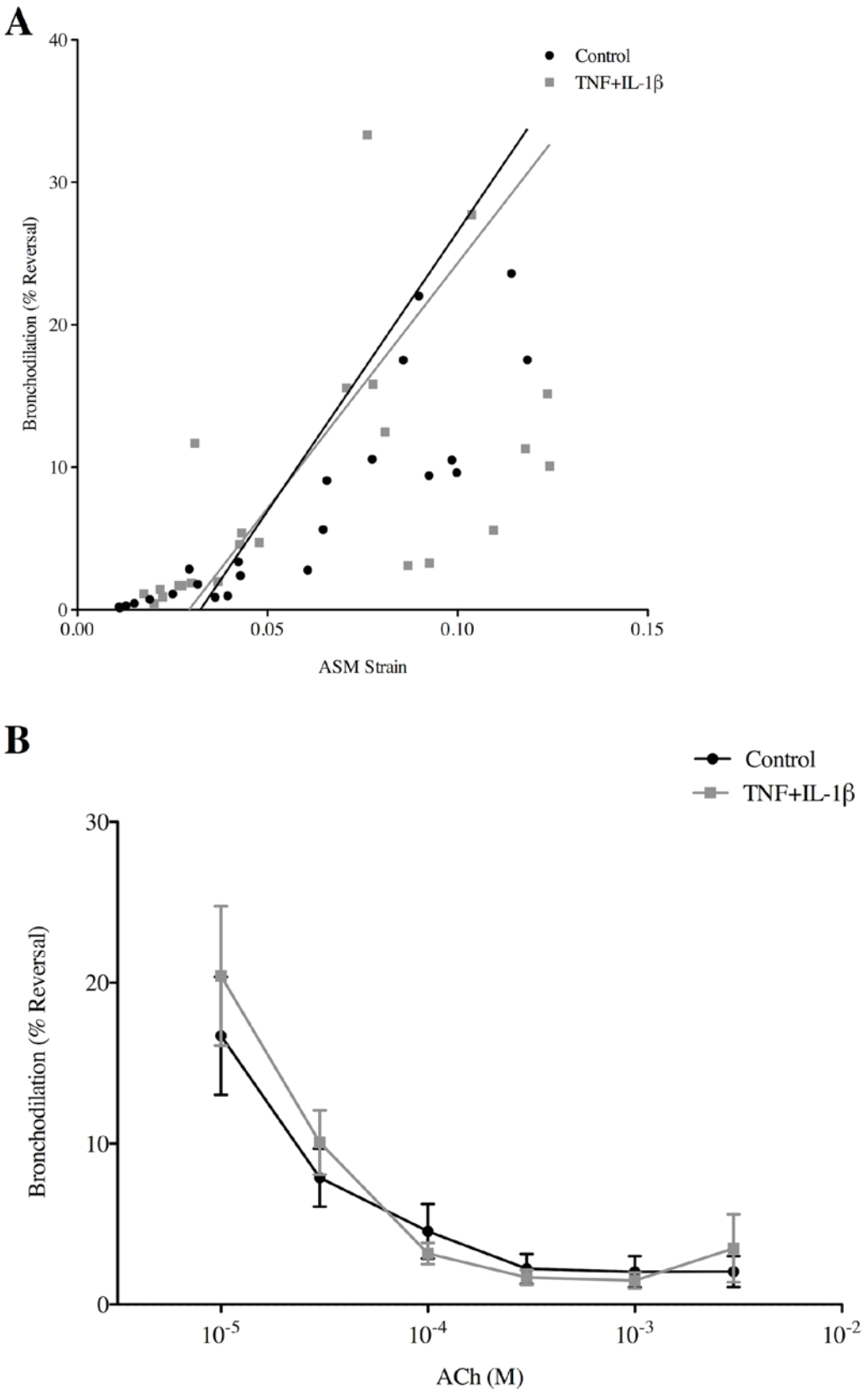

A
Control
TNF+IL-1β
Bronchodilation (% Reversal)
ASM Strain
0
10
20
30
40
0.00
0.05
0.10
0.15
B
Control
TNF+IL-1β
Bronchodilation (% Reversal)
ACh (M)
0
10
20
30
10-5
10-4
10-3
10-2

Figure 5. Scatter plots of bronchodilation (%Reversal) against ASM strain (A) produced by DI and bronchodilation to DI (%Reversal, B) in airways narrowed to ACh (10^{-5} to $3x10^{-3}$M) in the control and TNF+IL-1β treatment groups. Plots comprise 6 measurements per airway in A. Linear lines-of-best fit were fitted to individual airways and average intercept and slope was used to construct an average linear line-of-best-fit for each group. Bronchodilation to DI was positively correlated with ASM strain. There was no difference in the average intercept (control group; 0.029±0.011, TNF+IL-1β treatment group; 0.032±0.007) and slope between groups (control group; 262.6±61.4, TNF+IL-1β treatment group; 284.8±83.5). Bronchodilation to DI fell substantially with increasing dose of ACh (control; 16.7±3.7% reversal at 10^{-5}M ACh to 2.0±1.0% reversal, $p<0.001$, TNF+IL-1β treatment; 20.4±4.3% reversal at 10^{-5}M ACh to 3.5±2.1% reversal, $p<0.001$). There was no difference in bronchodilation to DI in both groups at comparable doses of ACh. n=6 per group. Mean±SEM in B.

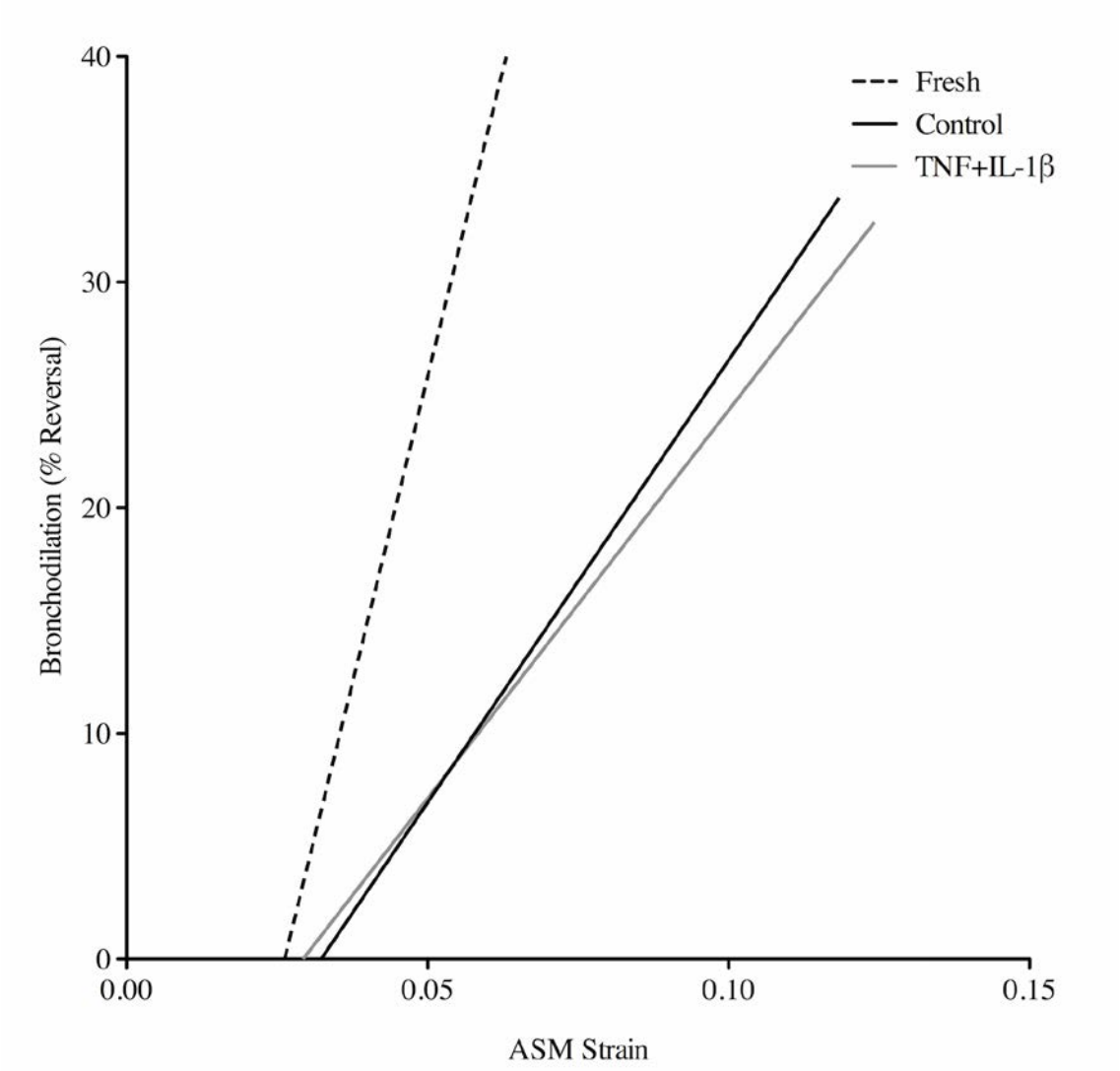


Figure 6. The effect of culture on strain-induced bronchodilation. Scatter plots of bronchodilation (%Reversal) against ASM strain produced by fixed–P_{tm} DI. Airways from the control and TNF+IL-1β treatment group in the present study are compared with fresh bronchial segments from our previous study[21]. Whilst there was no difference between the control and TNF+IL-1β treatment groups, both groups had a reduced bronchodilatory response to DI, compared with fresh bronchial segments. The average slope was greater in the fresh bronchial segments than the control ($p<0.05$) and TNF+IL-1β treatment ($p<0.05$) groups. n=6.