Title: Reproducibility of the bronchoconstrictive response to eucapnic voluntary hyperpnoea

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Abbreviations: EVH, eucapnic voluntary hyperpnoea; HIB, hyperpnoea-induced bronchoconstriction; EIB, exercise-induced bronchoconstriction; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s; MVV, maximal voluntary ventilation; $\dot{V}_E$, minute ventilation; AUC, area under the curve; CV, coefficient of variation; SMC, smallest meaningful change.

Abstract

Background: Eucapnic voluntary hyperpnoea (EVH) is considered an effective bronchoprovocation challenge for identifying exercise-induced bronchoconstriction (EIB). However, the reproducibility of the hyperpnoea-induced bronchoconstriction (HIB) response elicited by EVH remains unknown and was therefore the focus of this study.

Methods: Two cohorts of 16 physically active males (each cohort comprised 8 controls and 8 with physician diagnosis of asthma) participated in two studies of the short- and long-term reproducibility of the bronchoconstrictive response to an EVH test with dry air. EVH was performed on days 0, 7, 14, and 21 (short-term study), and 0, 35, and 70 (long-term study). HIB was diagnosed by a ≥10% fall in forced expiratory volume in 1 s (FEV_1) after EVH.

Results: On day 0 of the short-term study, FEV_1 fell by 2 ± 1% ($P < 0.05$) and 27 ± 18% ($P < 0.01$) from pre- to post-EVH in control and HIB-positive groups respectively. The post-EVH fall in FEV_1 did not differ across the short-term study test days. In the HIB-positive group, the day-to-day coefficient of variation, reproducibility, and smallest meaningful change for the fall in FEV_1 were 12%, 328 mL, and 164 mL, respectively. On day 0 of the long-term study, FEV_1 fell by 2 ± 2% and 25 ± 18% ($P < 0.01$) after EVH in control and HIB-positive groups respectively. The post-EVH fall in FEV_1 did not differ across the long-term study test days. In the HIB-positive group, the day-to-day coefficient of variation, reproducibility, and smallest meaningful change for the fall in FEV_1 were 10%, 196 mL, and 98 mL respectively.

Conclusion: The EVH test elicits a reproducible bronchoconstrictive response in physically active males with physician diagnosed asthma. These data thus support the clinical utility of the EVH test for EIB screening and monitoring.

Key words: Exercise-induced bronchoconstriction; asthma; eucapnic voluntary hyperpnoea; reproducibility.

Introduction

Asthma affects 5-10% of the population in developed countries[1] and is the most common chronic medical condition reported among Olympic athletes with a prevalence of around 8%.[2] At least 80% of individuals with clinically diagnosed asthma[3] and up to 50% of particular elite athlete populations[4] will also experience exercise-induced bronchoconstriction (EIB). EIB is characterised by transient airway narrowing during or after exercise and is ascribed to airway drying and subsequent changes in airway osmolality, which results in degranulation of inflammatory cells and release of inflammatory mediators.[5,6]

Diagnosis of EIB should not be based exclusively on self-reported symptoms[7-9] as they lack sensitivity and specificity.[10-14] Instead, EIB diagnosis should be objective and based on a fall in forced expiratory volume in 1 s (FEV_1) after an exercise challenge, or a surrogate for exercise such as eucapnic voluntary hyperpnoea (EVH).[7,9] Exercise challenges are highly specific, but they lack sensitivity and thus the rate of false-negative diagnoses can be high.[13,15] Exercise challenges are also difficult to standardise due to changing environmental conditions and ventilatory responses, which determine the degree of EIB.[15,16] The EVH test comprises 6 min of voluntary hyperpnoea using a dry gas and high minute ventilation ($\dot{V}_E$). Hyperpnoea-induced bronchoconstriction (HIB) is diagnosed when FEV_1 falls by $\geq$10%.[17,18] The EVH test can be tightly controlled, has high specificity and sensitivity for diagnosing EIB, and results in fewer false-negative diagnoses compared with exercise challenges.[15,19-21] It is therefore considered a superior bronchoprovocation challenge for identifying EIB.[15,22]

The clinical utility of the EVH test critically depends on the extent to which it elicits a reproducible day-to-day fall in FEV_1. Reproducibility is determined by the measurement error and within-individual fluctuation, which may increase with longer time intervals.[23] However, few studies have examined the reproducibility of HIB elicited by EVH across different time

intervals and using robust methods. Some studies failed to use appropriate statistical techniques to evaluate reproducibility,[17] whereas others examined elite swimmers[14] who may have a unique EIB pathophysiology.[24,25] Price et al.[26] reported poor reproducibility for the fall in FEV_1 (95% limits of agreement: -10.7% to 9.5%) after EVH in 32 individuals (6 with physician diagnosed asthma) with borderline HIB (~10% fall in FEV_1). The reproducibility of HIB elicited by EVH in individuals with physician diagnosed asthma and more severe HIB is therefore unknown.

Thus, the aim of the present study was to evaluate the short- (21 days) and long- (70 days) term test-retest reproducibility of HIB elicited by EVH in individuals with physician diagnosed asthma who were also positive for HIB during initial screening.

Methods

Following approval from the Nottingham Trent University Human Ethics Committee, two cohorts of 16 physically active (completing 4-6 hours of aerobic exercise per week) males provided written informed consent to participate in a short-term (4 EVH tests each separated by 7 days) or long-term (3 EVH tests each separated by 35 days) study of HIB reproducibility. All participants were non-smokers and had no history of smoking. Each cohort comprised 8 control participants and 8 HIB-positive participants. Inclusion criteria for HIB-positive participants included physician diagnosis of asthma, a baseline FEV_1 >65% of predicted,[17] and a ≥10% fall in FEV_1 following EVH.[9] On commencing the short-term study, HIB-positive participants were taking the following prescribed medication: N = 5, short acting β2 agonists; N = 1, combination of short acting β2 agonists and inhaled corticosteroids; N = 1, combination of short and long acting β2 agonists and inhaled corticosteroids; N = 1, combination of short acting β2 agonists, inhaled corticosteroids, and leukotriene modifiers. On commencing the long-term study, HIB-positive participants were taking the following

prescribed medication: N = 5, short acting β2 agonists; N = 1 short acting β2 agonists and inhaled corticosteroids; N = 2, short and long acting β2 agonists and inhaled corticosteroids. Inclusion criteria for the HIB-positive group did not consider changes of medication prior to starting the study, but exclusion criteria included a change in medication during the study. Throughout the study, participants adhered to their usual habitual exercise regime and avoided strenuous exercise during the 48 h prior to testing.[18]

Pulmonary function and EVH test

Baseline pulmonary function (forced vital capacity (FVC) and FEV_1) was assessed according to published guidelines[27] using a pneumotachograph (Pneumotrac, Vitalograph, Buckinghm, UK) as previously described.[28,29]

For the 2 weeks prior to each EVH test, participants were free from any chest or upper respiratory tract infection.[19] On EVH test days participants abstained from caffeine and alcohol as they can influence asthma exacerbations,[30,31] and arrived at the laboratory at least 2 h post-prandial. For each participant, EVH tests were performed at the same time of day. Participants with asthma ceased their medication prior to each EVH test (inhaled corticosteroids and leukotriene modifiers: 4 days; inhaled long acting β2 agonists: 2 days; anti-histamines: 2 days; inhaled short acting β2 agonists: the day of the test).[18,19]

The EVH test comprised 6 min of voluntary hyperpnoea at a target $\dot{V}_E$ of 85% of the predicted maximal voluntary ventilation (MVV) (30 x baseline FEV_1).[17,18] Participants breathed through a flanged mouthpiece (Series 9060; Hans Rudolph, Missouri, USA) connected to a flow sensor (ZAN variable orifice pneumotach; Nspire Health, Oberthulba, Germany) that was calibrated using a 3 L syringe. Gas concentrations were measured using fast responding laser diode absorption spectroscopy sensors, which were calibrated using gases of known concentration (5% CO_2, 15% O_2, balance N_2; BOC, Guilford, UK), and

ventilatory and pulmonary gas exchange variables were measured breath-by-breath (ZAN 600USB; Nspire Health) as previously described.[28,29] A two-way non-rebreathing valve (2700 Series; Hans Rudolph) was connected distally to the flow sensor and the inspiratory port was connected via a 1.2 m length of corrugated tubing (internal diameter: 35 mm) to a 150 L capacity Douglas bag. Participants inspired from the Douglas bag which was continuously filled with gases of known concentration (21% O_2, 5% CO_2, balance N_2; BOC).[32] The inspired gas was at room temperature (19-21°C) and of low humidity (<3%). During EVH, participants faced a computer monitor and received real-time visual feedback of $\dot{V}_E$, and end-tidal CO_2 was continuously monitored to ensure that isocapnia was maintained. After EVH, pulmonary function was assessed in duplicate at 3, 6 and 16 min, and the highest values recorded were used for subsequent analysis. After the EVH test, HIB-positive participants were supervised in the laboratory until their FEV_1 was within 10% of their baseline FEV_1.

Statistical analysis

The short- and long-term reproducibility studies were analysed separately using the Statistical Package for the Social Sciences (SPSS, Chicago, IL). Between-group comparisons (HIB-positive vs. control) for baseline FVC and FEV_1 were made using independent samples t-tests. One-way repeated measures ANOVA followed by Bonferroni adjusted pairwise comparisons was used to evaluate the within-group effects of day (short-term study: day 0, 7, 14, and 21; long-term study: day 0, 35, and 70) on baseline FVC and FEV_1. One-way repeated measures ANOVA followed by Bonferroni adjusted pairwise comparisons was used to evaluate the within-group effects of time after EVH (3, 6, and 16 min) on FVC and FEV_1. On all occasions there were no differences between these time points for FVC and FEV_1 and, therefore, the three values were averaged and used for further analyses, including reproducibility statistics. One-way repeated measures ANOVA followed by Bonferroni adjusted pairwise comparisons was used to evaluate the within-group effects of day on the

average FVC and FEV_1 measured after EVH. In HIB-positive participants the area under the curve for $\%\Delta FEV_1$ during the 16 min period after EVH ($AUC_{0\text{-}16}\%\Delta FEV_1$) was calculated using the trapezoidal rule.

Day-to-day variation in baseline FVC and FEV_1, and the fall in FEV_1 after EVH, was calculated as the within-participant coefficient of variation (CV). Measurement error and reproducibility were calculated for FVC and FEV_1 measured before and after EVH. The same statistics were also calculated for the absolute change in FVC and FEV_1 from baseline to post-EVH. The smallest meaningful change was subsequently determined.[33,34] Statistical significance was set at $P < 0.05$. Results are presented as mean ± SD, unless otherwise indicated.

Results

No participants were excluded during the course of the study. Participant characteristics and baseline (mean across study days) pulmonary function before EVH are shown in Table 1. In both short- and long-term studies FVC before EVH did not differ between HIB-positive and control groups. In the short-term study there was a trend for baseline FEV_1 to be lower in the HIB-positive group compared with the control group ($P = 0.056$). In the long-term study baseline FEV_1 was lower in the HIB-positive group compared with the control group ($P < 0.05$). The mean target $\dot{V}_E$ during EVH was therefore lower in the HIB-positive group compared with the control group in both short-term (121 ± 25 vs. 141 ± 12 $L{\cdot}min^{-1}$, $P = 0.056$) and long-term (113 ± 25 vs. 139 ± 11 $L{\cdot}min^{-1}$, $P < 0.05$) studies. In both studies and in both groups FVC and FEV_1 measured before EVH, and therefore the target $\dot{V}_E$ during EVH, were not different between test days. The achieved $\dot{V}_E$ during EVH was not different between test days in both short-term (HIB-positive: 121 ± 21 $L{\cdot}min^{-1}$, range

72-156 L·min^{-1}, 86 ± 11% MVV; control: 139 ± 12 L·min^{-1}, range 118-156 L·min^{-1}, 84 ± 2% MVV) and long-term (HIB-positive: 114 ± 23 L·min^{-1}, range 71-154 L·min^{-1}, 88 ± 9% MVV; control: 139 ± 12 L·min^{-1}, range 118-146 L·min^{-1}, 82 ± 4% MVV) studies. End-tidal CO_2 during EVH (39.5 ± 1.7 mmHg) was not different from rest (38.7 ± 1.8 mmHg) with a mean difference of 0.8 ± 1.4 mmHg (range: -3.5-3.5 mmHg) (data pooled from both groups and both studies).

Short term study

Control group

On day 0 of the short-term study, FVC was unchanged after EVH in the control group. Conversely, FEV_1 fell 3 ± 2% 3 min after EVH ($P < 0.05$) and remained below baseline throughout the post-EVH period ($P < 0.05$). The fall in FEV_1 after EVH was not different between the 4 short-term study days (Table 2).

HIB-positive group

On day 0 of the short-term study, FVC fell 11 ± 14% 3 min after EVH in the HIB-positive group ($P < 0.05$) and remained below baseline throughout the post-EVH period ($P < 0.05$). The fall in FVC after EVH was not different between the 4 short-term study days (Table 2). On day 0 of the short-term study, FEV_1 fell 27 ± 18% 3 min after EVH ($P < 0.01$) and remained below baseline throughout the post-EVH period (6 min: $P < 0.01$; 16 min: $P < 0.05$). The fall in FEV_1 after EVH was not different between the 4 short-term study days (Table 2). All HIB-positive participants experienced a >10% fall in FEV_1 after every EVH test and the consistency of the fall between days is shown in Figure 1. The $AUC_{0\text{-}16}$%ΔFEV_1 on day 0 of the short-term study was 390 ± 263. The $AUC_{0\text{-}16}$%ΔFEV_1 was not different between the 4 short-term study days. Between-day reproducibility in pulmonary function for

the short-term study is shown in Table 3. In the HIB-positive group, the smallest meaningful change for the fall in FEV_1 after EVH represented ~17% of the absolute fall.

Long term study

Control group

On day 0 of the long-term study, FVC was unchanged after EVH in the control group, whereas FEV_1 fell 3 ± 1% 6 min after EVH ($P < 0.01$). FEV_1 at 3 and 16 min after EVH was not different from baseline. The fall in FEV_1 was not different between the 3 long-term study days (Table 4).

HIB-positive group

On day 0 of the long-term study, FVC fell 20 ± 15% 6 min after EVH in the HIB-positive group ($P < 0.05$). There was also a trend for FVC to be lower than baseline 3 ($P = 0.07$) and 16 min ($P = 0.09$) after EVH. The fall in FVC after EVH was not different between the 3 long-term study days (Table 4). On day 0 of the long-term study, FEV_1 fell 27 ± 19% 3 min after EVH ($P < 0.05$) and remained below baseline throughout the post-EVH period (6 min: $P < 0.01$; 16 min: $P < 0.05$). The fall in FEV_1 was not different between the 3 long-term study days (Table 4). All HIB-positive participants experienced a >10% fall in FEV_1 after every EVH test and the consistency of the fall between days is shown in Figure 2. The $AUC_{0\text{-}16}\%\Delta FEV_1$ on day 0 of the long-term study was 364 ± 256. The $AUC_{0\text{-}16}\%\Delta FEV_1$ was not different between the 3 long-term study days. Between-day reproducibility in pulmonary function for the long-term study is shown in Table 5. In the HIB-positive group, the smallest meaningful change for the fall in FEV_1 after EVH represented ~11% of the absolute fall.

Discussion

The present study is the first to report the reproducibility of the bronchoconstrictive response elicited by the 6 min EVH test in recreationally active males with physician diagnosed asthma. The main finding was that the EVH test elicits a reproducible fall in FEV_1 when evaluated over short- (21 days) and long-term (70 days) periods. These data therefore support the clinical utility of the EVH test for EIB screening and monitoring.

The EVH test is an indirect bronchoprovocation test that is considered a suitable objective surrogate for identifying EIB.[9,35] The test can be tightly controlled and standardised, results in fewer false-negative EIB diagnoses compared with exercise challenges, and can diagnose EIB in previously undiagnosed and asymptomatic individuals.[15,19-21] The clinical utility of the EVH test is, in part, dependent on the extent to which it elicits a reproducible fall in FEV_1. Two previous studies reported good reproducibility for the fall in FEV_1 after two EVH tests performed on separate occasions; however, one failed to use appropriate statistical techniques to evaluate reproducibility,[17] and the other studied competitive swimmers[14] who may have a unique EIB pathophysiology.[24,25] The degree of HIB can be classified as mild (10-19.9% fall in FEV_1), moderate (20-29.9% fall in FEV_1), or severe (≥30% fall in FEV_1),[18] and Price et al.[26] have raised concerns regarding the reproducibility of HIB in individuals demonstrating a mild or borderline (~10%) post-EVH fall in FEV_1, which may result in misdiagnosis of EIB. Conversely, in the present study the post-EVH fall in FEV_1 was highly reproducible for all HIB-positive participants, including those with mild HIB (Figures 1 and 2). Although the reasons for this inter-study discrepancy remain unclear, only 6 of 32 participants studied by Price et al.[26] had physician diagnosed asthma and the post-EVH fall in FEV_1 (10 ± 8%) was much smaller than in the present study (~27%). Our data show that in

recreationally active males with physician diagnosed asthma the degree of HIB elicited by EVH is reproducible for up to 70 days irrespective of HIB severity.

Based on patient perception of change, the minimal important difference for improvement or worsening in FEV_1 is ~10% from baseline.[36] The post-EVH fall in FEV_1 in the control groups is thus unlikely to have clinical significance. In the HIB-positive groups the mean FEV_1 at baseline was 4.03 and 3.75 L in the short- and long-term studies respectively, and thus an improvement or worsening in FEV_1 of 403 and 375 mL respectively would be perceptible. The smallest meaningful change for the post-EVH fall in FEV_1 in the short- (164 mL) and long-term (98 mL) studies therefore demonstrates that the EVH test is sufficiently sensitive to detect clinically relevant changes in HIB that might occur with, for example, a therapeutic intervention.

It is essential that isocapnia is maintained during EVH because hyperpnoea-induced hypocapnia can cause bronchoconstriction in individuals with asthma, whereas hypercapnia can cause bronchodilation in individuals with and without asthma.[37] The gold standard EVH gas mixture (4.5-5% CO_2) is based on the work of Phillips et al.[32] who showed that isocapnia is maintained over a range of $\dot{V}_E$ from 40-105 $L \cdot min^{-1}$. Although in the present study the achieved $\dot{V}_E$ during EVH exceeded this range, end-tidal CO_2 scarcely deviated from rest and, therefore, the observed HIB was not influenced by hypocapnia or hypercapnia. The present study therefore extends the findings of Phillips et al.[32] by showing that an EVH gas mixture containing 5% CO_2 can maintain isocapnia over a range of $\dot{V}_E$ from 71-156 $L \cdot min^{-1}$.

Limitations

Caution is warranted when generalising the present findings to women. Changes in sex hormones during the menstrual cycle have been linked with cyclic changes in lung function in women with and without asthma,[38] and around half of women with asthma

experience an aggravation of asthma symptoms and increased medication use during the premenstrual or menstrual phase.[39,40] Women are also 2.38 times less likely than males to achieve a $\dot{V}_E$ of 60% MVV during EVH (a criterion for an adequate test), although they may also experience bronchoconstriction at a lower $\dot{V}_E$ than men.[41] These confounders may influence the ability of the EVH test to elicit a reproducible fall in FEV_1 in women with asthma. Further studies are therefore needed to establish the utility of the EVH test in women with asthma, including whether a reproducible bronchoconstrictive response is elicited across different time intervals and phases of the menstrual cycle.

The EVH test is very provocative and may not represent the ventilatory and environmental challenges associated with most forms of exercise.[19] However, exercise challenges are difficult to standardise and they lack sensitivity, which can result in false-negative diagnoses. Thus, a positive HIB diagnosis from the EVH test identifies that an individual is at risk of EIB and should therefore explore appropriate pharmacological treatment.

Conclusions

In conclusion, the degree of HIB elicited by the EVH test is reproducible over short- (21 days) and long-term (70 days) periods in recreationally active males with physician diagnosed asthma. These data support the clinical utility of the EVH test for EIB screening and monitoring, and for evaluating the effectiveness of therapeutic interventions for reducing EIB.

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Table 1. Participant characteristics and baseline pulmonary function (pooled data across all test days) for short- and long-term studies. Values are mean ± SD. *Significant difference between groups in the long-term study ($P < 0.05$).

	Short-term study		Long-term study	
	HIB-positive	Control	HIB-positive	Control
Age (yr)	28 ± 7	25 ± 3	31 ± 9	26 ± 4
Height (cm)	177 ± 6	181 ± 6	175 ± 2	181 ± 5
Body mass (kg)	76 ± 10	79 ± 8	73 ± 10	78 ± 9
FVC (L)	5.14 ± 0.93	5.56 ± 0.44	4.66 ± 0.68	5.25 ± 0.40
% predicted	100 ± 12	102 ± 7	94 ± 10	97 ± 6
FEV_1 (L)	4.03 ± 0.84	4.71 ± 0.41	3.75 ± 0.84*	4.63 ± 0.37
% predicted	93 ± 15	103 ± 6	91 ± 14	102 ± 5

HIB, hyperpnoea-induced bronchoconstriction; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s.

Table 2 Relative and absolute changes in pulmonary function after eucapnic voluntary hyperpnoea (EVH) performed on 4 separate days in the short-term study. Values represent the mean ± SD of the three post-EVH measurements.

Day		0		7		14		21	
Change		%	mL	%	mL	%	mL	%	mL
EIB-positive	ΔFVC	-11 ± 13	-511 ± 540	-16 ± 18	-735 ± 785	-14 ± 15	-663 ± 662	-14 ± 14	-612 ± 557
	ΔFEV_1	-27 ± 18	-973 ± 514	-27 ± 20	-1000 ± 616	-27 ± 19	-962 ± 558	-26 ± 19	-939 ± 607
Control	ΔFVC	-1 ± 1	-52 ± 67	-1 ± 1	-54 ± 39	-2 ± 2	-88 ± 91	-0 ± 1	-10 ± 49
	ΔFEV_1	-2 ± 1	-105 ± 64	-2 ± 2	-105 ± 91	-2 ± 2	-115 ± 86	-2 ± 2	-106 ± 79

FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s.

Table 3 Between-day reproducibility in baseline pulmonary function and the fall in pulmonary function after eucapnic voluntary hyperpnoea performed on 4 separate days (day 0, 7, 14, and 21) in the short-term study.

	Day-to-day CV (%)	Measurement error	Reproducibility	SMC
HIB-positive				
Baseline FVC (mL)	3	182	503	252
Baseline FEV_1 (mL)	2	95	263	131
Fall in FEV_1 (mL)	12	118	328	164
Control				
Baseline FVC (mL)	1	77	215	107
Baseline FEV_1 (mL)	2	100	277	139

CV, coefficient of variation; SMC, smallest meaningful change; HIB, hyperpnoea-induced bronchoconstriction; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s.

Table 4 Relative and absolute changes in pulmonary function after eucapnic voluntary hyperpnoea (EVH) in the long-term study. Values represent the mean ± SD of the three post-EVH measurements.

Day		0		35		70	
Change		%	mL	%	mL	%	mL
EIB-positive	ΔFVC	-17 ± 14	-747 ± 584	-16 ± 11	-697 ± 437	-16 ± 14	-701 ± 563
	ΔFEV_1	-25 ± 18	-893 ± 524	-25 ± 16	-888 ± 441	-25 ± 16	-833 ± 446
Control	ΔFVC	-1 ± 2	-57 ± 87	-2 ± 2	-89 ± 87	-1 ± 2	-39 ± 114
	ΔFEV_1	-2 ± 2	-101 ± 84	-2 ± 2	-72 ± 64	-3 ± 2	-115 ± 71

FVC, forced vital capacity; FEV_1, forced expiratory volume in 1.

Table 5 Between-day reproducibility in baseline pulmonary function and the fall in pulmonary function after eucapnic voluntary hyperpnoea performed on 3 separate days (day 0, 35, and 70) in the long-term study.

	Day-to-day CV (%)	Measurement error	Reproducibility	SMC
HIB-positive				
Baseline FVC (mL)	3	141	392	196
Baseline FEV_1 (mL)	2	89	248	124
Fall in FEV_1 (mL)	10	71	196	98
Control				
Baseline FVC (mL)	2	114	316	158
Baseline FEV_1 (mL)	2	84	232	116

CV, coefficient of variation; SMC, smallest meaningful change; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s.

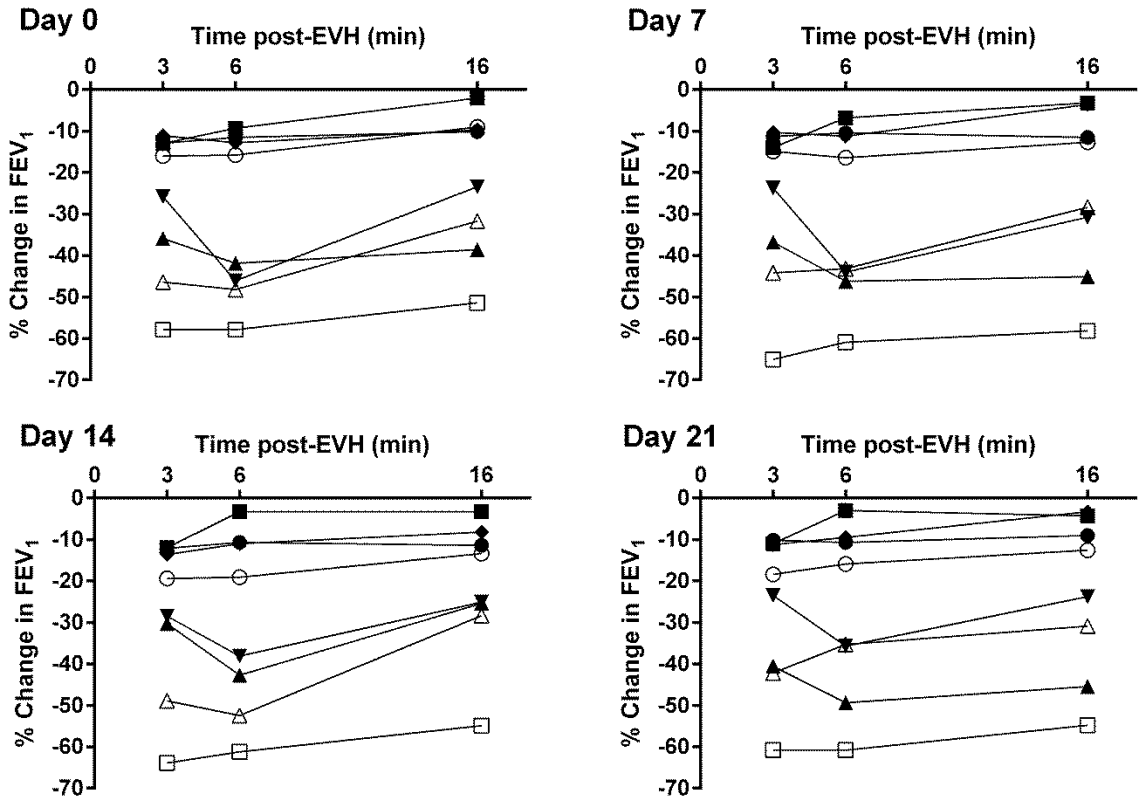

Figure 1. Individual falls in forced expiratory volume in 1 s (FEV_1) after eucapnic voluntary hyperpnoea (EVH) in the short-term study. Data are for HIB-positive participants only, with identical symbols representing the same HIB-positive participant.

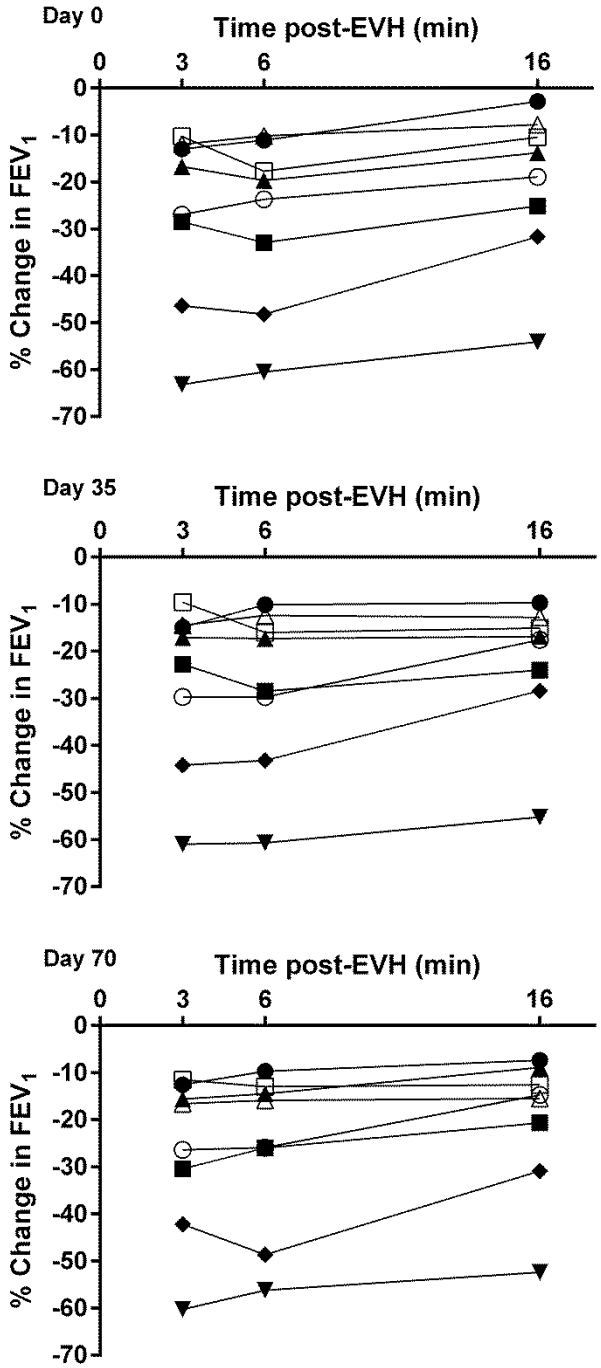

Figure 2. Individual falls in forced expiratory volume in 1 s (FEV_1) after eucapnic voluntary hyperpnoea (EVH) in the long-term study. Data are for HIB-positive participants only, with identical symbols representing the same HIB-positive participant.