

Diesel exhaust particles sensitise airway TRPV1 and shift cardiac autonomic balance in healthy humans: a randomised, double-blind, crossover controlled exposure study of genotype-dependent susceptibility

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Abstract

Background. Traffic-derived particulate triggers cardiorespiratory events within hours, a latency more compatible with a neurogenic than an inflammatory mechanism. The transient receptor potential vanilloid 1 (TRPV1) channel on bronchopulmonary vagal C-fibres is the candidate interface: it is directly activated by diesel exhaust particles (DEP) in vitro and is highly polymorphic. Whether DEP modulates TRPV1 function in humans in vivo, and whether genotype modifies that response, is unknown.

Methods. Twenty-three healthy non-smoking adults (12 men; 32.6 ± 8.6 years) completed a randomised, double-blind, two-period crossover inhalation study comparing a standardised DEP suspension (SRM 2975; 288 μg delivered dose) with its diluent, by dosimeter-controlled nebulisation. Capsaicin cough challenge, heart rate variability, blood pressure and redox biomarkers were assessed, and an additive capsaicin-responsiveness score was derived from four informative TRPV1 loci. Within-person contrasts used permutation-based paired t tests with Benjamini–Hochberg adjustment; genotype effect modification was examined in Bayesian multilevel and covariate-adjusted models.

Results. Compared with diluent, DEP halved the capsaicin concentration evoking two coughs (79.8 vs 155.1 μM ; $p = 0.029$), raised the LF/HF ratio from 1.07 to 1.87 ($p = 0.005$) with a reciprocal shift in normalised low- and high-frequency power (both $q = 0.030$) and unchanged heart rate, and increased systolic blood pressure ($p = 0.040$). Biological antioxidant potential and the redox index fell (both $q < 0.001$), hydroperoxides unchanged. No outcome differed between diluent and baseline. The exposure-by-score interaction was credible for rMSSD ($\beta = -0.32$; 95% CrI – 0.54 to – 0.10) but not for log C2, and survived adjustment for the post-diluent value, sequence, washout, age and sex ($\beta = -0.26$; 95% CrI – 0.49 to – 0.02).

Conclusions. A single inhaled dose of diesel particulate, below the elemental carbon burden of one working shift at the current occupational limit, sensitised airway TRPV1, tilted cardiac autonomic balance towards sympathetic predominance and consumed antioxidant defences in healthy adults. This first human demonstration of a pathway hitherto confined to cells and animals unfolds on the same timescale as the excess of cardiovascular events following pollution peaks. Susceptibility was genetically graded: the vagal displacement scaled with the burden of capsaicin-responsive TRPV1 alleles, variants that are silent towards indirect endogenous sensitisers. TRPV1 genotype thus emerges as a candidate susceptibility biomarker for traffic-exposed populations.

1. Introduction

Epidemiological studies consistently show that a rise in ambient particulate matter is followed by an excess of cardiovascular and respiratory events within hours [1–3], and the effect is most consistent for the traffic-derived fraction [3, 4]. This latency is the crux of the mechanistic problem: it is too short to be accounted for by the systemic inflammatory cascade, whose kinetics fit sub-chronic and chronic exposure far better [5, 6], and it therefore points towards a reflex pathway in which inhaled particles act on sensory nerve endings in the airways and the resulting afferent traffic is integrated centrally into an

altered autonomic drive to the heart [7]. Diesel exhaust particles (DEP) are the fraction for which this hypothesis is most developed, both because they represent a major component of urban particulate [6] and because their respirable size range deposits at the level of the terminal airways, where the relevant sensory endings lie [8].

The transient receptor potential vanilloid 1 (TRPV1) channel is the most plausible molecular interface of this pathway. TRPV1 is a non-selective, highly calcium-permeable cation channel expressed by unmyelinated bronchopulmonary vagal C-fibres, where its activation initiates the cough reflex and the release of the neuropeptides that drive neurogenic inflammation [9, 10]. Because capsaicin is a selective TRPV1 agonist, the standardised capsaicin cough challenge provides a non-invasive functional read-out of airway TRPV1 activity in vivo in humans, and the concentration evoking two coughs (C2) is an established quantitative index of channel responsiveness [11]. Several lines of experimental evidence indicate that particulate matter engages this channel directly rather than merely sensitising it: DEP, coal fly ash and silica activate heterologously expressed TRPV1, with a rank order of potency that identifies particulate as an agonist [12, 13]; pulmonary DEP increases susceptibility to myocardial ischaemia–reperfusion injury through sensory TRPV1 in rodents [14]; and inhalation of concentrated ambient particulate alters cardiac rhythm through TRP-dependent pulmonary reflexes [15].

Human evidence has so far stopped one step short of this conclusion. We previously showed that inhalation of the endogenous mediators prostaglandin E2 (PGE2) and bradykinin (BK), at doses that do not evoke cough on their own, shifts the capsaicin dose–response curve to the left and concomitantly moves cardiac autonomic balance towards sympathetic predominance, thereby establishing in humans the functional link between airway TRPV1 activity and autonomic regulation of cardiac rhythm [16]. In the same work, DEP — unlike PGE2 and BK — directly increased capsaicin-evoked calcium responses in TRPV1-transfected cells [16]. What has never been tested is the corresponding experiment in vivo: whether inhaling the pollutant itself, rather than a surrogate mediator, modifies TRPV1 function and cardiac autonomic regulation in humans, a gap we have recently identified as the principal open question of this field [17].

A second, independent strand of our work concerns why individuals differ so markedly in this response. TRPV1 is highly polymorphic, and six non-synonymous variants of its coding region have been functionally characterized [13, 18]. We demonstrated that in vivo capsaicin cough sensitivity is not explained by any single variant but by the combined burden of capsaicin-responsive alleles carried at several sites, and that a simple additive score counting these alleles ranks unselected healthy subjects along an approximately ten-fold range of C2 [19]; consistent findings have been reported for single variants in relation to cough and to capsaicin-induced sensory responses [20, 21], and the loss-of-function 585V variant is associated with a lower risk of wheeze and cough in children with asthma [22]. Critically, in the PGE2/BK study the same polymorphisms modified neither the cough nor the autonomic response [16]. That negative result has a mechanistic explanation rather than being a failure of the score: PGE2 and BK do not bind TRPV1, but act indirectly through EP3 and B2 G-protein-coupled receptors [23, 24] and downstream diacylglycerol and protein kinase C signalling [25], a route that

bypasses the channel domains affected by these variants. This expectation is not merely inferential: TRPV1 variants have been shown in heterologous expression systems to differ in their activation by particulate material itself [13], so that the same polymorphisms that are silent towards an indirect sensitiser discriminate between individuals when the stimulus is a particle acting on the channel. A direct channel agonist should, by the same logic, engage them, which yields a specific and falsifiable prediction: TRPV1 genotype should modify the human response to inhaled particulate even though it does not modify the response to indirect endogenous sensitisers.

Oxidative stress constitutes the third arm of the pathway and is mechanistically entangled with the first two, since reactive oxygen species both sensitise TRPV1 and are generated downstream of neurogenic inflammation [15, 26]. Systemic redox status can be characterised non-invasively by measuring circulating hydroperoxides, representing reactive oxygen metabolites (d-ROMs) together with the biological antioxidant potential (BAP) [27], whose joint interpretation discriminates an increase in pro-oxidant production from a consumption of antioxidant defences— a distinction that matters within the short time frame of an acute exposure experiment.

We therefore carried out a randomised, double-blind, crossover controlled inhalation study in healthy volunteers, in which each participant served as their own control, in order to test three hypotheses: that acute inhalation of DEP sensitises airway TRPV1 *in vivo*, as detected by an increase in capsaicin cough sensitivity; that the same exposure shifts cardiac autonomic balance towards sympathetic predominance and perturbs systemic redox balance; and that the magnitude of these responses, measured as the within-participant contrast between the two exposures, is modified by the individual burden of capsaicin-responsive TRPV1 alleles, which would identify TRPV1 genotype as a candidate susceptibility biomarker for the acute effects of traffic-related particulate.

2. Materials and methods

2.1 Study design

This was a single-centre, randomised, double-blind, two-period crossover controlled human inhalation study conducted at the Occupational Medicine Unit of the University Hospital of Padua between May 2024 and December 2025. The design is summarised in Fig. 1. Each participant attended three visits. At the baseline visit (BS) eligibility was confirmed, questionnaires were administered, venous blood was drawn for TRPV1 genotyping, resting blood pressure and heart rate variability (HRV) were recorded and a full capsaicin cough challenge was performed. At the two subsequent experimental visits participants inhaled, in randomised order, either the DEP suspension or its diluent; the HRV recording and the capsaicin cough challenge were repeated after the last breath of the test aerosol, and blood pressure was measured immediately before and after each administration. Blood for redox biomarkers was drawn immediately before and immediately after the DEP session only. Because each participant received both exposures, the design controls by construction for the stable interindividual characteristics — including genotype — that dominate the variability of both cough sensitivity and HRV.

The study was approved by the Ethics Committee of the University Hospital of Padua (protocol 4057/AO/17), with subsequent amendments approved on 13 September 2018, introducing the assessment of heart rate variability, and on 21 October 2021, transferring the role of principal investigator. All participants gave written informed consent, and all procedures conformed to the Declaration of Helsinki and to the applicable national regulations. Although the protocol incorporated the methodological features of a randomised controlled trial – double-blind allocation, crossover design and randomised exposure order – it was not prospectively registered in a public clinical trial registry, having been designed as a mechanistic physiological study in healthy volunteers rather than as an interventional clinical trial, consistently with the practice of comparable controlled human exposure studies in this field [28]. Reporting follows the CONSORT extension for randomised crossover trials [29].

2.2 Participants

Thirty volunteers were assessed for eligibility and twenty-three were enrolled and randomised (12 men, 11 women; age range 22–62 years, mean 32.6 ± 8.6 years). Three were excluded before consent because they did not meet the inclusion criteria (obesity, allergic sensitisation and gastro-oesophageal reflux disease, respectively) and four after the baseline visit but before randomisation, three because they did not complete the baseline cough challenge and one who withdrew for personal reasons. No participant discontinued after randomisation and all twenty-three completed both exposure visits. Inclusion criteria were age of at least 18 years, non-smoking status, absence of acute or chronic disease on the basis of medical history and physical examination, and no regular medication. Participants were excluded if they were affected by, or had a history of, arterial hypertension, diabetes mellitus, cardiovascular disease (moderate to severe valvular disease, coronary artery disease or arrhythmia), respiratory disease (asthma or atopy, chronic obstructive pulmonary disease or sleep apnoea), respiratory tract infection within the four weeks preceding the study, severe obesity, renal failure, thyroid disease, chronic liver disease, systemic inflammatory or autoimmune disorders, neurological disorders (cerebrovascular disease, Parkinson's disease, Guillain-Barre syndrome, polyneuropathy or multiple sclerosis), or malignancy. Women who were pregnant or breastfeeding were also excluded, as were participants taking angiotensin-converting enzyme inhibitors or any drug affecting the cough reflex or autonomic function, and current or former smokers. Participants abstained from alcohol for 48 h, from caffeine for 2 h and from heavy meals for 2–3 h before each visit, were asked to have slept at least 8 h and were not studied after a night shift; they also abstained from spicy food, garlic and cinnamon and from outdoor sporting activity in the 24 h preceding each visit.

The sample size was not based on a formal power calculation. It was determined on the basis of previous capsaicin cough challenge studies conducted with the same protocol in our laboratory, in which within-participant effects of comparable magnitude were detected in groups of similar size [16, 19], and the study is accordingly to be regarded as exploratory in its genetic component.

2.3 Randomisation, allocation concealment and blinding

Participants were allocated by simple randomisation, using a computer-generated list consulted at enrolment, to the sequence DEP→diluent (n = 14) or diluent→DEP (n = 9); the imbalance between sequences is a consequence of simple randomisation in a small sample and not of differential attrition, since no participant was lost after allocation. The protocol prescribed a minimum washout of seven days between the two exposure visits and no pair of visits was closer than this interval; the observed interval was 25.5 ± 25.5 days, and that between the baseline visit and the first exposure visit 25.7 ± 23.7 days. Both the participant and the investigator who performed the cough challenge and the HRV recording were blind to the content of the nebuliser. Blinding was achieved by covering the nebuliser ampoules with aluminium foil throughout preparation and administration, so that the visual difference between the dark particulate suspension and the clear diluent was not perceptible, and by assigning the preparation of the two solutions to an operator not involved in outcome assessment. No formal assessment of the success of blinding was undertaken, in line with CONSORT guidance and with established methodological recommendations [30], which discourage such testing because it is heavily confounded by perceived biological efficacy or by side effects rather than reflecting actual physical unblinding: blinding rested on the physical measures described above and on the crossover design itself, in which every participant received both aerosols.

2.4 Questionnaires and baseline clinical assessment

Cough-related quality of life was assessed with the validated Italian version of the Leicester Cough Questionnaire (LCQ; total score 0–21, higher scores indicating less impact of cough) [31, 32] and airway reflux symptoms with the Italian version of the Hull Airway Reflux Questionnaire (HARQ; 0–70, higher scores indicating more symptoms) [33, 34]. Habitual physical activity was quantified with the Italian version of the International Physical Activity Questionnaire and expressed in MET-minutes per week [35, 36]. Systolic and diastolic blood pressure were measured once in the sitting position with an automated electronic device (Omron 705IT, Omron Healthcare Europe, the Netherlands) after at least 5 min of rest, in accordance with the recommendations of the European Society of Hypertension [37]; the same single-reading approach was applied consistently at every session. Fasting capillary blood glucose was determined at the baseline visit using a plasma-calibrated Glucofix Tech glucometer (A. Menarini Diagnostics, Florence, Italy).

2.5 Diesel exhaust particle exposure and diluent

Participants inhaled a standardised suspension of diesel particulate matter (Standard Reference Material 2975, National Institute of Standards and Technology, Gaithersburg, MD, USA; Certificate of Analysis revised 2 July 2021) [38] at 3 mg/mL in sterile 0.9% sodium chloride, or the matched diluent alone. The suspension was vortexed for approximately 10 min immediately before use (ALC 110.4E vortex mixer), without sonication, and delivered from a Mefar MB2 ampoule connected to a Mefar MB3 dosimeter (Mefar S.p.A., Brescia, Italy), which released 16 μ L per breath over 1.5 s. Each participant inhaled six vital-capacity breaths, corresponding to a total delivered dose of 288 μ g of diesel particulate.

This dose was selected to reproduce a realistic occupational peak exposure. At a resting minute ventilation of 8 L/min [39], 288 µg corresponds to the amount inhaled during two hours at an airborne concentration of 300 µg/m³. Given the elemental carbon content of SRM 2975 (39.8%), the delivered dose corresponds to 112.3 µg of elemental carbon, that is 58% of the amount that would be inhaled over a full shift at the Italian occupational exposure limit for diesel engine exhaust of 50 µg/m³ as an eight-hour time-weighted average, set by the Interministerial Decree of 11 February 2021 implementing EU Directive 2019/130 [40], and to 78% of the dose corresponding to the short-term exposure limit of 1200 µg/m³ proposed by the IFA [41]. The exposure was well tolerated: no adverse event occurred, and all twenty-three participants completed the six breaths at both visits.

2.6 Characterisation of the particulate material

SRM 2975 is a diesel particulate matter reference material collected from an industrial forklift filtering system and certified by NIST for its content of polycyclic aromatic hydrocarbons and nitro-substituted polycyclic aromatic hydrocarbons. Its elemental carbon content is 39.8%, the total extractable organic mass is $2.7 \pm 0.2\%$ and the certified 1-nitropyrene content is 35.2 ± 2.2 mg/kg [38]. The particle size distribution of suspensions prepared with the same protocol was characterised in a previous study from our laboratory, and was not re-measured on the preparations used here with a laser analyser (DIPA 2000, Donner Technologies, Or Aquiva, Israel), giving a median diameter of 0.9 µm with a range of 0.2–6 µm and no change in the diameter distribution after dispersion, indicating that the particles do not aggregate under these conditions; the NIST certificate reports a mean number-based diameter of 1.62 ± 0.01 µm. The size range therefore corresponds to the respirable fraction expected to deposit at the level of the terminal airways.

2.7 Capsaicin cough challenge

The cough challenge was performed according to the European Respiratory Society guidelines [11, 42] and identically to our previous studies, beginning ten minutes after the end of the test aerosol, that is after the HRV recording. Single vital-capacity breaths of incremental concentrations of capsaicin (1, 3, 10, 30, 100, 300 and 1000 µM (Sigma-Aldrich, Milan, Italy) were inhaled at 2-min intervals from the dosimeter-controlled nebuliser. Each concentration was inhaled four times with a 30-s pause between inhalations, and both participant and investigator were blind to the concentration administered. The number of coughs in the 15 s following each inhalation was recorded with a digital audio recorder and counted off-line. The average number of coughs at each concentration was used to construct the individual concentration–response curve, fitted with dedicated software (Dr Fit v1.042) [43], from which the concentrations evoking at least two coughs (C2) and at least five coughs (C5) were derived; lower values denote greater sensitivity. C2 was the pre-specified primary cough outcome.

No imputation rule was applied: participants who did not reach C2 or C5 at the highest concentration inhaled were treated as non-responders and excluded from the corresponding analysis.

2.8 Assessment of heart rate variability

HRV was assessed from a resting electrocardiogram recorded in the supine position for 10 min immediately after the end of the test aerosol, using a three-electrode Bluetooth acquisition system (BT16plus, FM, Monza, Italy) with a sampling rate of 250 Hz and 16-bit resolution, and analysed with Kubios HRV Scientific 4.0.3 [44]. A stable five-minute segment was selected for analysis and recordings containing more than 5% abnormal beats were excluded. Acquisition and analysis followed the same protocol as in our previous studies of cardiac autonomic modulation [45, 46] and in accordance with current guidance on the use of heart rate variability in occupational medicine [47]. Power spectral density was estimated with an autoregressive model of order 16; the areas under the spectral peaks in the 0.04–0.15 Hz and 0.15–0.40 Hz bands defined low-frequency (LF) and high-frequency (HF) power, which were normalised to total power in the 0.01–0.4 Hz range (LFnu, HFnu) [48] so as to minimise the influence of changes in heart rate. The LF/HF ratio was taken as index of sympathovagal balance and HFnu as index of vagal modulation, while SDNN and rMSSD quantified overall and short-term, parasympathetically mediated variability. Mean heart rate was obtained from the same recordings. Respiratory frequency was not controlled but was very unlikely to fall outside the HF band. An autoregressive model was preferred to the fast Fourier transform used in our previous study because it affords better spectral resolution on short recordings and derives the power of each component directly from the residuals of the model poles, without manual integration over fixed frequency bands; this difference in spectral estimation should be borne in mind whenever absolute values are compared between the two studies.

2.9 Redox biomarkers

Circulating hydroperoxides d-ROMs and BAP were measured in serum samples obtained from blood collected in K₃EDTA tubes (BD Vacutainer, BD, USA) immediately before and after the DEP session. After centrifugation to obtain serum, samples were stored at – 20°C within 1 hour of collection. All measurements were performed in serum using the d-ROMs test and BAP test (Diacron, Grosseto, Italy), colorimetric assays miniaturized in microplates and automated on the Thunderbolt GSD platform (Tecan Italia, Milan, Italy), according to the manufacturer's instructions (Diacron, Grosseto, Italy). For both assays, the lyophilized calibrators and two-level matrix controls provided with the respective kits were used. d-ROMs concentrations were expressed in Carratelli units, whereas BAP values were expressed in $\mu\text{mol/L}$ of reduced iron. The oxidative balance index (Index) was calculated using the OSI algorithm [27], which directly relates d-ROMs and BAP values. The algorithm was modified by normalizing the respective values to the medians calculated in a reference population, according to the following formula: $(\text{BAP}/\text{median})/(\text{d-ROMs}/\text{median})$, with higher values denoting a more favourable balance between antioxidant capacity and pro-oxidant load. No corresponding measurements were obtained at the diluent session; this asymmetry of the design is stated explicitly and taken into account in the interpretation of the corresponding results.

2.10 TRPV1 genotyping and construction of the responsiveness score

Genomic DNA was extracted from 400 μ L of whole blood collected in K₃EDTA tubes (BD Vacutainer, BD, USA), using the QIAamp DNA Mini kit on the QIAcube system (QIAGEN, Milan, Italy) according to the manufacturer's instructions as previously described [49, 50]. DNA concentration and purity were assessed using the QIAxpert system (QIAGEN, Milan, Italy), and DNA quality was confirmed by an A260/A280 ratio ranging from 1.78 to 1.87. Genomic DNA was genotyped for eight TRPV1 variants: the six nonsynonymous functional polymorphisms previously characterized [19] (K2N rs9894618, P91S rs222749, I315M rs222747, T469I rs224534, T505A rs17633288 and I585V rs8065080) and two additional variants identified in the investigated regions of the TRPV1 gene (rs8078936, rs748639589). Sequencing was performed using a next-generation sequencing (NGS) approach on the MiSeq i100 platform (Illumina, San Diego, CA, USA).

The additive capsaicin-responsiveness score was computed as in our previous work [19], with the capsaicin-responsive alleles for rs222747 and rs222749 represented on the complementary strand, by counting for each participant the number of capsaicin-responsive alleles carried at each informative site, with a theoretical range from 0 to 8. Two of the six non-synonymous variants (rs9894618 and rs17633288) were monomorphic in this population, as in our previous cohorts, and the score was therefore based on the four remaining informative loci (585V, 469I, 315M, 91S); this indicator is referred to throughout as *Score SNPs* [1]. In short, a score of 0 indicates the absence of all SNP variants and 0 level of responsiveness, while 8 indicates the presence of all variants on both alleles and the maximum level of responsiveness [19]. An extended score additionally incorporating rs8078936 and rs748639589 was computed but discarded from the inferential analysis because of its negligible variability in this sample. Deviation of genotype frequencies from Hardy–Weinberg equilibrium was assessed by chi-square test.

2.11 Statistical analysis

Continuous variables are summarised as mean $\pm$ standard deviation and categorical variables as counts and percentages; C2 and C5, whose distributions are markedly right-skewed, are additionally reported as median with interquartile range. Within-participant differences between conditions – post-diluent versus baseline, post-DEP versus post-diluent, and post-DEP versus pre-DEP for redox biomarkers – were tested with two-sided paired t tests whose null distribution was obtained by permutation with 20,000 replications; standardised effect sizes are reported alongside p values. The association between *Score SNPs* [1] and each outcome was quantified with Kendall's rank correlation coefficient tau-b, which is robust to non-normality and to the ties inherent to an ordinal score, in three families: with the outcomes measured after diluent, with those measured after DEP, and with the within-participant changes induced by DEP.

Analyses were organised according to a hierarchy specified in advance. The primary hypotheses were the effect of DEP, relative to diluent, on C2 and on the LF/HF ratio, and the modification of the DEP-induced response by the TRPV1 responsiveness score; all remaining comparisons were exploratory. Every test performed is reported in full, including the non-significant ones, and Benjamini–Hochberg adjusted p values are reported alongside the unadjusted ones within each family of tests [51].

Participants with missing values for a given outcome were excluded from that analysis only: three participants lacked one or both post-exposure C2 values, and C5 could be determined in twelve participants, so that C5 is reported descriptively and was not used for inferential conclusions. Statistical significance was set at a two-sided $p < 0.05$.

Effect modification by genotype was examined in a Bayesian framework, which was preferred to frequentist mixed models and analysis of covariance because, at this sample size, the ratio between model complexity and the number of observations would have yielded imprecise estimates and a substantial risk of overfitting; weakly informative priors stabilise the estimates and regularise inference under small-sample constraints. Two complementary sets of models were fitted. The first was a multilevel model with a participant-level random intercept fitted to the values observed at both exposure visits, including exposure, the responsiveness score and their interaction, the interaction coefficient constituting the test of effect modification. The second consisted of regression models fitted to each post-DEP outcome, adjusted for the corresponding value observed after diluent, for crossover sequence, for the washout interval between the two visits, and for age and sex; this formulation holds the pre-exposure level constant instead of subtracting it, and therefore protects against regression to the mean. Outcomes were log-transformed C2 and rMSSD as primary and SDNN, heart rate and log-transformed LF/HF as secondary; all continuous variables were standardised, a standard normal prior was assigned to each regression coefficient, and Gaussian response distributions were used except for log LF/HF, modelled with a Student t distribution to accommodate heavy tails. Posterior distributions were estimated by Markov chain Monte Carlo with four independent chains of 10,000 iterations each and 50% burn-in, yielding 20,000 post-warm-up samples. Redox biomarkers could not be analysed in this framework, no measurement having been obtained at the diluent visit. Analyses were performed in R 4.6.0 [52] with the packages `flip` [53], for permutation testing, and `brms`, for Bayesian regression [54–56].

3. Results

3.1 Participants

Twenty-three healthy volunteers completed both exposure visits and were included in the analysis; their characteristics are reported in Table 1 and the flow of the study is shown in Fig. 1. Mean age was 32.6 ± 8.6 years and the sample was balanced by sex, with 12 men (52.2%). All participants were free of cough at baseline: the mean LCQ score was 20.7 ± 0.3 out of 21, essentially at the ceiling of the instrument, and the mean HARQ score was 2 ± 2 out of 70, far below the threshold indicative of airway reflux. Habitual physical activity was moderate to high, with 78.3% of participants classified as sufficiently or very active. Baseline blood pressure and fasting capillary blood glucose were consistent with the participants' declared health status. Fourteen participants (60.9%) were allocated to the DEP→diluent sequence and nine (39.1%) to the diluent→DEP sequence.

Table 1
Characteristics of the 23 participants at the baseline visit.

Variable	n (%)	Mean $\pm$ SD
Age, years		32.6 $\pm$ 8.6
Sex – male	12 (52.2)	
Sex – female	11 (47.8)	
Sequence DEP $\rightarrow$ diluent	14 (60.9)	
Sequence diluent $\rightarrow$ DEP	9 (39.1)	
Days between baseline and visit 1		25.7 $\pm$ 23.7
Days between visit 1 and visit 2		25.5 $\pm$ 25.5
Physical activity, MET-min/week		3323.3 $\pm$ 3375.9
Very active	6 (26.1)	
Sufficiently active	12 (52.2)	
Active	3 (13.0)	
Inactive	2 (8.7)	
Leicester Cough Questionnaire (0–21)		20.7 $\pm$ 0.3
Hull Airway Reflux Questionnaire (0–70)		2 $\pm$ 2
Fasting capillary blood glucose, mg/dL		104.3 $\pm$ 16.1
Systolic blood pressure, mmHg		122.7 $\pm$ 14.6
Diastolic blood pressure, mmHg		75.4 $\pm$ 10.1
<i>MET, metabolic equivalent of task. No missing values.</i>		

3.2 TRPV1 genotype distribution and responsiveness score

Genotype frequencies are reported in Table 2 and the distribution of the responsiveness score is shown in Fig. 2. As in our previous cohorts, rs17633288 (T505A) and rs9894618 (K2N) were monomorphic, all participants being homozygous wild type, whereas the four remaining non-synonymous variants were informative, with heterozygosity ranging from 17.4% for rs222749 (P91S) to 52.2% for rs224534 (T469I). There was no evidence that the genotype frequencies of these four variants deviated from Hardy–Weinberg equilibrium (chi-square p = 0.897, 0.913, 0.774 and 0.430 for rs8065080, rs224534, rs222747 and rs222749 respectively). The percentage distribution of alleles closely reproduced that observed in our previous, independent cohort of unselected healthy subjects. Score SNPs [1] ranged from 2 to 6, with most participants clustered at values of 3 and 4, and no participant reached either extreme of the

theoretical range. Of the two additional variants, rs8078936 was polymorphic, with 47.8% heterozygotes, whereas rs748639589 was carried by a single participant.

Table 2
Distribution of TRPV1 genotypes in the 23 participants.

Variant (amino-acid change)	Included in score	Responsive allele	Genotype	n (%)
rs8065080 (I585V)	Yes	T	TT	10 (43.5)
			TC	11 (47.8)
			CC	2 (8.7)
rs224534 (T469I)	Yes	A	GG	7 (30.4)
			GA	12 (52.2)
			AA	4 (17.4)
rs222747 (I315M)	Yes	G	GG	11 (47.8)
			CG	10 (43.5)
			CC	2 (8.7)
rs222749 (P91S)	Yes	A	GG	19 (82.6)
			GA	4 (17.4)
rs17633288 (T505A)	No (monomorphic)		TT	23 (100)
rs9894618 (K2N)	No (monomorphic)		CC	23 (100)
rs8078936	No (extended score)		CC	10 (43.5)
			CT	11 (47.8)
			TT	2 (8.7)
rs748639589	No (extended score)		CC	22 (95.7)
			CT	1 (4.3)
Score SNPs [1] counts capsaicin-responsive alleles (585V, 469I, 315M, 91S) at the four informative loci; theoretical range 0–8, observed range 2–6. Hardy–Weinberg chi-square $p = 0.897, 0.913, 0.774$ and 0.430 respectively				

3.3 Acute DEP inhalation increases cough sensitivity to capsaicin

Inhalation of DEP produced a marked leftward shift of the capsaicin concentration–response curve (Table 3, Supplementary Figure S1). The concentration of capsaicin required to evoke two coughs (C2) fell from $155.1 \pm 162.2 \mu\text{M}$ after diluent to $79.8 \pm 88.0 \mu\text{M}$ after DEP (Fig. 3), a reduction of 48.6% (effect

size - 0.520; $p = 0.029$, $q = 0.051$). The corresponding median [IQR] C2 values were 79.0 [35.5–275.0] μM at baseline, 81.7 [40.0–232.3] μM after diluent, and 57.55 [25.1–104.8] μM after DEP. The corresponding value after diluent was superimposable on the baseline value of 171.2 ± 174.5 μM (effect size 0.024; $p = 0.916$), which demonstrates that neither the repetition of the challenge nor the nebulisation procedure in itself modified cough sensitivity, and that the change observed after DEP is attributable to the particulate. C5 moved in the same direction, from 493.0 ± 339.0 μM after diluent to 285.6 ± 210.9 μM after DEP, but could be determined in twelve participants only and is reported descriptively. Median [IQR] C5 values were 288.0 [122.85–503.5] μM at baseline, 354.0 [293.0–712.0] μM after diluent, and 205.0 [151.0–361.0] μM after DEP.

Table 3

Cough, cardiac autonomic and blood pressure parameters at baseline and after inhalation of diluent and of DEP.

Variable	Baseline	Post diluent	Post DEP	Diluent vs baseline ES (p)	DEP vs diluent ES (p; q)
Cough response					
C2, μM $\boxtimes$	171.2 $\pm$ 174.5	155.1 $\pm$ 162.2	79.8 $\pm$ 88.0	0.024 (0.916)	-0.520 (0.029; 0.051)
C5, μM $\boxtimes$	353.5 $\pm$ 276.7	493.0 $\pm$ 339.0	285.6 $\pm$ 210.9	—	—
Heart rate variability					
HR, bpm	65.8 $\pm$ 10.7	63.4 $\pm$ 11.3	64.4 $\pm$ 9.1	-0.433 (0.048)	0.176 (0.433; 0.505)
LFnu	47.2 $\pm$ 13.4	47.4 $\pm$ 15.6	57.1 $\pm$ 18.4	0.020 (0.927)	0.564 (0.013; 0.030)
HFnu	52.8 $\pm$ 13.4	52.6 $\pm$ 15.6	42.8 $\pm$ 18.4	-0.020 (0.929)	-0.564 (0.013; 0.030)
LF/HF	1.02 $\pm$ 0.54	1.07 $\pm$ 0.64	1.87 $\pm$ 1.65	0.162 (0.443)	0.498 (0.005; 0.030)
SDNN, ms	36.0 $\pm$ 13.7	41.0 $\pm$ 24.1	40.9 $\pm$ 20.4	0.318 (0.138)	-0.006 (0.976; 0.976)
rMSSD, ms	36.5 $\pm$ 14.9	43.1 $\pm$ 27.8	40.0 $\pm$ 23.9	0.386 (0.062)	-0.205 (0.342; 0.479)
Blood pressure $\boxtimes$					
Systolic, mmHg	122.7 $\pm$ 14.6	119.4 $\pm$ 12.6	124.4 $\pm$ 12.6	-0.278 (0.205)	0.459 (0.040; 0.073)
Diastolic, mmHg	75.4 $\pm$ 10.1	74.4 $\pm$ 11.0	75.5 $\pm$ 9.9	0.119 (0.595)	0.123 (0.619; 0.696)
Mean $\pm$ SD. ES, standardised effect size; p from permutation-based paired t tests; q, Benjamini–Hochberg adjusted p within the DEP-versus-diluent family. $\boxtimes$ n = 20. $\boxtimes$ n = 12, descriptive only. $\boxtimes$ Pre-exposure values 121.6 $\pm$ 11.0 / 74.0 $\pm$ 10.1 mmHg before diluent and 121.4 $\pm$ 12.2 / 74.1 $\pm$ 8.9 mmHg before DEP;					

The collective concentration–response curves are reported as Supplementary Figure S1 rather than in the main text. Because the curves are fitted to the mean number of coughs at each concentration, whereas C2 and C5 are derived from individual curves and then summarised, the two quantities do not coincide; the halving of C2 corresponds to only 0.29 log-10 units on the concentration axis, and the inclusion of non-responders in the group means further attenuates the apparent displacement. The

modest graphical separation of the collective curves is itself consistent with the absence of a credible genotype-by-exposure interaction on the airway sensory axis, in contrast with the vagal axis.

3.4 Acute DEP inhalation shifts cardiac autonomic balance towards sympathetic predominance

DEP inhalation was followed by a consistent redistribution of spectral power (Table 3, Fig. 3).

Normalised low-frequency power rose from 47.4 ± 15.6 to 57.1 ± 18.4 (effect size 0.564; $p = 0.013$, $q = 0.030$) and normalised high-frequency power fell symmetrically from 52.6 ± 15.6 to 42.8 ± 18.4 (effect size - 0.564; $p = 0.013$, $q = 0.030$), so that the sympathovagal balance expressed by the LF/HF ratio increased from 1.07 ± 0.64 to 1.87 ± 1.65 (effect size 0.498; $p = 0.005$, $q = 0.030$), that is by approximately 75%. None of these indices differed between diluent and baseline (all $p \geq 0.44$), which again excludes an effect of the procedure. Mean heart rate was unchanged by DEP (64.4 ± 9.1 versus 63.4 ± 11.3 bpm; $p = 0.433$), so that the redistribution of spectral power cannot be a mathematical consequence of a change in cycle length; the small reduction of heart rate observed between baseline and diluent ($p = 0.048$) is compatible with progressive habituation to the laboratory setting.

The time-domain indices SDNN and rMSSD did not change appreciably at group level ($p = 0.976$ and $p = 0.342$). This apparent absence of effect is itself informative once the analysis is stratified by genotype, since both indices proved to be the parameters most closely related to the responsiveness score, as reported in sections 3.6 and 3.7: at group level, opposite individual responses cancel one another. Systolic blood pressure rose after DEP, from 121.4 ± 12.2 to 124.4 ± 12.6 mmHg, in contrast with the slight decrease observed across the diluent session, from 121.6 ± 11.0 to 119.4 ± 12.6 mmHg. No significant differences in any outcome were observed between men and women.

3.5 Acute DEP inhalation depletes antioxidant capacity without increasing hydroperoxide production

Redox biomarkers changed in a specific and internally consistent pattern (Table 4). The biological antioxidant potential decreased from 1992.5 ± 162.2 to 1835.1 ± 126.9 $\mu\text{mol/L}$ (effect size - 1.084; $p < 0.001$, $q < 0.001$) and the redox index from 1.13 ± 0.25 to 1.04 ± 0.21 (effect size - 1.016; $p < 0.001$, $q < 0.001$), whereas circulating hydroperoxides remained essentially unchanged, at 313.4 ± 66.6 versus 309.6 ± 64.8 Carratelli units (effect size - 0.253; $p = 0.243$). A pronounced fall in antioxidant capacity with a stable pro-oxidant load indicates consumption of antioxidant defences rather than an increase in the production of reactive species detectable within the time frame of the experiment, and these are the largest effect sizes observed in the study. As stated in section 2.9, however, redox biomarkers were measured before and after DEP only, without the corresponding measurements at the diluent session, so that a contribution of the procedure, of venepuncture or of circadian variation cannot be formally excluded; this is the single comparison in the study that is not protected by the crossover design.

Table 4
Redox biomarkers before and after DEP inhalation.

Biomarker	Pre DEP	Post DEP	ES (p)	q (BH)
BAP, $\mu\text{mol/L}$	1992.5 $\pm$ 162.2	1835.1 $\pm$ 126.9	-1.084 (< 0.001)	< 0.001
Redox index	1.13 $\pm$ 0.25	1.04 $\pm$ 0.21	-1.016 (< 0.001)	< 0.001
d-ROMs, U.CARR	313.4 $\pm$ 66.6	309.6 $\pm$ 64.8	-0.253 (0.243)	0.243
<i>n = 23. No measurement was obtained at the diluent session, so this contrast is not protected by the crossover design.</i>				

3.6 Rank associations between the TRPV1 responsiveness score and the response to DEP

The associations between *Score SNPs* [1] and the outcomes measured after each exposure are reported in Table 5. After diluent, none of the cough, cardiac or redox variables was significantly associated with the score. After DEP, the score was associated with cough sensitivity, participants carrying a higher number of capsaicin-responsive alleles requiring a lower concentration of capsaicin to cough ($\tau = -0.418$; $p = 0.020$), although this association did not survive adjustment for multiplicity within its family ($q = 0.200$). The analysis of the within-participant changes is reported in Table 6 and Fig. 4. The higher the number of capsaicin-responsive alleles carried, the greater the DEP-induced reduction in SDNN ($\tau = -0.417$; $p = 0.012$, $q = 0.075$) and in rMSSD ($\tau = -0.405$; $p = 0.015$, $q = 0.075$), and the greater the DEP-induced increase in heart rate ($\tau = 0.360$; $p = 0.034$, $q = 0.113$). Since rMSSD reflects short-term, parasympathetically mediated variability and SDNN overall variability, and since heart rate rises as vagal restraint is withdrawn, the three associations describe a single coherent phenomenon: participants with a higher burden of capsaicin-responsive TRPV1 alleles underwent a more pronounced vagal withdrawal upon exposure. A concordant, borderline association was observed for the change in circulating hydroperoxides ($\tau = 0.324$; $p = 0.052$), whereas the change in C2 was not associated with the score ($\tau = 0.121$; $p = 0.516$).

Table 5

Association between Score SNPs [1] and the variables measured after diluent, and before and after DEP inhalation (Kendall's tau-b).

Variable	tau (diluent)	p	q	tau (DEP)	p	q
C2	-0.247	0.152	0.304	-0.418	0.020	0.200
HR	-0.244	0.148	0.304	-0.125	0.469	0.752
LFnu	-0.182	0.279	0.398	-0.082	0.637	0.752
HFnu	0.191	0.255	0.398	0.082	0.637	0.752
LF/HF	-0.191	0.255	0.398	-0.082	0.637	0.752
SDNN	0.245	0.141	0.304	-0.073	0.677	0.752
rMSSD	0.281	0.090	0.304	-0.082	0.637	0.752
BAP	-0.018	0.934	0.934	0.014	0.956	0.956
Redox index	0.191	0.255	0.398	0.118	0.488	0.752
d-ROMs	-0.250	0.134	0.304	-0.137	0.421	0.752
<i>n = 20 for C2, n = 23 otherwise. q, Benjamini–Hochberg adjusted p within each family of ten tests. For the redox biomarkers the “diluent” column reports the pre-DEP measurement. All computed correlations are reported.</i>						

Table 6

Association between Score SNPs [1] and the within-participant changes induced by DEP inhalation (Kendall's tau-b).

Change	tau	p	q (BH)
SDNN (post DEP – post diluent)	-0.417	0.012	0.075
rMSSD (post DEP – post diluent)	-0.405	0.015	0.075
HR (post DEP – post diluent)	0.360	0.034	0.113
d-ROMs (post DEP – pre DEP)	0.324	0.052	0.130
Redox index (post DEP – pre DEP)	-0.227	0.174	0.348
C2 (post DEP – post diluent)	0.121	0.516	0.708
BAP (post DEP – pre DEP)	0.091	0.598	0.708
LFnu (post DEP – post diluent)	0.082	0.637	0.708
HFnu (post DEP – post diluent)	-0.082	0.637	0.708
LF/HF (post DEP – post diluent)	-0.027	0.890	0.890
<i>n = 20 for C2, n = 23 otherwise. Rows ordered by increasing p value.</i>			

3.7 Bayesian analysis of effect modification

In the multilevel model fitted to the values observed at both exposure visits, the interaction between exposure and responsiveness score was credible for rMSSD ($\beta = -0.32$; 95% credible interval -0.54 to -0.10 , Supplementary Figure S2), indicating that the vagal response became progressively more negative as the score increased, while the score showed no association with rMSSD after diluent ($\beta = 0.16$; 95% CrI -0.29 to 0.60). For log-transformed C2 the interaction was in the expected direction but not credible ($\beta = -0.14$; 95% CrI -0.57 to 0.30 , Supplementary Figure S2), against a background association of the score with the cough threshold across both conditions ($\beta = -0.38$; 95% CrI -0.80 to 0.05). In the regression models fitted to each post-DEP outcome with adjustment for the corresponding post-diluent value, crossover sequence, washout interval, age and sex, the responsiveness score retained a credible negative association with rMSSD ($\beta = -0.26$; 95% CrI -0.49 to -0.02) (Table 7). Concordant associations that did not exclude the null were observed for SDNN ($\beta = -0.24$; 95% CrI -0.55 to 0.10) and, in the opposite and physiologically consistent direction, for heart rate ($\beta = 0.15$; 95% CrI -0.09 to 0.40), whereas no association was found for log LF/HF ($\beta = -0.05$; 95% CrI -0.44 to 0.35). For log-transformed C2 the association with the score disappeared entirely once the post-diluent value was held constant ($\beta = -0.05$; 95% CrI -0.53 to 0.39). The value observed after diluent was strongly predictive of the value observed after DEP for heart rate ($\beta = 1.02$), rMSSD ($\beta = 0.85$), SDNN ($\beta = 0.78$) and log C2 ($\beta = 0.60$), but not for log LF/HF ($\beta = 0.09$). Among the covariates, rMSSD after DEP tended to be lower in men ($\beta = -0.39$; 95% CrI -0.80 to 0.03) and with increasing age ($\beta = -0.21$; 95% CrI -0.52 to 0.11), neither association excluding the null. For log-transformed C2, an interaction between crossover sequence and washout interval approached credibility ($\beta = -1.03$; 95% CrI -2.05 to 0.08), participants allocated to the diluent→DEP sequence showing lower post-DEP cough thresholds as the interval between visits lengthened; no comparable signal was detected for any cardiac outcome.

Table 7

Bayesian regression models of the post-DEP outcomes, adjusted for the corresponding post-diluent value, crossover sequence, washout interval, age and sex. Standardised coefficients with 95% credible intervals.

Outcome (post DEP)	β score	95% CrI	β post-diluent value	95% CrI
rMSSD	-0.26	-0.49 to - 0.02	0.85	0.62 to 1.08
SDNN	-0.24	-0.55 to 0.10	0.78	0.44 to 1.06
Heart rate	0.15	-0.09 to 0.40	1.02	0.76 to 1.25
log LF/HF	-0.05	-0.44 to 0.35	0.09	-0.42 to 0.61
log C2	-0.05	-0.53 to 0.39	0.60	0.12 to 1.04
<i>Coefficients are expressed in standard deviation units. The exposure-by-score interaction estimated in the multilevel models was $\beta = -0.32$ (95% CrI -0.54 to -0.10) for rMSSD and $\beta = -0.14$ (95% CrI -0.57 to 0.30) for log C2.</i>				

Taken together, the two sets of models converge on a single conclusion: effect modification by TRPV1 genotype is supported for the vagal axis, where it survives both the interaction test and adjustment for the pre-exposure value, and is not supported for the airway sensory axis, where the rank association observed after exposure is accounted for by the correlation between the two visits.

4. Discussion

In this randomised, double-blind, crossover controlled exposure study, a single acute inhalation of standardised diesel exhaust particles produced three concurrent effects in healthy adults: it halved the concentration of capsaicin required to evoke cough, it displaced cardiac autonomic balance towards sympathetic predominance without altering heart rate, and it consumed circulating antioxidant capacity without a detectable rise in hydroperoxides. None of these changes occurred after inhalation of the diluent, whose values were superimposable on baseline, so that the procedure itself can be excluded as their source. Beyond the average effect, the magnitude of the vagal response varied systematically with the individual burden of capsaicin-responsive TRPV1 alleles, an association that survived both a formal test of interaction and adjustment for the pre-exposure value. Taken together, these observations provide the first in vivo human evidence for a pathway so far demonstrated only in transfected cells and in animal models, and they introduce the individual TRPV1 genotype as a candidate determinant of who converts an environmental particulate exposure into a measurable autonomic response (Fig. 5).

4.1 From cells and animals to humans: the DEP–TRPV1 axis in vivo

That particulate matter engages TRPV1 directly, rather than merely lowering its activation threshold, is well established outside the human setting. Heterologous expression studies rank the potency of particulate species on the channel with coal fly ash at least as effective as diesel exhaust particulate and both more effective than silica, a pattern that identifies particles as agonists [12, 13] rather than as passive carriers. In rodents, pulmonary diesel particulate raises blood pressure and increases the vulnerability of the myocardium to ischaemia–reperfusion injury through the joint activation of sensory TRPV1 and β 1 adrenoceptors [14], and inhalation of concentrated ambient particles alters cardiac rhythm and electrocardiographic morphology through TRP-dependent pulmonary reflexes [15]. In our own cell model, diesel particulate — unlike prostaglandin E2 and bradykinin — increased capsaicin-evoked calcium responses in TRPV1-transfected cells.

The present study closes the remaining step. Using the capsaicin cough challenge as a functional and channel-selective read-out, we found that a single exposure lowered C2 by 48.6%, a shift of the same order as the one we previously obtained in the same laboratory by inhaling prostaglandin E2 or bradykinin, but obtained here with the pollutant itself and at a dose corresponding to a realistic occupational peak. The internal control provided by the crossover is essential to this interpretation: because C2 after diluent was indistinguishable from C2 at baseline, the change cannot be attributed to repetition of the challenge, to the nebulisation procedure, or to a learning effect on the cough response.

One caveat must be stated plainly. Capsaicin is a selective TRPV1 agonist, so a leftward shift of the capsaicin dose–response curve is a channel-specific measure; but our design did not include a TRPV1 antagonist arm, and mediation by TRPV1 is therefore inferred rather than demonstrated pharmacologically. The most compelling argument available in a human study is in fact the genetic one developed below: if the response to the particulate were independent of TRPV1, there would be no reason for the number of capsaicin-responsive TRPV1 alleles to track its magnitude. A contribution of TRPA1, which is also expressed on airway C-fibres and is a candidate target for the soluble organic fraction adsorbed on the particle core [57], cannot be excluded, although in our previous work the cough response to cinnamaldehyde was milder than that to capsaicin and its dose–response curve was almost flat.

4.2 Autonomic dysregulation and its coherence with the experimental and observational literature

The autonomic signature we observed – an increase in normalised low-frequency power, a symmetrical fall in normalised high-frequency power and a 75% rise in the LF/HF ratio, with heart rate unchanged – corresponds closely to what has been described in animals exposed to traffic-derived particulate. In a rat model of metabolic syndrome, inhalation of ambient-level traffic-derived particles decreased cardiac vagal influence and baroreflex sensitivity and increased arrhythmia [58]; in aged, spontaneously hypertensive rats, on-road highway aerosol altered autonomic responses [59]; and the same phenomenology has since been reproduced with particles of entirely different origin, including printer-emitted nanoparticles, which impaired cardiac conduction, haemodynamics and autonomic regulation and induced electrical remodelling [60], and the constituents of electronic cigarettes, which produced conduction defects and arrhythmia [61]. The recurrence of the same autonomic phenotype across sources as different as diesel exhaust, laser printers and electronic cigarettes argues that the determinant is the inhalation of respirable particles engaging airway sensory pathways, rather than a chemical signature peculiar to one fuel.

In humans, the closest precedent is the demonstration that acute exposure to diesel exhaust increases muscle sympathetic nerve activity within ten minutes and peaks at thirty [62], a direct neurographic measure that converges with our indirect spectral one. Our findings also supply the experimental counterpart to a large observational literature: panel studies relating ambient particulate to heart rate variability have repeatedly reported reductions in vagally mediated indices, but with substantial heterogeneity attributable to exposure misclassification, co-pollutants and confounding [63], and a recent meta-analysis restricted to ultrafine particles reaches the same conclusion [64]. A randomised crossover with a placebo aerosol removes those sources of variability by construction. At the clinical end of the same chain, long-term exposure to particulate matter has been associated with an increased incidence of arrhythmias in cohort studies [65], which is precisely the outcome that a sustained or repeated autonomic displacement of the kind we measured would be expected to produce. That heart rate did not change is informative rather than incidental. The redistribution of spectral power therefore cannot be a mathematical artefact of a shortened cycle length, and the finding reproduces what we

previously observed after bradykinin, which sensitised TRPV1 and shifted heart rate variability without inducing tachycardia. The autonomic displacement we describe is a subclinical shift of sympathovagal balance in healthy young adults – the kind of change that carries little consequence in this population but that, in individuals with reduced cardiac reserve, ischaemic disease or metabolic comorbidity, corresponds to the substrate on which triggered arrhythmias arise.

4.3 Why genotype mattered on the vagal axis

The most novel observation of this study is that the individual autonomic response to the particulate was graded by TRPV1 genotype. The additive count of capsaicin-responsive alleles at four non-synonymous loci was associated with the magnitude of the DEP-induced change in three concordant indices, and this association survived the two analyses designed to challenge it: the interaction between exposure and score was credible for rMSSD in a multilevel model, and the score retained a credible negative association with post-exposure rMSSD once the corresponding post-diluent value, the crossover sequence, the washout interval, age and sex were held constant. Because the score was unrelated to rMSSD after diluent, genotype was associated not with the resting autonomic phenotype but with the extent of the vagal withdrawal elicited by the exposure. This result acquires its meaning from the contrast with our previous study, in which the very same polymorphisms modified neither the cough nor the autonomic response to inhaled prostaglandin E2 and bradykinin. That negative finding was not a failure of the score but a consequence of pharmacology. Prostaglandin E2 and bradykinin do not bind TRPV1; they act on EP3 and B2 G-protein-coupled receptors and sensitise the channel indirectly through diacylglycerol and protein kinase C, a route that operates largely independently of the channel domains altered by these variants. A direct channel agonist should behave differently, and the *in vitro* literature supports this expectation specifically for particulate: TRPV1 variants differ in their activation by coal fly ash particles, so that the same polymorphisms that are silent towards an indirect sensitiser discriminate between individuals when the stimulus is a particle. Our data extend that observation from transfected cells to intact humans, and offer a coherent explanation for an apparent inconsistency between two of our own studies: indirect sensitisers do not reveal the effect of genotype, whereas a direct agonist does.

The airway sensory axis behaved differently, and the distinction must be drawn explicitly. The rank association between the score and the capsaicin threshold measured after DEP, which was absent after diluent, did not survive either adjustment for multiplicity within its family or adjustment for the corresponding post-diluent value, and the change in C2 was unrelated to the score. Part of that association is therefore attributable to the correlation between the two visits rather than to a differential response to the particulate. Two readings remain compatible with the data. The first is a floor effect of peripheral origin, in which genetically hypersensitive participants already cough at low capsaicin concentrations under control conditions and have limited room for a further fall in threshold. The second is central and invokes the well-documented placebo and nocebo responsiveness of the cough reflex [66–68], a behaviour that is not expected to distribute according to TRPV1 genotype and would therefore dilute any genotype-dependent peripheral signal. Distinguishing between them will require a larger sample with adequate representation at the extremes of the score.

Two further qualifications apply. The score spanned only the central portion of its theoretical range, from two to six, so that the individuals in whom the effect would be expected to be largest were not represented; and the analyses rest on twenty-three participants, which is why we have reported them as exploratory and have deliberately subjected them to the most conservative available checks rather than to the most favourable.

4.4 Redox balance: consumption of defences rather than oxidative damage

The redox pattern we observed is internally consistent and, in its specificity, informative: the biological antioxidant potential fell markedly while circulating hydroperoxides remained unchanged. A drop in antioxidant capacity at a stable pro-oxidant load indicates that defences were being consumed within the short time frame of the experiment, before any measurable accumulation of systemic oxidative damage. This is what one would expect of a mechanism operating over minutes rather than days, and it is consistent with experimental evidence that the cardiac oxidative stress produced by inhaled particulate is itself mediated by TRP-dependent pulmonary reflexes rather than by systemic inflammation. The tendency of the change in hydroperoxides to track the responsiveness score, although it did not reach significance, points in the same direction. The toxicological relevance of the administered dose is further corroborated by this response: that a dose equivalent to only 58% of the elemental carbon inhaled over a full shift at the occupational exposure limit was sufficient to deplete circulating antioxidant capacity indicates that a realistic particulate peak triggers a systemic compensatory response, and that this response operates over minutes rather than days, before any measurable accumulation of oxidative damage. This is nonetheless the least protected comparison in the study, because no corresponding measurements were obtained at the diluent session, and the effect sizes, which are the largest we observed, should be read with that reservation.

4.5 Implications for risk assessment and preventive strategy

If the acute cardiovascular effects of particulate peaks are mediated in part by a sensory reflex, then the determinants of individual susceptibility are to be sought not only in cardiovascular reserve but also in the sensory apparatus itself. Susceptibility to inhaled particulate has so far been characterised in terms of acquired conditions — metabolic syndrome, obesity, advanced age, pre-existing cardiac disease — each of which amplifies the cardiovascular consequences of exposure in experimental models [58, 59, 69]. Our data suggest that a constitutional, and readily measurable, determinant should be added to that list: a four-locus genotype obtainable from a single blood sample identified, in this small sample, the individuals in whom a given inhaled dose produced the largest vagal withdrawal.

The practical implications are most immediate in occupational settings, where exposure to traffic-derived particulate is both intense and predictable, as in professional drivers and in workers operating diesel-powered equipment in confined environments, and where health surveillance programmes already collect biological samples. It is relevant in this respect that the dose administered here, which was

sufficient to displace autonomic balance in healthy young adults, corresponds to little more than half of the elemental carbon that would be inhaled during a full shift at the current occupational exposure limit. A susceptibility marker of this kind would not justify exclusion from work, but could inform the prioritisation of engineering controls, the design of surveillance intervals and the interpretation of individual cardiovascular findings. In parallel, TRPV1 antagonists are already in clinical development [70], and a genotype-stratified design would be the natural way to test whether blocking the channel abolishes the autonomic response to particulate in humans – an experiment that would also settle the question of mediation left open by the present study.

4.6 Strengths and limitations

The principal strength of this study is its design. A randomised, double-blind crossover in which each participant receives both the particulate and its diluent removes, by construction, the interindividual variability that dominates both cough sensitivity and heart rate variability, and it provides two independent internal controls: the comparison of diluent with baseline, which was null for every outcome, and the comparison of the particulate with its own diluent within the same individual. Blinding was maintained by concealing the nebuliser ampoules, and no participant discontinued after randomisation. The exposure was standardised through a certified reference material, delivered by dosimeter and quantified as an absolute inhaled dose that can be related to occupational exposure limits, and every outcome was assessed with methods already validated in two previous studies from the same laboratory.

The limitations are of four kinds. The first concerns the genetic analyses, which involve a small number of participants and a score spanning only part of its range; they are explicitly exploratory, and the association with the airway sensory axis did not survive the confirmatory analyses. The second concerns mechanism: without a TRPV1 antagonist arm, mediation by the channel is inferred from the selectivity of the capsaicin probe and from the genotype dependence, and a contribution of other sensory channels, or a direct cardiac effect of translocated particles of the kind described for mitochondrial permeability transition in animal models [71], cannot be excluded. The third concerns the exposure: a nebulised suspension of a reference particulate delivered in six breaths reproduces neither the physical state nor the temporal profile of ambient diesel aerosol, and although the total dose corresponds to two hours at 300 $\mu\text{g}/\text{m}^3$, its delivery as a bolus implies a dose rate and a regional deposition pattern that differ from a continuous exposure; the particle size distribution, moreover, derives from the previous characterisation of suspensions prepared with the same protocol rather than from a measurement on these preparations. Particulate matter is moreover not a uniform entity, and the chemical speciation of ambient PM_{2.5} varies substantially between urban centres, reflecting different local sources and atmospheric processing [72]; a certified reference material such as SRM 2975 guarantees experimental reproducibility but may lack components typical of contemporary urban aerosols, in particular secondary organic aerosol, so that our findings speak to the effect of a defined diesel particulate rather than to that of any specific urban mixture. The fourth concerns generalisability and scope: the participants were healthy, young and non-smoking, the observation window was short, no arrhythmic endpoint was assessed, the redox

comparison lacked its diluent counterpart, C5 could be determined in twelve participants only, and the two exposure sequences were unbalanced with a variable interval between visits, for which sequence and washout interval were included as covariates in the adjusted models.

5. Conclusions

A single controlled inhalation of diesel exhaust particles, at a dose corresponding to a realistic occupational peak, sensitises airway TRPV1 and displaces cardiac autonomic balance towards sympathetic predominance in healthy humans, while consuming systemic antioxidant capacity. Obtained under randomised, double-blind, crossover conditions with an internally consistent placebo, these effects provide the first in vivo human demonstration of a neurogenic pathway previously documented only in cells and in animal models, and offer a mechanism whose time course matches the excess of cardiovascular events observed within hours of particulate peaks. The magnitude of the vagal response was graded by the individual burden of capsaicin-responsive TRPV1 alleles and survived adjustment for the pre-exposure value, whereas the same polymorphisms are known to be silent towards indirect endogenous sensitisers of the channel — a contrast coherent with the direct agonist action of particulate on TRPV1 and one that identifies TRPV1 genotype as a candidate susceptibility biomarker for traffic-related particulate exposure. Confirmation in larger, genotype-stratified studies, and pharmacological verification with a TRPV1 antagonist, are the natural next steps.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the University Hospital of Padua (protocol 4057/AO/17), with amendments approved on 13 September 2018 and on 21 October 2021. All participants gave written informed consent before any study procedure, and all procedures were performed in accordance with the Declaration of Helsinki and with the applicable national regulations.

Consent for publication

Not applicable: the manuscript contains no data relating to an identifiable individual person.

Competing interests

The authors declare that they have no competing interests.

Supplementary Information

Additional file 1. Supplementary Figures S1 and S2. Supplementary Figure S1. Capsaicin concentration–response curves expressed as mean $\pm$ standard deviation of the number of coughs against the logarithm

of the capsaicin concentration, at baseline, after inhalation of the diluent and after inhalation of diesel exhaust particles. Supplementary Figure S2: posterior distributions of the exposure-by-score interaction parameters for the log-transformed C2 and rMSSD outcomes in the Bayesian multilevel models.

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Author Contribution

S.P. and F.L. conceived and designed the study, obtained ethical approval and secured funding. F.L. conducted the controlled exposure sessions, the capsaicin cough challenges and the heart rate variability recordings, and drafted the Methods. L.C. performed the TRPV1 genotyping and the redox biomarker analyses and curated the data. B.H., I.B. and A.B. carried out the laboratory analyses and contributed to data validation. S.D.V. performed the statistical analysis, including the permutation-based tests and the Bayesian models, and prepared Figures 2–4 and Supplementary Figure S1. F.L. prepared Supplementary Figure S2. S.P. prepared Figures 1 and 5, wrote the main manuscript text and coordinated the work of the team throughout. F.L. and S.P. supervised the project. All authors reviewed and approved the final manuscript.

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Data Availability

The datasets generated and analysed during the current study are not publicly available because they contain individual genotype data from a small number of participants, which could compromise participant privacy, but are available from the corresponding author on reasonable request.

References

1. Pope CA, Dockery DW. Health Effects of Fine Particulate Air Pollution: Lines that Connect. *J Air Waste Manage Assoc* Taylor Francis Group. 2006;56:709–42. <https://doi.org/10.1080/10473289.2006.10464485>.
2. Sun Q, Hong X, Wold LE. Cardiovascular effects of ambient particulate air pollution exposure. *Circulation* Lippincott Williams Wilkins. 2010;121:2755–65. <https://doi.org/10.1161/CIRCULATIONAHA.109.893461>.
3. Peters A, von Klot S, Heier M, Trentinaglia I, Hörmann A, Wichmann HE, et al. Exposure to Traffic and the Onset of Myocardial Infarction. *New Engl J Med* New Engl J Med (NEJM/MMS). 2004;351:1721–30. <https://doi.org/10.1056/NEJMOA040203>.
4. Folino F, Buja G, Zanutto G, Marras E, Allocca G, Vaccari D, et al. Association between air pollution and ventricular arrhythmias in high-risk patients (ARIA study): a multicentre longitudinal study. *Lancet Planet Health* Elsevier. 2017;1:e58–64. [https://doi.org/10.1016/S2542-5196\(17\)30020-7](https://doi.org/10.1016/S2542-5196(17)30020-7).
5. Scapellato ML, Lotti M. Short-Term Effects of Particulate Matter: An Inflammatory Mechanism? *Crit Rev Toxicol*. Taylor Francis. 2007;37:461–87. <https://doi.org/10.1080/10408440701385622>.
6. Rajagopalan S, Brook RD, Salerno PRVO, Bourges-Sevenier B, Landrigan P, Nieuwenhuijsen MJ, et al. Air pollution exposure and cardiometabolic risk. *Lancet Diabetes Endocrinol* Elsevier. 2024;12:196–208. [https://doi.org/10.1016/S2213-8587\(23\)00361-3](https://doi.org/10.1016/S2213-8587(23)00361-3).
7. Lee LY, Widdicombe JG. Modulation of airway sensitivity to inhaled irritants: role of inflammatory mediators. *Environ Health Perspect Public Health Serv US Dept Health Hum Serv*. 2001;109:585. <https://doi.org/10.1289/EHP.01109S4585>.
8. Hamanaka RB, Mutlu GM. Particulate matter air pollution: effects on the respiratory system. *J Clin Invest Am Soc Clin Invest*. 2025;135. <https://doi.org/10.1172/JCI194312>.
9. Grace MS, Baxter M, Dubuis E, Birrell MA, Belvisi MG. Transient receptor potential (TRP) channels in the airway: Role in airway disease. *Br J Pharmacol* John Wiley Sons Inc. 2014;171:2593–607. <https://doi.org/10.1111/BPH.12538>.
10. Liviero F, Campisi M, Mason P, Pavanello S. Transient Receptor Potential Vanilloid Subtype 1: Potential Role in Infection, Susceptibility, Symptoms and Treatment of COVID-19. *Front Med*

- (Lausanne). *Front Media S A*. 2021;8:753819. <https://doi.org/10.3389/FMED.2021.753819>.
11. Morice AH, Fontana GA, Belvisi MG, Birring SS, Chung KF, Diczpinigaitis PV, et al. ERS guidelines on the assessment of cough. *Eur Respiratory J Eur Respiratory Soc*. 2007;29:1256–76. <https://doi.org/10.1183/09031936.00101006>.
 12. Deering-Rice CE, Johansen ME, Roberts JK, Thomas KC, Romero EG, Lee J, et al. Transient Receptor Potential Vanilloid-1 (TRPV1) Is a Mediator of Lung Toxicity for Coal Fly Ash Particulate Material. *Mol Pharmacol Elsevier*. 2012;81:411–9. <https://doi.org/10.1124/MOL.111.076067>.
 13. Deering-Rice CE, Stockmann C, Romero EG, Lu Z, Shapiro D, Stone BL, et al. Characterization of Transient Receptor Potential Vanilloid-1 (TRPV1) Variant Activation by Coal Fly Ash Particles and Associations with Altered Transient Receptor Potential Ankyrin-1 (TRPA1) Expression and Asthma. *J Biol Chem Elsevier*. 2016;291:24866–79. <https://doi.org/10.1074/JBC.M116.746156>.
 14. Robertson S, Thomson AL, Carter R, Stott HR, Shaw CA, Hadoke PWF et al. Pulmonary diesel particulate increases susceptibility to myocardial ischemia/reperfusion injury via activation of sensory TRPV1 and β 1 adrenoreceptors. *Particle and Fibre Toxicology* 2014 11:1. *BioMed Central*; 2014;11:12-. <https://doi.org/10.1186/1743-8977-11-12>
 15. Ghelfi E, Rhoden CR, Wellenius GA, Lawrence J, Gonzalez-Flecha B. Cardiac oxidative stress and electrophysiological changes in rats exposed to concentrated ambient particles are mediated by TRP-dependent pulmonary reflexes. *Toxicol Sci Toxicol Sci*. 2008;102:328–36. <https://doi.org/10.1093/TOXSCI/KFN005>.
 16. Liviero F, Scarpa MC, De Stefani D, Folino F, Campisi M, Mason P et al. Modulation of TRPV-1 by prostaglandin-E2 and bradykinin changes cough sensitivity and autonomic regulation of cardiac rhythm in healthy subjects. *Scientific Reports* 2020 10:1. *Nature Publishing Group*; 2020;10:15163-. <https://doi.org/10.1038/s41598-020-72062-y>
 17. Liviero F, Pavanello S. Epidemiological and mechanistic links between PM2.5 exposure and type 2 diabetes: focus on the TRPV1 receptor. *Front Endocrinol (Lausanne) Front Endocrinol (Lausanne)*. 2025;16. <https://doi.org/10.3389/FENDO.2025.1653375>.
 18. Xu H, Tian W, Fu Y, Oyama TT, Anderson S, Cohen DM. *Am Physiological Soc*. 2007;293:1865–76. <https://doi.org/10.1152/AJPRENAL.00347.2007>. <https://doi.org/10.1152/ajprenal003472007>. Functional effects of nonsynonymous polymorphisms in the human TRPV1 gene.
 19. Liviero F, Campisi M, Scarpa MC, Mason P, Guarnieri G, Maestrelli P, et al. Multiple single nucleotide polymorphisms of the transient receptor potential vanilloid 1 (TRPV1) genes associate with cough sensitivity to capsaicin in healthy subjects. *Pulm Pharmacol Ther Acad Press*. 2020;61. <https://doi.org/10.1016/j.pupt.2020.101889>.
 20. Sadofsky LR, Cantero-Recasens G, Wright C, Valverde MA, Morice AH. TRPV1 polymorphisms influence capsaicin cough sensitivity in men. *J Thorac Dis AME Publishing Co*. 2017;9:839. <https://doi.org/10.21037/JTD.2017.03.50>.
 21. Forstenpointner J, Förster M, May D, Hofschulte F, Cascorbi I, Wasner G, et al. Short Report: TRPV1-polymorphism 1911 A > G alters capsaicin-induced sensory changes in healthy subjects. *PLoS One*

- PLoS One. 2017;12. <https://doi.org/10.1371/JOURNAL.PONE.0183322>.
22. Cantero-Recasens G, Gonzalez JR, Fandos C, Duran-Tauleria E, Smit LAM, Kauffmann F, et al. Loss of function of transient receptor potential vanilloid 1 (TRPV1) genetic variant is associated with lower risk of active childhood asthma. *J Biol Chem Am Soc Biochem Mol Biology Inc.* 2010;285:27532–5. <https://doi.org/10.1074/jbc.C110.159491>.
 23. Grace M, Birrell MA, Dubuis E, Maher SA, Belvisi MG. Transient receptor potential channels mediate the tussive response to prostaglandin E2 and bradykinin. *Thorax Thorax.* 2012;67:891–900. <https://doi.org/10.1136/THORAXJNL-2011-201443>.
 24. Maher SA, Birrell MA, Belvisi MG. Prostaglandin E2 mediates cough via the EP3 receptor: implications for future disease therapy. *Am J Respir Crit Care Med Am J Respir Crit Care Med.* 2009;180:923–8. <https://doi.org/10.1164/RCCM.200903-0388OC>.
 25. Grace MS, Dubuis E, Birrell MA, Belvisi MG. Pre-clinical studies in cough research: Role of Transient Receptor Potential (TRP) channels. *Pulm Pharmacol Ther.* 2013;26:498. <https://doi.org/10.1016/J.PUPT.2013.02.007>.
 26. Brook RD, Rajagopalan S, Pope CA, Brook JR, Bhatnagar A, Diez-Roux AV, et al. Particulate matter air pollution and cardiovascular disease: An update to the scientific statement from the American Heart Association. *Circulation Circulation.* 2010;121:2331–78. <https://doi.org/10.1161/CIR.0B013E3181DBECE1>.
 27. Sánchez-Rodríguez MA, Mendoza-Núñez VM. Oxidative Stress Indexes for Diagnosis of Health or Disease in Humans. *Oxid Med Cell Longev. Oxid Med Cell Longev;* 2019;2019. <https://doi.org/10.1155/2019/4128152>
 28. Cardenas A, Fadadu RP, Van Der Laan L, Ward-Caviness C, Granger L, Diaz-Sanchez D, et al. Controlled human exposures to diesel exhaust: a human epigenome-wide experiment of target bronchial epithelial cells. *Environ Epigenet.* Volume 7. Oxford University Press; 2021. p. dvab003. <https://doi.org/10.1093/EEP/DVAB003>.
 29. Dwan K, Li T, Altman DG, Elbourne D. CONSORT 2010 statement: extension to randomised crossover trials. *BMJ BMJ.* 2019;366. <https://doi.org/10.1136/BMJ.L4378>.
 30. Sackett DL, Commentary. Measuring the success of blinding in RCTs: don't, must, can't or needn't? *Int J Epidemiol Oxf Acad.* 2007;36:664–5. <https://doi.org/10.1093/IJE/DYM088>.
 31. Birring SS, Prudon B, Carr AJ, Singh SJ, Morgan L, Pavord ID. Development of a symptom specific health status measure for patients with chronic cough: Leicester Cough Questionnaire (LCQ). *Thorax Thorax.* 2003;58:339–43. <https://doi.org/10.1136/THORAX.58.4.339>.
 32. Sorano A, Fumagalli C, Cinelli E, Birring SS, Fontana GA, Lavorini F. Development of an Italian version of the Leicester cough questionnaire and its relationship with other symptom-specific measures for patients with chronic cough. *Respir Med.* W.B. Saunders Ltd; 2024;227. <https://doi.org/10.1016/j.rmed.2024.107642>
 33. Morice AH, Faruqi S, Wright CE, Thompson R, Bland JM. Cough Hypersensitivity Syndrome: A Distinct Clinical Entity. *Springer.* 2010;189:73–9. <https://doi.org/10.1007/S00408-010-9272-1>. Lung

34. International Society for the Study of Cough. Hull Cough Hypersensitivity Questionnaire, Italian Version. International Society for the Study of Cough.
<https://www.issc.info/HullCoughHypersensitivityQuestionnaire.html>. Accessed 3 Sep 2026.
35. Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc Med Sci Sports Exerc*. 2003;35:1381–95. <https://doi.org/10.1249/01.MSS.0000078924.61453.FB>.
36. Mannocci A, Di Thiene D, Del Cimmuto A, Masala D, Boccia A, De Vito E, et al. Int Phys Activity Questionnaire: validation Assess Italian sample. 2010;7:369–76. <https://doi.org/10.2427/5694>.
37. Mancia G, Kreutz R, Brunström M, Burnier M, Grassi G, Januszewicz A, et al. 2023 ESH Guidelines for the management of arterial hypertension The Task Force for the management of arterial hypertension of the European Society of Hypertension: Endorsed by the International Society of Hypertension (ISH) and the European Renal Associa...*J Hypertens*. *J Hypertens*; 2023;41:1874–2071. <https://doi.org/10.1097/HJH.0000000000003480>.
38. National Institute of Standards and Technology. Standard Reference Materials | NIST. Certificate of Analysis, Standard Reference Material 2975: Diesel Particulate Matter (Industrial Forklift).
<https://www.nist.gov/srm>. Accessed 3 Sep 2026.
39. LeBaron TW, Ohno K, Salomez-Ihl C, Cinquin P, Boucher F, Sano M et al. Respiratory-physiology modeling of therapeutic hydrogen inhalation: defining the fraction of inspired hydrogen (FiH₂) and flow-rate requirements. *Respiratory Research* 2026 27:1. *BioMed Central*; 2026;27:259-.
<https://doi.org/10.1186/S12931-026-03664-9>
40. Folesani G, Gherardi M, Galetti M, Petronini PG, Pasquale F, De, Cavallo D, et al. Critical Issues in Assessing Occupational Exposure to Diesel Dust Exhaust. *Med Lav Med Lav*. 2024;115.
<https://doi.org/10.23749/MDL.V115I4.16100>.
41. IFA. GESTIS International Limit Values: diesel engine emissions. IFA.
<https://limitvalue.ifa.dguv.de/substances>. Accessed 3 Sep 2026.
42. Morice AH, Millqvist E, Bieksiene K, Birring SS, Dicpinigaitis P, Ribas CD, et al. ERS guidelines on the diagnosis and treatment of chronic cough in adults and children. *Eur Respir J*. 2020;55.
<https://doi.org/10.1183/13993003.01136-2019>.
43. Di Veroli GY, Fornari C, Goldlust I, Mills G, Koh SB, Bramhall JL et al. An automated fitting procedure and software for dose-response curves with multiphasic features. *Scientific Reports* 2015 5:1. *Nature Publishing Group*; 2015;5:14701-. <https://doi.org/10.1038/srep14701>
44. Tarvainen MP, Niskanen JP, Lipponen JA, Ranta-aho PO, Karjalainen PA. Kubios HRV - Heart rate variability analysis software. *Comput Methods Programs Biomed Comput Methods Programs Biomed*. 2014;113:210–20. <https://doi.org/10.1016/j.cmpb.2013.07.024>.
45. Liviero F, Scapellato ML, Folino F, Moretto A, Mason P, Pavanello S. Persistent Increase of Sympathetic Activity in Post-Acute COVID-19 of Paucisymptomatic Healthcare Workers. *Int J*

- Environ Res Public Health Int J Environ Res Public Health. 2023;20.
<https://doi.org/10.3390/IJERPH20010830>.
46. Liviero F, Scapellato ML, Volpin A, Battistella M, Fabris L, Brischigliaro L, et al. Long term follow-up of heart rate variability in healthcare workers with mild COVID-19. *Front Neurol Front Neurol*. 2024;15.
<https://doi.org/10.3389/FNEUR.2024.1403551>.
 47. Sammito S, Thielmann B, Klussmann A, Deußen A, Braumann KM, Böckelmann I. Guideline for the application of heart rate and heart rate variability in occupational medicine and occupational health science. *J Occup Med Toxicol J Occup Med Toxicol*. 2024;19. <https://doi.org/10.1186/S12995-024-00414-9>.
 48. Malik M, John Camm A, Thomas Bigger J, Breithardt G, Cerutti S, Cohen RJ, et al. Heart rate variability: Standards of measurement, physiological interpretation, and clinical use. *Circulation Lippincott Williams Wilkins*. 1996;93:1043–65. <https://doi.org/10.1161/01.CIR.93.5.1043>.
 ;WEBSITE:WEBSITE:AHA-SITE;ISSUE:ISSUE:DOI.
 49. Pavanello S, Campisi M, Fabozzo A, Cibir G, Tarzia V, Toscano G, et al. The biological age of the heart is consistently younger than chronological age. *Sci Rep Sci Rep*. 2020;10.
<https://doi.org/10.1038/S41598-020-67622-1>.
 50. Campisi M, Cannella L, Paccagnella O, Brazzale AR, Agnolin A, Grothe T, et al. Unveiling the geroprotective potential of *Monarda didyma* L.: insights from in vitro studies and a randomized clinical trial on slowing biological aging and improving quality of life. *Geroscience Geroscience*. 2025;47:4253–90. <https://doi.org/10.1007/S11357-025-01580-2>.
 51. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *J R Stat Soc Series B Stat Methodol. Oxf Acad*. 1995;57:289–300.
<https://doi.org/10.1111/J.2517-6161.1995.TB02031.X>.
 52. R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria 2026. Vienna, Austria: R Foundation for Statistical Computing; 2026;
<https://doi.org/10.32614/R.MANUALS>
 53. Finos L. Multivariate Permutation Tests [R package flip version 2.5.1]. CRAN: Contributed Packages., Comprehensive R. Archive Network (CRAN); 2025; <https://doi.org/10.32614/CRAN.PACKAGE.FLIP>
 54. Bürkner PC. brms: An R Package for Bayesian Multilevel Models Using Stan. *J Stat Softw Am Stat Association*. 2017;80:1–28. <https://doi.org/10.18637/JSS.V080.I01>.
 55. Bürkner PC. Advanced Bayesian multilevel modeling with the R package brms. *R Journal. Technische Universitaet Wien*. 2018;10:395–411. <https://doi.org/10.32614/RJ-2018-017/>.
 56. Bürkner PC. Bayesian Item Response Modeling in R with brms and Stan. *J Stat Softw Am Stat Association*. 2021;100:1–54. <https://doi.org/10.18637/JSS.V100.I05>.
 57. Robinson RK, Birrell MA, Adcock JJ, Wortley MA, Dubuis ED, Chen S, et al. Mechanistic link between diesel exhaust particles and respiratory reflexes. *J Allergy Clin Immunol Mosby*. 2018;141:1074–e10849. <https://doi.org/10.1016/J.JACI.2017.04.038>.

58. Carll AP, Crespo SM, Filho MS, Zati DH, Coull BA, Diaz EA, et al. Inhaled ambient-level traffic-derived particulates decrease cardiac vagal influence and baroreflexes and increase arrhythmia in a rat model of metabolic syndrome. *Part Fibre Toxicol*. BioMed Cent Ltd. 2017;14:16. <https://doi.org/10.1186/S12989-017-0196-2>.
59. Elder A, Couderc JP, Gelein R, Eberly S, Cox C, Xia X et al. Effects of on-road highway aerosol exposures on autonomic responses in aged, spontaneously hypertensive rats. *Inhal Toxicol* [2007];19:1–12. <https://doi.org/10.1080/08958370600985735>
60. Carll AP, Salatini R, Pirela SV, Wang Y, Xie Z, Lorkiewicz P, et al. Inhalation of printer-emitted particles impairs cardiac conduction, hemodynamics, and autonomic regulation and induces arrhythmia and electrical remodeling in rats. *Part Fibre Toxicol*. 2020;17. <https://doi.org/10.1186/S12989-019-0335-Z>.
61. Carll AP, Arab C, Salatini R, Miles MD, Nystoriak MA, Fulghum KL, et al. E-cigarettes and their lone constituents induce cardiac arrhythmia and conduction defects in mice. *Nat Commun*. 2022;13. <https://doi.org/10.1038/S41467-022-33203-1>.
62. Rankin GD, Kabéle M, Brown R, Macefield VG, Sandström T, Bosson JA. Acute Exposure to Diesel Exhaust Increases Muscle Sympathetic Nerve Activity in Humans. *J Am Heart Assoc*. 2021;10. <https://doi.org/10.1161/JAHA.120.018448>.
63. Buteau S, Goldberg MS. A structured review of panel studies used to investigate associations between ambient air pollution and heart rate variability. *Environ Res*. 2016;148:207–47. <https://doi.org/10.1016/J.ENVRES.2016.03.013>.
64. Zhang S, Breitner S, Pickford R, Lanki T, Okokon E, Morawska L, et al. Short-term effects of ultrafine particles on heart rate variability: A systematic review and meta-analysis. *Environmental Pollution*. Elsevier Ltd; 2022. p. 314. <https://doi.org/10.1016/j.envpol.2022.120245>.
65. Zhang Z, Kang J, Hong YS, Chang Y, Ryu S, Park J, et al. Long-Term Particulate Matter Exposure and Incidence of Arrhythmias: A Cohort Study. *J Am Heart Assoc*. 2020;9. <https://doi.org/10.1161/JAHA.120.016885>.
66. Leech J, Mazzone SB, Farrell MJ. The effect of placebo conditioning on capsaicin-evoked urge to cough. *Chest*. 2012;142:951–7. <https://doi.org/10.1378/chest.12-0362>.
67. Drake MG, McGarvey LP, Morice AH. From bench to bedside: The role of cough hypersensitivity in chronic cough. *Clin Transl Med*. 2023;13. <https://doi.org/10.1002/CTM2.1343>.
68. Zhang M, Zhang B, Morice AH. Decoding the impact of the placebo response in clinical trials for chronic cough. *ERJ Open Res*. 2024;10. <https://doi.org/10.1183/23120541.00335-2024>.
69. Wagner JG, Allen K, Yang HY, Nan B, Morishita M, Mukherjee B, et al. Cardiovascular Depression in Rats Exposed to Inhaled Particulate Matter and Ozone: Effects of Diet-Induced Metabolic Syndrome. *Environ Health Perspect*. 2013;122:27. <https://doi.org/10.1289/EHP.1307085>.
70. Gram DX, Holst JJ, Szallasi A. TRPV1: A Potential Therapeutic Target in Type 2 Diabetes and Comorbidities? *Trends Mol Med*. Elsevier Ltd. 2017;23:1002–13.

71. Wu T, Tong M, Chu A, Wu K, Niu X, Zhang Z. PM2.5-Induced Programmed Myocardial Cell Death via mPTP Opening Results in Deteriorated Cardiac Function in HFpEF Mice. *Cardiovasc Toxicol*. 2022;22:746–62. <https://doi.org/10.1007/S12012-022-09753-7>.

72. Farahani VJ, Pirhadi M, Sioutas C. Are standardized diesel exhaust particles (DEP) representative of ambient particles in air pollution toxicological studies? *Science of the Total Environment*. Elsevier B V. 2021;788. <https://doi.org/10.1016/j.scitotenv.2021.147854>.

Figures

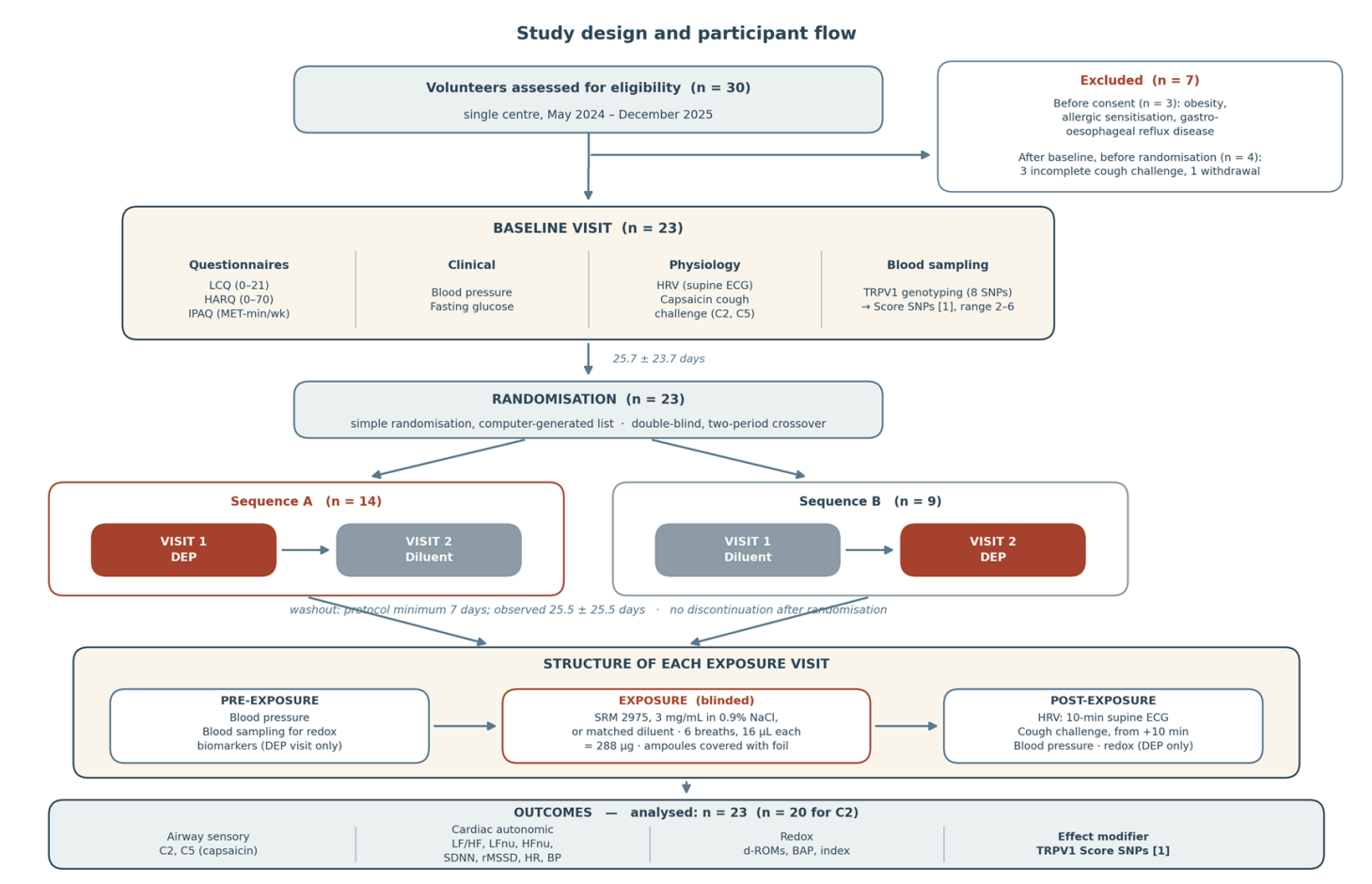


Figure 1

Study design and participant flow. Study design and participant flow. Of thirty volunteers assessed for eligibility, twenty-three were enrolled and randomised to one of two exposure sequences in a double-blind, two-period crossover; none discontinued after randomisation. Each exposure visit comprised a pre-exposure assessment, the blinded administration of the DEP suspension or of the matched diluent by dosimeter-controlled nebulisation, and a post-exposure assessment in which the heart rate variability recording preceded the capsaicin cough challenge. Redox biomarkers were measured before and after the DEP session only. LCQ, Leicester Cough Questionnaire; HARQ, Hull Airway Reflux Questionnaire;

IPAQ, International Physical Activity Questionnaire; HRV, heart rate variability; C2 and C5, capsaicin concentrations evoking at least two and at least five coughs.

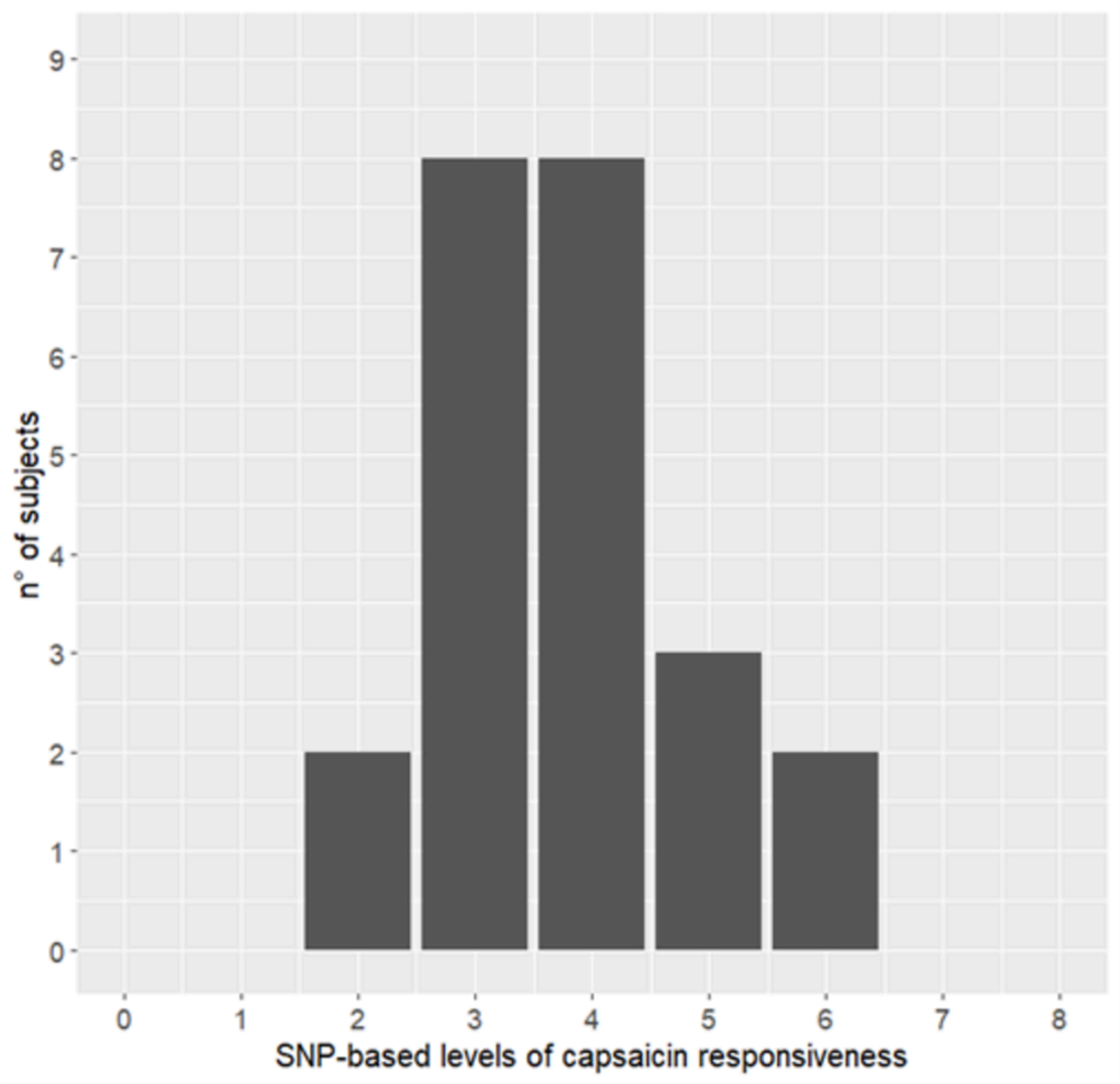


Figure 2

Distribution of SNP-based levels of capsaicin responsiveness in 23 participants. Level was calculated by counting the number of capsaicin-responsive alleles present in each of the SNP sites considered. Data is presented ranging from 0 to 8, representing the four SNP sites on two alleles, as indicated in Table 2.

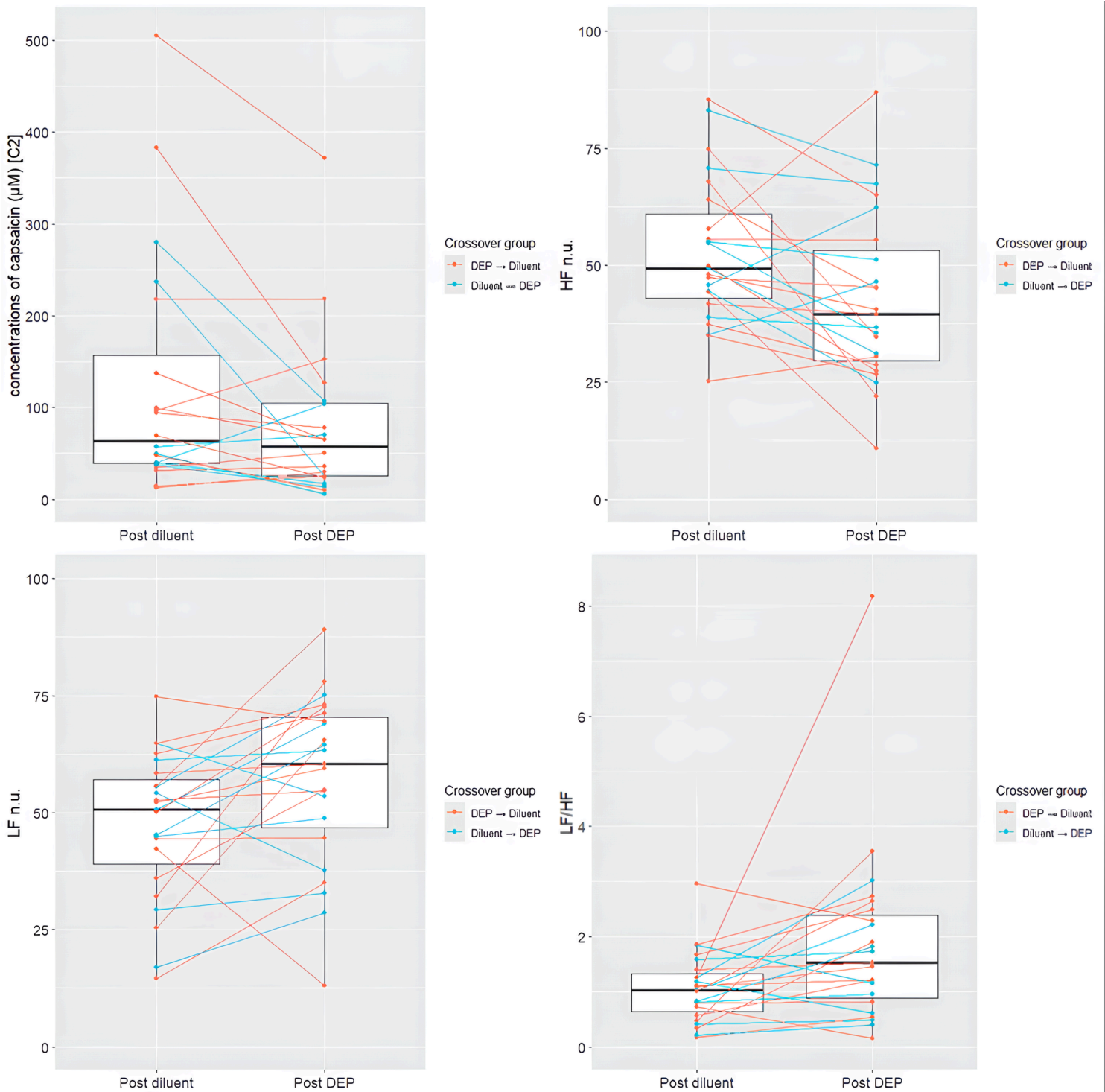


Figure 3

Individual paired plots for LFnu, HFnu, LF/HF and C2 after diluent and after DEP, with the median indicated by a horizontal bar.

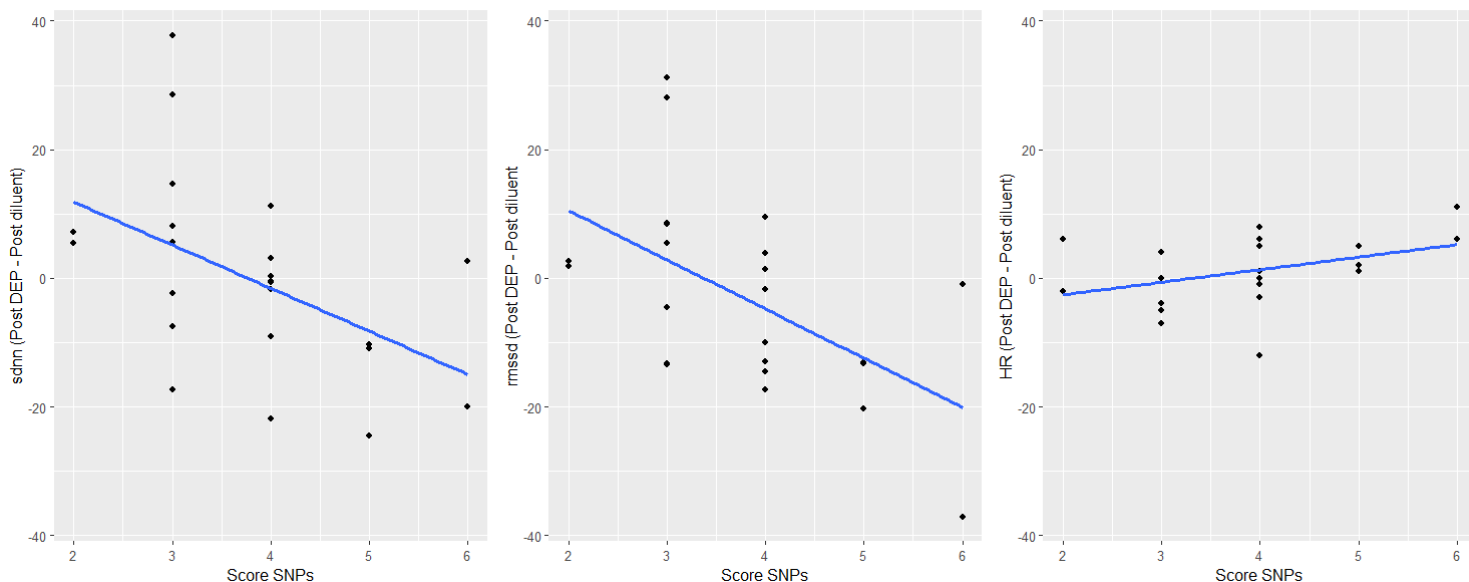


Figure 4

Association between Score SNPs [1] and the DEP-induced changes in rMSSD, SDNN and heart rate.

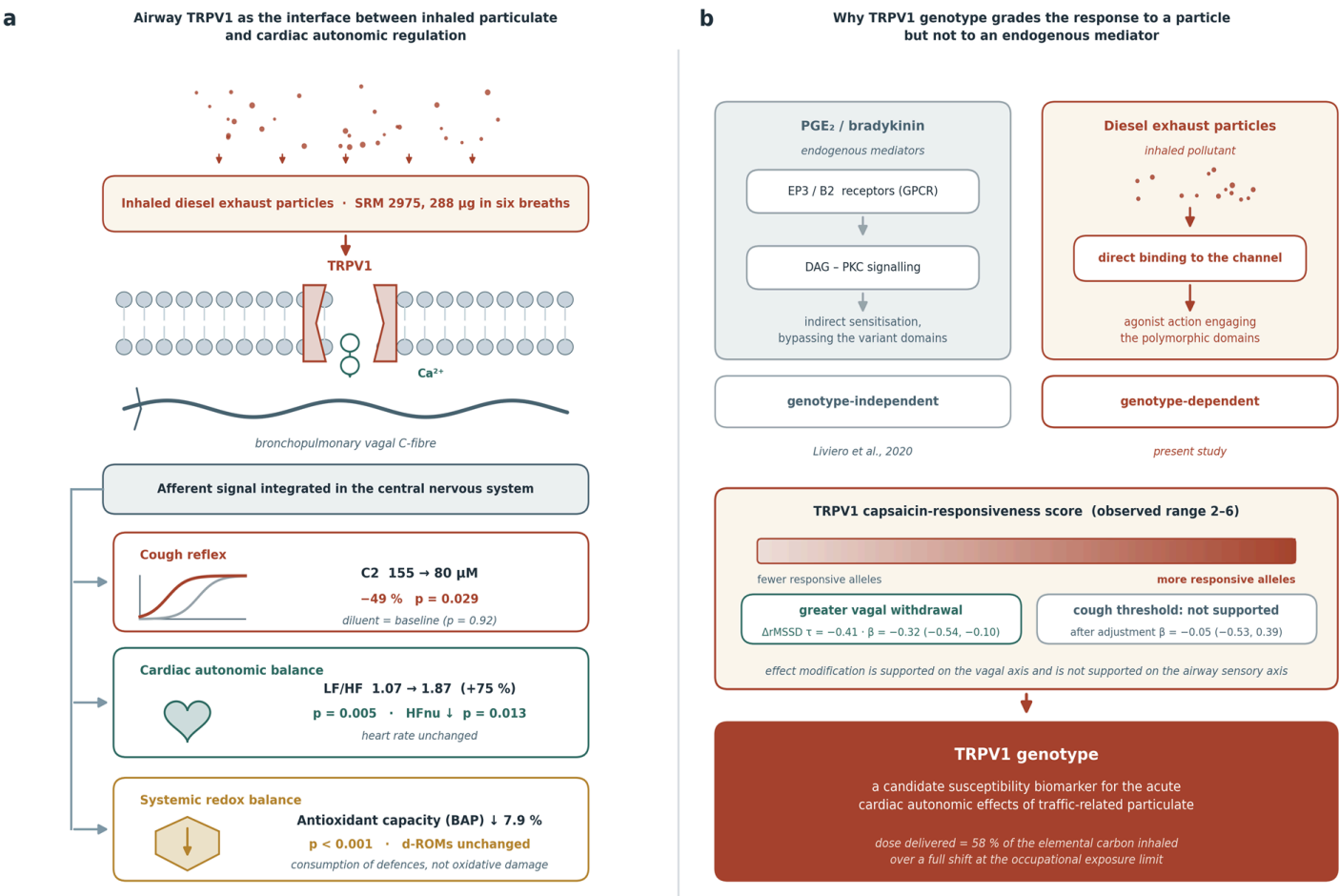


Figure 5

Proposed mechanism and summary of the findings. Proposed mechanism and summary of the findings. (a) Inhaled diesel exhaust particles reach the terminal airways and act as direct agonists of TRPV1 on bronchopulmonary vagal C-fibres; the resulting afferent signal, integrated centrally, is followed by the three effects measured in this study – an increase in cough sensitivity to capsaicin, a displacement of cardiac autonomic balance towards sympathetic predominance at unchanged heart rate, and a consumption of systemic antioxidant capacity without a rise in circulating hydroperoxides. Values are those reported in Tables 3 and 4; the comparison of diluent with baseline was null for every outcome. (b) Contrast between two ways of engaging the same channel. Prostaglandin E2 and bradykinin do not bind TRPV1 but sensitise it indirectly through EP3 and B2 G-protein-coupled receptors and downstream diacylglycerol and protein kinase C signalling, a route that bypasses the domains altered by the non-synonymous variants, and in our previous study the responsiveness score modified neither the cough nor the autonomic response; diesel particulate, acting as a direct agonist, engages those domains, and the magnitude of the response varied with the score. Effect modification was supported on the vagal axis, where it survived both the test of interaction and adjustment for the post-diluent value, and was not supported on the airway sensory axis. The left-hand column of panel b summarises a comparison with our previously published study and not an experiment performed here.

Supplementary Files

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