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Supplementary Materials

Vitamin B6 (pyridoxal 5' phosphate) antagonizes carotid body P2X3 receptors in hypertension

Igor S A Felipe¹, Thalia L Babbage¹, Rajaa Shaheen², Marcella Bassetto³, Jui-Lin Fan¹, Audrys Pauza¹, Olivia Gold¹, Pratik Thakkar¹, Matthew Dawes⁴, Melissa L. Bates⁵, Fiona McBryde¹, Samuel J Fountain², James P Fisher¹†, Julian F R Paton¹†

1- Department of Physiology, Manaaki Manawa – The Centre for Heart Research, Faculty of Medical & Health Sciences, University of Auckland, New Zealand.

2- School of Biological Sciences, University of East Anglia, United Kingdom

3- School of Pharmacy and Pharmaceutical Sciences, Cardiff University, United Kingdom

4- Department of Medicine, Faculty of Medical & Health Sciences, University of Auckland, New Zealand

5- Department of Internal Medicine, Division of Hematology, Oncology, and Blood & Marrow Transplantation and Department of Pediatrics, Division of Neonatology, University of Iowa, United States of America

This supplemental material is divided into 4 sections:

- Section I - Material & Methods: Pages 1-13
- Section II - Detailed statistical analysis per dataset: Pages 13-15
- Section III - Additional Results: Pages 16-23
- Section IV - Statistical output: Pages 24-100

Section I - Material & Methods:

Pre-clinical work

Sample size calculation

For preclinical work, no sample size calculation a priori was performed. The minimum sample size per protocol was defined as 5 animals. This sample size was defined based on previous work from the group, in which we demonstrated it to be enough to detect translatable outcomes in CB interventions. The preclinical work was unblind and no method of randomization was carried out. This occurred due to the nature of the experiments as animals were not allocated into different groups; rather we assessed the effect of PLP over time, i.e., comparing our dependent variable before and after the intervention on the same experimental unit (i.e. rat).

In vitro studies

A 1321N1 cell line stably expressing the human P2X2/3 receptor²⁰ was maintained in Dulbecco's Modified Eagle Medium containing 10% (v/v) fetal bovine serum, 2mM L-glutamine, 50 U/mL penicillin, 50 µg/mL streptomycin and 250 ng/mL puromycin. Cells were cultured at 37°C with 5% CO₂ in a humidified incubator. Intracellular Ca²⁺ assays were performed in salt buffered saline (SBS) containing (mM): NaCl, 130; KCl, 5; MgCl₂, 1.2; CaCl₂, 1.5; D-glucose, 8; HEPES, 10; pH 7.4 with NaOH. 1321N1 cells were seeded at 25,000 cells/well in clear bottomed 96-well plates. Cells were cultured overnight following replacement of culture medium with SBS containing 0.01%

(w/v) Pluronic acid and 2 μ M Fura-2AM (Abcam, Cambridge). Cells were loaded with Fura-2 for 1 hour at 37°C. Following loading, SBS was removed and replaced with 0.2 mL SBS. Fura-2 was excited at 340 and 380 nm and emission at 520 nm collected using a Flexstation 3 instrument (Molecular Devices) and 2s sampling. F ratio was calculated as 520 nm at 340 / 380 nm. Cells were incubated with PLP for 20 minutes before α,β -methylene ATP application. All experiments were performed at 37°C.

Concentration-response curves were fitted using a modified Hill equation:

$$Y = Start + (End - Start) \frac{X^n}{k^n + X^n}$$

Where K = Michaelis constant and n = number of cooperative sites.

In silico studies

Molecular modelling experiments were performed on an Asus WS X299 PRO Intel® i9-10980XE CPU @ 3.00GHz $\times$ 36 running Ubuntu 18.04 (graphic card: GeForce RTX 2080 Ti). Molecular Operating Environment (MOE, 2022.02, Montreal, QC, Canada) and Maestro (Schrödinger Release 2024-4, New York, NY, USA) were used as molecular modelling software.

Molecular docking

The crystal structure of hP2X3R in complex with MK-7264 was downloaded from the PDB (<http://www.rcsb.org/>; PDB code 5YVE). The protein was prepared using the MOE Protein Preparation tools and the protein–ligand complex was saved in mae format. The structure was preprocessed using Schrödinger Protein Preparation Wizard, by assigning bond orders, adding hydrogens and performing a restrained energy minimisation of the added hydrogens using the OPLS4 force field. A 10 Å docking grid was prepared, using as centroid the coordinates of the co-crystallised MK-7264. The structure of PLP was built in MOE, saved in the sdf format and prepared using the Maestro LigPrep tool by energy minimising the structure (OPLS4 force field), generating possible ionisation states at pH 7 ± 2 (Epik), generating tautomers and low-energy ring conformers. The ligand was analysed for its predicted binding to the hP2X3R using the Glide XP docking algorithm, using the default parameters, and performing a post-docking minimisation. 30 output poses were visually inspected in MOE, to evaluate the most likely binding mode to the negative allosteric site. To validate the suitability of the Glide XP docking algorithm for this receptor site, the structure of co-crystallised MK-7264 was re-docked to the site, following the same protocol described above for PLP (Figure S4).

Molecular dynamics and MM-GBSA calculations

Molecular dynamics simulations were performed using the Desmond package for MD simulation, employing the OPLS4 force field in the explicit solvent and the TIP3 water model. The initial coordinates for the MD simulations were taken from the 5YVE crystal structure for MK-7264, and from the best docking pose obtained for PLP. A cubic water box was used for the solvation of the system, ensuring a buffer distance of approximately 12 Å between each box side and the complex atoms. The systems were neutralised adding 12 chlorine counter ions for MK-7264, and 10 chlorine counter ions for PLP. The system was minimised and pre-equilibrated using the default relaxation routine implemented in Desmond. A 100 ns MD simulation was performed for each system, during which the equation of motion was integrated using a 2 fs time step in the NPT ensemble, with a temperature (300 K) and pressure (1 atm) constant. All other parameters were set using the Desmond default values. Data were collected every 8.5 ps (energy) and every 33.3 ps (trajectory). Each simulation was performed in triplicate, every time using a random seed as a starting point. Visualisation of the protein–ligand complex and MD trajectory analyses were carried out using Maestro. RMSD, secondary structure and protein–ligand interactions analyses were performed using the Simulation Event Analysis tool and the Simulation Interaction Diagram of Desmond. The $\Delta G_{\text{binding}}$ values for each protein–ligand complex were calculated using the MM/GBSA method, as implemented in the Prime module from Maestro, using the default settings and the Maestro script `thermal_mmgbsa.py`. Briefly, the script takes in the MD trajectory from the last 80 ns of simulation, splits it into individual frame snapshots (extracted every 1 ns, for a total of 801 frames), and runs each one through MMGBSA (after deleting waters and separating the ligand from the receptor). For each simulation triplicate, an average $\Delta G_{\text{binding}}$ value for the final 80 ns was calculated.

In vitro CSN recording.

Four- to five-week-old (n=8) and six- to eight-week-old (n=6) male SHR were deeply anesthetized with 5% isoflurane in O₂ (1 L.min⁻¹) and killed by exsanguination. The left and right intact carotid artery bifurcations containing the CSN, and the CB were exposed via a longitudinal incision on the ventral surface of the neck; the salivary glands, sternomastoid (STM) and sternohyoid (SHM) muscles were cut away and retracted. The bifurcation was surgically resected and subsequently placed in a recording chamber and superfused continuously with Ringer's solution (composition in mmol/L as follows: NaCl, 125; NaHCO₃, 24; KCl, 3.75; CaCl₂, 2.5; MgSO₄, 1.25; KH₂PO₄ 1.25 and D-glucose 10; Sigma-Aldrich). The superfusate was gassed with carbogen (5% CO₂ in 95% O₂), warmed to 36-37°C (TC-324C, Temperature Controller – Warner Instruments), filtered (nylon mesh, 25 µm; Millipore) and pumped at 5 mL/min (Gilson). Under a binocular microscope, the common carotid artery (CCA) was cannulated using the polyethylene tubing (PE-10) whereby the Ringer's solution was being perfused; thereafter, all perfusate reaching the bath would first flow through the CCA. After cannulation, the CSN was carefully dissected, while keeping the superior cervical ganglion intact. When true normoxia was desired, the perfusate was gassed with 10-12% O₂, 5% CO₂ in N₂ using a gas mixer (Pegas 4000, Columbus instrument) to maintain the perfusate's partial pressure of oxygen (PO₂) in the range of 90-105 mmHg; the solution PO₂ was continuously

monitored using an in-line flow-through oxygen sensor (FTC-PST3, OXY-1 SMA, PreSens). Sensory afferent activity from the CSN was recorded from the cut end of the nerve using a glass suction electrode. The signal was amplified x 10,000 (AM systems, model 1700 amplifier), band-pass filtered (100 Hz -1 kHz), and digitized (20 kHz, Micro1401-3 Cambridge Electronic Design, Cambridge). Acquisition and analysis were performed using Spike2 software (Cambridge Electronic Design, Cambridge). To differentiate nerve activity from background noise, lignocaine (Nopaine 2%; 300 μ L bolus) was administered at the end of each experimental trial to establish a baseline noise level. This baseline was subtracted from the recorded carotid sinus nerve activity in analyses focusing on firing frequency; spikes were detected as nerve discharge events above this noise threshold.

Experimental protocol.

Potassium cyanide (KCN, 0.08%; 100 μ L) was injected as a bolus from a 1 mL syringe, via a side port connected to the perfusing line, close to the CCA, to generate a control CB sensory response. Protocol 1) was carried out under hyperoxia (i.e., perfusate gassed with 5% CO₂ in 95% O₂; PO₂ > 400 mmHg). After a first KCN injection to check nerve viability, the CB was challenged again 2 or 3 times until we had doses that were similar in magnitude. Then, we perfused 5 mL of PLP (5 mM) through our system and challenged the CB twice more with KCN. This high PLP dose was used as a proof-of-principle to test whether PLP would be capable of inhibiting KCN-evoked CSN discharge. Protocol 2) was carried out under true normoxia (i.e., PO₂= 90-105 mmHg); achieved as previously explained. In pilot experiments, we screened (20-200 μ M) for the lower dose of PLP, at constant infusion (15 min), capable of attenuating the resting CSN firing under true normoxia. The experiment was then carried out with the chosen concentration of 50 μ M. The experimental timeline for these protocols is depicted in figure S1.

Experimental timeline

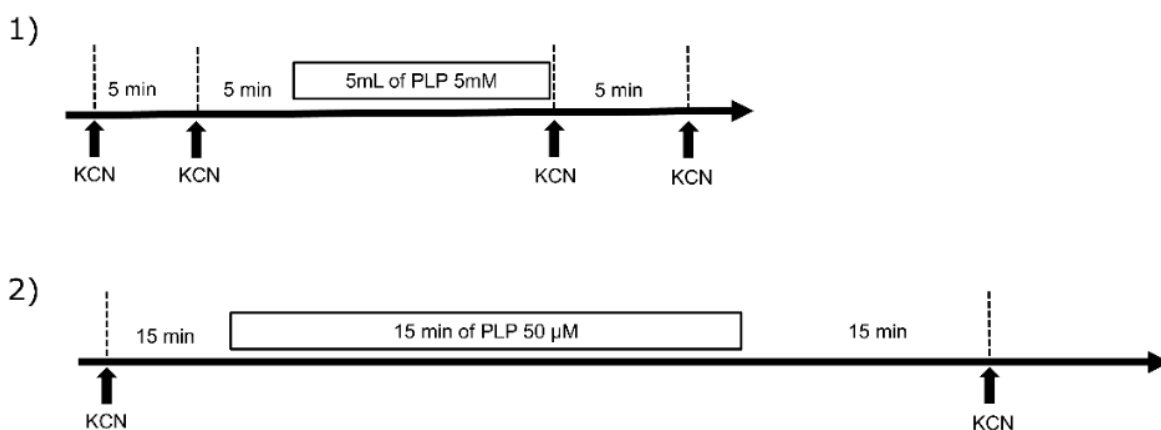


Figure S1: Experimental timeline of Pyridoxal 5' Phosphate (PLP) injection in the *in situ* CSN recording preparation. Protocol 1) KCN injections were carried out until we had two responses of equivalent level as a control; the third occurred right after the 5 mL PLP (5 mM) infusion. The fourth injection is used to check whether responses returned to basal levels (i.e., Washout). Protocol 2) After a first KCN response to check viability of the nerve, we waited 15 min to allowed CSN firing to stabilize. Subsequently, continuous PLP infusion commenced (15 min, 50 μ M) to assess its effect on resting CSN firing.

In situ Working Heart-brainstem Preparation (WHBP)

Juvenile SHRs (n=15, 3-6 weeks old, 50-90 g) were anesthetized deeply with isoflurane (5% in O₂, 1L min⁻¹, via inhalation) until loss of paw and tail withdrawal reflexes, and then heparinized (350 UI i.p.; Pfizer). Subsequently, animals were euthanized via exsanguination following bisection below the diaphragm. After cooling the upper body in Ringer's solution (composition in mmol/L as follows: NaCl, 125; NaHCO₃, 24; KCl, 3.75; CaCl₂, 2.5; MgSO₄, 1.25; KH₂PO₄ 1.25 and D-glucose 10; Sigma-Aldrich), animals were decerebrated pre-collicularly, the lungs were removed, and the descending aorta isolated and cannulated with a double-lumen catheter. Retrograde perfusion of the thorax and head restored viability based on the return of a ramp-like phrenic nerve discharge pattern. The perfusate was the Ringer's solution above plus an oncotic agent (1.5%, polyethylene glycol, 95172-250G-F, Sigma- Aldrich, Australia), gassed with carbogen (5% CO₂, 95% O₂), warmed to 31-32°C, filtered with nylon mesh (25 µm; Millipore) and recirculated. The second lumen of the cannula was connected to a Neurolog pressure transducer (NL108T2, Digitimer) and amplifier (NL108A, Digitimer) to monitor perfusion pressure (PP) in the aorta. The PP was maintained between 55-90 mmHg adjusting the peristaltic pump flow (20-25 mL/min; Watson-Marlow 530s) and the addition of vasopressin (2-2.5 nmol/L - V9879-5MG, Sigma-Aldrich) into the perfusate. Neuromuscular blockade to arrest respiratory-related movement was established using vecuronium bromide added into the reservoir (10 mg/mL, Mylan). Simultaneous recordings of combinations of the following nerves were made using bipolar glass suction electrodes from: phrenic (PN) and thoracic sympathetic chain (tSNA; between T13 and L3). In addition, heart rate (HR) was derived from the inter R-wave of the electrocardiogram (ECG) recorded through two electrodes from each forelimb of the preparation. All signals were amplified x10,000 (A-M Systems model 1700), filtered (bandwidth 10 Hz–5 kHz, A-M Systems), digitized (20 kHz, Micro1401-3, Cambridge Electronic Design), and saved using Spike2 software (Cambridge Electronic Design). Background noise was determined 15 min after the peristaltic pump had been turned off and subtracted from the tSNA signal.

Peripheral chemoreflex response in situ.

Potassium cyanide (KCN, 0.04%; 50-100 µL) was injected as a bolus directly into the ICA from a pre-calibrated 100 µL Hamilton syringe to stimulate the CB chemoreceptors. The chemoreflex consists of increased phrenic activity, bradycardia, sympathoexcitation and increased PP. We quantified the chemoreflex in two ways: first, calculating the percentage increase in respiratory rate (i.e., tachypnoea) and sympathoexcitation relative to the baseline immediately before the stimulus with noise subtracted; the period of baseline used for this calculation was the same time-length approximately as the chemoreflex response (e.g., 5s). Second, the maximum bradycardia and increase in PP were calculated as the change (Δ) in HR (bpm) and PP (mmHg) from baseline. At least 5 min elapsed between consecutive KCN doses.

Experimental protocol.

In the WHBP, the left common carotid artery (CCA) was ligated to ensure only the CB chemoreceptors on the ipsilateral intervention side were stimulated. We cannulated the right ICA

with a fine cannula having a dead space of 10 μ L, which was accounted for in all injections. The tip of this cannula pointed towards the CCA with its tip just rostral to the bifurcation and juxtapositioned to the CB artery; its other end was connected to a Hamilton syringe (100 μ L). The proper position of the tip of the cannula close to the CB artery and the integrity of the CB and its CSN connection were confirmed by the presence of the chemoreflex evoked by 50 μ L of KCN (0.04%) locally injected into the ICA to stimulate the CB as previously described.²⁵ Before switching to different drugs, the Hamilton syringe was disconnected and the perfusate was permitted to flow through the catheter to rinse it out.

At least two consistent control chemoreflex responses were evoked by injections of KCN into the ICA, then we injected 50 μ L of PLP at various doses (i.e., 1-5 mM), which was immediately followed by another dose of KCN, and another one 5 min thereafter to assess washout. Because we were concerned about possible residual effects of the PLP, only one dose was given per preparation (Figure S2).

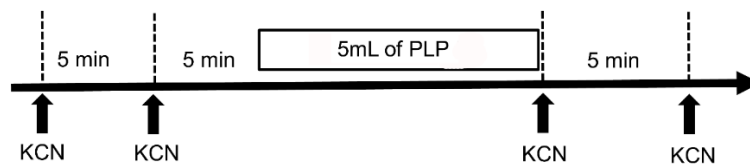


Figure S2: Experimental timeline of Pyridoxal 5' Phosphate (PLP) injection in the *in situ* Working Heart-Brainstem Preparation. Two KCN injections were performed to activate the chemoreflex as a control, and the third occurred right after the PLP injection. The last injection is used to check whether responses returned to basal levels (i.e., Washout).

In vivo telemetered SHR:

Radio-telemetry instrumentation

Under anesthesia with isoflurane (2-5% in O₂, 1 L min⁻¹, via inhalation), adult male SHRs (n= 5, 30-34 weeks old, 300-350 g) were given a single abdominal subcutaneous injection of analgesic (0.05 mg/Kg of Vetergesic - buprenorphine), anti-inflammatory (2 mg/Kg of Meloxicam – Metacam), local anesthetic (6 mg/Kg Bupivacaine 0.25%, 2.5 mg/ml+/-1:400,000 epinephrine - Marcaine), and antibiotic (4 mg/Kg of Baytril - enrofloxacin). During anesthesia, animals were kept warmed (37°C) using a heating pad, petroleum-based artificial tear ointment was applied onto the eyes to keep corneal lubrication, and subcutaneous injections of warmed (35-37°C) saline (0.9% NaCl, 5 mL/Kg hourly) were used to maintain animal's hydration. Surgical fields were trimmed and disinfected using chlorhexidine scrub solution. Under aseptic technique, a midline abdominal incision of 3-4 cm was made, and the descending abdominal aorta was exposed and dissected free of surrounding tissue. The aorta was briefly occluded, then pierced using a bent 23G needle to insert the blood pressure (BP) catheter of the transmitter (either HD-S10, DSI or TRM54P, Kaha Science -AD Instruments). The catheter was advanced so that the tip was positioned just below the left renal artery. Blood flow was restored through the aorta once the probe was secured in place using tissue

adhesive (Histoacryl®, B Braun) and polypropylene mesh (Small Parts Ltd). The transmitter body was placed in the abdominal cavity, and the abdominal muscle layer was closed with silk sutures.

After the BP telemeter was implanted, the right femoral vein was exposed via a 1 cm incision. The vein line was composed of two catheters of polyurethane connecting 3 cm of MRE-033 (Braintree Scientific) pre-coated with heparin (TDMAC, Polysciences, Eppelheim) with 16 cm of MRE040 (Braintree Scientific). The line was pre-filled with a locking solution (50 U/mL heparin + 2000 U/mL of penicillin G dissolved in sterile saline) and the catheter was inserted 1.5 cm into the femoral vein. The vein line was secured in place with tissue adhesive and polypropylene mesh. The catheter was tunneled subcutaneously and connected to a capped intrascapular port. After the surgery, analgesic (0.05 mg/Kg - buprenorphine) and anti-inflammatory (2 mg/Kg of Meloxicam – Metacam) were given subcutaneously once a day for a minimum of 3 days, and the femoral line was flushed with heparinized saline solution every 2 days throughout the time of experiments. Animals were allowed a 7-day recovery period before any experiment was conducted. At the end of the experiments, animals were euthanized via intravenous injection of Pentobarb 300 (800 mg/kg—Sodium Pentobarbitone—Provet NZ Pty Ltd, New Zealand).

In vivo blood pressure recordings

Blood pressure recordings were carried out using either PowerLab (ADInstruments) through the software LabChart 8 (ADInstruments) or Spike2 and DSI talker (Cambridge Electronic Design). Systolic (SBP), diastolic (DBP), mean blood pressure (MBP), and heart rate (HR) were derived offline from pulsatile pressure.

Barometric whole-body Plethysmography

Animals were placed inside a custom-made chamber (height: 25 cm × width: 160 cm × length: 23 cm) with controlled air inflow and outflow. The chamber has 3 mm Fluro orange panels to line the inside and prevent animals from seeing the experimenter in the room. Ventilatory parameters were measured through oscillations in pressure caused by breathing movements using a differential air pressure sensor (FE141 Spirometer – ADInstruments). The pressure signal was sampled at 1 kHz in LabChart 8. Tidal volume (V_T) was calculated using the barometric method of Drorbaugh & Fenn²⁶ (Equation 1). To do so, Auckland barometric pressure was daily collected from New Zealand MetService's website (<https://www.metservice.com/towns-cities/locations/auckland>); chamber's relative humidity was measured using a humidity sensor (HIH-4000-004, Honeywell), whilst chamber's temperature was continuously measured using a T-type thermocouple (Thermalert Monitoring Thermometer, Analog Output - TH 5A-120V, Braintree Scientific). Furthermore, to reduce noise from pressure changes in the room, the reference pressure unit from the two-point measurement of our sensor was connected to a separate reference chamber. The respiratory frequency (f_R) was derived from pressure oscillations, and the minute ventilation (V_E) calculated using Equation 3.

Equation 1:

$$V_T = \frac{P}{P_K} \times V_K \times \frac{T_A(P_B - P_C)}{T_A(P_B - P_C + P) - T_C(P_B - P_A + P)}$$

Where,

P : (Tidal pressure),

PK : (change in pressure oscillation due to calibration),

VK : (Calibration Volume),

TA : (Temperature of the animal in °C),

PB : (Barometric pressure in mmHg),

PC : (Vapor pressure of water in the chamber; Equation 2),

TC : (Temperature of the chamber in °C), and

PA : (Vapor pressure of water in the animal's lung).

PS: (Vapor pressure of Water in a given temperature)

Equation 2:

$$PC = \frac{\text{Relative Humidity}}{100} \times PS$$

Equation 3:

$$V_E = V_T \times f_R$$

The fraction of inspired oxygen (FIO₂) and carbon dioxide (FICO₂) were manipulated via manual gas mixing with real-time reading using an oxygen/carbon dioxide gas analyzer (Vmax 29 analyzer, SensorMedics). 2 L/min of inflow 21% O₂ in N₂ (i.e., FIO₂= 0.21) was delivered by mixing 100% O₂ and 100% N₂ gases at different flows (i.e., 0.42 L/min of O₂ plus 1.58 L/min of N₂). Inflow and outflow rates were measured with FE141 spirometers connected to flow heads (MLT10L, ADInstruments). The rate of outflow was kept the same as inflow using a vacuum (i.e., negative pressure). Oxygen consumption (VO₂) and carbon dioxide production (VCO₂) were calculated using Equations 4 and 5, respectively.³¹

Equation 4:

$$VO_2 = V_I(F_I O_2 - F_E O_2) / [1 - (1 - R)F_E O_2]$$

Equation 5:

$$VCO_2 = V_I(F_I CO_2 - F_I CO_2) / [1 - (1 - 1/R)F_E CO_2]$$

Where,

Vi : (Flow rate into the chamber)

F_iO₂ : (Fraction of oxygen in the inlet)

F_eO₂ : (Fraction of oxygen in the outlet)

F_iCO₂ : (Fraction of carbon dioxide in the inlet)

F_eCO₂ : (Fraction of carbon dioxide in the outlet)

RER: (Respiratory exchange ratio, Equation 6)

Equation 6:

$$RER = (F_E CO_2 - F_I CO_2) / (F_I O_2 - F_E O_2)$$

Peripheral chemoreflex - *in vivo*.

Rats were challenged with KCN injections ($1 \mu\text{g}/\mu\text{L}$; either 15 or 30 μg per rat, i.v., regardless of body weight) to evoke chemoreflex responses. The dose of 15 μg was used in SHR and 30 μg in Wistar rats. These doses were chosen because they would evoke visible cardiovascular with minimum behavioral response. The latter is important, so it won't interfere with respiratory measurements. The maximum CB-evoked pressor, bradycardic, and respiratory responses were analyzed.

Experimental protocol *in vivo*.

During 3 days before the experiment, animals were placed into the plethysmography chamber for habituation for 30 min. On the day of the experiment, animals ($n=5$) had their i.v. line connected to an external cannula for PLP infusion. After placing the animal into the chamber, we waited for at least 30 min, so animals could explore the new environment before starting our protocol. The latter was initiated with animals being challenged with a control KCN response; this was followed by a 15 min recovery time, then PLP was infused at rate of 48 mg/Kg/h (30 min) to reach the final dose of 24 mg/Kg. At the end of the infusion a second dose of KCN was given and a third 30 minutes thereafter to test washout (Figure S3). To assess the effect of PLP infusion on resting blood pressure, breathing, and metabolism, we compared epochs of 1 min immediately before onset of infusion against the last minute of infusion, i.e., prior the second KCN injection.

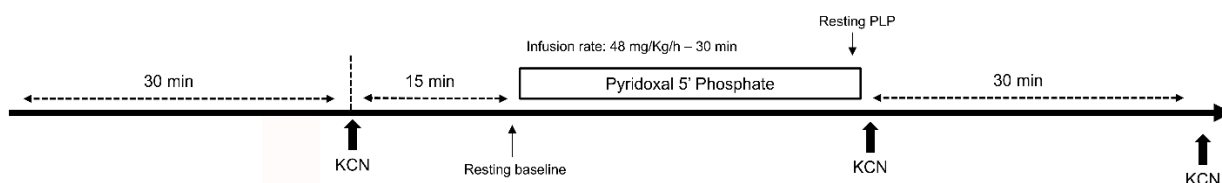


Figure S3: Experimental timeline of Pyridoxal 5' Phosphate (PLP) infusion *in vivo* conscious adult spontaneously hypertensive rats (SHRs). A first potassium cyanide (KCN) injection was given to activate the chemoreflex as a control response. After a recovery time, a 30 min PLP infusion started at an infusion rate of 48mg/Kg/h. At the end of the infusion, a second KCN injection was given. At the end of the infusion, followed by a third one after 30 min. Resting baseline and PLP arrows indicate when resting cardiovascular and respiratory parameters were collected to assess the effect of PLP infusion.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) for ALP genes expression.

Steady-state gene expression analysis in the carotid bodies was performed on $n=8$ male, 4-6 weeks old Wistar and SHR rats. CBs were micro-dissected from the common carotid artery bifurcation, placed into homogenizer tubes (Cat. # 9790B, Takara) and snap-frozen in liquid nitrogen. Samples were stored at -80°C until batch processed. Samples were homogenized in QIAzol Lysis Reagent (Cat. # 79306, Qiagen). RNA was separated using phenol-chloroform phase separation method. Aspirated aqueous phase was supplemented with glycogen (Cat. #10899232103, Roche) to the final

concentration of 0.05 µg/mL and RNA precipitated in x2.5 volumes of absolute ethanol overnight at -80°C. Resulting RNA pellets were rinsed twice in ice- cold 70% ethanol, air-dried for 30 min and resuspended in 15 µL nuclease-free water. RNA concentration was determined using Qubit™ RNA High Sensitivity Assay (Cat. #Q32852; Thermo-Fisher Scientific). On average, 125 ng of total RNA was recovered from a single carotid body. RNA integrity was determined using Agilent 2100 Bioanalyzer System (Cat. # G2939BA; Agilent) using RNA 6000 Pico Kit (Cat. # 5067-1513; Agilent). Only samples with RIN >7 were processed for further analysis.

RNA was reverse transcribed using iScript™ gDNA Clear cDNA Synthesis Kit (#1725035, Bio-Rad). gDNA removal step was included for every sample. RT-qPCR was carried out in triplicates using Luna® Universal qPCR Master Mix (Cat. # M3003; New England Biolabs) on a QuantStudio 12K Flex Real-Time PCR System (Applied Biosystems, USA). Eukaryotic translation initiation factor 4B (Eif4b; ENSRNOG00000010103) was used as a housekeeping control as described previously.²⁷

Primers for Alpg, NCBI Reference Sequence: XM_039084682.2 (5'-CGTGACCCCAAGTACCGGC-3' and 5'- CGCGAATATGGCCACGTCCT-3'); Alpl, NCBI Reference Sequence: NM_013059.3 (5'- CTGCCTGCAGGATCGGAA-3' and 5'-TGCCGATGGCCAGTACTAAAA-3'); Alpp, NCBI Reference Sequence: XM_002730058.5 (5'-CCTCAGCCTACAGATGTCCCT -3' and 5'- TATCCGGGTAGCCGTTACTGT -3'); a primer pair recognising multiple Alp isoforms (Alpg, Alpp, Alpi) (5'-TCACCAGTGAGAAGGACACGAT-3' and 5'- CGCGAATATGGCCACGTCCT-3'), and housekeeper Eif4b, Rnor_6.0 ENSRNOG00000010103 (5'-CCGGGATCGCTATGATGACC -3' and 5'- GTCCCCACCTCCTCTGTAGT -3'). No amplification was detected in no template controls (NTC) processed in parallel. Melt curve was performed at the end of each run to confirm a single amplicon was produced in each well. Housekeeping genes were always run on the same plate as target genes. For relative quantification of gene expression, the $2^{-\Delta\Delta CT}$ method was used.²⁸ Hypothesis testing was performed using ΔCT values.

Double-blind Randomized Clinical Trial

Sample size calculation

To calculate the sample size a priori, we use the work of Bock et al.²⁹ who observed a 38% reduction in the hypoxic ventilatory response to 10% inspired O₂ (as a proxy for peripheral chemoreflex sensitivity) with dietary nitrate supplementation in older adults. Assuming acute B6 supplementation will have a greater effect than dietary nitrate, a sample size of 15 per group (30 total) at 80% power and 5% alpha would be required to detect a difference of 56% in peripheral chemoreflex sensitivity.

Inclusion criteria

Participants were eligible for inclusion if they were aged over 18 years with Stage 2 or above hypertension (untreated office SBP $\geq$ 140 mmHg or DBP $\geq$ 90 mmHg). Participants were ineligible

for inclusion if they were current smokers or abusers of alcohol, had a body mass index $>35 \text{ kg.m}^{-2}$, or had any significant medical conditions including chronic lung and heart disease.

Participants

Eighteen participants who met the inclusion criteria were recruited from local community and attended both visits to the laboratory during 2022-2023; 4 participants did not successfully complete the isocapnic hypoxic rebreathing due to frequent ectopics (n=1) and technical issues (n=3). Thus, fourteen participants (4 men) completed the study.

Experimental Protocol

A randomized placebo-controlled double-blind crossover study was conducted. Randomization of the treatment was performed by a biostatistician at the University of Auckland. Participants attended the laboratory at the University of Auckland Clinical Research Centre on three separate occasions for a familiarization visit and two experimental visits.

Familiarization

Participants attended the lab for an initial screening and familiarization visit. All participants provided written informed consent and completed a health history questionnaire followed by measurement of height and weight. Participants then completed a short breathing test.

Experimental visits

Participants were asked to abstain from caffeine for 12 hours prior, alcohol on the day before the study and day of the study, exercise after 2000h the evening before the study and on the day of the study, and any 'over the counter' (e.g., paracetamol) or cardioactive medications (beta-blocker, ACE inhibitor, angiotensin receptor blockers, calcium channel antagonists, diuretics [e.g., spironolactone], alpha blockers) on the morning of the experimental study visits. Upon arrival, a venous blood sample was drawn from a forearm antecubital vein (10 mL) for analysis of plasma biochemistry and PHC and its metabolites (e.g., PLP). Either placebo or vitamin B6 (600 mg in 24 mL liquid) was then consumed by the participant, followed by a wait period of 2 hr. After 2 hr, a second venous blood sample was collected. Participants were then instrumented for collection of cardiorespiratory variables. After instrumentation, participants performed an isocapnic hypoxic rebreathing protocol. Briefly, this included a 5-minute baseline period, followed by switching using a three-way stopcock to a closed-circuit filled with room air at the end of a normal expiration (21% O₂, balance N₂). The closed circuit allowed for progressive decrease in PO₂ with continued rebreathing, while isocapnia was maintained via a soda lime scrubber. A second three-way stopcock allowed for airflow to be directed either via the soda lime scrubber or bypassing the scrubber, to maintain isocapnia. The test was terminated when P_{ET}O₂ reached 45 mmHg.

Experimental Measures

Heart rate (HR) was measured continuously using a lead II electrocardiogram (BioAmp, FE231, ADInstruments). Beat-to-beat blood pressure was measured via finger photoplethysmography (Finometer MIDI, Finapres Medical Systems, Amsterdam, Netherlands). Blood pressure values from the finger were validated against brachial artery blood pressure measurements (BP+, Uscom). Arterial oxygen saturation (S_pO_2) was measured using finger pulse oximetry (Radical-7 Pulse CO-Oximeter, Masimo, Irvine).

Participants were instrumented with a mouthpiece and nose clip, connected to a low resistance bacterial-viral filter (disposable filter, MLA304, ADInstruments) and a pneumotachometer (3830 Series, Heated Linear E Pneumotachometer, Hans Rudolph Inc., Kansas City, MO, USA). A sample line was connected to the mouthpiece and allowed continuous sampling of expired partial pressures of oxygen ($P_{ET}O_2$) and carbon dioxide ($P_{ET}CO_2$) (Respiratory Gas Analyzer, ML206, ADInstruments). Ventilation ($\dot{V}_E$), respiratory rate (R_f) and tidal volume (V_T) were measured breath-by-breath with the pneumotachometer.

Blood samples

Venous blood samples were spun at 4000 revolutions per minute for 10 minutes at 4°C, and plasma was then pipetted using disposable pipette tips into 1mL Nunc tubes for storage at -80°C in secure storage within the University of Auckland Clinical Research Centre. Plasma B vitamins were analysed by The Liggins Institute, The University of Auckland, New Zealand, according to Andraos et al.³⁰ using Vanquish ultra-high-pressure liquid chromatography (UHPLC+) and TSQ Quantiva triple quadrupole mass spectrometer (Thermo Scientific). Plasma biochemistry was analysed by The Liggins Institute, The University of Auckland, New Zealand using a cobas c 311 analyser (Roche Diagnostics International Ltd.) using photometric assays.

Data Analysis

Cardiorespiratory signals were recorded via analogue-to-digital conversion at 1000 Hz (PowerLab 16/35 and LabChart Version 8, ADInstruments). HR was obtained from the ECG trace beat-to-beat, and the finger photoplethysmography waveform was used to identify systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MBP) beat-to-beat. f_R , V_T , $\dot{V}_E$, $P_{ET}O_2$, and $P_{ET}CO_2$ were obtained breath-by-breath, and erroneous breaths (i.e., due to cough or swallow) were removed. Central blood pressures were calculated using a model-based approach.³³

Arterial oxygen saturation (S_aO_2) was calculated from $P_{ET}O_2$ using the Severinghaus equation.³² The index of peripheral chemoreflex sensitivity was calculated as the delta change in $\dot{V}_E$ from baseline (5-min average) to peak rebreathing (final 15s of rebreathing) divided by the equivalent change in estimated S_aO_2 :

$$\text{Peripheral chemoreflex sensitivity (L/min/\%)} = \frac{\dot{V}_{E_{\text{peak rebreathing}}} - \dot{V}_{E_{\text{baseline}}}}{S_{aO_{2_{\text{peak rebreathing}}}} - S_{aO_{2_{\text{baseline}}}}}$$

Patients were classified as having a “sensitized” peripheral chemoreflex index if their slopes were steeper than -0.5 L/min/% during placebo treatment. This categorization was based on the work of Narkiewicz et al.¹⁶ who were able to sort patients undergoing unilateral CB resection into responders and non-responders based on their baseline HVR (-0.5 ± 0.05 and -0.32 ± 0.06 L/min/%, respectively).

Statistical Analysis

Graphic and statistical analyses were performed using GraphPad Prism (version 9.3.1, USA) and Jamovi (Version 2.4.8) [Computer Software]. The Jamovi project (2023). Retrieved from <https://www.jamovi.org>. Paired and unpaired Student’s t-test, and mixed regression models were used accordingly (For details, see *detailed statistical analysis per dataset*). In our analysis, we fitted the data in 2 - 3 different models to streamline which one best described it. The criteria to choose the final model was based, first, on the analysis of residuals and, second, on the value of Akaike Information Criterion (AIC) goodness of fit. As a general approach, we first fitted our data using a linear distribution model (i.e., assuming normal distribution). However, if the analysis of residuals exposed a violation of the assumption of normality and/or heteroskedasticity of residuals, then we would fit our data using a generalized gamma distribution model and link function identity, which can accommodate a variety of data with skewed continuous distribution profile. The level of significance was set at 5%, and data were expressed as mean $\pm$ standard deviation (SD).

Section II - Detailed statistical analysis per dataset.

Pre-clinical work

In vitro CSN recording.

KCN response:

The data was fitted using mixed regression model to investigate the effect of PLP infusion over KCN-evoked CSN discharge of pre-hypertensive SHR (4–5-week-old). The analysis of residuals exposed a violation of the assumption of normality of residuals; thus, we continue our analysis using a generalized mixed model with gamma distribution and link function identity. In our model, the dependent variable CSN (μ V) was explained by the categorical independent variable “Condition” with 3 levels (“KCN first”, “PLP 5mM”, and “KCN washout”). The responses were controlled by their respective baseline, i.e., CSN baseline was added as a covariate. To account for between-rat variations, we allowed each Rat ID to assume different intercepts randomly (i.e., random effects). Our full model equations were run as follows: $\text{CSN} \sim 1 + \text{Condition} + \text{CSN baseline} + (1 \mid \text{Rat ID})$; the equation was written according to the R code for the lme4 package.

Resting CSN firing:

The data was fitted using mixed regression model to investigate the effect of PLP infusion over resting CSN firing of pre-hypertensive SHR (6–8-week-old). We fitted our data using a mixed linear model (i.e.,

assuming a normal distribution of residuals) with residual covariance matrix “autoregressive one AR(1)” In our model, the dependent variable CSN rate (impulse/s) was explained by the categorical independent variable “Time” with 3 levels (“Before PLP”, “After PLP”, and “washout”). To account for between-rats variations, we allowed each Rat ID to assume different intercepts and slopes for “Time” randomly (i.e., random effects). Our full model equations were run as follows: $CSN \sim 1 + Time + (1 + Time | Rat ID)$; the equation was written according to the R code for the lme4 package.

In situ WHBP.

The data was fitted using mixed regression model to investigate the effect of PLP infusion over the KCN-evoked motor response of adult SHR. We fitted our data using a mixed linear model (i.e., assuming a normal distribution of the data) with residual covariance matrix “autoregressive one AR(1)”. In our model, the dependent variable either SNA, HR, PB amp or PN rate (i.e., generally represented as DV) were explained by the following independent variables: the categorical fixed effects “Time” with 3 levels (“Control”, “PLP”, “Washout”) and “PLP dose” with 5 levels (1-5 mM). The response was controlled by its respective baseline, e.g., if SNA was the dependent variable, then SNA baseline was added as a covariate. To account for between-rats variations, we allowed each Rat ID to assume different intercepts randomly (i.e., random effect). Our full model equation was run as follows: $DV \sim 1 + Time + PLP\ dose + DV's\ baseline + (1 | Rat ID)$; the equation was written according to the R code for the lme4 package.

For the dependent variable, inspiratory drive (PN amp/Ti), the model was fitted using gamma distribution.

In vivo Whole-body plethysmography and BP telemetry

KCN

SBP, DBP, MBP, HR, f_R , V_T , V_E , and V_E/CO_2

The data was fitted using mixed regression model to investigate the effect of PLP infusion over the KCN-evoked motor response of adult SHR. First, we fitted our data using a mixed linear model (i.e., assuming a normal distribution of the data) with residual covariance matrix “unstructured”. In our model, the dependent variable either SBP, DBP, MBP or f_R (i.e., generally represented as DV) were explained by the following independent variable: a categorical fixed effect “Condition” with 3 levels (“First KCN”, “KCN with PLP”, “Third KCN”). The response was controlled by its respective baseline, e.g., if SBP was the dependent variable, then SBP baseline was added as a covariate. To account for between-rats variations, we allowed each Rat ID to assume different intercepts and slopes for “condition” randomly (i.e., random effects). Our full model equation was run as follows: $DV \sim 1 + Condition + DV's\ baseline + (1 + condition | Rat ID)$; the equation was written according to the R code for the lme4 package.

Resting blood pressure, breathing and metabolism

Systolic, diastolic and mean blood pressures (SBP, DBP, MBP), as well as hear rate (HR)

Tidal volume, minute ventilation, respiratory frequency, inspiratory time, breathing inspiratory drive (V_T , V_E , f_R , T_i , V_T/T_i)

Oxygen consumption, carbon dioxide production, respiratory exchange rate (VO_2 , VCO_2 , R)

The data was fitted using mixed regression model to investigate the effect of PLP infusion over the blood pressure of adult SHRs. First, we fitted our data using a mixed linear model (i.e., assuming a normal distribution) with residual covariance matrix “unstructured”. In our model, the dependent variable (generally represented as DV) were explained by the following independent variable: a categorical fixed effect “Time” with 2 levels (“Before” and “After PLP”). To account for between-rats variations, we allowed each Rat ID to assume different intercepts randomly (i.e., random effects). Our full model equation was run as follows: $DV \sim 1 + \text{Time} + (1 | \text{Rat ID})$; the equation was written according to the R code for the lme4 package.

Respiratory efficiency (V_E/VCO_2)

The data was fitted using mixed regression model to investigate the effect of PLP infusion over the V_E/VCO_2 of adult SHRs. The data was analyzed using a generalized mixed model with gamma distribution and link function identity. In our model, the dependent variable V_E/VCO_2 was explained by the following independent variable: a categorical fixed effect “Time” with 2 levels (“Before” and “After PLP”). To account for between-rats variations, we allowed each Rat ID to assume different intercepts randomly (i.e., random effects). Our full model equation was run as follows: $DV \sim 1 + \text{Time} + (1 | \text{Rat ID})$; the equation was written according to the R code for the lme4 package.

Randomized Clinical Trial

The data was fitted using mixed regression model to investigate the effect of oral PHC hydrochloride supplementation over the peripheral chemoreflex response in patients with hypertension. The data was analyzed using a linear mixed model (i.e., assuming a normal distribution). In our model, the dependent variable (DV) either “peripheral chemoreflex index”, “chemoreflex-evoked ΔSBP ” or “chemoreflex-evoked ΔDBP ” was explained by the following independent variables: categorical fixed effects “Level of sensitization” with 2 levels (“Normal” and “sensitized”), and “treatment” with two levels (“Placebo” and “PHC”). Patients were classified as having a “sensitized” peripheral chemoreflex index if their slopes were steeper than -0.5 during placebo treatment. To account for between-patients variations, we allowed each patient to assume different intercepts randomly (i.e., random effects). Our full model equation was run as follows: $DV \sim 1 + \text{Treatment} + \text{Level of sensitization} + \text{Treatment:level of sensitization} + (1 | \text{Patient ID})$; the equation was written according to the R code for the lme4 package

Section III - Additional Results:

Pre-clinical work

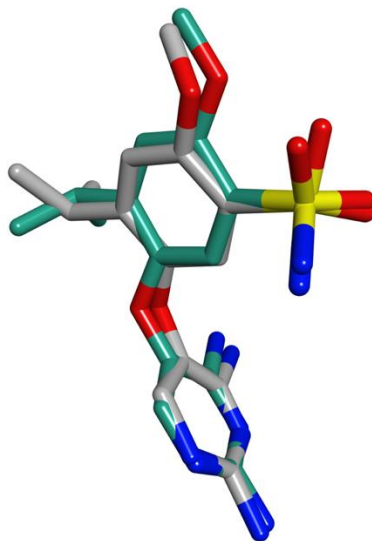


Figure S4: Superposition between the structure of MK-7264 co-crystallized with P2X3R (PDB ID 5YVE, carbon atoms in light grey) and the lowest-energy docking pose of MK-7264 to the same site of the 5YVE crystal structure (carbon atoms in sage), obtained with the Glide XP docking tool. The overall RMSD value between the two structures is 0.5 Å.

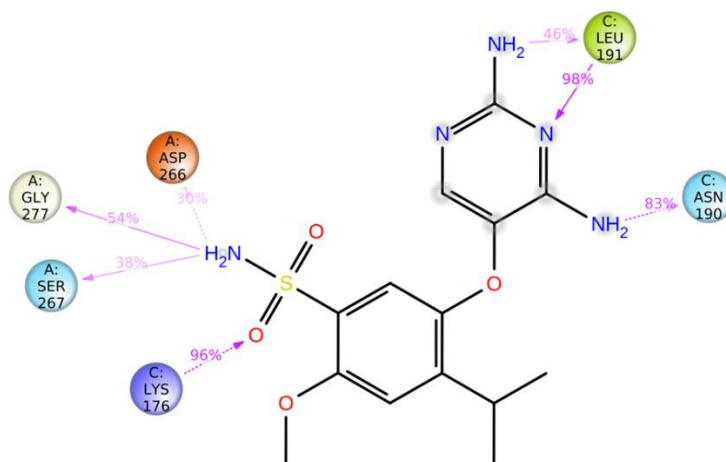


Figure S5: A schematic of the predicted interactions of MK-7264 with the protein residues observed during the molecular dynamic simulations. Interactions that occur more than 30% of the simulation time (0.00 through 100.00 nsec) are shown. Obtained with the Simulation Interaction Diagram of Desmond.

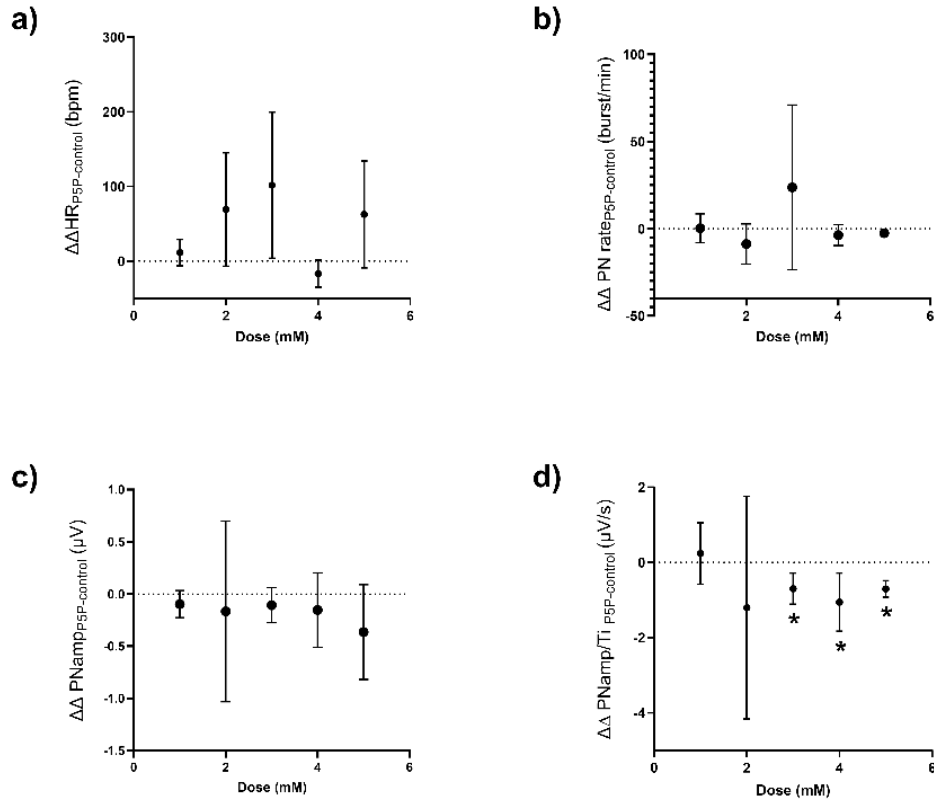


Figure S6: Effect of Pyridoxal 5'Phosphate (PLP) on carotid body (CB) activity in the working heart-brainstem preparation. focal injections of PLP (1-5 mM) into the internal carotid artery showed an attenuating trend ($P=0.056$) on CB-evoked **a)** bradycardia whereas it had no effect on **b)** tachypnea nor **c)** phrenic amplitude (PN amp). However, PLP successfully attenuated the **d)** neural inspiratory drive (PN amp/ inspiratory time – PN amp/ Ti). Data are shown as $\Delta\Delta$, which means the difference between the Δ responses from PLP versus the first KCN (i.e., control response). The further the data departs from the dotted line at zero, the more attenuated the response. Data were analyzed using a mixed regression model with either linear (i.e., normal) or gamma distribution. Mean $\pm$ SD, * $P<0.05$, ** $P<0.01$.

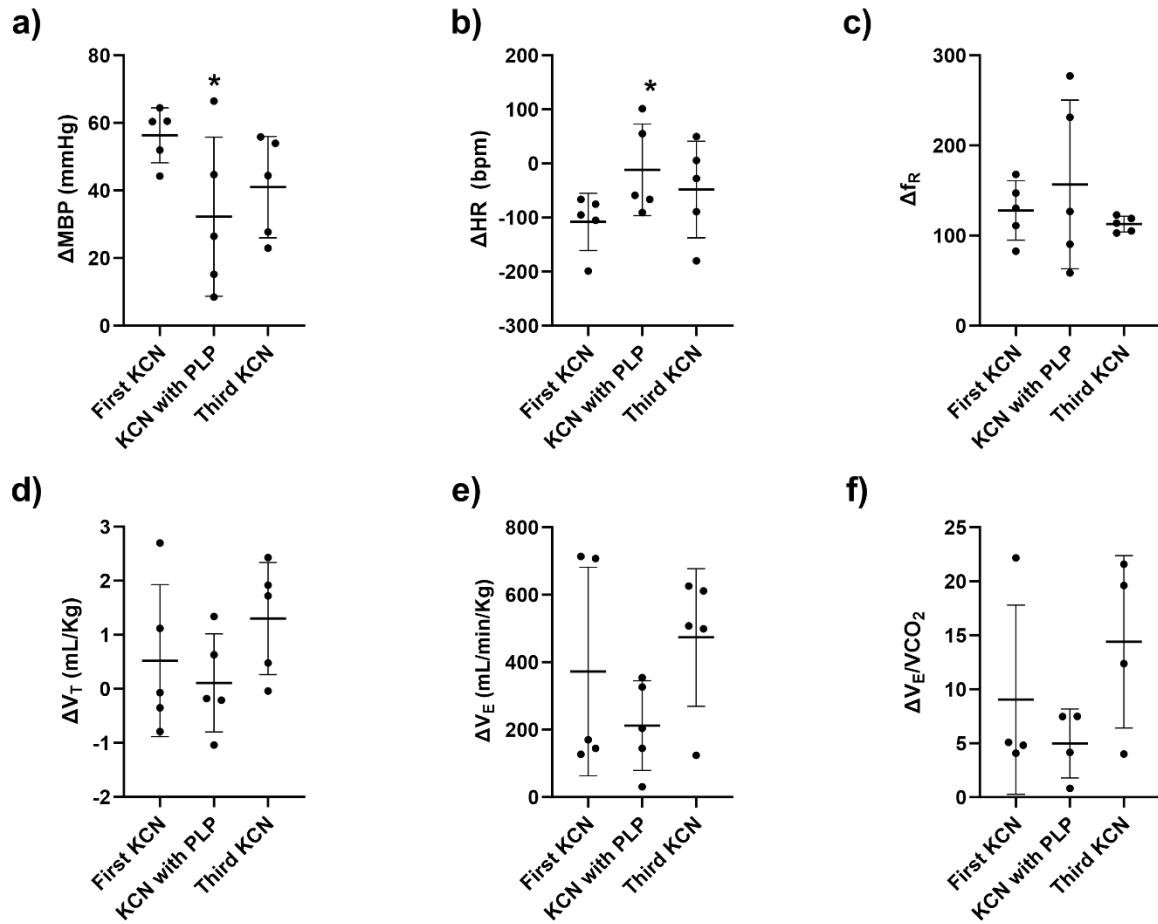


Figure S7: Effect of Pyridoxal 5' Phosphate (PLP) on carotid body (CB) activity in normotensive rats. a) In adult telemetered Wistar rats ($n=5$), we used potassium cyanide (KCN, 30 µg/rat; bolus injection i.v.) to stimulate the peripheral chemoreceptors either in the presence or absence of PLP (intravenous infusion - 48 mg/kg/h, 30 min i.v.). PLP attenuated but did not abolish the KCN-evoked increase in mean blood pressure (MBP, a). Other KCN-evoked responses quantified are bradycardia (HR, b), tachypnea (f_R, c), changes in tidal volume (VT, d), minute ventilation (VE, e), and respiratory efficiency (VE/VCO₂, f). Data are shown as Δ response relative to the immediate baseline. Data were analyzed using mixed regression model with either linear (i.e., normal) or gamma distribution. Mean $\pm$ SD, * $P < 0.05$.

Table S1: Average effect of PLP infusion on resting blood pressure and respiratory parameters in telemetered SHR.

	Condition	N	Missing	Mean	SD
SBP *	Baseline	5	0	201	23.5
	PLP	5	0	184	26.6
DBP *	Baseline	5	0	136	16.6
	PLP	5	0	121	16.4
MBP *	Baseline	5	0	164	23.9
	PLP	5	0	148	25.3
V_T (mL/Kg)	Baseline	5	0	1.65	1.15
	PLP	5	0	1.48	0.99
V_E (mL/min/Kg)	Baseline	5	0	175	124
	PLP	5	0	136	102
f_R (breaths/min)	Baseline	5	0	107	8
	PLP	5	0	88	34
Ti (s)	Baseline	5	0	0.30	0.02
	PLP	5	0	0.32	0.05
V_T/Ti (mL/s/Kg)	Baseline	5	0	5.3	3.5
	PLP	5	0	4.4	2.9
VO_2 (mL/min/Kg)	Baseline	5	0	32	9
	PLP	5	0	29	5
VCO_2 (mL/min/Kg) *	Baseline	5	0	35	6.5
	PLP	5	0	26	2.4
RER *	Baseline	5	0	1.1	0.20
	PLP	5	0	0.9	0.14
V_E/VCO_2	Baseline	5	0	4.8	2.8
	PLP	5	0	5.0	3.6

SBP= systolic blood pressure, DBP= diastolic blood pressure, MBP= mean blood pressure, V_T = tidal volume, V_E = minute ventilation, f_R = respiratory frequency, Ti= inspiratory time, V_T/Ti = breathing inspiratory drive, VO_2 = oxygen consumption, VCO_2 =carbon dioxide production, RER= respiratory exchange ratio, V_E/VCO_2 = respiratory efficiency, SD = standard deviation. Data was analyzed using mixed regression models with either linear (i.e., normal) or gamma distribution. Mean $\pm$ SD, * $P<0.05$, * $P=0.054$.

Table S2: Average effect of PLP infusion on resting blood pressure and respiratory parameters in telemetered Wistar rats.

	Condition	N	Missing	Mean	SD
SBP *	Baseline	5	0	132.7	10.51
	PLP	5	0	128.1	11.68
DBP *	Baseline	5	0	94.2	9.61
	PLP	5	0	90.6	10.57
MBP *	Baseline	5	0	106.9	9.08
	PLP	5	0	102.9	10.12
V_T (mL/Kg)	Baseline	5	0	1.8	0.59
	PLP	5	0	1.6	0.49
V_E (mL/min/Kg)	Baseline	5	0	190.0	58.92
	PLP	5	0	160.7	68.73
f_R (breaths/min)	Baseline	5	0	108.0	28.64
	PLP	5	0	97.9	24.86
Ti (s)	Baseline	5	0	0.3	0.03
	PLP	5	0	0.3	0.05
V_T/Ti (mL/s/Kg) *	Baseline	5	0	2.8	0.65
	PLP	5	0	2.4	0.81
VO_2 (mL/min/Kg)	Baseline	5	0	23.5	1.38
	PLP	5	0	24.1	5.49
VCO_2 (mL/min/Kg)	Baseline	5	0	31.7	2.11
	PLP	5	0	31.5	7.34
RER	Baseline	5	0	1.3	0.01
	PLP	5	0	1.3	0.05
V_E/VCO_2	Baseline	5	0	5.8	2.16
	PLP	5	0	4.4	1.58

SBP= systolic blood pressure, DBP= diastolic blood pressure, MBP= mean blood pressure, V_T = tidal volume, V_E = minute ventilation, f_R = respiratory frequency, Ti= inspiratory time, V_T/Ti = breathing inspiratory drive, VO_2 = oxygen consumption, VCO_2 =carbon dioxide production, RER= respiratory exchange ratio, V_E/VCO_2 = respiratory efficiency, SD = standard deviation. Data was analyzed using mixed regression models with either linear (i.e., normal) or gamma distribution. Mean $\pm$ SD, * $P<0.05$, * $P=0.054$.

Double-blind Randomized Clinical Trial

Participant recruitment and characteristics

Twenty-two hypertensive participants expressed interest in the study from local community groups and by word of mouth attended the lab for screening and familiarization. Three participants were excluded after screening due to not meeting the inclusion criteria (current smoker [n=1], history of migraine [n=1], frequent ectopy [n=1]) and one participant withdrew after attending the study familiarization visit. Thus, eighteen participants attended both experimental study visits; 4 participants did not successfully complete the isocapnic hypoxic rebreathing due to frequent ectopics (n=1) and technical issues (n=3). Hence, fourteen participants completed both experimental study visits. Participant demographics and current medications are included for all 18 participants who attended both experimental study visits Table 1.

Baseline (i.e., prior to chemoreflex assessment during the placebo visit) HR, BP and blood biochemistry results are presented in Table S3. Both blood glucose (5.78 ± 1.29 mmol/L vs. normal fasting glucose 3.5-5.4 mmol/L) and total cholesterol were elevated (5.40 ± 1.27 mmol/L vs. normal <5.0 mmol/L). 39% of participants were prescribed a statin for treatment of hyperlipidemia. All other blood biochemistry markers were normal (Table S3). Assessment of plasma vitamin B6 (PHC, Pyridoxamine, and PLP) and other B vitamins is presented in Table S4. Most notably, oral supplementation of PHC significantly increased plasma PHC concentration 2-hr after supplementation ($P<0.001$). Additionally, Pantothenic Acid (vitamin B5) was significantly increased 2-hr post-oral supplementation with PHC ($P=0.008$). 4-Pyridoxic Acid was also significantly greater 2-hr post-oral supplementation of PHC ($P<0.001$). PLP and pyridoxamine (other metabolites of vitamin B6) were also significant higher 2-hr following supplementation of PHC ($P<0.001$). Riboflavin (vitamin B2) and Thiamine (vitamin B1) were reduced with time ($P=0.005$ and $P=0.046$, respectively). Nicotinamide vitamin B3) and Trimethylamine N-oxide were unchanged with supplementation of PHC or 2-hrs post the initial blood sample. Half the minimum detectable limit was used for pyridoxal and pyridoxamine reporting in cases where the result was “not found”.

The BP+ device utilized to assess brachial BP also provided an assessment of central BPs. Values provided in Table 2 show peripheral and central BPs prior to any chemoreflex measurements taking place, under placebo and PHC conditions. There were no significant differences observed in any of the measurements with PHC supplementation (SBP, DBP, MBP, pulse rate, pulse pressure, central SBP, central DBP, central MBP, or central pulse pressure, $P>0.05$).

Table S3: Baseline hemodynamic variables and blood biochemistry in hypertensive participants

Resting cardiovascular variables	
HR (bpm)	62 ± 8
SBP (mmHg)	149 ± 21
DBP (mmHg)	82 ± 8
MBP (mmHg)	104 ± 11
Blood Biochemistry	
Albumin (g/L)	44.4 ± 2.0
Urea (mmol/L)	5.6 ± 1.2
High-density lipoprotein (mmol/L)	1.68 ± 0.49
Low-density lipoprotein (mmol/L)	3.13 ± 1.18
Total protein (g/L)	71.6 ± 3.8
Alanine transaminase (U/L)	21.5 ± 11.1
Aspartate transaminase (U/L)	25.5 ± 9.9
Creatinine (μmol/L)	68 ± 13
Bilirubin (μmol/L)	8.8 ± 4.2
Glucose (mmol/L)	5.78 ± 1.29
Triglyceride (mmol/L)	1.65 ± 1.20
Cholesterol (mmol/L)	5.40 ± 1.27

Values are expressed as mean ± SD for continuous variables. HR: heart rate, SBP: systolic blood pressure, DBP: diastolic blood pressure, MBP: mean arterial pressure

Table S4: Plasma B vitamer analysis under placebo and PHC conditions

		Pre-treatment	2-hrs post-treatment	Time	Treatment	Interaction
Pyridoxine (nM)	Placebo	1.60 ± 0.37	1.31 ± 0.36	<0.001	<0.001	<0.001
	PHC	1.26 ± 0.44	18369.45 ± 6295.27‡§			
Pyridoxal (nM)	Placebo	14.05 ± 10.46	15.09 ± 12.78	<0.001	<0.001	<0.001
	PHC	16.65 ± 13.62	5812.61 ± 3061.44‡§			
Pyridoxamine (nM)	Placebo	1.25 ± 0.00	1.25 ± 0.00	<0.001	<0.001	<0.001
	PHC	1.25 ± 0.00	19.25 ± 3.28‡§			
Pantothenic acid (nM)	Placebo	233.98 ± 145.78	233.09 ± 153.28	0.012	0.005	0.008
	PHC	236.15 ± 149.96	264.40 ± 150.86‡§			
Pyridoxic acid (nM)	Placebo	36.75 ± 42.90	33.99 ± 36.65	<0.001	<0.001	<0.001
	PHC	40.17 ± 51.40	8374.28 ± 2177.08‡§			
Nicotinamide (nM)	Placebo	97.34 ± 32.13	96.23 ± 32.74	0.438	0.284	0.367
	PHC	99.57 ± 35.02	113.64 ± 60.73			
Trimethylamine N-oxide (nM)	Placebo	12.94 ± 15.25	10.00 ± 8.75	0.367	0.378	0.812
	PHC	10.20 ± 14.06	8.39 ± 9.03			
Riboflavin (nM)	Placebo	24.38 ± 11.57	19.99 ± 10.31	0.005	0.174	0.753
	PHC	26.07 ± 16.98	22.28 ± 13.34			
Thiamine (nM)	Placebo	12.29 ± 1.69	12.14 ± 1.71	0.046	0.115	0.146
	PHC	12.96 ± 2.60	12.18 ± 2.48			

Values are expressed as mean±SD for continuous variables. The main effects of time, treatment and their interaction were examined using a mixed regression model. Where a significant interaction was observed, differences identified during post hoc analysis (t tests with Bonferroni correction) are identified as ‡ P<0.05 vs. pre-treatment, § P<0.05 vs. placebo.

Section IV - Statistical output:

Table S5: Fixed Effects Parameter Estimates CSN

Names	Effect	Estimate	SE	95% Confidence Interval		z	p
				Lower	Upper		
(Intercept)	(Intercept)	1.030	0.0487	0.935	1.126	21.14	< .001
CSN Baseline	CSN Baseline	1.517	0.6631	0.217	2.816	2.29	0.022
Condition1	PLP 5mM - KCN first	-0.863	0.1051	-1.069	-0.657	-8.21	< .001
Condition2	KCN washout - KCN first	-0.744	0.1076	-0.955	-0.533	-6.92	< .001

Random Components

Groups	Name	SD	Variance	ICC
ID	(Intercept)	0.0569	0.00323	
Residual		0.1772	0.03140	
Residuals		0.1772	0.03140	.

Note. Number of Obs: 24 , groups: ID 8

Table S6: Fixed Effects Parameter Estimates resting CSN firing

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	26.20	4.60	15.8	36.61	9.00	5.693	< .001
time1	After P5P - Before P5P	-11.44	4.13	-20.8	-2.11	9.00	-2.773	0.022
time2	Washout - Before P5P	2.61	6.80	-12.8	17.99	9.00	0.385	0.709

Random Components

Groups	Name	Variance	SD	ICC	Phi
Rat ID	(Intercept)	141.37	11.89	0.945	2.19e-4
	time1	89.42	9.46		
	time2	269.91	16.43		
Residual		8.16	2.86		

Note. Number of Obs: 18 , Number of groups: Rat ID 7

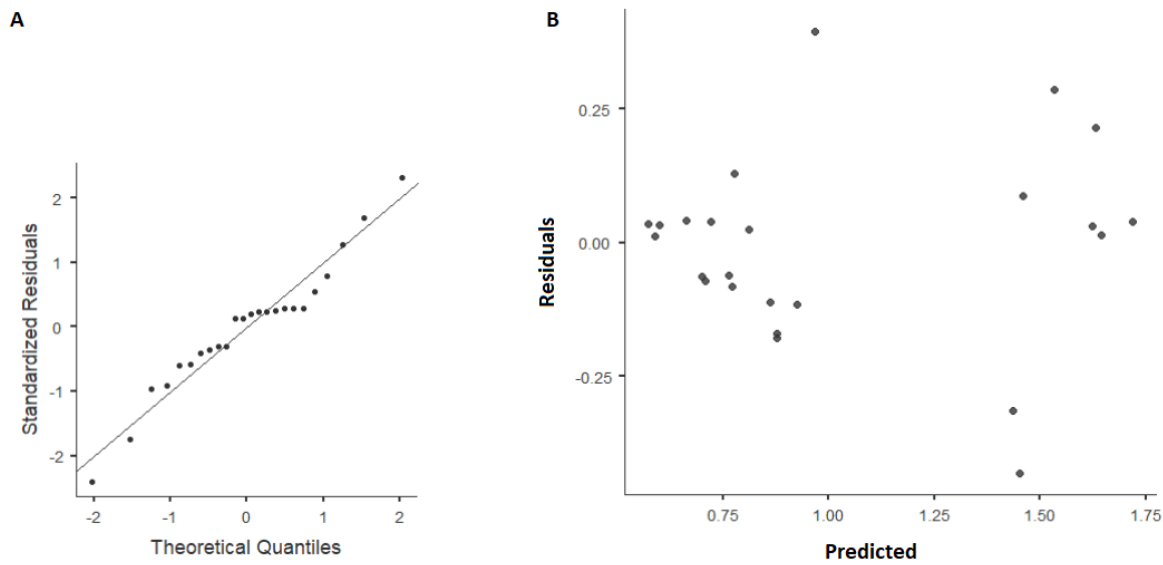


Figure S8: Residual analysis of the chosen generalized mixed model for CSN response to KCN in pre-hypertensive SHR. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

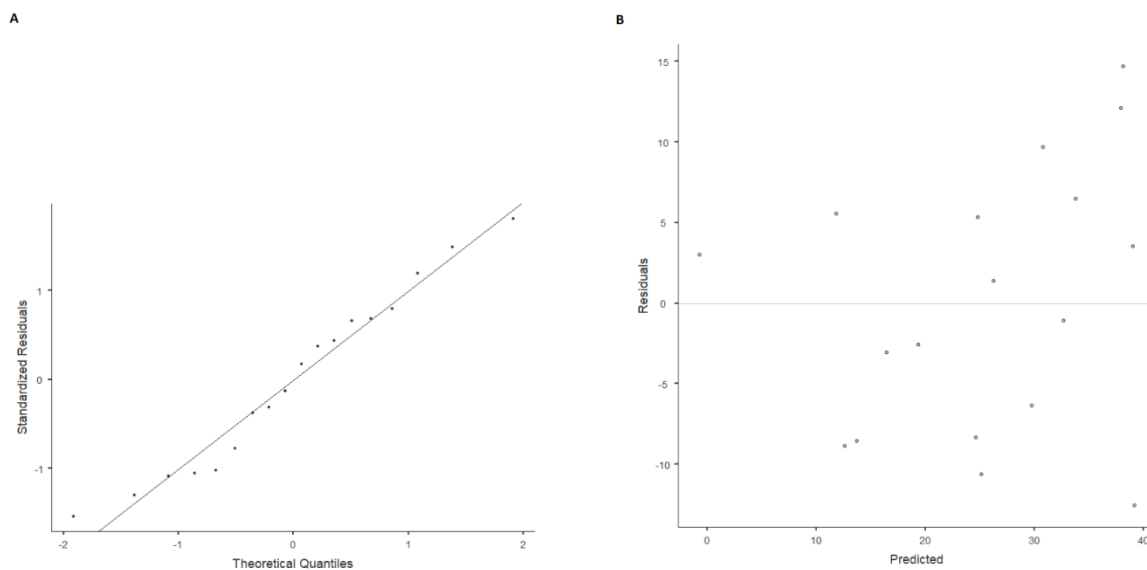


Figure S9: Residual analysis of the linear mixed model for CSN resting firing in pre-hypertensive SHR. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

Table S7: Parameter Estimates (Fixed coefficients) SNA

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	2.36468	0.0502	2.2615	2.4679	26.0	47.089	< .001
PLP Dose1	2mM - 1mM	-0.23423	0.1571	-0.5844	0.1159	10.0	-1.491	0.167
PLP Dose2	3mM - 1mM	-0.32397	0.1657	-0.6931	0.0451	10.0	-1.956	0.079
PLP Dose3	4mM - 1mM	-0.10637	0.1765	-0.4997	0.2869	10.0	-0.603	0.560
PLP Dose4	5mM - 1mM	-0.37723	0.1584	-0.7302	-0.0243	10.0	-2.382	0.039
Time1	PLP - control	-0.12531	0.0519	-0.2319	-0.0187	26.0	-2.416	0.023
Time2	washout - control	0.00327	0.0295	-0.0574	0.0640	26.0	0.111	0.913
Baseline SNA	Baseline SNA	1.12299	0.0519	1.0162	1.2297	26.0	21.626	< .001

Random Components

Groups	Name	Variance	SD	ICC	Phi
Rat ID	(Intercept)	0.0359	0.190	0.752	-0.697
Residual		0.0119	0.109		

Note. Number of Obs: 44 , Number of groups: Rat ID 15

Table S8: Parameter Estimates (Fixed coefficients) HR

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	271.326	17.472	235.412	307.24	26.0	15.5294	< .001
PLP Dose1	2mM - 1mM	130.849	68.858	-22.577	284.27	10.0	1.9003	0.087
PLP Dose2	3mM - 1mM	127.498	56.286	2.085	252.91	10.0	2.2652	0.047
PLP Dose3	4mM - 1mM	88.232	55.529	-35.494	211.96	10.0	1.5889	0.143
PLP Dose4	5mM - 1mM	0.931	55.748	-123.283	125.14	10.0	0.0167	0.987
Time1	PLP - control	41.996	20.997	-1.164	85.16	26.0	2.0001	0.056
Time2	washout - control	-3.796	18.482	-41.785	34.19	26.0	-0.2054	0.839
Baseline HR	Baseline HR	1.193	0.463	0.242	2.14	26.0	2.5796	0.016

Random Components

Groups	Name	Variance	SD	ICC	Phi
Rat ID	(Intercept)	3956	62.9	0.605	-0.272
Residual		2579	50.8		

Note. Number of Obs: 44 , Number of groups: Rat ID 15

Table S9: Fixed Effects Parameter Estimates PN amp/Ti

Names	Effect	Estimate	SE	95% Confidence Interval		z	p
				Lower	Upper		
(Intercept)	(Intercept)	15.762	1.0118	13.78	17.745	15.578	< .001
Time1	PLP - control	-0.675	0.1892	-1.05	-0.304	-3.566	< .001
Time2	washout - control	-0.827	0.1946	-1.21	-0.445	-4.249	< .001
PLP Dose1	2mM - 1mM	-3.890	4.0119	-11.75	3.974	-0.970	0.332
PLP Dose2	3mM - 1mM	-7.100	2.6105	-12.22	-1.984	-2.720	0.007
PLP Dose3	4mM - 1mM	-5.288	2.4645	-10.12	-0.458	-2.146	0.032
PLP Dose4	5mM - 1mM	-4.216	2.6551	-9.42	0.988	-1.588	0.112
Baseline PN Amp/TI	Baseline PN Amp/TI	1.242	0.0987	1.05	1.435	12.587	< .001

Random Components

Groups	Name	SD	Variance	ICC
Rat ID	(Intercept)	1.5792	2.49372	
Residual		0.0873	0.00762	
Residuals		0.0873	0.00762	.

Note. Number of Obs: 44 , groups: Rat ID 15

Table S10: Parameter Estimates (Fixed coefficients) PN amp

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	7.9104	0.2087	7.481	8.3393	26.0	37.909	< .001
Baseline PN amp	Baseline PN amp	1.0711	0.0666	0.934	1.2079	26.0	16.088	< .001
PLP Dose1	2mM - 1mM	-0.6855	0.7031	-2.252	0.8811	10.0	-0.975	0.353
PLP Dose2	3mM - 1mM	-0.9916	0.6590	-2.460	0.4766	10.0	-1.505	0.163
PLP Dose3	4mM - 1mM	-0.6039	0.6598	-2.074	0.8663	10.0	-0.915	0.382
PLP Dose4	5mM - 1mM	-0.5410	0.6784	-2.053	0.9707	10.0	-0.797	0.444
Time1	PLP - control	-0.1773	0.1105	-0.404	0.0498	26.0	-1.605	0.121
Time2	washout - control	0.0176	0.0863	-0.160	0.1951	26.0	0.204	0.840

Random Components

Groups	Name	Variance	SD	ICC	Phi
Rat ID	(Intercept)	0.6406	0.800	0.909	-0.430
Residual		0.0640	0.253		

Note. Number of Obs: 44 , Number of groups: Rat ID 15

Table S11: Parameter Estimates (Fixed coefficients) PN rate

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	48.19	11.015	25.81	70.579	5.88	4.376	0.005
Baseline PN rate	Baseline PN rate	-1.09	0.750	-2.62	0.433	35.97	-1.455	0.154
Time1	PLP - control	2.26	4.595	-7.08	11.603	21.14	0.493	0.627
Time2	washout - control	5.95	4.748	-3.70	15.601	21.35	1.254	0.224
PLP Dose1	2mM - 1mM	36.67	34.895	-34.25	107.583	5.91	1.051	0.334
PLP Dose2	3mM - 1mM	17.06	35.028	-54.13	88.243	5.99	0.487	0.644
PLP Dose3	4mM - 1mM	-7.73	34.921	-78.70	63.239	5.93	-0.221	0.832
PLP Dose4	5mM - 1mM	20.33	34.953	-50.70	91.365	5.95	0.582	0.582

Random Components

Groups	Name	Variance	SD	ICC
Rat ID	(Intercept)	1765	42.0	0.918
Residual		158	12.6	

Note. Number of Obs: 44 , Number of groups: Rat ID 15

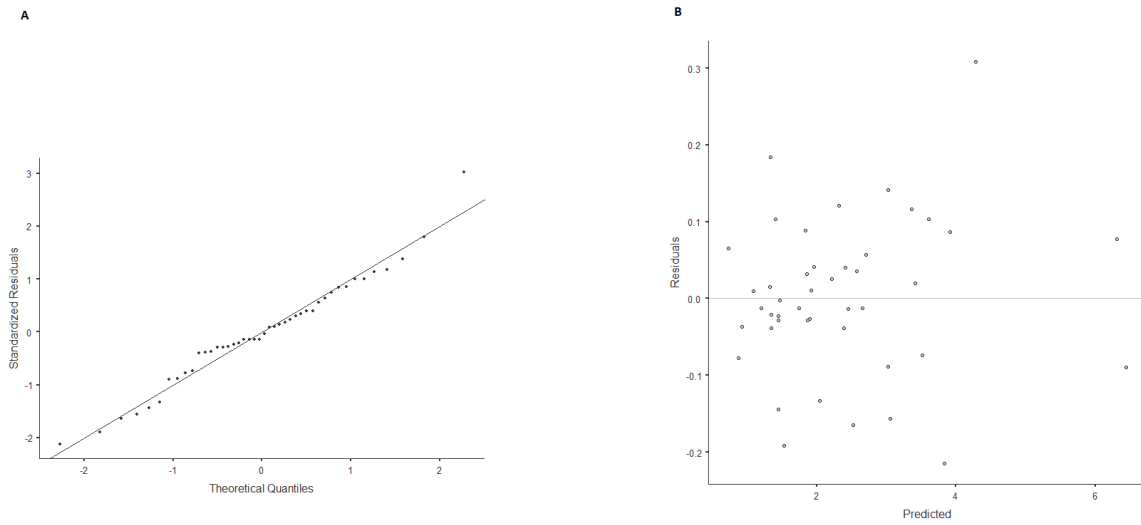


Figure S10: Residual analysis of the chosen mixed linear model for SNA in pre-hypertensive SHRs in the WHBP. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

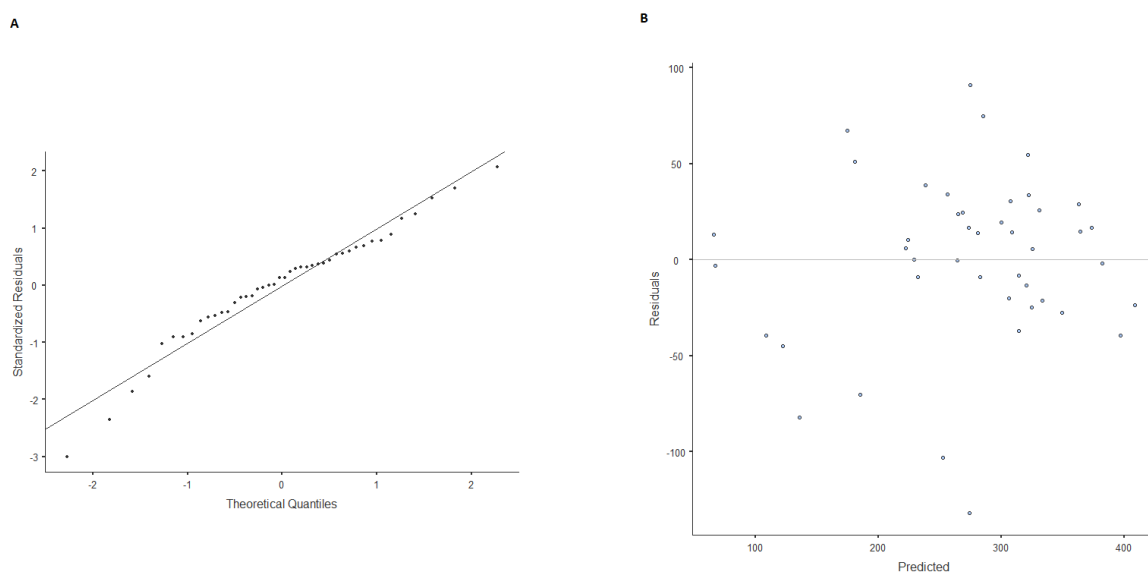


Figure S11: Residual analysis of the chosen mixed linear model for HR in pre-hypertensive SHRs in the WHBP. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

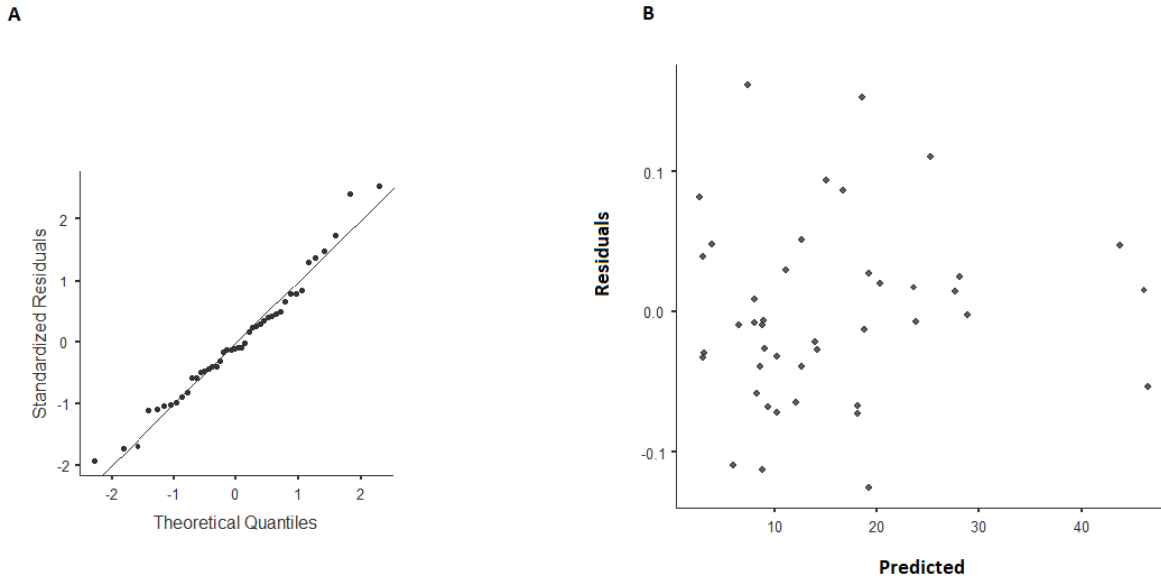


Figure S12: Residual analysis of the chosen generalized mixed model for PN amp/Ti in pre-hypertensive SHRs in the WHBP. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

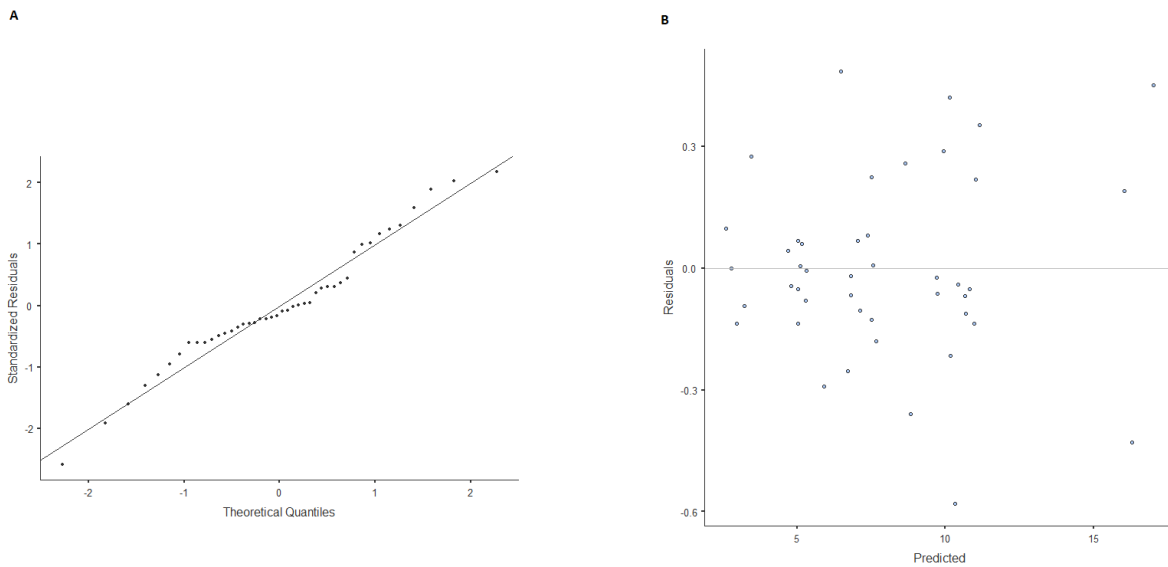


Figure S13: Residual analysis of the chosen mixed linear model for PN amp in pre-hypertensive SHRs in the WHBP. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

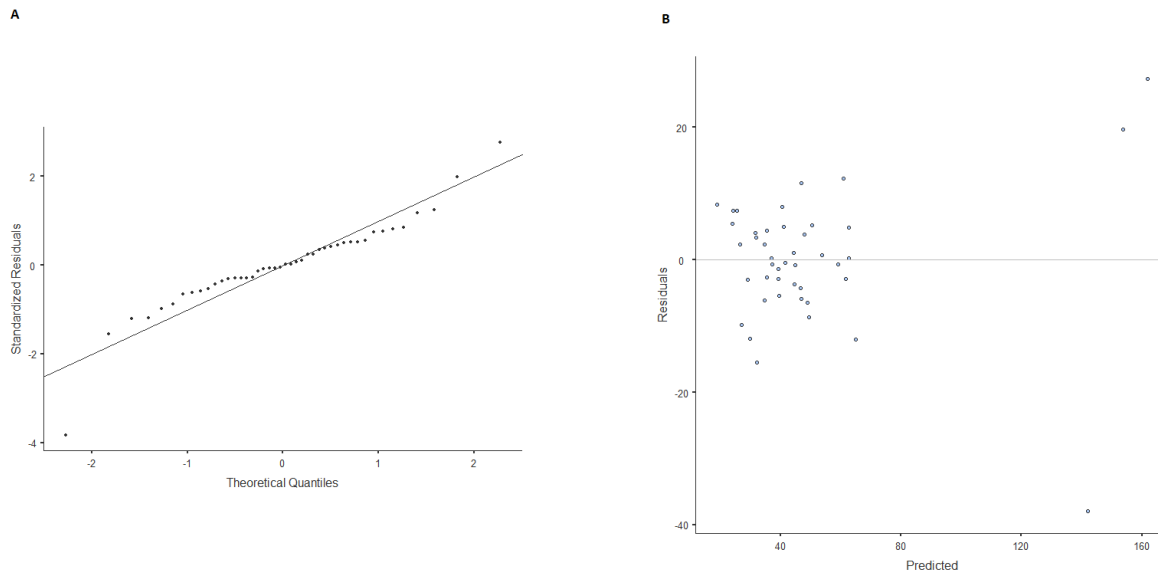


Figure S14: Residual analysis of the chosen mixed linear model for PN rate in pre-hypertensive SHRs in the WHBP. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

Table S12: Parameter Estimates (Fixed coefficients) SBP

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	42.3	6.66	26.9	57.62	8.00	6.35	< .001
Condition1	KCN with PLP - First KCN	-56.1	11.38	-82.3	-29.85	8.00	-4.93	0.001
Condition2	Third KCN - First KCN	-23.6	6.79	-39.3	-7.99	8.00	-3.48	0.008

Random Components

Groups	Name	Variance	SD	ICC	Phi
ID	(Intercept)	194	13.9	0.496	-0.644
Residual		197	14.0		

Note. Number of Obs: 15 , Number of groups: ID 5

Table S13: Parameter Estimates (Fixed coefficients) DBP

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	31.3	3.38	23.5	39.07	8.00	9.25	< .001
Condition1	KCN with PLP - First KCN	-44.9	10.94	-70.2	-19.71	8.00	-4.11	0.003
Condition2	Third KCN - First KCN	-14.6	5.43	-27.1	-2.10	8.00	-2.69	0.027

Random Components

Groups	Name	Variance	SD	ICC	Phi
ID	(Intercept)	35.9	5.99	0.174	-0.754
Residual		170.7	13.06		

Note. Number of Obs: 15 , Number of groups: ID 5

Table S14: Parameter Estimates (Fixed coefficients) ΔMBP SHR

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	34.6	4.42	24.4	44.79	8.00	7.83	< .001
Condition1	KCN with PLP - First KCN	-48.3	10.83	-73.3	-23.34	8.00	-4.46	0.002
Condition2	Third KCN - First KCN	-19.5	6.54	-34.6	-4.44	8.00	-2.99	0.017

Random Components

Groups	Name	Variance	SD	ICC	Phi
ID	(Intercept)	72.4	8.51	0.288	-0.636
Residual		179.3	13.39		

Note. Number of Obs: 15 , Number of groups: ID 5

Table S15: Parameter Estimates (Fixed coefficients) ΔfR SHR

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	129.6	16.3	91.9	167.24	8.00	7.94	< .001
Condition1	KCN with PLP - First KCN	-85.8	41.2	-180.8	9.23	8.00	-2.08	0.071
Condition2	Third KCN - First KCN	-43.5	26.5	-104.7	17.69	8.00	-1.64	0.140

Random Components

Groups	Name	Variance	SD	ICC	Phi
ID	(Intercept)	933	30.5	0.258	-0.585
Residual		2676	51.7		

Note. Number of Obs: 15 , Number of groups: ID 5

Table S16: Fixed Effects Parameter Estimates ΔHR SHR

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	-16.1	24.8	-73.3	41.1	8.00	-0.649	0.534
Condition1	KCN with PLP - First KCN	45.8	61.9	-96.8	188.4	8.00	0.740	0.480
Condition2	Third KCN - First KCN	30.4	40.2	-62.2	123.0	8.00	0.757	0.471

Random Components

Groups	Name	Variance	SD	ICC	Phi
ID	(Intercept)	2161	46.5	0.263	-0.579
Residual		6059	77.8		

Note. Number of Obs: 15 , Number of groups: ID 5

Table S17: Fixed Effects Parameter Estimates Δ VT SHR

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	-0.0954	0.252	-0.677	0.487	8.00	-0.378	0.715
Condition1	KCN with PLP - First KCN	-0.0815	0.344	-0.874	0.711	8.00	-0.237	0.819
Condition2	Third KCN - First KCN	0.3470	0.322	-0.394	1.088	8.00	1.079	0.312

Random Components

Groups	Name	Variance	SD	ICC	Phi
ID	(Intercept)	0.245	0.495	0.482	-0.124
Residual		0.263	0.512		

Note. Number of Obs: 15 , Number of groups: ID 5

Table S18: Fixed Effects Parameter Estimates Δ VE SHR

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	155.3	36.9	70.1	240.4	8.00	4.203	0.003
Condition1	KCN with PLP - First KCN	-150.6	91.0	-360.5	59.3	8.00	-1.654	0.137
Condition2	Third KCN - First KCN	23.0	60.8	-117.2	163.1	8.00	0.378	0.715

Random Components

Groups	Name	Variance	SD	ICC	Phi
ID	(Intercept)	4751	68.9	0.263	-0.554
Residual		13331	115.5		

Note. Number of Obs: 15 , Number of groups: ID 5

Table S19: Fixed Effects Parameter Estimates Δ VE/VCO2 SHR

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	4.84	0.987	2.5640	7.12	8.00	4.90	0.001
Condition1	KCN with PLP - First KCN	-3.27	2.157	-8.2484	1.70	8.00	-1.52	0.168
Condition2	Third KCN - First KCN	3.04	1.353	-0.0802	6.16	8.00	2.25	0.055

Random Components

Groups	Name	Variance	SD	ICC	Phi
ID	(Intercept)	3.82	1.96	0.345	-0.607
Residual		7.24	2.69		

Note. Number of Obs: 15 , Number of groups: ID 5

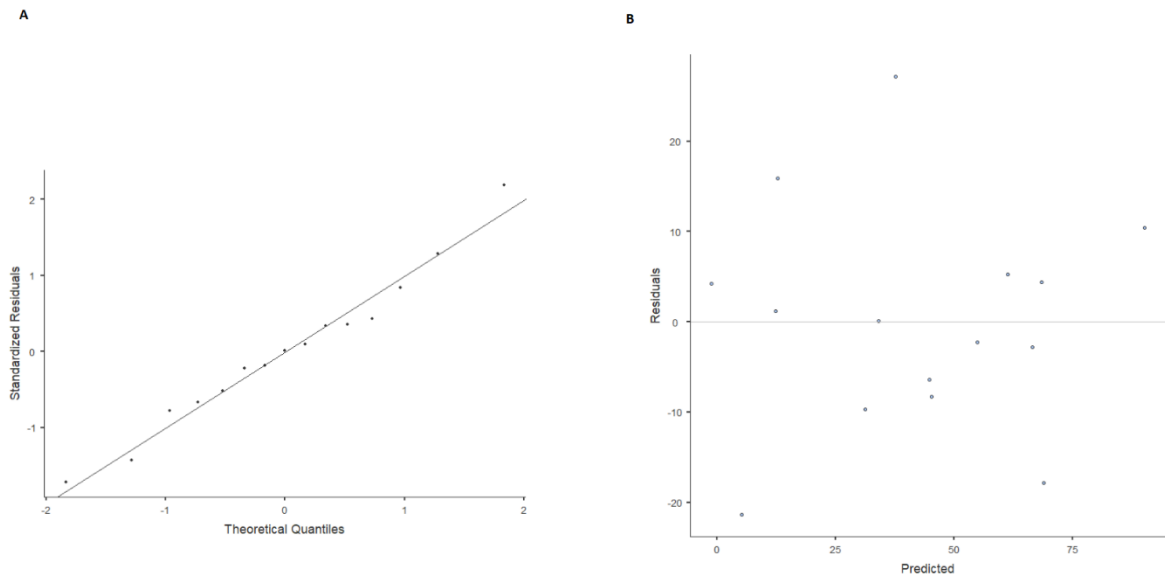


Figure S15: Residual analysis of the chosen mixed linear model for KCN-evoked SBP response in adults SHR. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

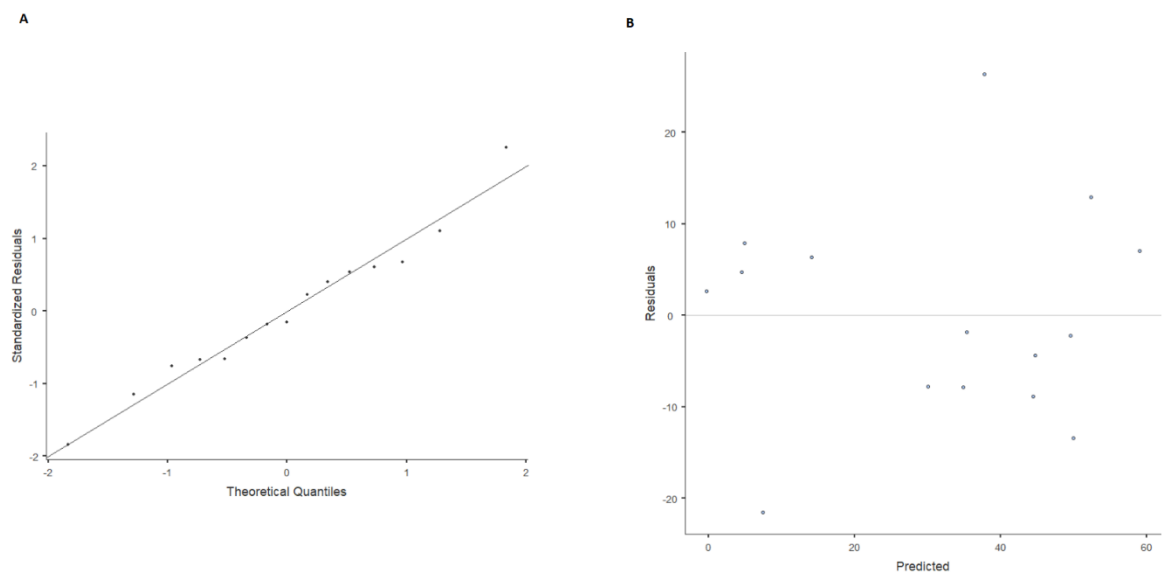


Figure S16: Residual analysis of the chosen mixed linear model for KCN-evoked DBP response in adults SHR. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

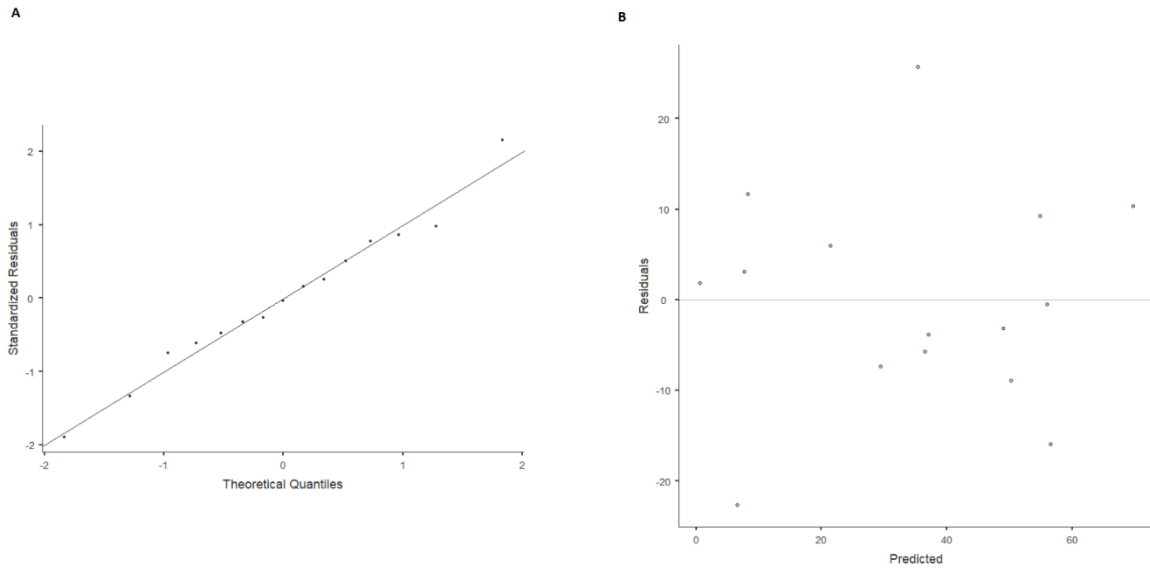


Figure S17: Residual analysis of the chosen mixed linear model for KCN-evoked MBP response in adults SHR. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

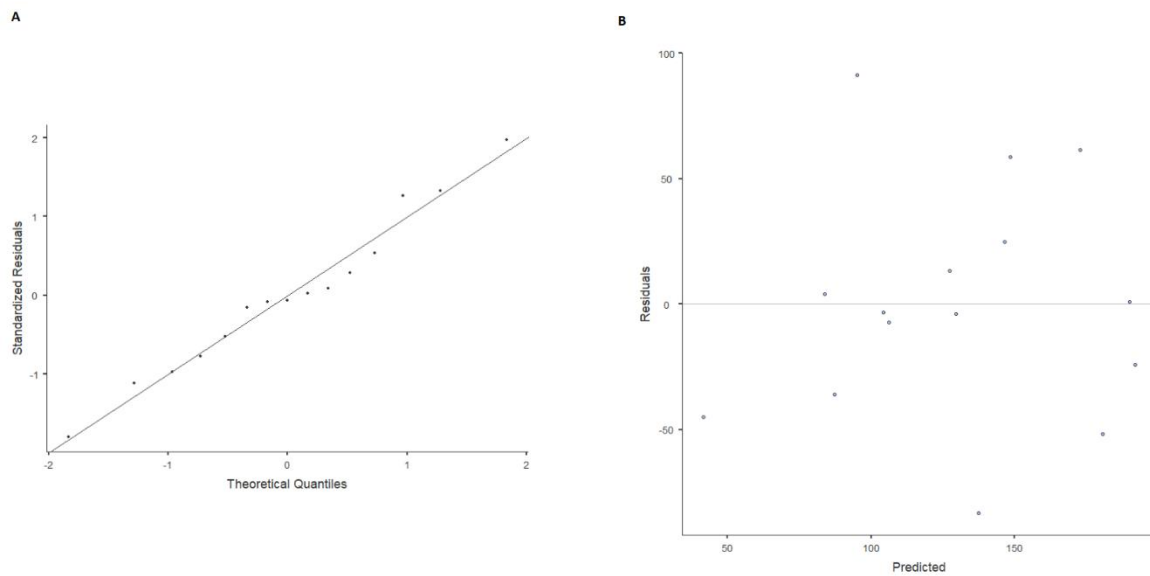


Figure S18: Residual analysis of the chosen mixed linear model for KCN-evoked f_R response in adults SHR. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

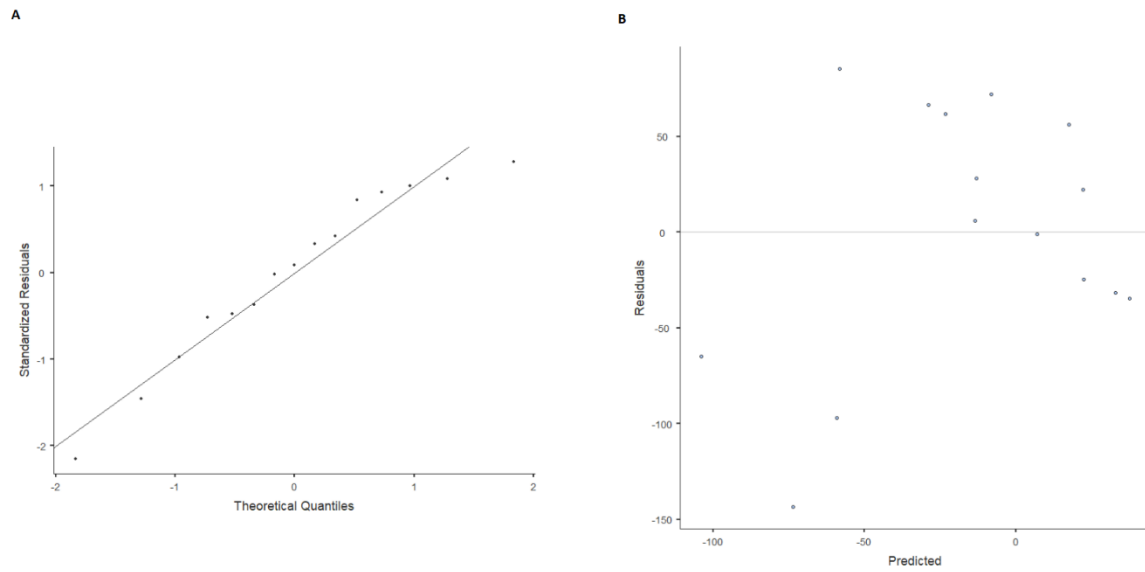


Figure S19: Residual analysis of the chosen generalized mixed model for KCN-evoked HR response in adults SHR. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

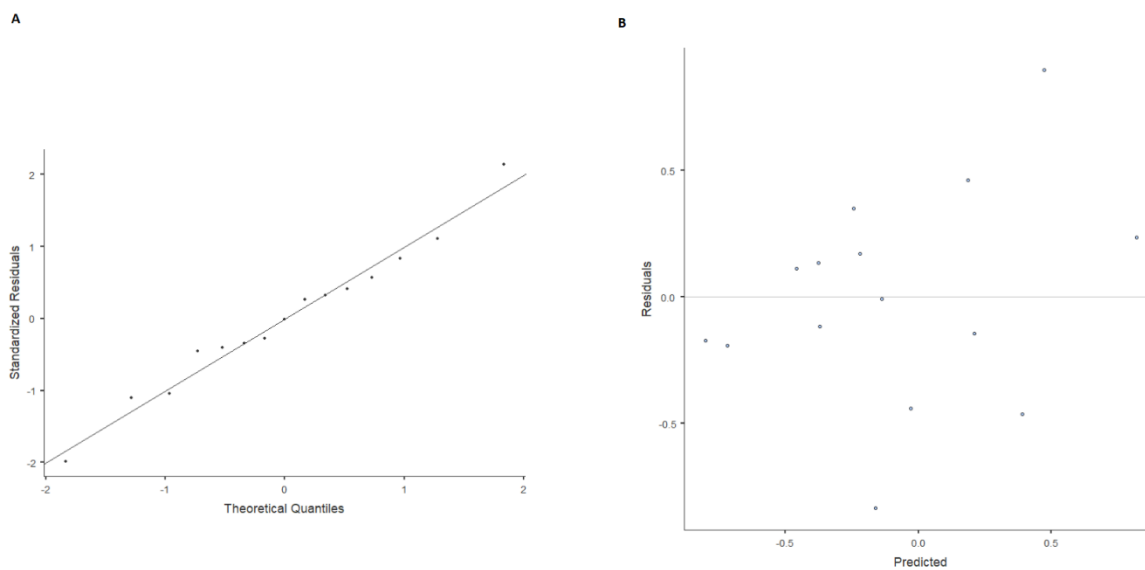


Figure S20: Residual analysis of the chosen generalized mixed model for KCN-evoked VT response in adults SHR. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

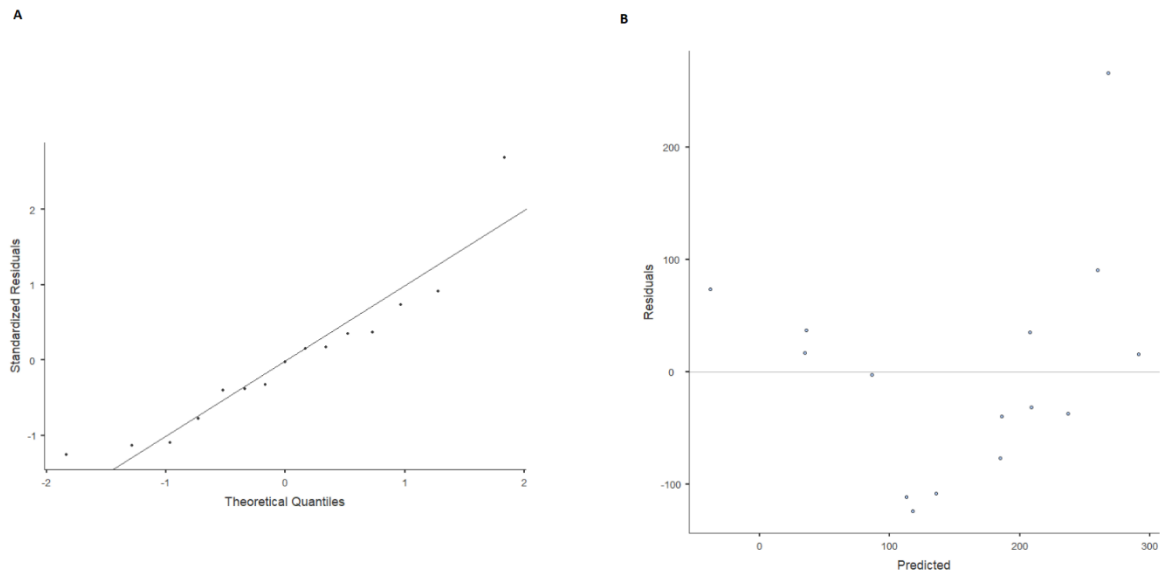


Figure S21: Residual analysis of the chosen generalized mixed model for KCN-evoked VE response in adults SHR. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

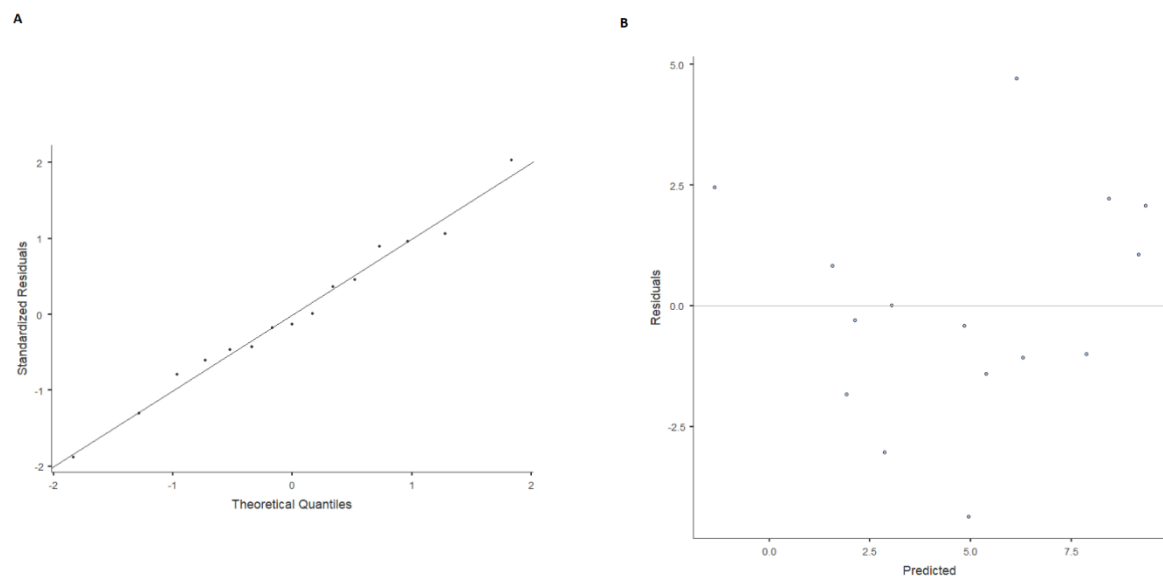


Figure S22: Residual analysis of the chosen generalized mixed model for KCN-evoked VE/VCO₂ response in adults SHR. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

Table S20: Parameter Estimates (Fixed coefficients) ΔMBP Wistar

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	43.2	6.22	29.3	57.040	4.00	6.94	0.002
Condition1	KCN with PLP - First KCN	-24.1	7.29	-40.3	-7.840	8.00	-3.30	0.011
Condition2	Third KCN - First KCN	-15.3	7.29	-31.6	0.886	8.00	-2.11	0.068

Random Components

Groups	Name	Variance	SD	ICC
ID	(Intercept)	149	12.2	0.529
Residual		133	11.5	

Note. Number of Obs: 15 , Number of groups: ID 5

Table S21: Parameter Estimates (Fixed coefficients) Δ HR Wistar

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	-56.1	29.5	-121.90	9.64	4.00	-1.90	0.130
Condition1	KCN with PLP - First KCN	96.4	31.3	26.54	166.19	8.00	3.08	0.015
Condition2	Third KCN - First KCN	59.9	31.3	-9.97	129.69	8.00	1.91	0.093

Random Components

Groups	Name	Variance	SD	ICC
ID	(Intercept)	3538	59.5	0.590
Residual		2455	49.6	

Note. Number of Obs: 15 , Number of groups: ID 5

Table S22: Parameter Estimates (Fixed coefficients) ΔfR Wistar

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	132.4	14.8	99.4	165.5	12.0	8.924	< .001
Condition1	KCN with PLP - First KCN	28.9	36.3	-52.1	109.9	12.0	0.795	0.442
Condition2	Third KCN - First KCN	-15.1	36.3	-96.1	65.9	12.0	-0.415	0.686

Random Components

Groups	Name	Variance	SD	ICC
ID	(Intercept)	0	0.0	0.00
Residual		3303	57.5	

Note. Number of Obs: 15 , Number of groups: ID 5

Table S23: Parameter Estimates (Fixed coefficients) ΔVT Wistar

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	0.644	0.442	-0.340	1.627	4.00	1.458	0.219
Condition1	KCN with PLP - First KCN	-0.416	0.438	-1.392	0.560	8.00	-0.949	0.370
Condition2	Third KCN - First KCN	0.779	0.438	-0.197	1.755	8.00	1.778	0.113

Random Components

Groups	Name	Variance	SD	ICC
ID	(Intercept)	0.815	0.903	0.629
Residual		0.480	0.693	

Note. Number of Obs: 15 , Number of groups: ID 5

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Table S23: Parameter Estimates (Fixed coefficients) Δ VE Wistar

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	353	88.5	155.5	549.7	4.00	3.99	0.016
Condition1	KCN with PLP - First KCN	-160	86.5	-353.2	32.3	8.00	-1.85	0.101
Condition2	Third KCN - First KCN	101	86.5	-91.6	293.9	8.00	1.17	0.276

Random Components

Groups	Name	Variance	SD	ICC
ID	(Intercept)	32894	181	0.637
Residual		18711	137	

Note. Number of Obs: 15 , Number of groups: ID 5

Table S24: Parameter Estimates (Fixed coefficients) Δ VE/VCO2 Wistar

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	7.58	2.95	1.01	14.15	4.00	2.57	0.062
Condition1	KCN with PLP - First KCN	-3.24	3.00	-9.93	3.45	8.00	-1.08	0.312
Condition2	Third KCN - First KCN	4.28	3.00	-2.41	10.98	8.00	1.43	0.192

Random Components

Groups	Name	Variance	SD	ICC
ID	(Intercept)	36.0	6.00	0.615
Residual		22.6	4.75	

Note. Number of Obs: 15 , Number of groups: ID 5

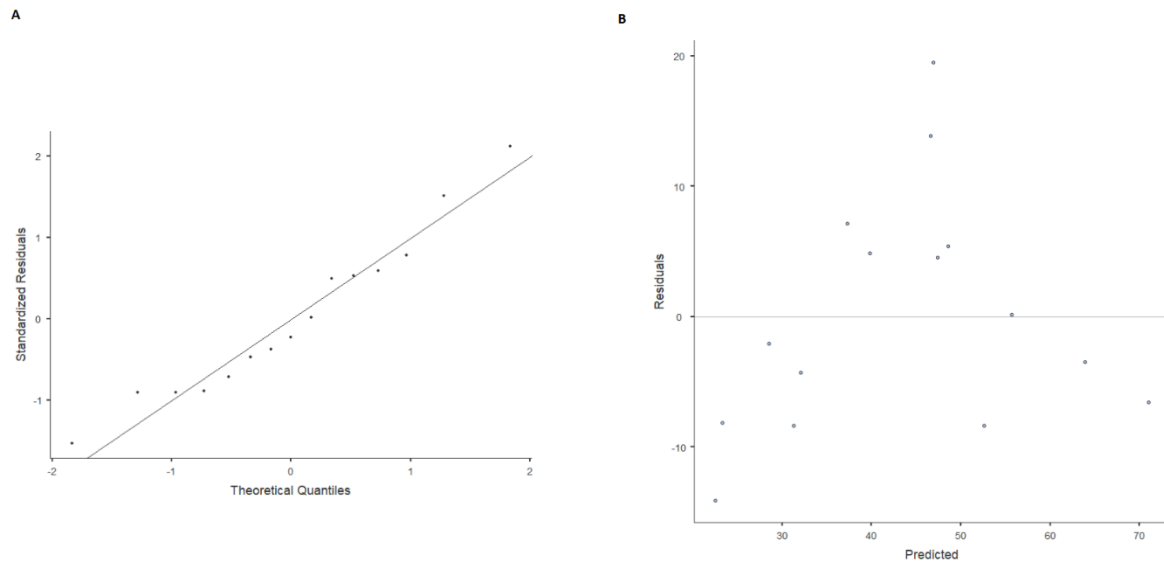


Figure S23: Residual analysis of the chosen generalized mixed model for KCN-evoked MBP response in adult Wistar rats. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

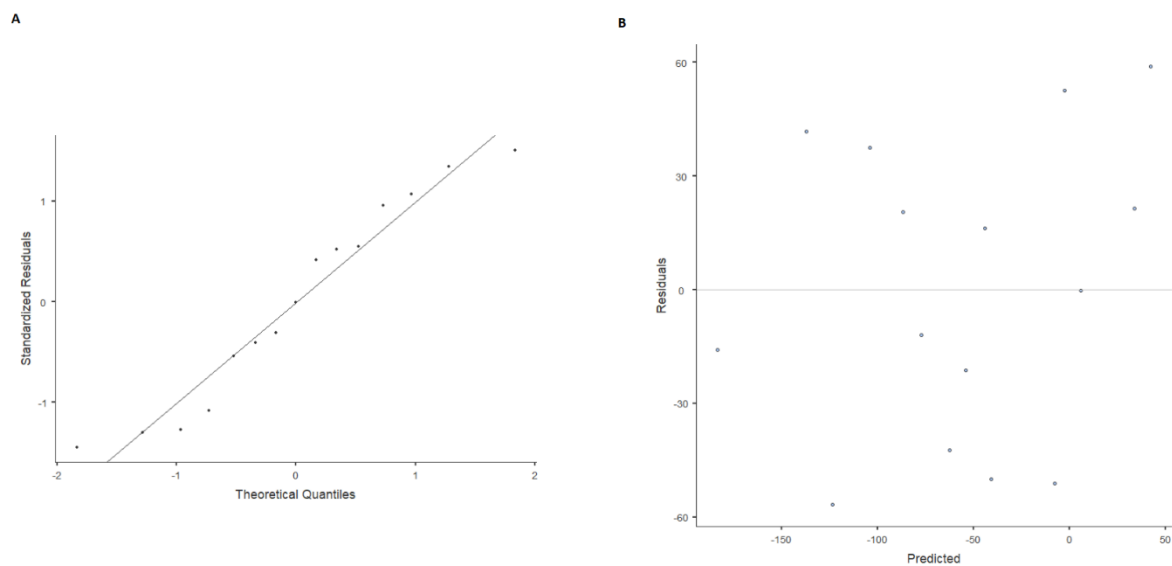


Figure S24: Residual analysis of the chosen generalized mixed model for KCN-evoked HR response in adult Wistar rats. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

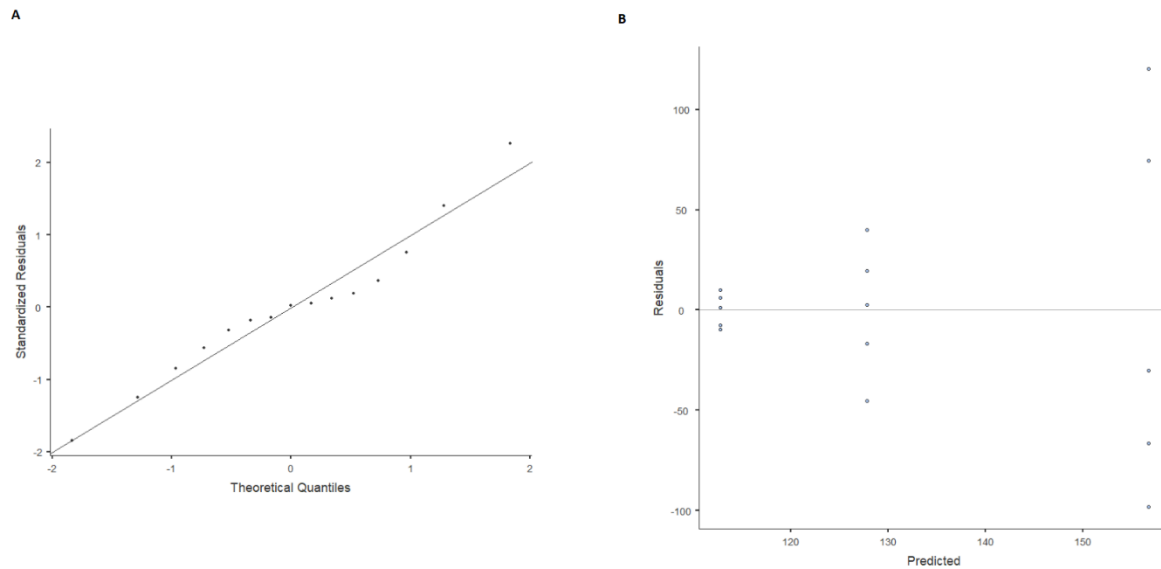


Figure S25: Residual analysis of the chosen generalized mixed model for KCN-evoked fR response in adult Wistar rats. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

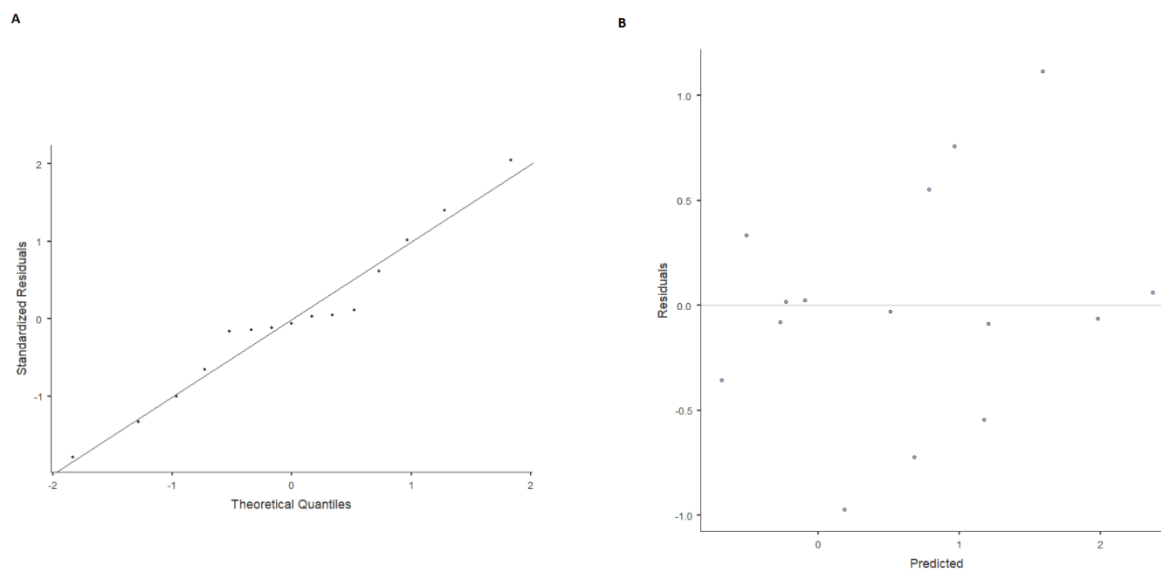


Figure S26: Residual analysis of the chosen generalized mixed model for KCN-evoked VT response in adult Wistar rats. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

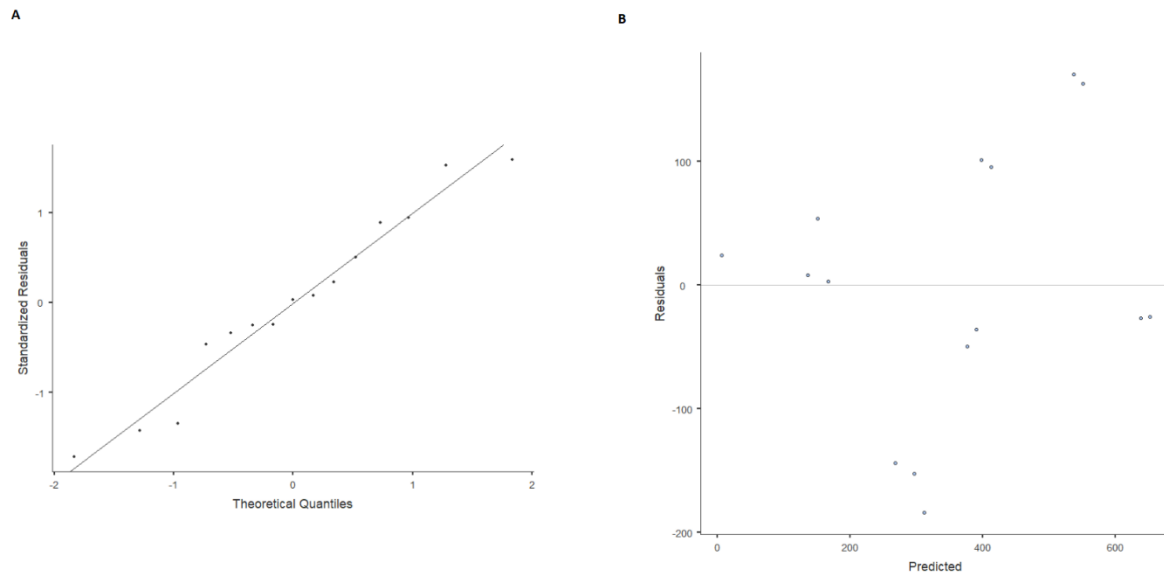


Figure S27: Residual analysis of the chosen generalized mixed model for KCN-evoked VE response in adult Wistar rats. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

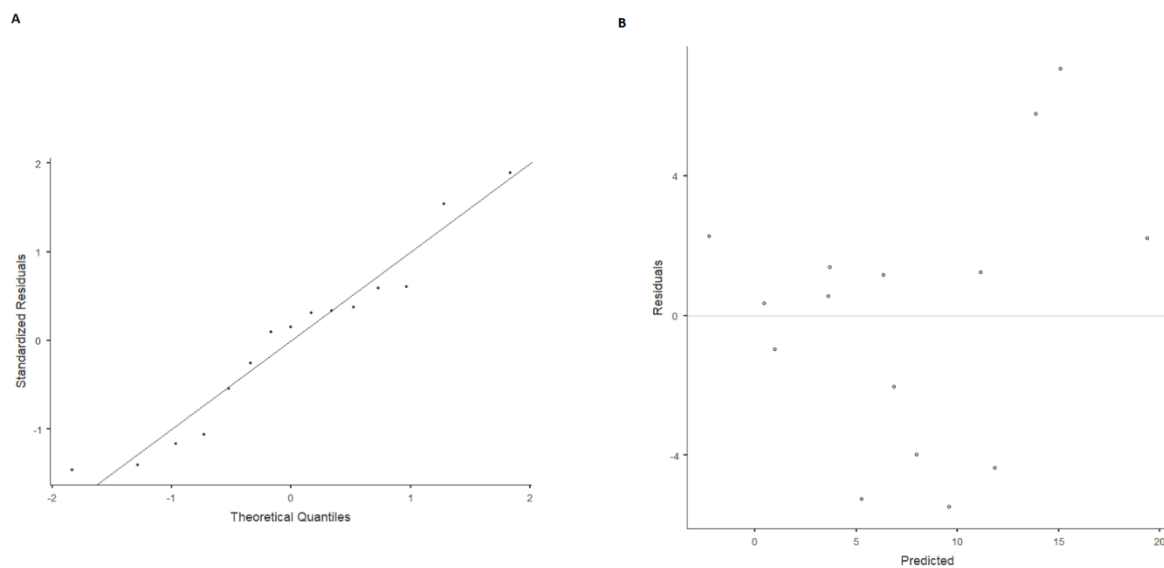


Figure S28: Residual analysis of the chosen generalized mixed model for KCN-evoked VE/VCO₂ response in adult Wistar rats. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

Table S25: Parameter Estimates (Fixed coefficients) resting SBP

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	192.6	11.01	162.1	223.20	4.00	17.49	< .001
Time1	PLP - Before	-16.9	4.41	-29.2	-4.65	4.00	-3.83	0.019

Random Components

Groups	Name	Variance	SD	ICC
ID	(Intercept)	582.0	24.12	0.923
Residual		48.7	6.98	

Note. Number of Obs: 10 , Number of groups: ID 5

Table S26: Parameter Estimates (Fixed coefficients) resting DBP

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	128.5	7.28	108.3	148.70	4.00	17.65	< .001
Time1	PLP - Before	-15.8	2.36	-22.4	-9.25	4.00	-6.69	0.003

Random Components

Groups	Name	Variance	SD	ICC
ID	(Intercept)	258.0	16.06	0.949
Residual		13.9	3.73	

Note. Number of Obs: 10 , Number of groups: ID 5

Table S27: Parameter Estimates (Fixed coefficients) resting MBP

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	155.9	10.91	125.6	186.16	4.00	14.29	< .001
Time1	PLP - Before	-16.2	2.93	-24.3	-8.02	4.00	-5.51	0.005

Random Components

Groups	Name	Variance	SD	ICC
ID	(Intercept)	584.2	24.17	0.964
Residual		21.5	4.64	

Note. Number of Obs: 10 , Number of groups: ID 5

Table S28: Parameter Estimates (Fixed coefficients) resting HR

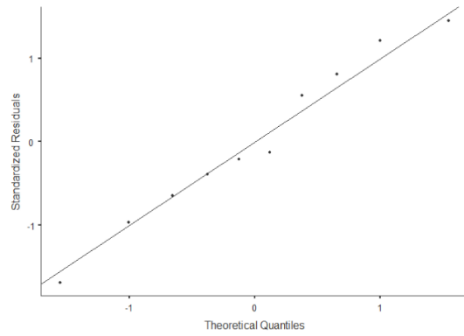
Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	332.0	12.35	297.7	366.3	4.00	26.88	< .001
Time1	PLP - Before	-35.1	8.09	-57.6	-12.7	4.00	-4.34	0.012

Random Components

Groups	Name	Variance	SD	ICC
ID	(Intercept)	681	26.1	0.807
	Residual	163	12.8	

Note. Number of Obs: 10 , Number of groups: ID 5

A



B

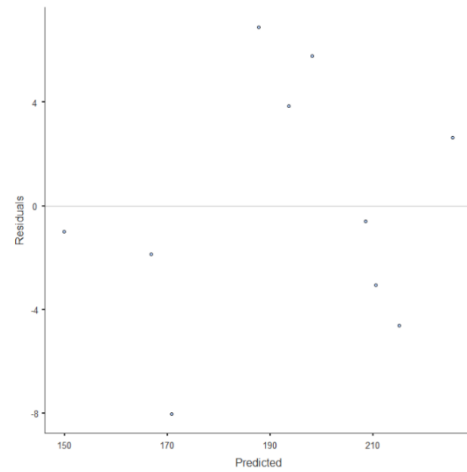
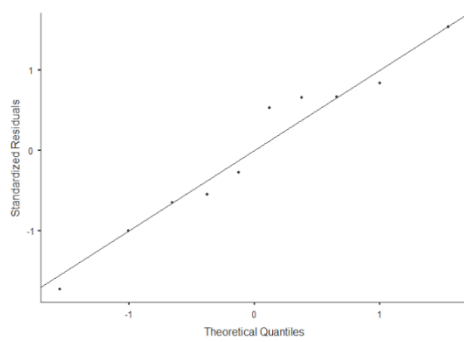


Figure S29: Residual analysis of the chosen mixed linear model for resting SBP in adults SHR. **A** QQ-Plot is used to analyze the normality of residuals, and **B** scatter plots residuals-predicted values to assess heteroskedasticity.

A



B

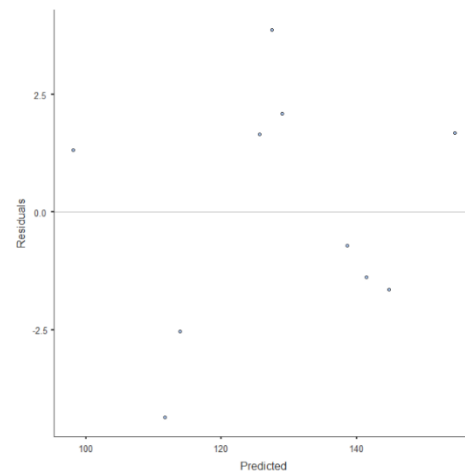


Figure S30: Residual analysis of the chosen mixed linear model for resting DBP in adults SHR. **A** QQ-Plot is used to analyze the normality of residuals, and **B** scatter plots residuals-predicted values to assess heteroskedasticity.

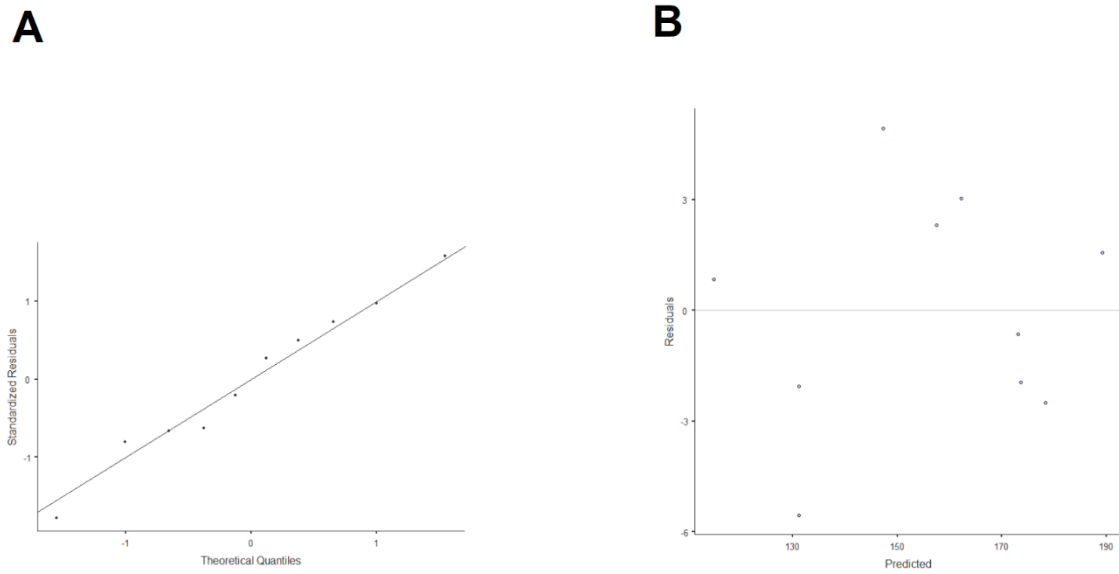


Figure S31: Residual analysis of the chosen mixed linear model for resting MBP in adults SHR. **A** QQ-Plot is used to analyze the normality of residuals, and **B** scatter plots residuals-predicted values to assess heteroskedasticity.

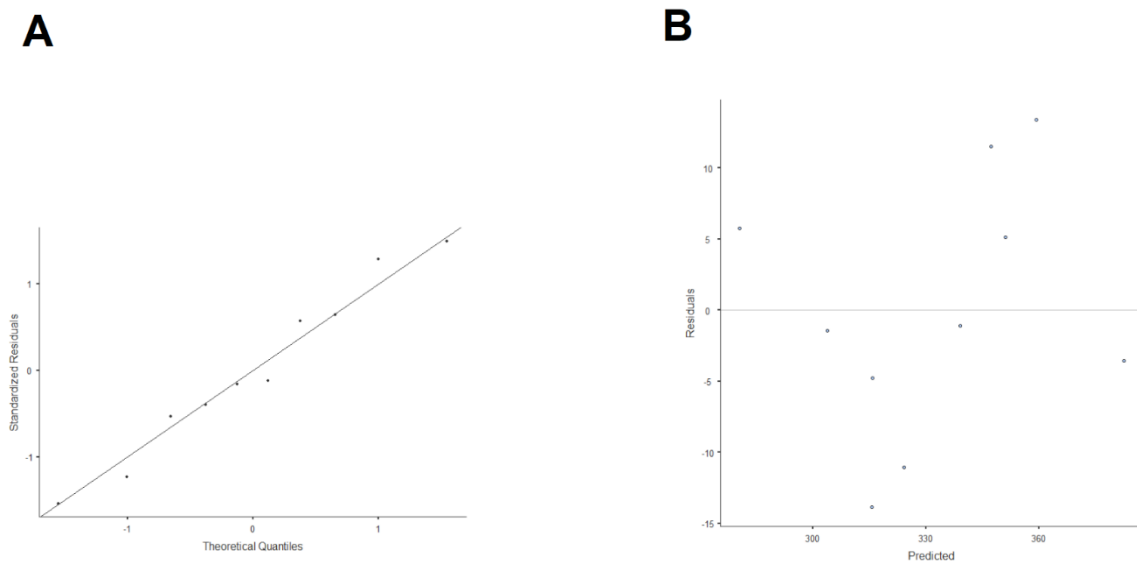


Figure S32: Residual analysis of the chosen mixed linear model for resting HR in adults SHR. **A** QQ-Plot is used to analyze the normality of residuals, and **B** scatter plots residuals-predicted values to assess heteroskedasticity.

Table S29: Parameter Estimates (Fixed coefficients) resting VT SHR

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	1.566	0.468	0.420	2.712	4.00	3.343	0.029
Time1	PLP - Before	-0.177	0.221	-0.717	0.363	4.00	-0.803	0.467

Random Components

Groups	Name	Variance	SD	ICC
ID	(Intercept)	1.036	1.018	0.895
Residual		0.122	0.349	

Note. Number of Obs: 10 , Number of groups: ID 5

Table S30: Parameter Estimates (Fixed coefficients) resting VE SHR

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	156.2	47.8	39.1	273.2	4.00	3.26	0.031
Time1	PLP - Before	-38.8	35.5	-125.7	48.2	4.00	-1.09	0.337

Random Components

Groups	Name	Variance	SD	ICC
ID	(Intercept)	9864	99.3	0.758
Residual		3155	56.2	

Note. Number of Obs: 10 , Number of groups: ID 5

Table S31: Parameter Estimates (Fixed coefficients) resting fR SHR

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	97.8	9.26	75.1	120.4	4.00	10.56	< .001
Time1	PLP - Before	-19.0	12.46	-49.5	11.5	4.00	-1.53	0.202

Random Components

Groups	Name	Variance	SD	ICC
ID	(Intercept)	234	15.3	0.376
Residual		388	19.7	

Note. Number of Obs: 10 , Number of groups: ID 5

Table S32: Parameter Estimates (Fixed coefficients) resting Ti SHR

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	0.3119	0.0159	0.2730	0.3508	4.00	19.63	< .001
Time1	PLP - Before	0.0250	0.0193	-0.0221	0.0722	4.00	1.30	0.263

Random Components

Groups	Name	Variance	SD	ICC
ID	(Intercept)	7.99e-4	0.0283	0.463
Residual		9.27e-4	0.0304	

Note. Number of Obs: 10 , Number of groups: ID 5

Table S33: Parameter Estimates (Fixed coefficients) resting VT/Ti SHR

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	4.867	1.397	1.45	8.284	4.00	3.48	0.025
Time1	PLP - Before	-0.908	0.681	-2.57	0.758	4.00	-1.33	0.253

Random Components

Groups	Name	Variance	SD	ICC
ID	(Intercept)	9.17	3.03	0.888
	Residual	1.16	1.08	

Note. Number of Obs: 10 , Number of groups: ID 5

Table S34: Parameter Estimates (Fixed coefficients) resting VO2 SHR

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	30.80	2.97	23.52	38.08	4.00	10.354	< .001
Time1	PLP - Before	-2.15	2.83	-9.07	4.78	4.00	-0.759	0.490

Random Components

Groups	Name	Variance	SD	ICC
ID	(Intercept)	34.2	5.85	0.631
Residual		20.0	4.48	

Note. Number of Obs: 10 , Number of groups: ID 5

Table S35: Parameter Estimates (Fixed coefficients) resting VCO2 SHR

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	31.02	1.90	26.4	35.68	4.00	16.29	< .001
Time1	PLP - Before	-8.53	2.27	-14.1	-2.98	4.00	-3.76	0.020

Random Components

Groups	Name	Variance	SD	ICC
ID	(Intercept)	11.7	3.42	0.475
Residual		12.9	3.59	

Note. Number of Obs: 10 , Number of groups: ID 5

Table S36: Parameter Estimates (Fixed coefficients) resting RER SHR

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	1.026	0.0683	0.859	1.1927	4.00	15.02	< .001
Time1	PLP - Before	-0.218	0.0810	-0.416	-0.0201	4.00	-2.69	0.054

Random Components

Groups	Name	Variance	SD	ICC
ID	(Intercept)	0.0151	0.123	0.480
	Residual	0.0164	0.128	

Note. Number of Obs: 10 , Number of groups: ID 5

Table S37: Parameter Estimates (Fixed coefficients) resting VE/VCO2 SHR

Names	Effect	Estimate	SE	95% Confidence Interval		z	p
				Lower	Upper		
(Intercept)	(Intercept)	5.653	1.278	3.149	8.157	4.42	< .001
Time1	PLP - Before	-0.375	0.308	-0.979	0.229	-1.22	0.223

Random Components

Groups	Name	SD	Variance	ICC
ID	(Intercept)	2.073	4.297	
Residual		0.447	0.200	
Residuals		0.447	0.200	.

Note. Number of Obs: 10 , groups: ID 5

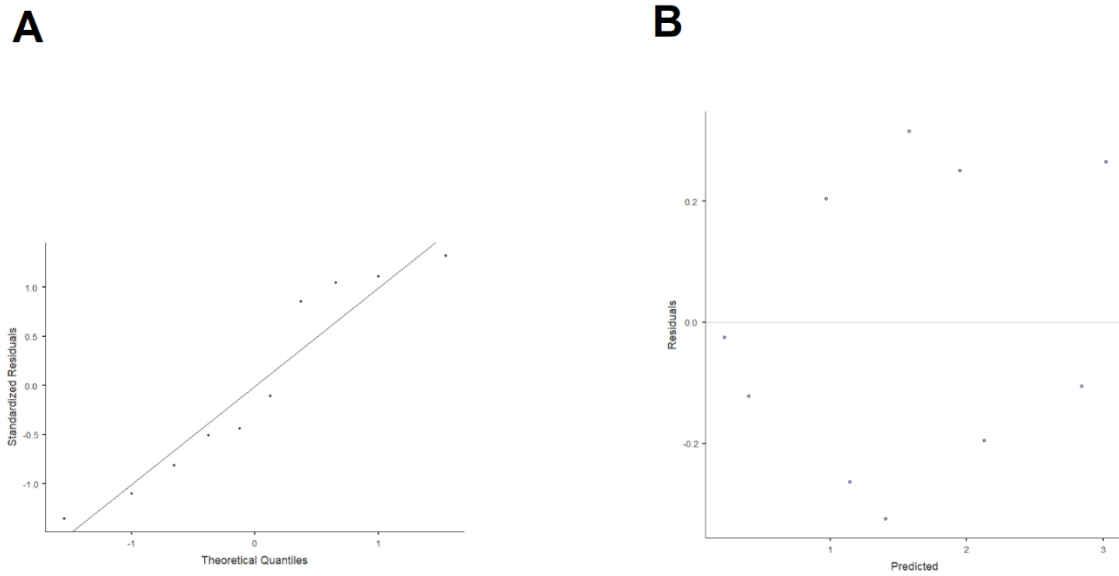


Figure S33: Residual analysis of the chosen mixed linear model for resting V_T in adults SHR. **A** QQ-Plot is used to analyze the normality of residuals, and **B** scatter plots residuals-predicted values to assess heteroskedasticity.

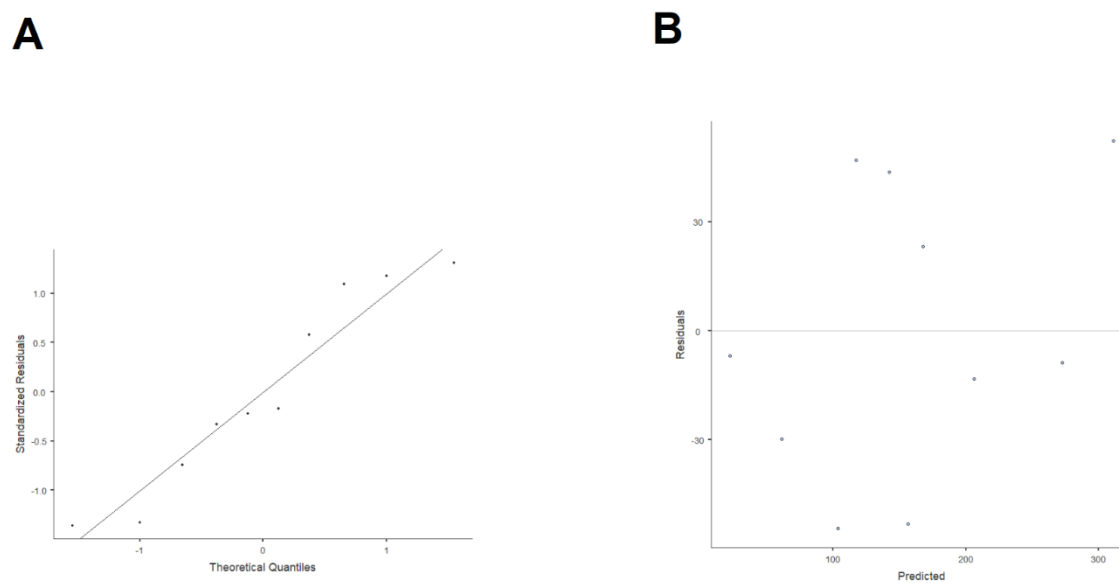


Figure S34: Residual analysis of the chosen mixed linear model for resting V_E in adults SHR. **A** QQ-Plot is used to analyze the normality of residuals, and **B** scatter plots residuals-predicted values to assess heteroskedasticity.

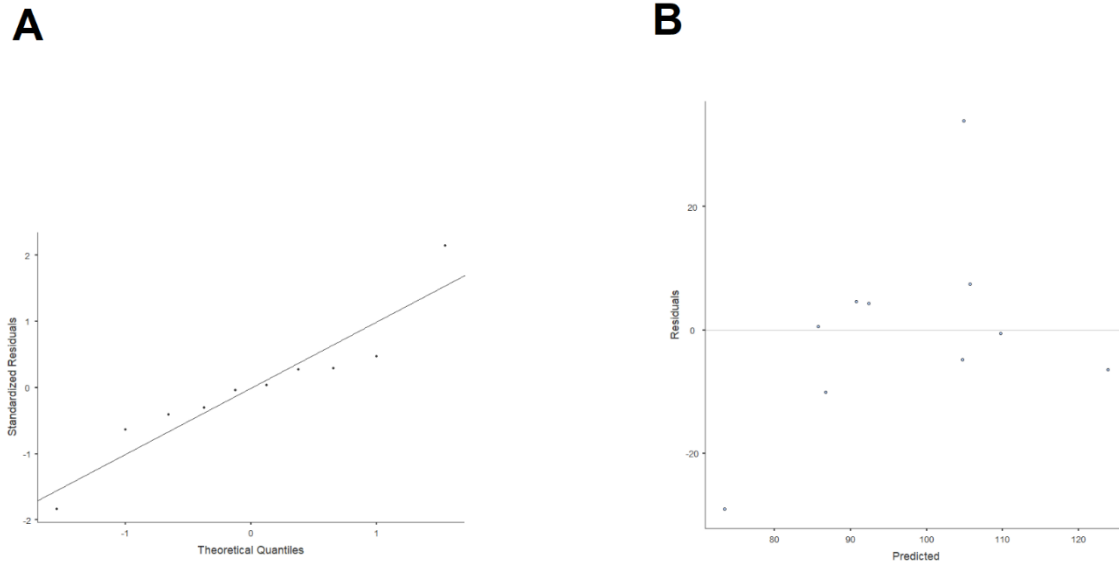


Figure S35: Residual analysis of the chosen mixed linear model for resting f_R in adults SHR. **A** QQ-Plot is used to analyze the normality of residuals, and **B** scatter plots residuals-predicted values to assess heteroskedasticity.

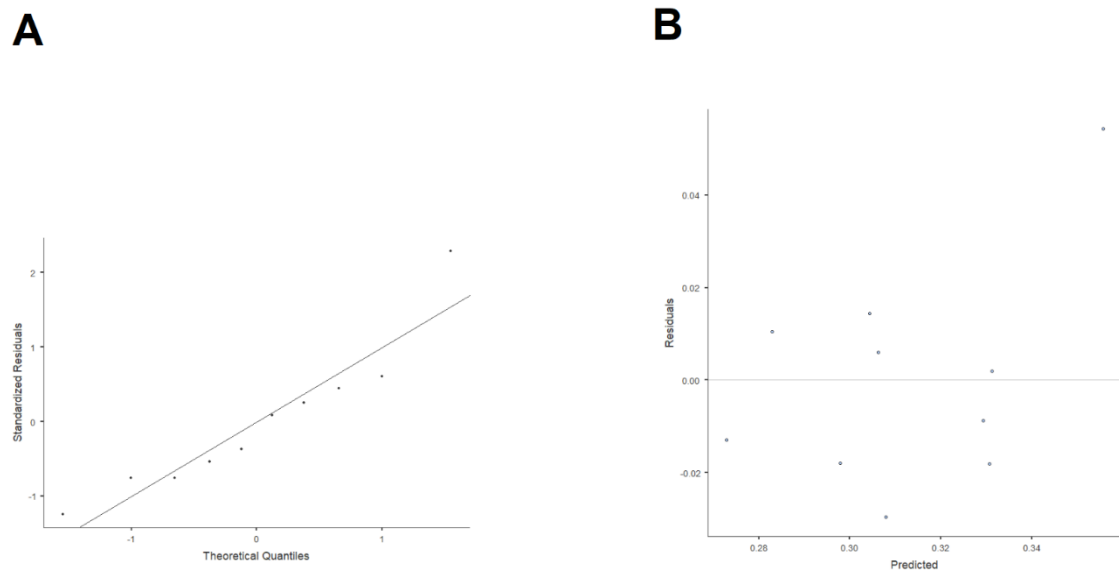
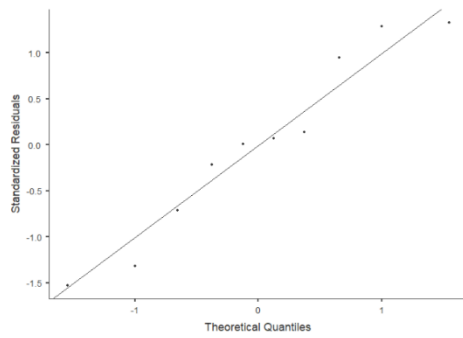


Figure S36: Residual analysis of the chosen mixed linear model for resting T_i in adults SHR. **A** QQ-Plot is used to analyze the normality of residuals, and **B** scatter plots residuals-predicted values to assess heteroskedasticity.

A



B

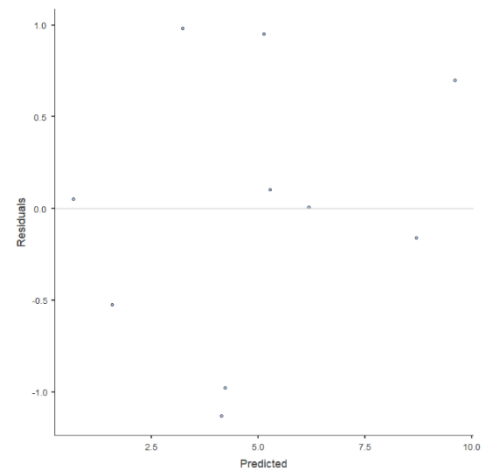
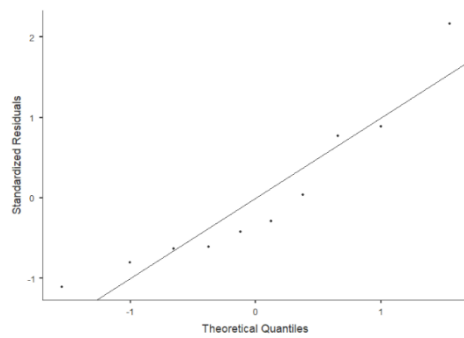


Figure S37: Residual analysis of the chosen mixed linear model for resting V_T/T_i in adults SHR. **A** QQ-Plot is used to analyze the normality of residuals, and **B** scatter plots residuals-predicted values to assess heteroskedasticity.

A



B

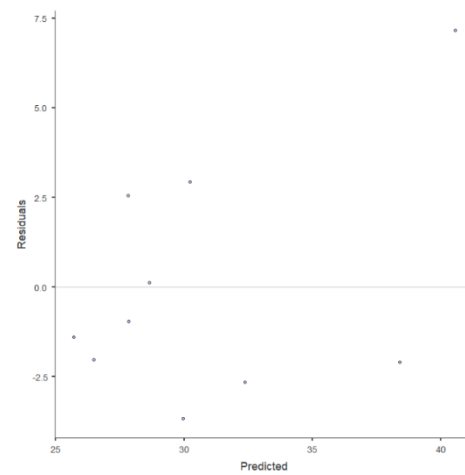
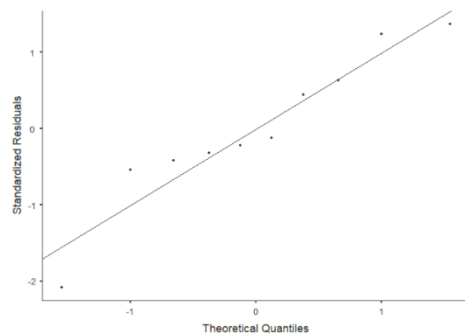


Figure S38: Residual analysis of the chosen mixed linear model for resting VO_2 in adults SHR. **A** QQ-Plot is used to analyze the normality of residuals, and **B** scatter plots residuals-predicted values to assess heteroskedasticity.

A



B

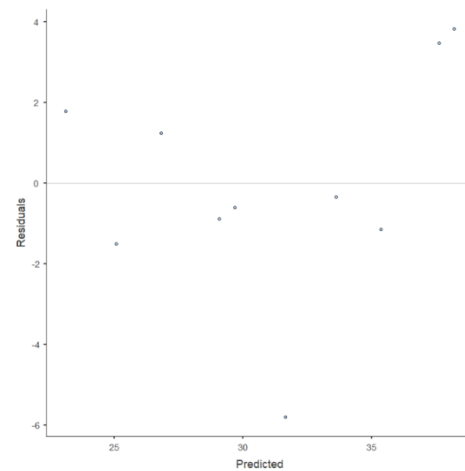
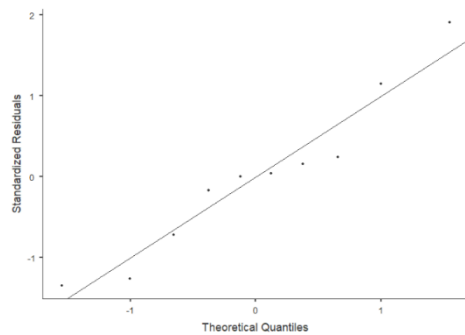


Figure S39: Residual analysis of the chosen mixed linear model for resting VCO_2 in adults SHR. **A** QQ-Plot is used to analyze the normality of residuals, and **B** scatter plots residuals-predicted values to assess heteroskedasticity.

A



B

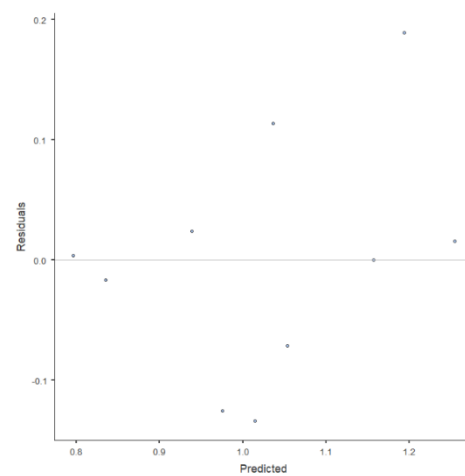


Figure S40: Residual analysis of the chosen mixed linear model for resting R in adults SHR. **A** QQ-Plot is used to analyze the normality of residuals, and **B** scatter plots residuals-predicted values to assess heteroskedasticity.

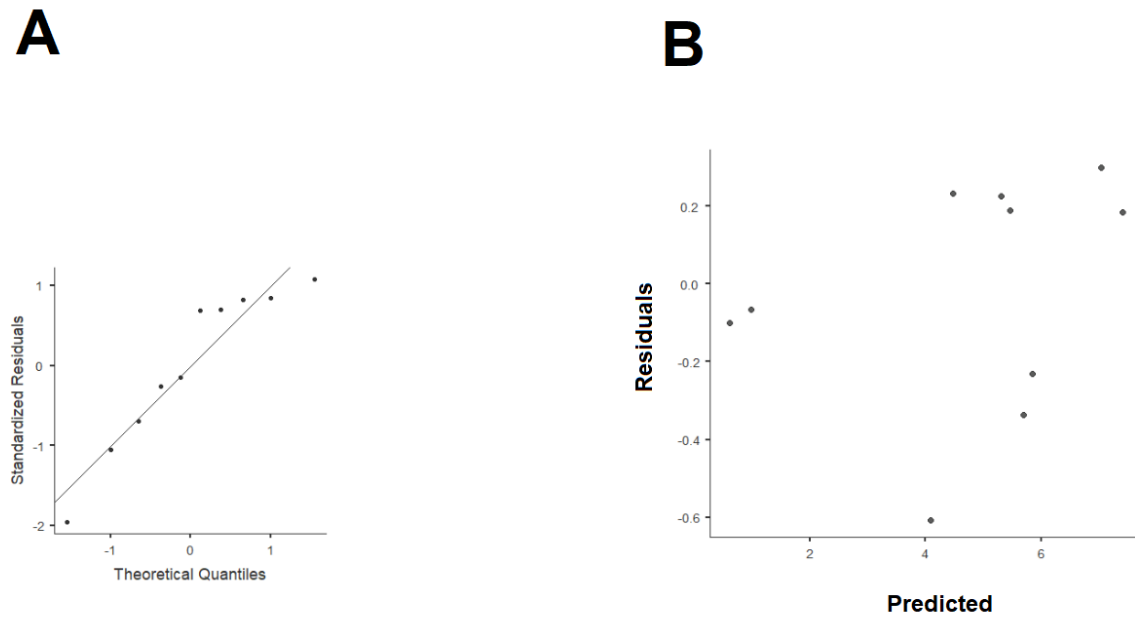


Figure S41: Residual analysis of the chosen mixed linear model for resting V_E/V_{CO_2} in adults SHR. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

Table S38: Parameter Estimates (Fixed coefficients) resting SBP Wistar

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	130.39	4.91	118.39	142.401	4.00	26.57	< .001
Time1	PLP - Before	-4.64	1.57	-8.49	-0.792	4.00	-2.95	0.042

Random Components

Groups	Name	Variance	SD	ICC
ID	(Intercept)	117.30	10.83	0.950
Residual		6.19	2.49	

Note. Number of Obs: 10 , Number of groups: ID 5

Table S39: Parameter Estimates (Fixed coefficients) resting DBP Wistar

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	92.39	4.48	81.43	103.356	4.00	20.62	< .001
Time1	PLP - Before	-3.57	1.12	-6.31	-0.827	4.00	-3.19	0.033

Random Components

Groups	Name	Variance	SD	ICC
ID	(Intercept)	98.84	9.94	0.969
Residual		3.14	1.77	

Note. Number of Obs: 10 , Number of groups: ID 5

Table S40: Parameter Estimates (Fixed coefficients) resting MBP Wistar

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	104.89	4.25	94.48	115.294	4.00	24.66	< .001
Time1	PLP - Before	-4.03	1.28	-7.17	-0.892	4.00	-3.14	0.035

Random Components

Groups	Name	Variance	SD	ICC
ID	(Intercept)	88.36	9.40	0.955
Residual		4.12	2.03	

Note. Number of Obs: 10 , Number of groups: ID 5

Table S41: Parameter Estimates (Fixed coefficients) resting HR Wistar

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	344.9	11.0	318.0	371.89	4.00	31.32	< .001
Time1	PLP - Before	-17.6	11.0	-44.4	9.20	4.00	-1.61	0.183

Random Components

Groups	Name	Variance	SD	ICC
ID	(Intercept)	457	21.4	0.603
Residual		300	17.3	

Note. Number of Obs: 10 , Number of groups: ID 5

Table S42: Parameter Estimates (Fixed coefficients) resting VT Wistar

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	1.714	0.234	1.143	2.286	4.00	7.34	0.002
Time1	PLP - Before	-0.231	0.142	-0.579	0.118	4.00	-1.62	0.180

Random Components

Groups	Name	Variance	SD	ICC
ID	(Intercept)	0.2473	0.497	0.830
Residual		0.0507	0.225	

Note. Number of Obs: 10 , Number of groups: ID 5

Table S43: Parameter Estimates (Fixed coefficients) resting VE Wistar

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	175.3	22.3	121	230.0	4.00	7.851	0.001
Time1	PLP - Before	-29.3	35.8	-117	58.4	4.00	-0.818	0.459

Random Components

Groups	Name	Variance	SD	ICC
ID	(Intercept)	890	29.8	0.217
Residual		3208	56.6	

Note. Number of Obs: 10 , Number of groups: ID 5

Table S44: Parameter Estimates (Fixed coefficients) resting fR Wistar

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	103.0	8.48	82.2	123.7	8.00	12.141	< .001
Time1	PLP - Before	-10.1	16.96	-51.6	31.4	8.00	-0.596	0.568

Random Components

Groups	Name	Variance	SD	ICC
ID	(Intercept)	0	0.0	0.00
Residual		719	26.8	

Note. Number of Obs: 10 , Number of groups: ID 5

Table S45: Parameter Estimates (Fixed coefficients) resting Ti Wistar

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	0.2714	0.0147	0.2354	0.3074	4.00	18.456	< .001
Time1	PLP - Before	0.0130	0.0205	-0.0370	0.0631	4.00	0.638	0.558

Random Components

Groups	Name	Variance	SD	ICC
ID	(Intercept)	5.58e-4	0.0236	0.348
Residual		0.00105	0.0323	

Note. Number of Obs: 10 , Number of groups: ID 5

Table S46: Parameter Estimates (Fixed coefficients) resting RER Wistar

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	1.3262	0.0127	1.291	1.3616	3.00	104.25	< .001
Time1	PLP - Before	-0.0445	0.0235	-0.110	0.0206	3.00	-1.90	0.154

Random Components

Groups	Name	Variance	SD	ICC
ID	(Intercept)	9.67e-5	0.00983	0.0807
Residual		0.00110	0.03319	

Note. Number of Obs: 8 , Number of groups: ID 4

Table S47: Parameter Estimates (Fixed coefficients) resting VO2 Wistar

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	23.824	1.43	19.85	27.80	3.00	16.655	< .001
Time1	PLP - Before	0.604	2.80	-7.17	8.38	3.00	0.216	0.843

Random Components

Groups	Name	Variance	SD	ICC
ID	(Intercept)	0.346	0.588	0.0216
Residual		15.677	3.959	

Note. Number of Obs: 8 , Number of groups: ID 4

Table S48: Parameter Estimates (Fixed coefficients) resting VCO2 Wistar

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	31.609	1.91	26.3	36.9	6.00	16.5547	< .001
Time1	PLP - Before	-0.241	3.82	-10.8	10.4	6.00	-0.0631	0.952

Random Components

Groups	Name	Variance	SD	ICC
ID	(Intercept)	1.23e-13	3.51e-7	4.22e-15
Residual		29.2	5.40	

Note. Number of Obs: 8 , Number of groups: ID 4

Table S49: Parameter Estimates (Fixed coefficients) resting VE/VCO2 Wistar

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	5.07	0.668	3.22	6.93	6.00	7.59	< .001
Time1	PLP - Before	-1.41	1.336	-5.12	2.30	6.00	-1.06	0.331

Random Components

Groups	Name	Variance	SD	ICC
ID	(Intercept)	0.00	0.00	0.00
	Residual	3.57	1.89	

Note. Number of Obs: 8 , Number of groups: ID 4

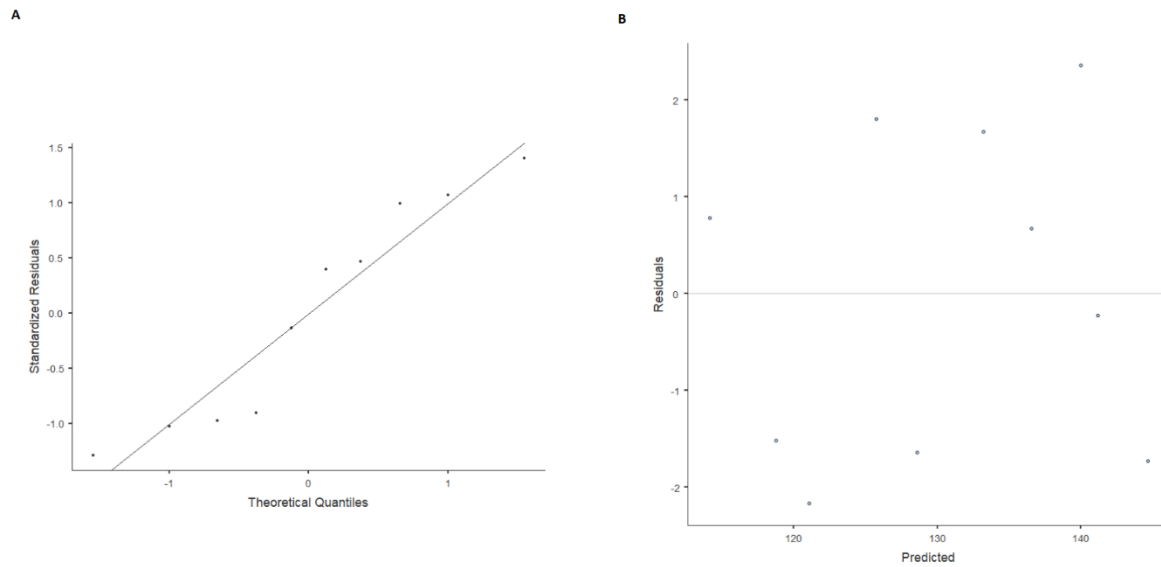


Figure S42: Residual analysis of the chosen mixed linear model for resting SBP in adult Wistar rats. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

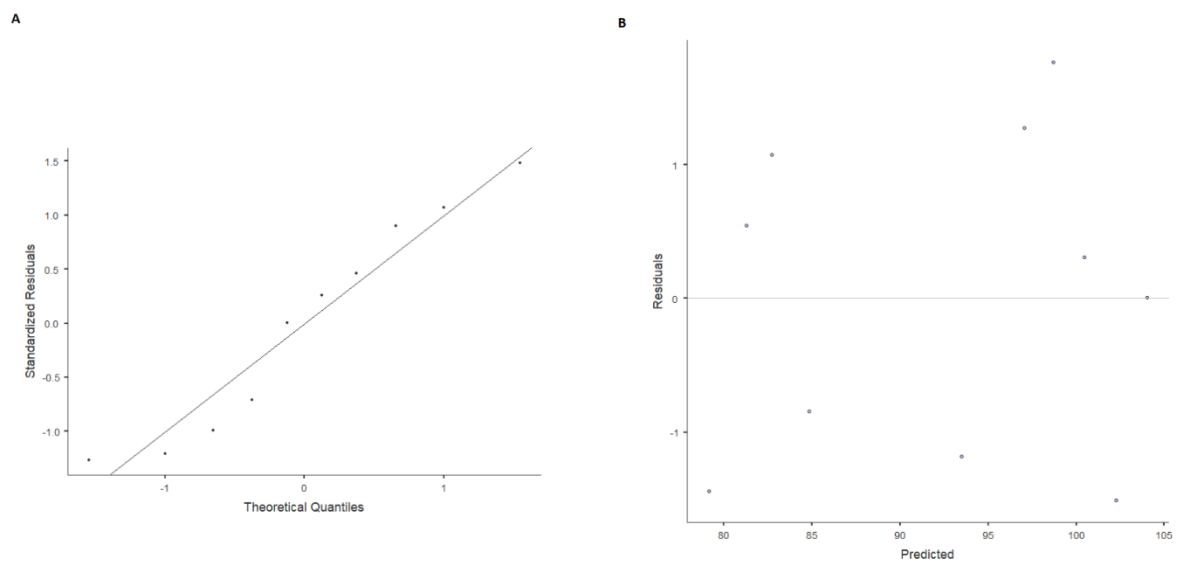


Figure S43: Residual analysis of the chosen mixed linear model for resting DBP in adult Wistar rats. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

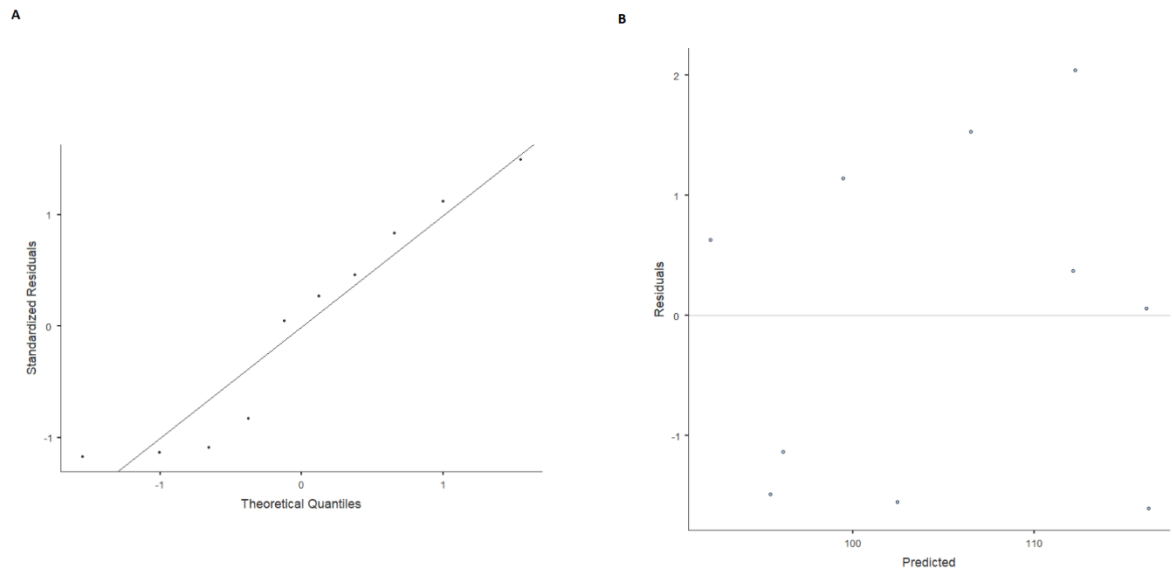


Figure S44: Residual analysis of the chosen mixed linear model for resting MBP in adult Wistar rats. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

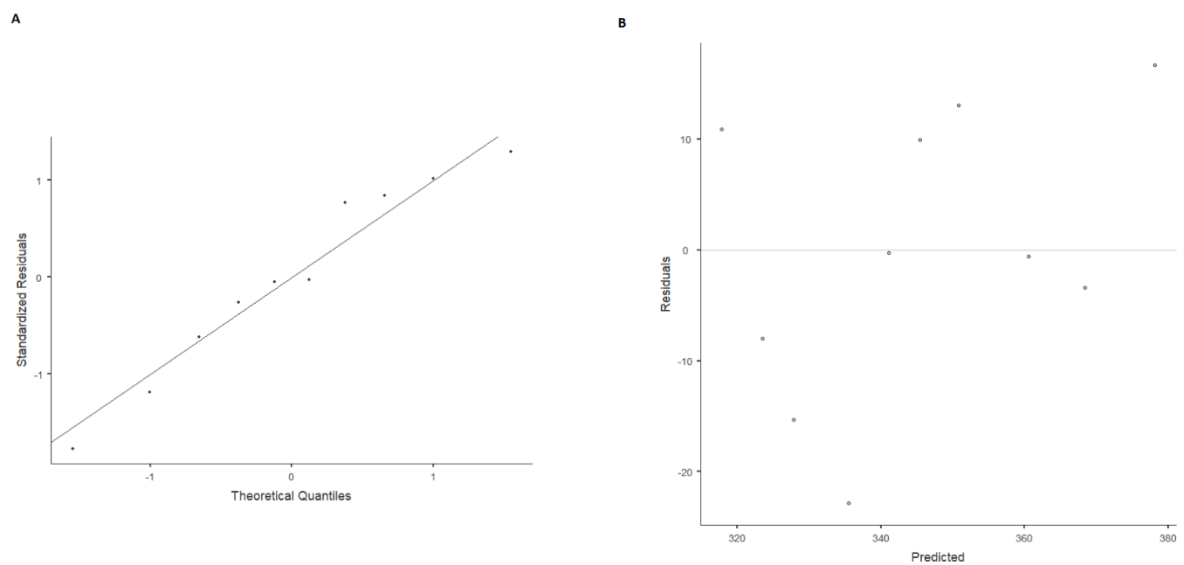


Figure S45: Residual analysis of the chosen mixed linear model for resting HR in adult Wistar rats. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

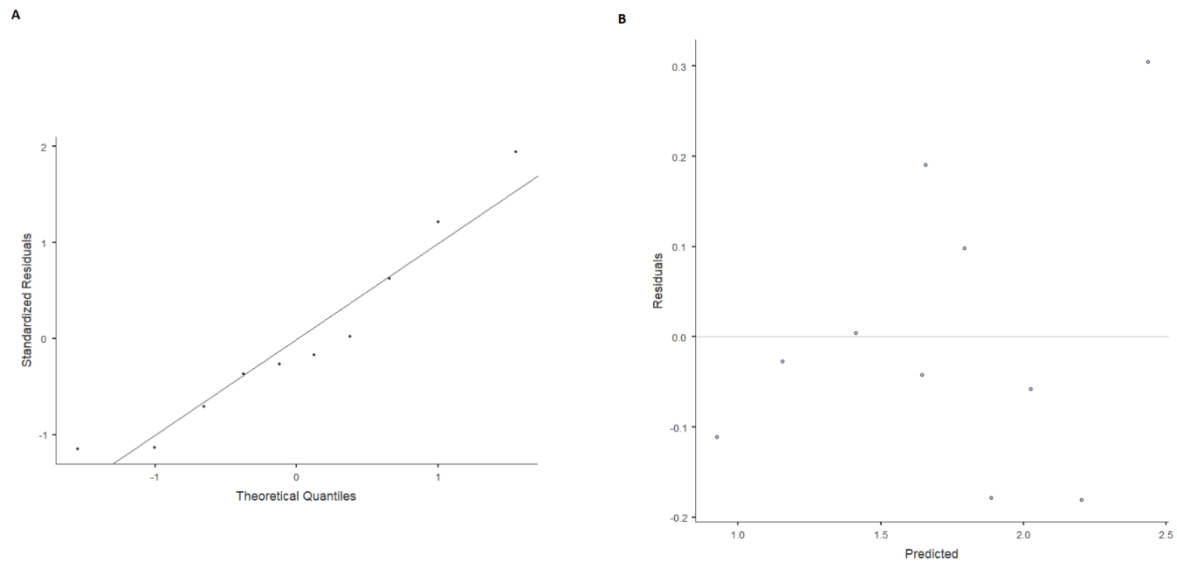


Figure S46: Residual analysis of the chosen mixed linear model for resting VT in adult Wistar rats. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

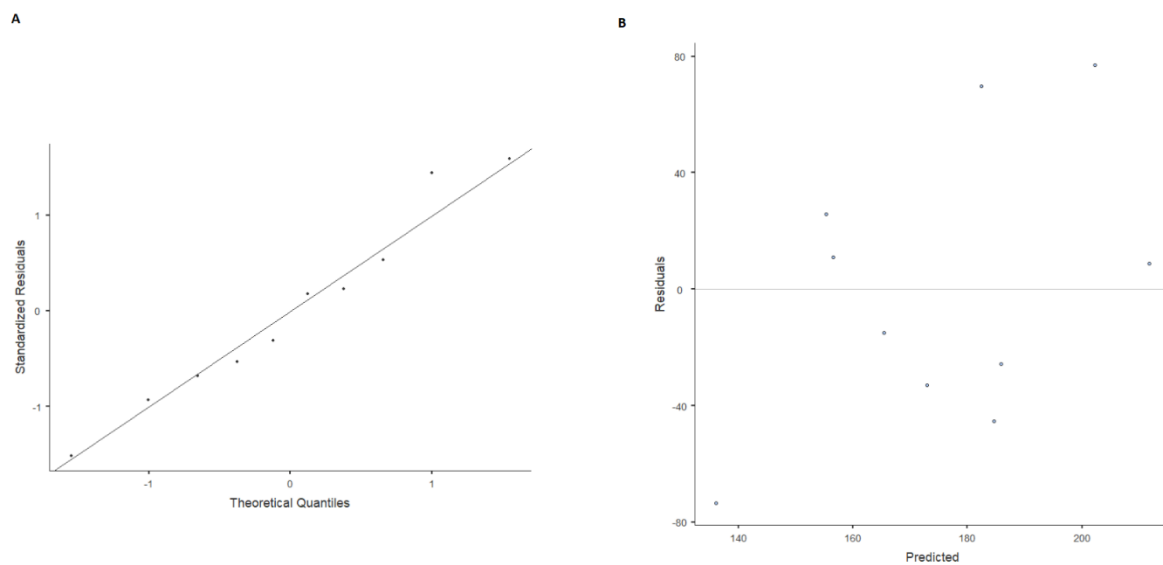


Figure S47: Residual analysis of the chosen mixed linear model for resting VE in adult Wistar rats. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

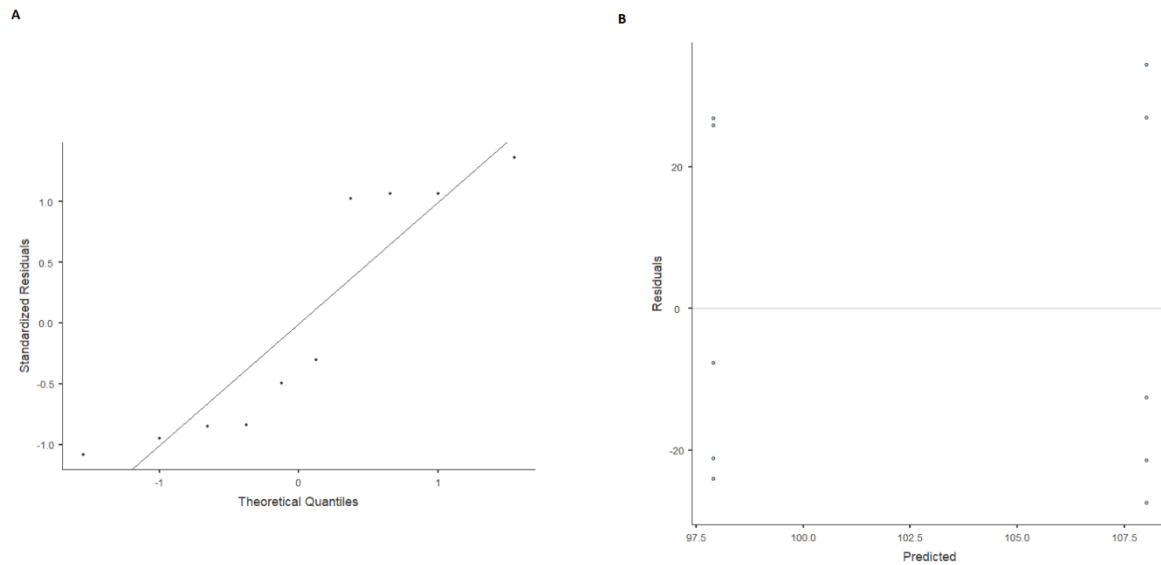


Figure S48: Residual analysis of the chosen mixed linear model for resting fR in adult Wistar rats. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

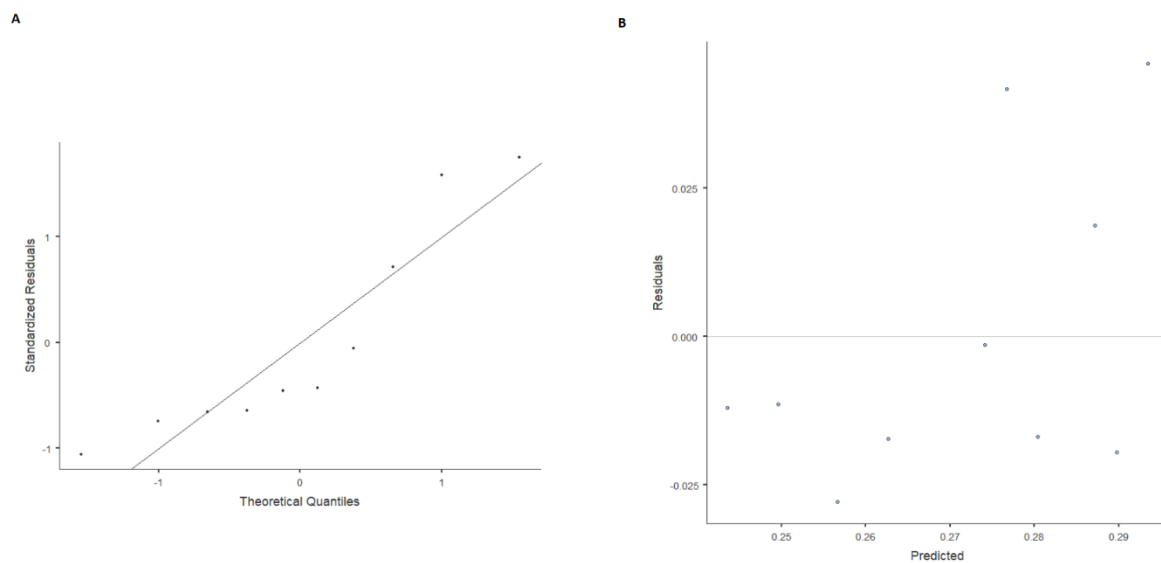


Figure S49: Residual analysis of the chosen mixed linear model for resting Ti in adult Wistar rats. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

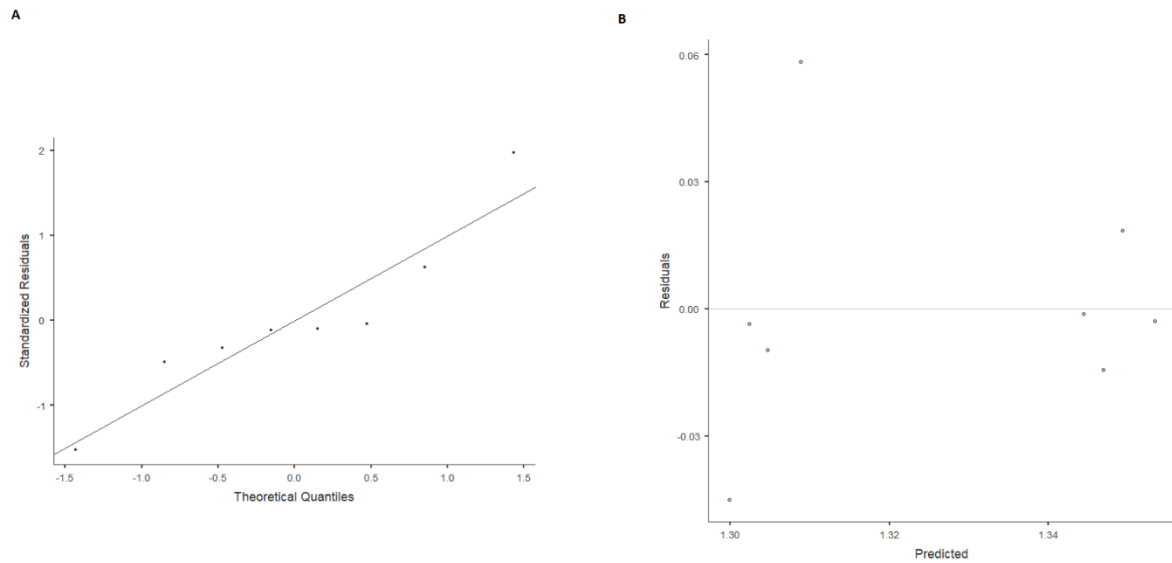


Figure S50: Residual analysis of the chosen mixed linear model for resting RER in adult Wistar rats. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

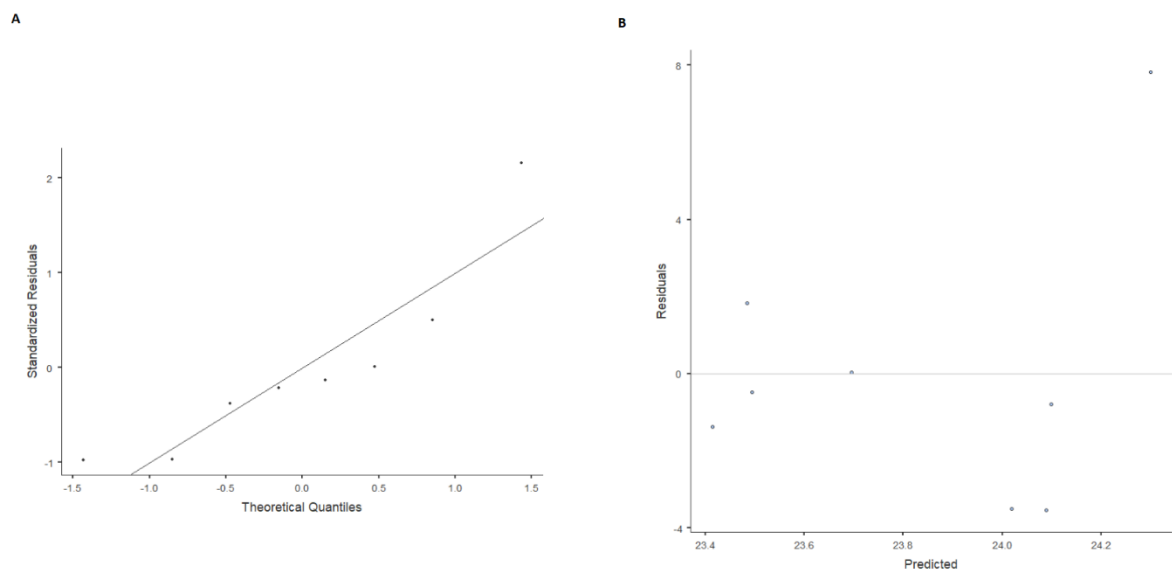


Figure S51: Residual analysis of the chosen mixed linear model for resting VO2 in adult Wistar rats. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

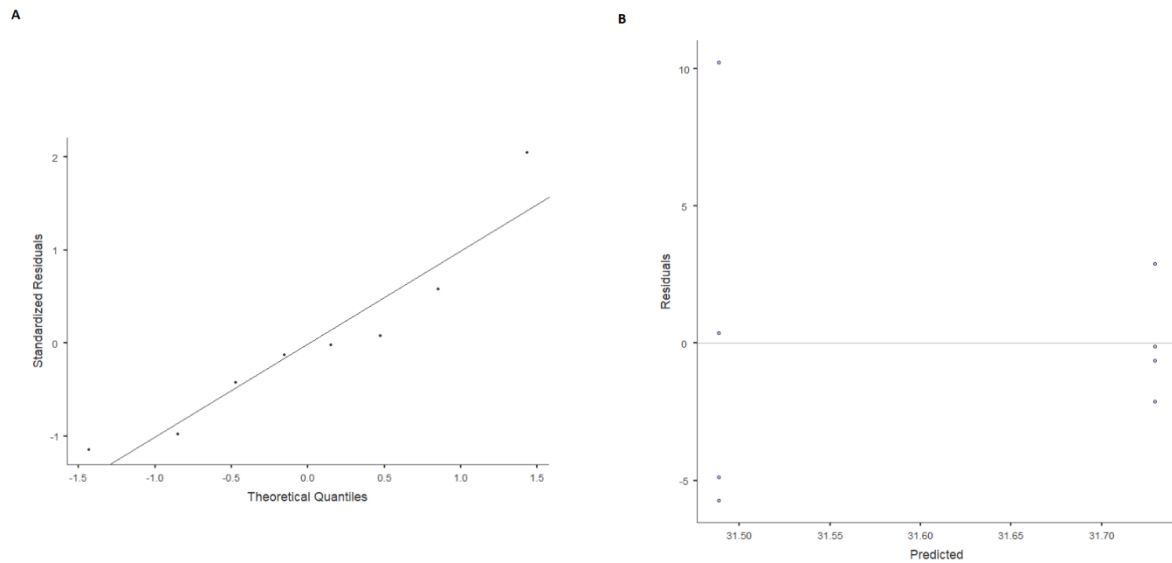


Figure S52: Residual analysis of the chosen mixed linear model for resting VCO₂ in adult Wistar rats. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

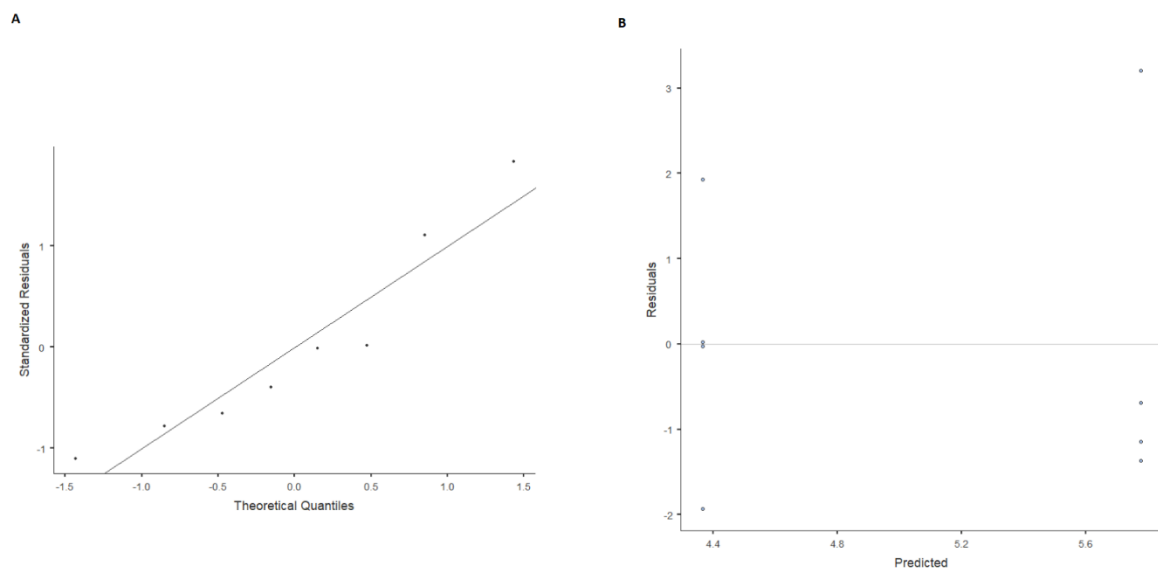


Figure S53: Residual analysis of the chosen mixed linear model for resting VE/VCO₂ in adult Wistar rats. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

Table S50: Parameter Estimates (Fixed coefficients) in human Chemoreflex index

		Estimate	SE	95% Confidence Intervals		df	t	p
Names	Effect			Lower	Upper			
(Intercept)	(Intercept)	-0.502	0.0692	-0.6531	-0.352	12.0	-7.26	< .001
Treatment1	PHC - Placebo	0.186	0.0930	-0.0168	0.389	12.0	2.00	0.069
level of sensitisation1	Sensitized - Normal	-0.423	0.1384	-0.7243	-0.121	12.0	-3.05	0.010
Treatment1 * level of sensitisation1	(PHC - Placebo) * (Sensitized - Normal)	0.496	0.1861	0.0906	0.901	12.0	2.67	0.021

Random Components

Groups	Name	Variance	SD	ICC
Patient ID	(Intercept)	0.0370	0.192	0.380
Residual		0.0604	0.246	

Note. Number of Obs: 28 , Number of groups: Patient ID 14

Table S51: Parameter Estimates (Fixed coefficients) in human Chemoreflex-evoked Δ SBP

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	10.31	3.62	2.42	18.2	12.0	2.847	0.015
Treatment1	PHC - Placebo	-5.81	7.96	-23.34	11.7	11.0	-0.730	0.481
level of sensitisation1	Sensitized - Normal	13.90	7.24	-1.88	29.7	12.0	1.920	0.079
Treatment1 * level of sensitisation1	(PHC - Placebo) * (Sensitized - Normal)	6.95	15.93	-28.11	42.0	11.0	0.436	0.671

Random Components

Groups	Name	Variance	SD	ICC
Patient ID	(Intercept)	35.2	5.93	0.0904
Residual		354.3	18.82	

Note. Number of Obs: 27 , Number of groups: Patient ID 14

Table S52: Parameter Estimates (Fixed coefficients) in human Chemoreflex-evoked ΔDBP

Names	Effect	Estimate	SE	95% Confidence Intervals		df	t	p
				Lower	Upper			
(Intercept)	(Intercept)	4.173	1.33	1.27	7.08	12.0	3.129	0.009
Treatment1	PHC - Placebo	-0.511	3.30	-7.77	6.74	11.0	-0.155	0.880
level of sensitisation1	Sensitized - Normal	6.918	2.67	1.11	12.73	12.0	2.594	0.023
Treatment1 * level of sensitisation1	(PHC - Placebo) * (Sensitized - Normal)	6.979	6.59	-7.53	21.49	11.0	1.059	0.312

Random Components

Groups	Name	Variance	SD	ICC
Patient ID	(Intercept)	4.08	2.02	0.0674
Residual		56.45	7.51	

Note. Number of Obs: 27 , Number of groups: Patient ID 14

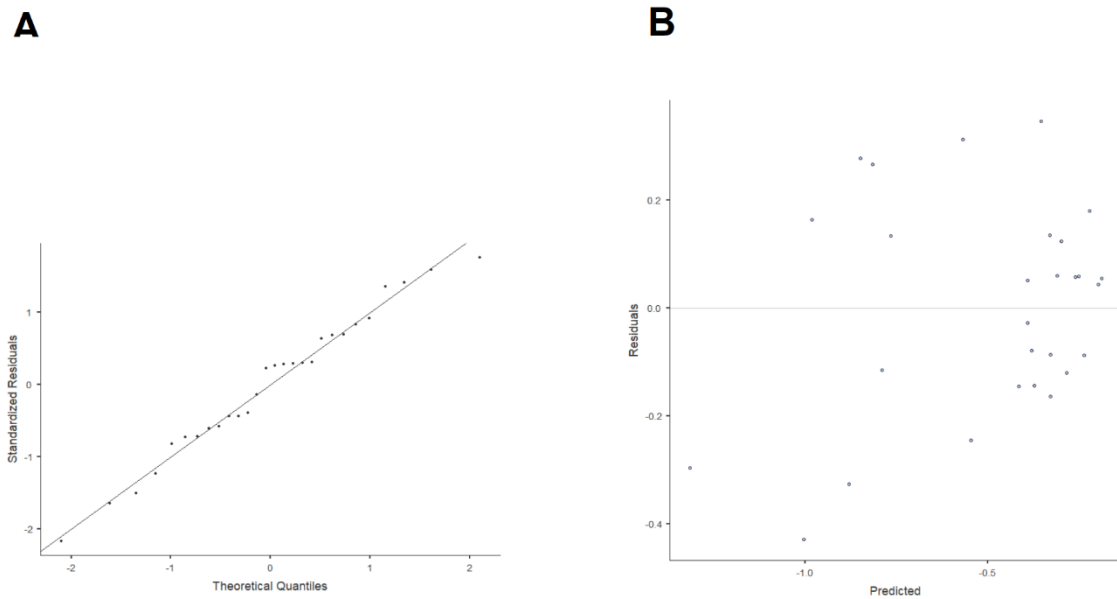


Figure S42: Residual analysis of the chosen mixed linear model for peripheral chemoreflex index in patients with hypertension. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

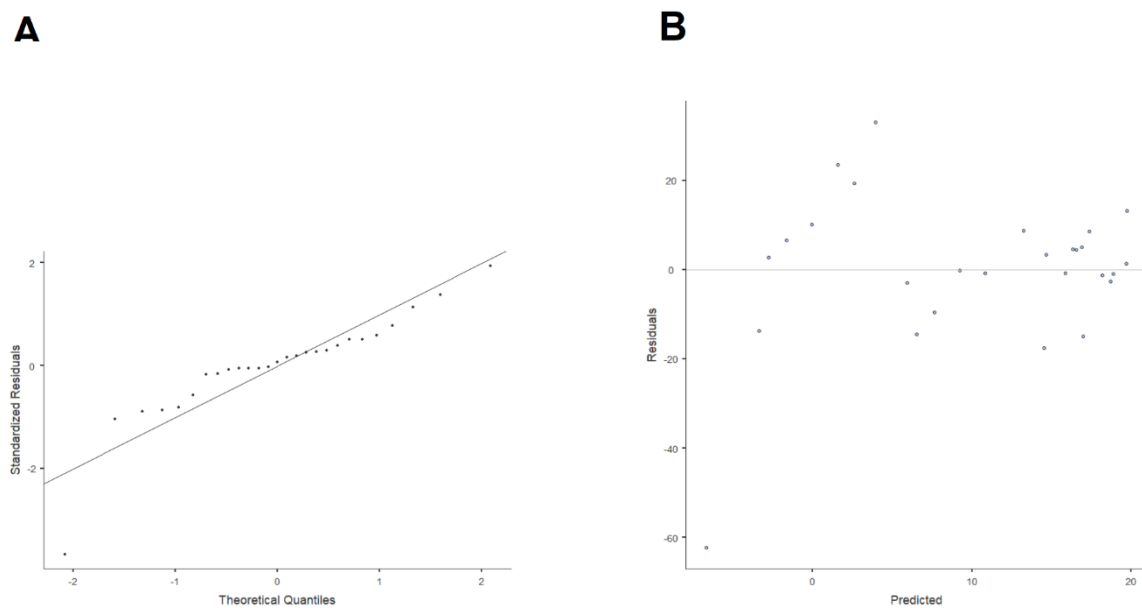


Figure S43: Residual analysis of the chosen mixed linear model for peripheral chemoreflex evoked Δ SBP in patients with hypertension. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.

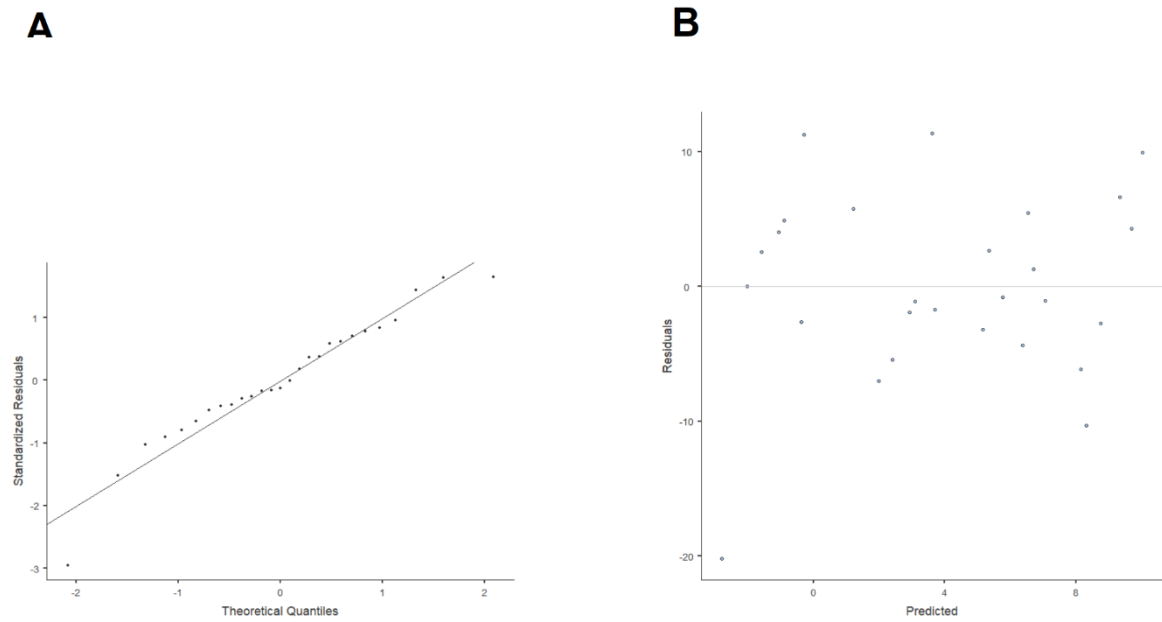


Figure S44: Residual analysis of the chosen mixed linear model for peripheral chemoreflex evoked Δ DBP in patients with hypertension. A QQ-Plot is used to analyze the normality of residuals, and B scatter plots residuals-predicted values to assess heteroskedasticity.