

## **Appendix 1: Written instructions for breathing exercises**

### **1. Exercise with prolonged retention of breath**

#### **Preparation:**

1. Sit down comfortably in a place where you are not disturbed.
2. It is permitted to drink water, coffee, or tea, but do not add sugar or milk.

#### **Exercise:**

1. Breathe in and out approximately 30 times. Do not force anything. Breathe preferably in through the nose and out through the mouth. Use your abdomen, diaphragm and chest. Try to find a rhythm, bring your attention to breathing in-and-out, in-and-out, as if you are inflating a balloon. During this phase you can start to feel tingling in the neck, finger, legs, etc. You have to end with a deep exhalation (empty lungs) and holding of your breath.
2. Breathe deeply in and out once and hold your breath after exhalation. Relax, do not force anything. Hold your breath until you feel the urge to breathe again, do not force anything.
3. Finally, take a deep breath and hold it for 10 seconds. Do not force anything. Now your lungs are filled with oxygen. Close your eyes and then breathe out.

*This is the end of the first cycle; now start immediately with a new cycle (point 1). Do not force anything. Perform a total of 3 or 4 cycles, whatever you feel comfortable with.*

### **2. Breathing exercise without retention of breath**

#### **Preparation:**

1. Sit down comfortably in a place where you are not disturbed.
2. It is permitted to drink water, coffee, or tea, but do not add sugar or milk.

#### **Exercise:**

1. Breathe in and out approximately 30 times. Do not force anything. Breathe preferably in through the nose and out through the mouth. Use your abdomen, diaphragm and chest. Try to find a rhythm, bring your attention to breathing in-and-out, in-and-out, as if you are inflating a balloon. During this phase you can start to feel tingling in the neck, finger, legs, etc.
2. Exhale and inhale vigorously once, and firmly tighten all your muscles for approximately 10 seconds.

*This is the end of the first cycle; now start immediately with a new cycle (point 1). Perform a total of 3 or 4 cycles, whatever you feel comfortable with.*

**Supplemental Table S1.** Accompanying statistics to Figure 3: Arterial blood gas parameters and plasma epinephrine levels during the breathing exercises study: influence of breathing exercise.

	<b>Panel A.</b> Oxygen saturation	<b>Panel B.</b> pO <sub>2</sub>	<b>Panel C.</b> pH	<b>Panel D.</b> CO <sub>2</sub>	<b>Panel E.</b> Epinephrine
Time x Column factor	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>0.0002</b>	<b>0.003</b>
<b>F(DFn,DFd)</b>	F(6,224) =31.50	F(6,225) =18.43	F(6,225) =7.96	F(6,225) =4.67	F(4,152) =4.19
<b>Time</b>					
0	>0.9999	0.9909	>0.9999	>0.9999	0.9805
0.5 EH	>0.9999	0.235	0.9979	0.9552	>0.9999
0.5 ER	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	0.228	0.774	
1.0 EH	>0.9999	0.2545	0.4551	0.7556	0.652
1.0 ER	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	0.0584	0.3722	
1.5 EH	0.9983	0.5801	0.1328	0.9225	<b>0.0006</b>
1.5 ER	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>0.0113</b>	<b>0.0057</b>	
3.0					0.9983

Reference group: without retention (-retention).

EH - end of hyperventilation. ER - end of retention of breath.

p-values of Time x Column factor was calculated using linear mixed models analysis.

p-values of timepoints represent adjusted p-values calculated using Sidak's multiple comparison tests.

**Supplemental Table S2.** Accompanying statistics to Figure 4: Arterial blood gas parameters and plasma epinephrine levels during human endotoxemia..

Group	Panel A. pH		Panel B. Oxygen saturation		Panel C. pCO <sub>2</sub>		Panel D. Epinephrine	
	BRT	CBR	BRT	CBR	BRT	CBR	BRT	CBR
Time x Column factor	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>0.0005</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>0.0378</b>	<b>0.0009</b>
<b>F(DFn, DFd)</b>	F(3,88) =27.80	F(3,66) =82.13	F(3,88) =12.67	F(3,88) =6.49	F(3,66) =95.23	F(3,66) =91.04	F(8,160) =2.11	F(8,160) =3.51
<b>Time</b>								
-1	0.6763	0.98	0.9281	0.3802	0.8801	0.6244	0.7062	0.6356
-0.25							0.5793	0.3439
0	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	>0.9999	0.9998
0.5							0.7874	0.6968
1							<b>0.0346</b>	<b>0.0006</b>
1.5	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>0.0009</b>	<b>0.0004</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	0.8164	0.849
2.5							0.9706	0.9983
3	0.1456	0.8799	0.5357	>0.9999	0.0933	0.1306	0.3644	0.9958
4							0.8192	>0.9999

Reference group: control (CON)

p-values of Time x Column factor was calculated using linear mixed models analysis.

p-values of timepoints represent adjusted p-values calculated using Sidak's multiple comparison tests.

CON: control group. BRT: breathing exercise group. CEX: cold exposure group. CBR: cold exposure and breathing exercise group.

**Supplemental Table S3.** Accompanying statistics to Figure 5: Cardiorespiratory parameters, tympanic temperature, and symptoms during human endotoxemia.

	<b>Panel A.</b> Heartrate		<b>Panel B.</b> MAP	<b>Panel C.</b> Temp	<b>Panel D.</b> Symptom score		
Group	BRT	CBR	BRT	BRT	BRT	CEX	CBR
Time x Column factor	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>0.0126</b>	<b>0.0112</b>	<b>&lt;0.0001</b>	<b>0.0111</b>	<b>0.0021</b>
<b>F(DFn, DFd)</b>	F(18,396)=4.78	F(18,396)=3.97	F(18,396)=1.93	F(18,396)=1.96	F(18,396)=4.47	F(18,396)=1.96	F(18,396)=2.29
<b>Time</b>							
-1	>0.9999	>0.9999	>0.9999	>0.9999	>0.9999	0.9993	>0.9999
-0.5	>0.9999	>0.9999	0.989	>0.9999	>0.9999	0.836	0.9896
0	0.0738	0.1062	>0.9999	>0.9999	0.9998	0.9566	0.9993
0.5	0.4048	0.1159	>0.9999	>0.9999	0.744	0.9907	>0.9999
1	0.4446	0.3435	0.9644	>0.9999	0.9203	0.7643	>0.9999
1.5	0.9997	0.9985	>0.9999	0.9997	>0.9999	0.3527	>0.9999
2	0.9754	0.9999	>0.9999	0.9959	0.9999	0.999	>0.9999
2.5	0.9911	>0.9999	>0.9999	0.7718	>0.9999	0.997	0.989
3	0.9998	>0.9999	0.9995	0.9973	0.9637	0.9936	0.9974
3.5	0.9823	>0.9999	>0.9999	>0.9999	0.6507	0.7147	0.238
4	0.9998	>0.9999	>0.9999	>0.9999	0.3383	0.6272	0.2923
4.5	0.9985	>0.9999	>0.9999	0.966	0.809	0.9166	0.4748
5	>0.9999	>0.9999	0.9028	0.9862	0.6643	0.8812	0.6546
5.5	>0.9999	>0.9999	0.8134	>0.9999	0.857	0.9466	0.8936
6	>0.9999	>0.9999	0.8417	>0.9999	>0.9999	0.9997	0.9546
6.5	>0.9999	>0.9999	0.9995	>0.9999	>0.9999	0.9979	0.9821
7	0.9998	0.9998	>0.9999	>0.9999	>0.9999	0.9984	0.9443
7.5	0.9998	>0.9999	>0.9999	>0.9999	>0.9999	0.9999	0.9957

8	>0.9999	>0.9999	0.4134	>0.9999	>0.9999	0.9997	0.9953
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Reference group: control (CON).

p-values of Time x Column factor was calculated using linear mixed models analysis.

p-values of timepoints represent adjusted p-values calculated using Sidak's multiple comparison tests.

CON: control group. BRT: breathing exercise group. CEX: cold exposure group. CBR: cold exposure and breathing exercise group.

**Supplemental Table S4.** Accompanying statistics to Figure 6: Plasma concentrations of inflammatory cytokines during human endotoxemia.

	<b>Panel A.</b> TNF- $\alpha$	<b>Panel B.</b> IL-6		<b>Panel C.</b> IL-8		<b>Panel D.</b> IL-10
Group	CBR	BRT	CBR	BRT	CBR	CBR
Time x Column factor	<b>0.0263</b>	<b>0.0402</b>	<b>0.0291</b>	<b>0.0161</b>	<0.0001	0.0190
<b>F(DFn, DFd)</b>	F(10,220) =2.09	F(10,220) =1.95	F(10,220) =2.06	F(10,220) =2.25	F(10,220) =3.97	F(10,220) =2.20
<b>Time</b>						
-1	0.7778	>0.9999	>0.9999	>0.9999	0.9812	>0.9999
0	0.8051	>0.9999	>0.9999	0.9491	0.8476	>0.9999
0.5	0.9999	0.9894	0.9998	0.8715	0.7003	>0.9999
1	0.9999	>0.9999	>0.9999	0.6979	0.8908	>0.9999
1.5	0.906	>0.9999	0.9986	0.7169	>0.9999	0.9254
2	0.4979	0.9747	0.7848	0.998	>0.9999	<b>0.0238</b>
2.5	0.1871	0.7648	0.4367	0.9979	0.4874	<b>0.0291</b>
3	0.4659	0.6841	0.9558	0.7793	0.1891	0.5524
4	0.313	0.8405	>0.9999	0.7155	0.4983	>0.9999
6	0.3456	0.7306	0.9998	0.9826	0.4558	>0.9999
8	0.556	0.9879	0.9431	0.9694	0.7952	>0.9999

Reference group: control (CON).

p-values of Time x Column factor was calculated using linear mixed models analysis.

p-values of timepoints represent adjusted p-values calculated using Sidak's multiple comparison tests.

CON: control group. BRT: breathing exercise group. CEX: cold exposure group. CBR: cold exposure and breathing exercise group.

**Supplemental Table S5.** Accompanying statistics to Supplemental figure 1: Arterial blood gas parameters and plasma epinephrine levels during the breathing exercises study: morning vs. afternoon session.

	<b>Panel D.</b> Epinephrine
Time x Column factor	<b>&lt;0.0001</b>
F (DFn, DFd)	F (4,312)=6.42
<b>Timepoint</b>	
0	0.9977
0.5	<b>&lt;0.0001</b>
1.0	0.2473
1.5	0.8104
3.0	0.9240

Reference group: afternoon session (afternoon).

p-values of Time x Column factor was calculated using linear mixed models analysis.

p-values of timepoints represent adjusted p-values calculated using Sidak`s multiple comparison tests.

**Supplemental Table S6.** Accompanying statistics to Supplemental figure 4: Plasma concentrations of inflammatory cytokines during human endotoxemia.

	Panel A. IP-10		Panel B. MCP1			Panel C. MIP1 $\alpha$		Panel D. MIP1 $\beta$	
Group	BRT	CBR	BRT	CEX	CBR	BRT	CBR	BRT	CBR
Time x Column factor	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0165	<0.0001
<b>F(DFn, DFd)</b>	F(10,220) =9.42	F(10,220) =7.99	F(10,220) =6.07	F(10,220) =4.75	F(10,220) =4.64	F(10,220) =6.59	F(10,220) =6.25	F(10,220) =2.24	F(10,220) =4.43
<b>Time</b>									
-1	0.3636	0.9768	0.9479	0.9981	>0.9999	>0.9999	0.9894	0.9835	0.9582
0	0.3869	0.9966	0.9929	0.9082	0.8822	>0.9999	0.9986	0.9283	0.9741
0.5	0.2833	0.9768	>0.9999	0.7094	0.1006	0.9831	0.9983	0.5339	0.9316
1	0.2993	0.966	0.6302	0.9881	0.0726	0.8953	0.9667	>0.9999	0.9999
1.5	0.9031	0.9989	>0.9999	0.7848	0.9997	0.6022	0.976	0.9988	0.9706
2	0.9549	0.9978	0.4732	0.6536	>0.9999	0.1366	0.0835	0.6774	0.9925
2.5	0.9869	>0.9999	0.105	0.0605	0.5088	<b>0.0157</b>	<b>0.0111</b>	0.6882	0.167
3	0.8111	0.7109	0.1681	<b>0.0425</b>	0.1422	<b>0.0466</b>	<b>0.0353</b>	0.6845	0.0651
4	<b>0.0153</b>	<b>0.0355</b>	0.0654	0.3481	0.2218	0.0591	<b>0.0486</b>	0.2352	0.1395
6	0.0926	0.1219	0.6838	>0.9999	0.6735	0.1894	0.1791	0.6955	0.6542
8	0.4848	0.2467	0.9966	0.9894	0.979	0.6916	0.1518	0.9708	0.9895

Reference group: control (CON).

p-values of Time x Column factor was calculated using linear mixed models analysis.

p-values of timepoints represent adjusted p-values calculated using Sidak's multiple comparison tests.

CON: control group. BRT: breathing exercise group. CEX: cold exposure group. CBR: cold exposure and breathing exercise group.



## Supplemental Figures and legends

### **Supplemental Figure S1: Arterial blood gas parameters and plasma epinephrine levels during the breathing exercises study: morning vs. afternoon session.**

**A.** Oxygen saturation. **B.** Oxygen partial pressure (pO<sub>2</sub>). **C.** pH. **D.** Carbon dioxide partial pressure (pCO<sub>2</sub>). **E.** Plasma epinephrine concentrations. Morning session: data from participants during the first breathing exercise on the experiment day (breathing exercise 1, Figure 1). Afternoon session: data from participants during the second breathing exercise on the experiment day (breathing exercise 2, Figure 1). Data are presented as mean ± 95% confidence interval (panels A-D) or median and interquartile range (panel E) of 20 participants per group and p-values depicted in the graphs represent the between-group comparison calculated using linear mixed models analysis (time\*column factor). Epinephrine data were log-transformed before analysis. For comparisons that yielded time\*column factor p-values <0.05, results of post-hoc analyses performed using Sidak's multiple comparison test are reported in Supplemental Table S5.

### **Supplemental Figure S2: arterial blood gas parameters and plasma epinephrine levels during the breathing exercise study: influence of trainer.**

**A.** Oxygen saturation. **B.** Oxygen partial pressure (pO<sub>2</sub>). **C.** pH. **D.** Carbon dioxide partial pressure (pCO<sub>2</sub>). **E.** Plasma epinephrine concentrations. Training by the creator of the intervention: data obtained during the first breathing exercise on the experiment day (breathing exercise 1, Figure 1) from participants were trained by the creator of the intervention. Training by independent trainer: data obtained during the first breathing exercise on the experiment day (breathing exercise 1, Figure 1) from participants were trained by the independent trainer. Data are presented as mean ± 95% confidence interval (panels A-D) or median and interquartile range (panel E) of 20 participants per group and p-values depicted in the graphs represent the between-group comparison calculated using linear mixed models analysis (time\*column factor). Epinephrine data were log-transformed before analysis.

### **Supplemental Figure S3: Arterial blood gas parameters and plasma epinephrine levels during the breathing exercise study: influence of length of training.**

**A.** Oxygen saturation. **B.** Oxygen partial pressure (pO<sub>2</sub>). **C.** pH. **D.** Carbon dioxide partial pressure (pCO<sub>2</sub>). **E.** Plasma epinephrine concentrations. Long training: data obtained during the first breathing exercise on the experiment day (breathing exercise 1, Figure 1) from participants that received four days of training. Short training: data obtained during the first breathing exercise on the experiment day (breathing exercise 1, Figure 1) from participants that received 2 hours of training. Data are presented as mean ± 95% confidence interval (panels A-D) or median and interquartile range (panel E) of 20 participants per group and p-values depicted in the graphs represent the between-group comparison calculated using linear mixed models analysis (time\*column factor). Epinephrine data were log-transformed before analysis.

### **Supplemental Figure S4: Plasma concentrations of inflammatory cytokines during human endotoxemia.**

**A.** Interferon Gamma-Induced Protein 10 (IP-10). **B.** Monocyte chemoattractant protein 1 (MCP-1). **C.** Macrophage Inflammatory Protein 1α (MIP-1α). **D.** Macrophage Inflammatory Protein 1β (MIP-1β). The grey box indicates the period during which the trained participants practiced the breathing exercise (BRT and CBR groups only). Data are presented as mean ± 95% CI of 12 participants per group. P-values depicted next to the legend represent the comparison of that group with the control group over time, calculated using linear mixed

models analysis (time\*column factor). Significant p-values are shown in bold. CON: control group. BRT: breathing exercise group. CEX: cold exposure group. CBR: cold exposure and breathing exercise group. For comparisons that yielded time\*column factor p-values <0.05, results of post-hoc analyses performed using Sidak's multiple comparison test are reported in Supplemental Table S6.

**Supplemental Figure S5: Area under the curve (AUC) of cytokine responses during human endotoxemia.**

**A.** Tumor necrosis factor (TNF)- $\alpha$ . **B.** Interleukin (IL)-6. **C.** IL-8. **D.** IL-10. Data are presented as scatterplot with bars representing the mean value of 12 participants per group. CON: control group. BRT: breathing exercise group. CEX: cold exposure group. CBR: cold exposure and breathing exercise group.

**Supplemental Figure S6: Area under the curve (AUC) of cytokine responses during human endotoxemia.**

**A.** Interferon Gamma-Induced Protein 10 (IP-10). **B.** Monocyte chemoattractant protein 1 (MCP-1). **C.** Macrophage Inflammatory Protein 1 $\alpha$  (MIP-1 $\alpha$ ). **D.** Macrophage Inflammatory Protein 1 $\beta$  (MIP-1 $\beta$ ). Data are presented as scatterplot with bars representing the mean value of 12 participants per group. CON: control group. BRT: breathing exercise group. CEX: cold exposure group. CBR: cold exposure and breathing exercise group.

**Supplemental Figure S7: CONSORT diagram of the breathing exercises study.**

**Supplemental Figure S8: CONSORT diagram of the human endotoxemia study.**

Figure S1.

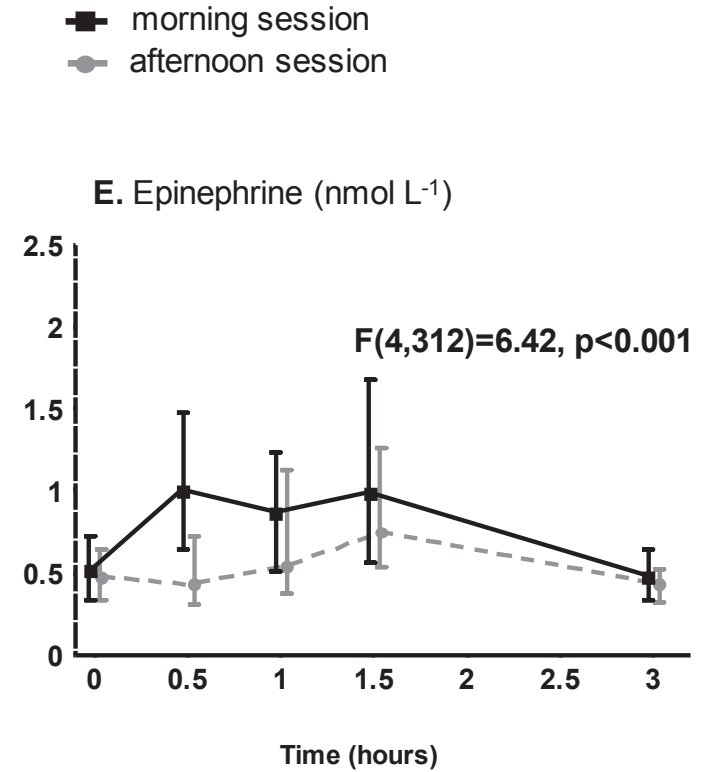
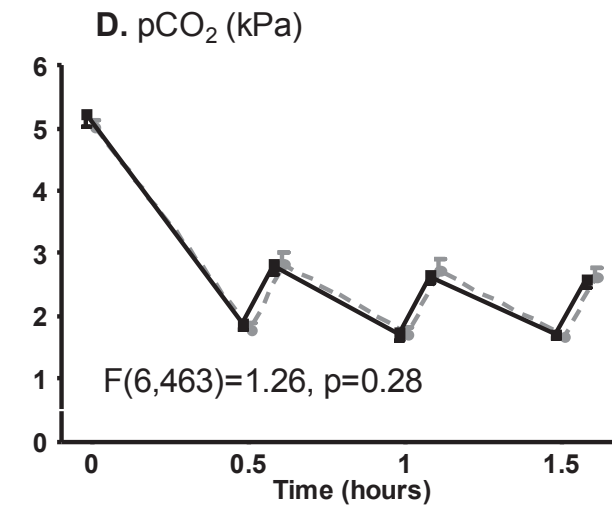
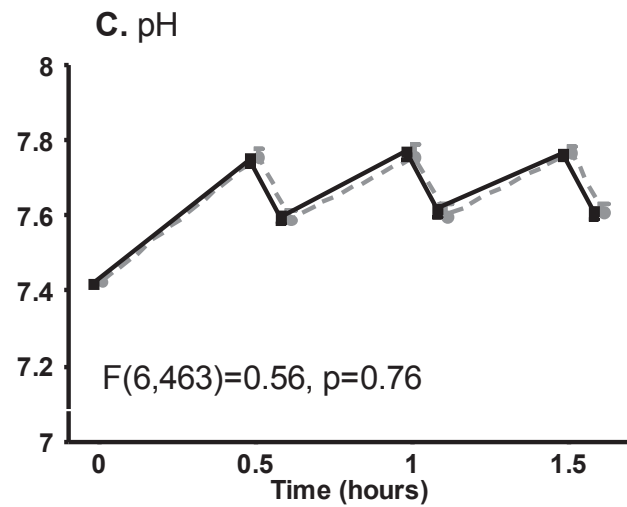
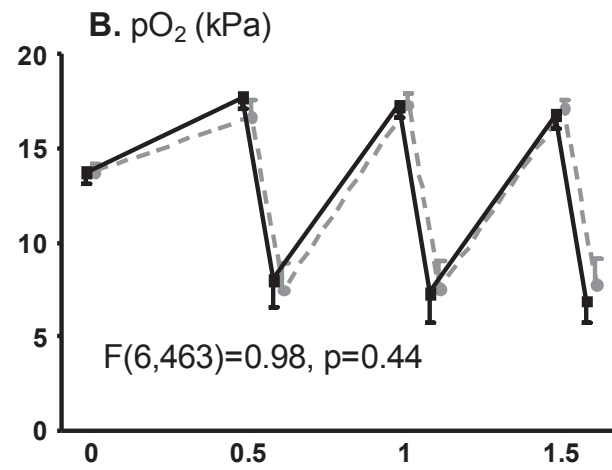
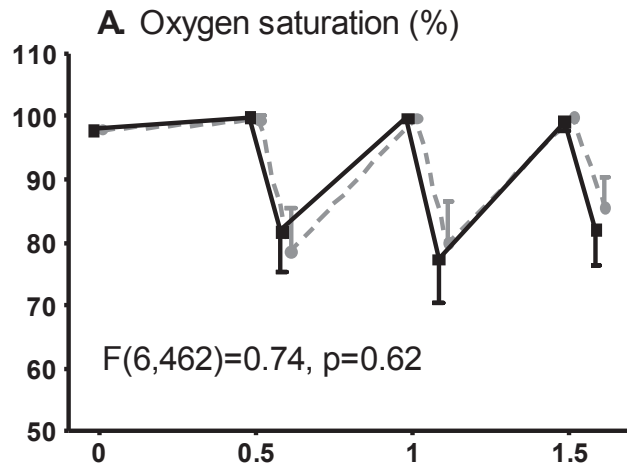


Figure S2.

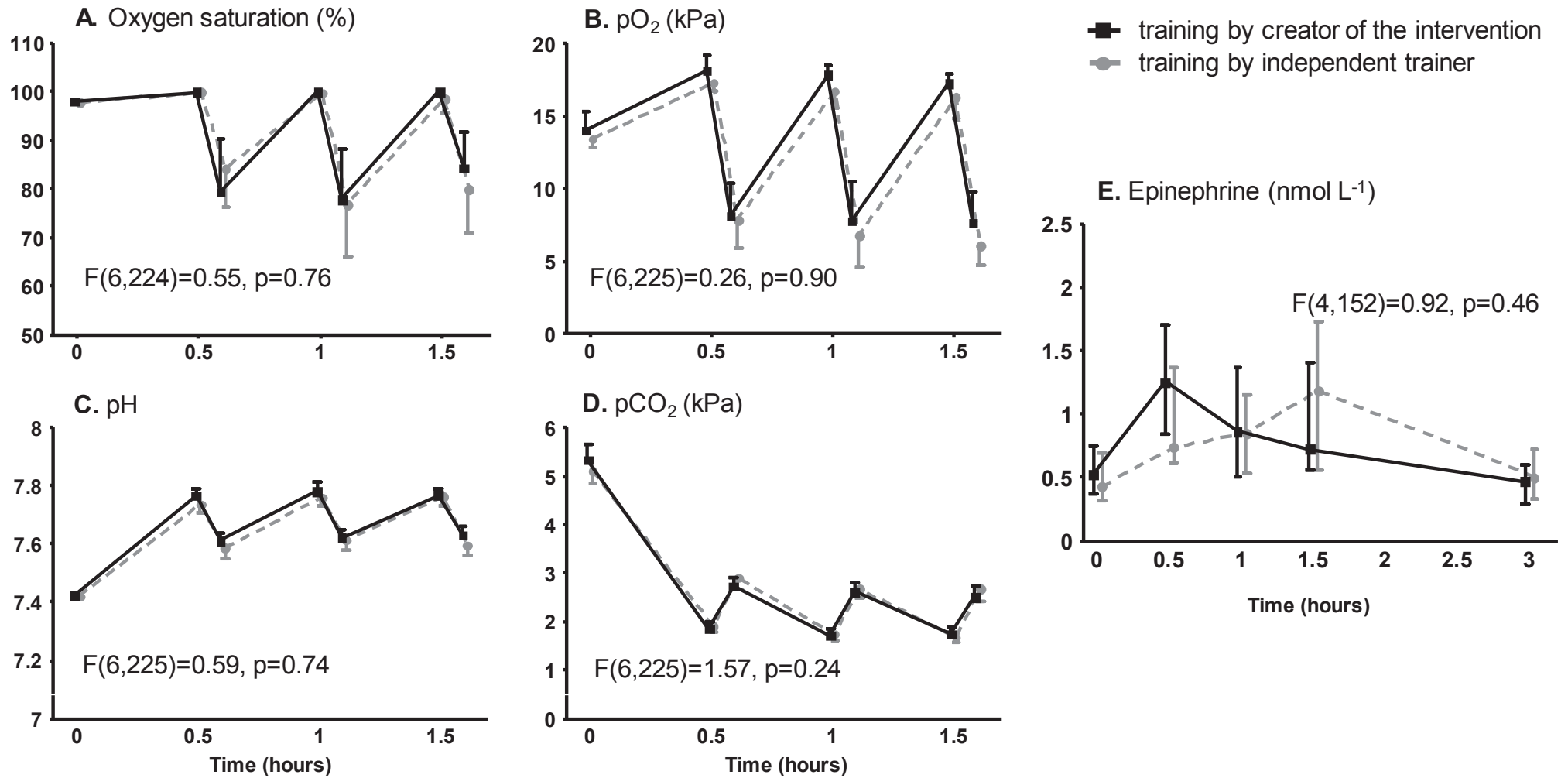
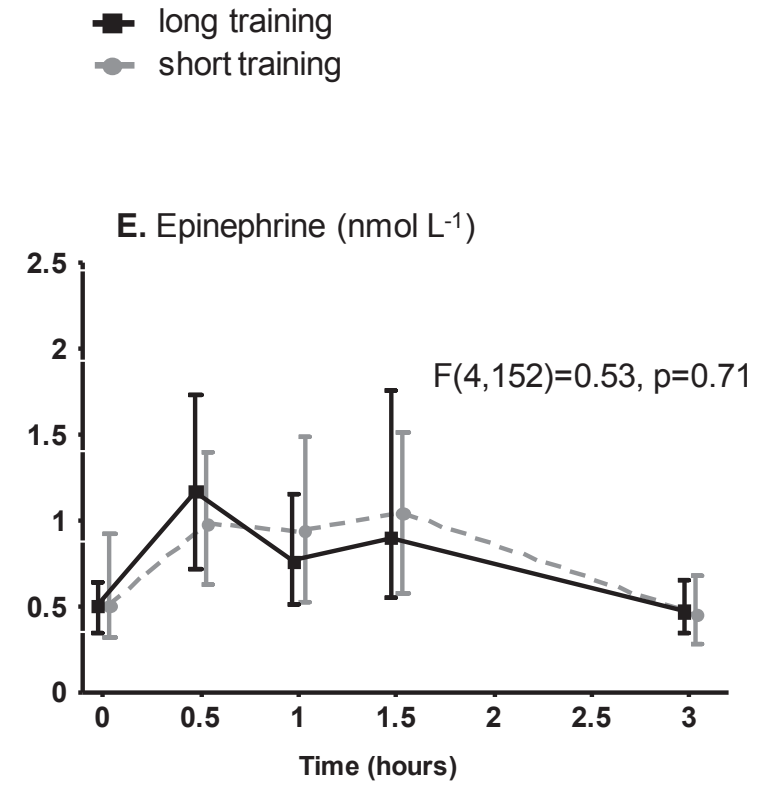
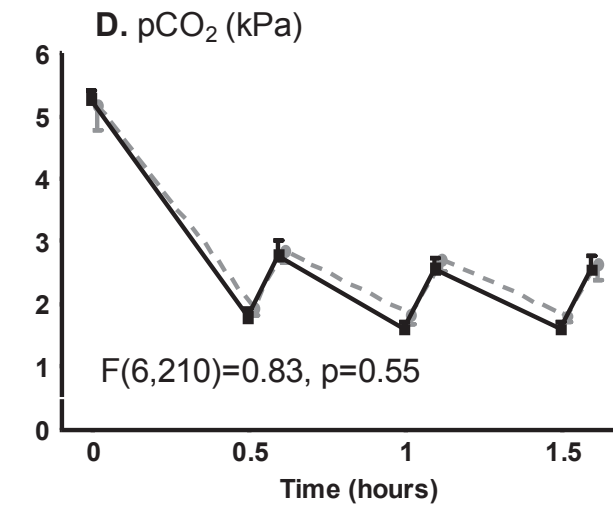
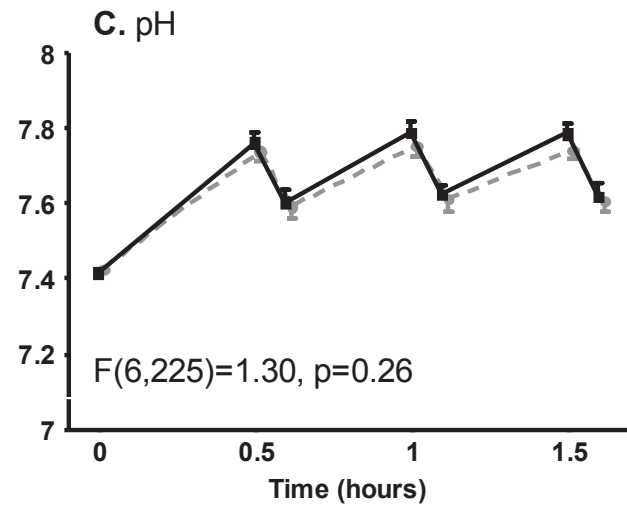
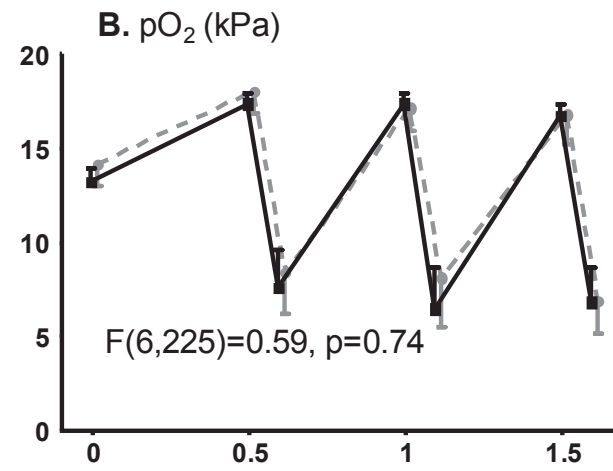
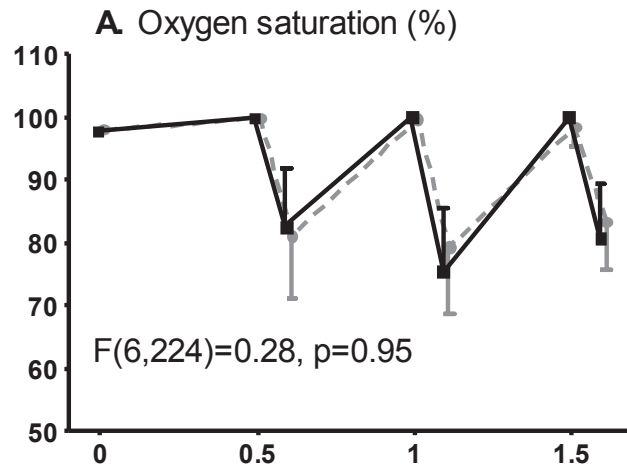


Figure S3.



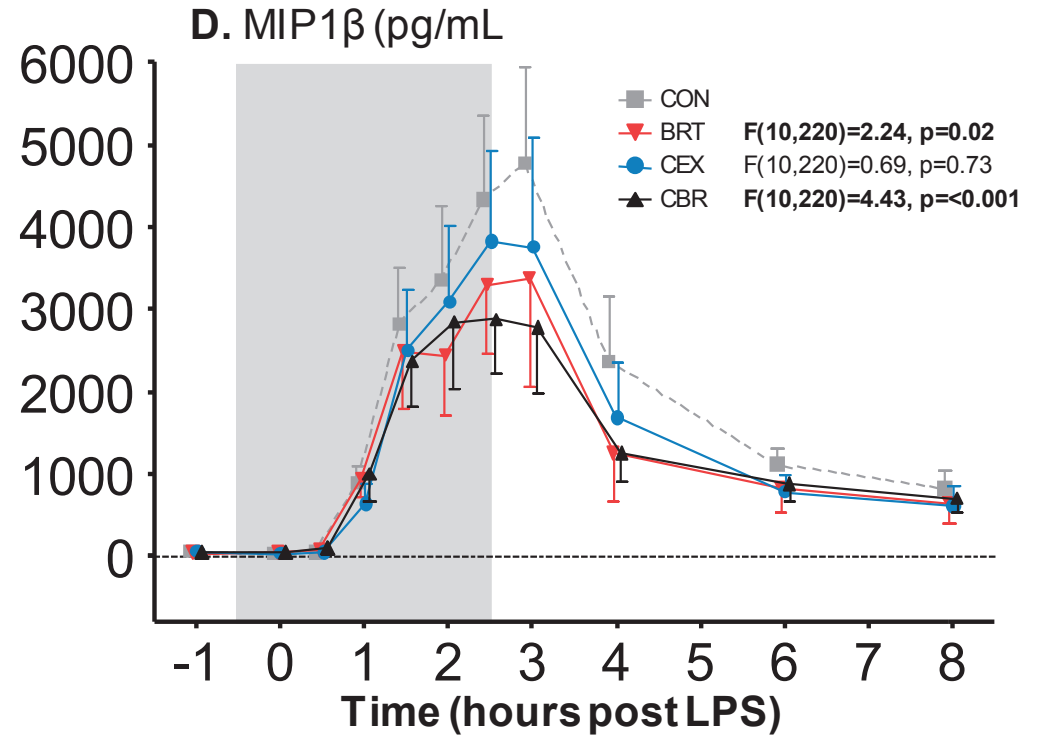
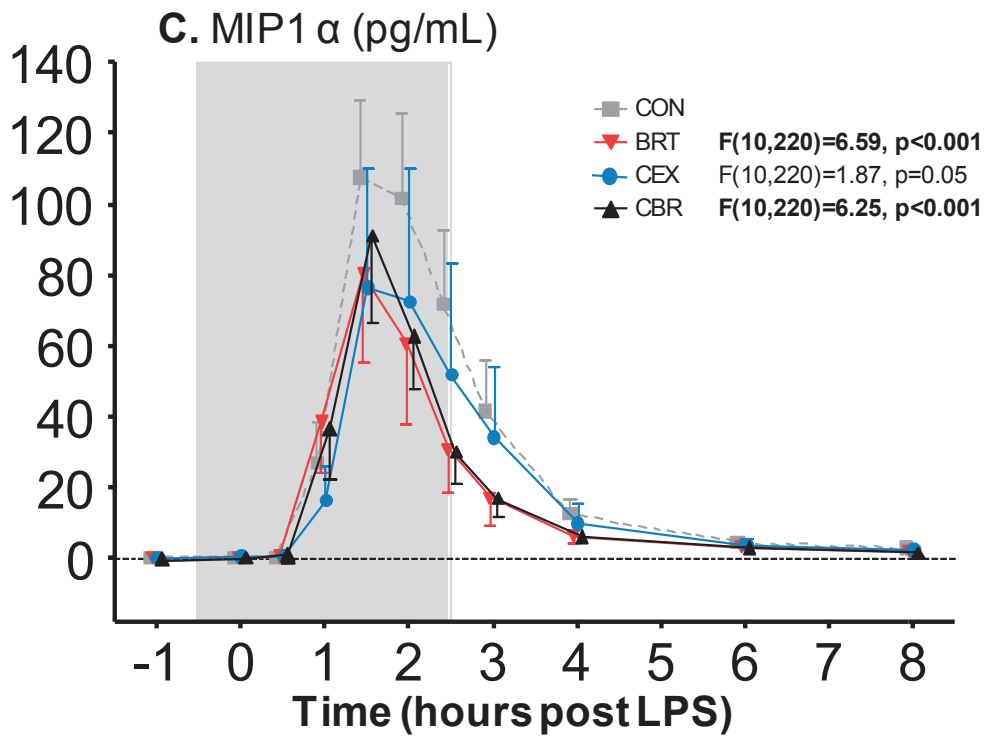
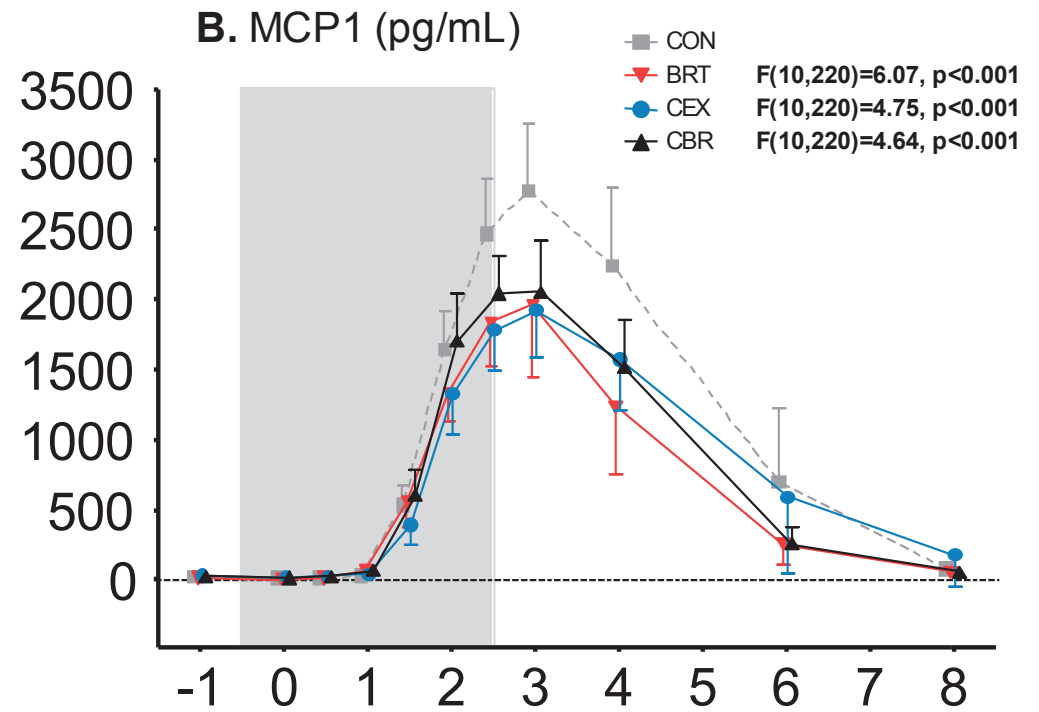
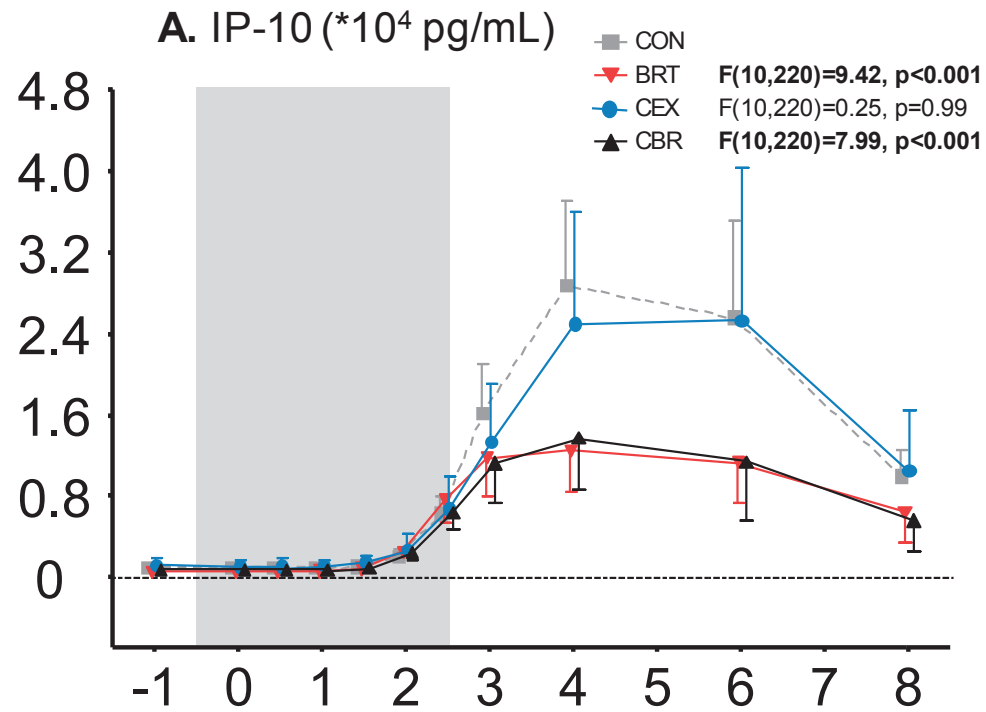
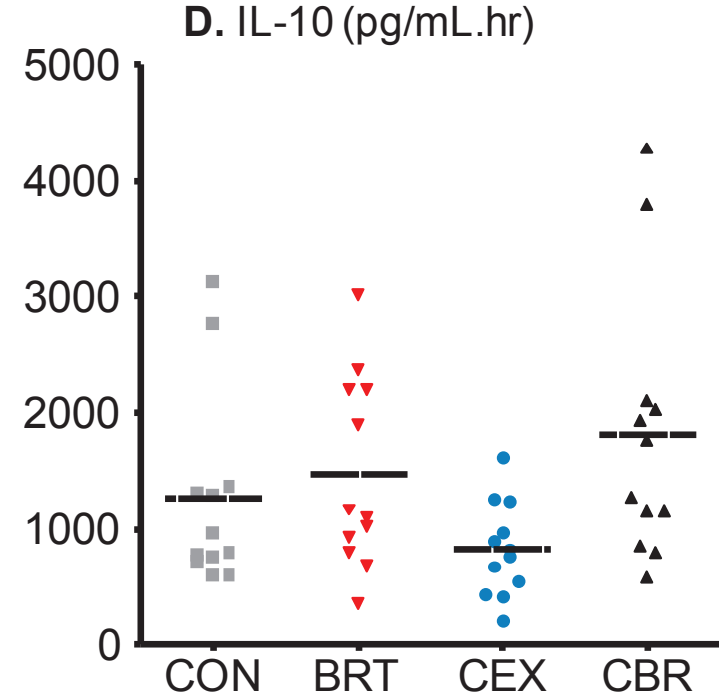
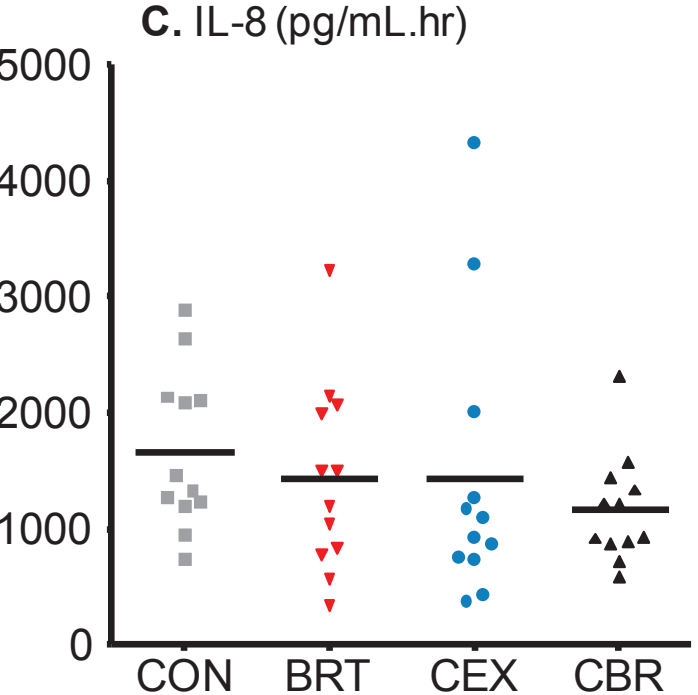
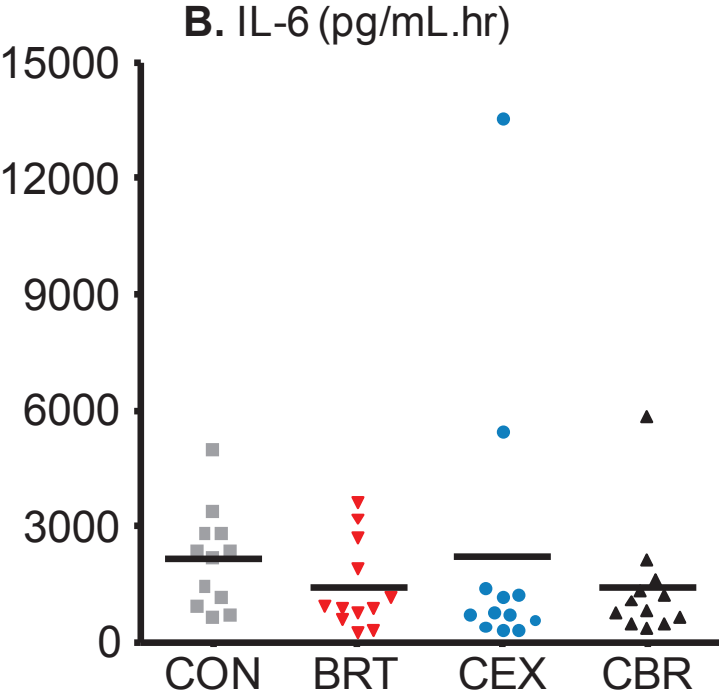
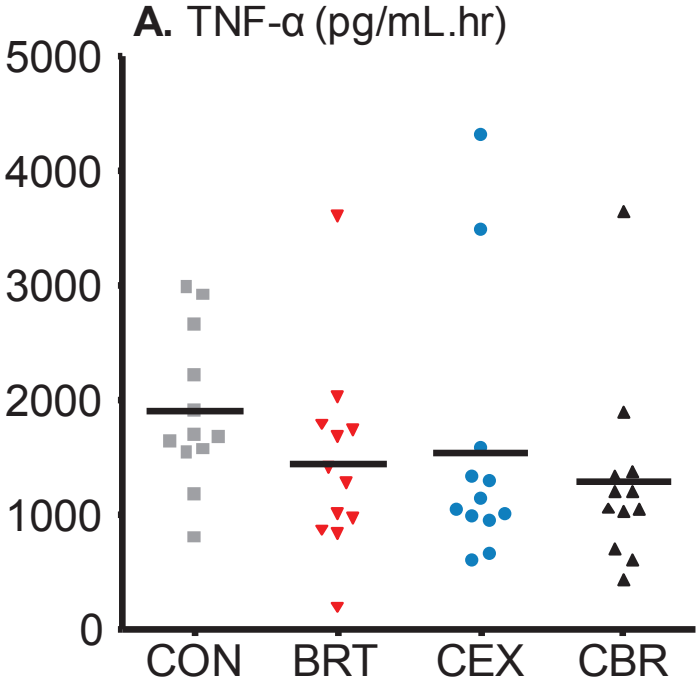
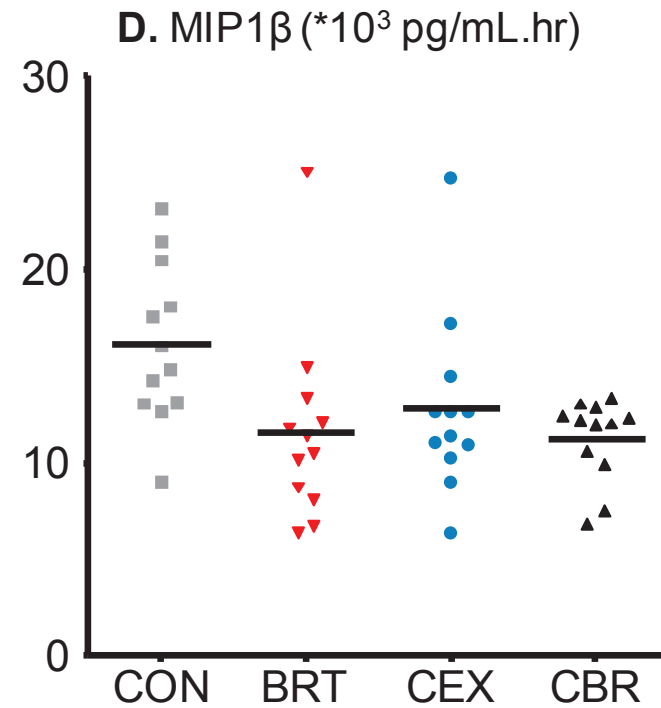
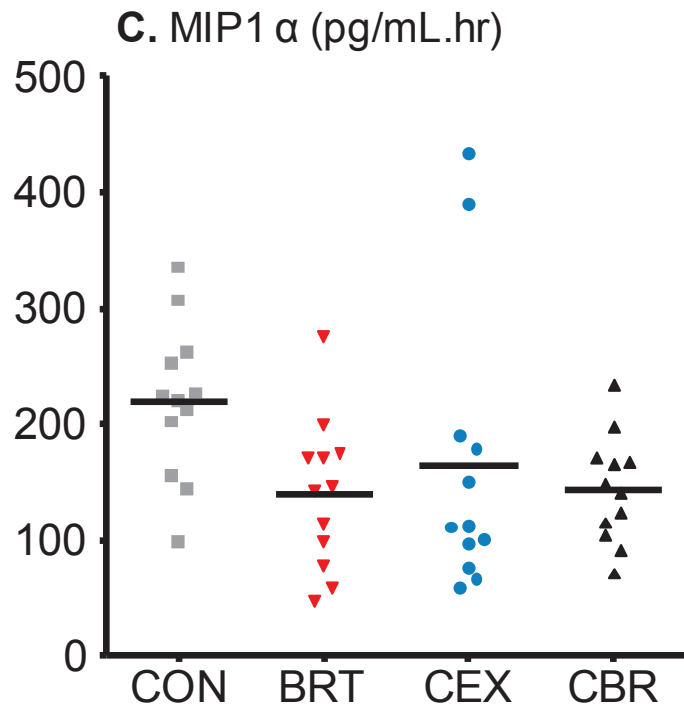
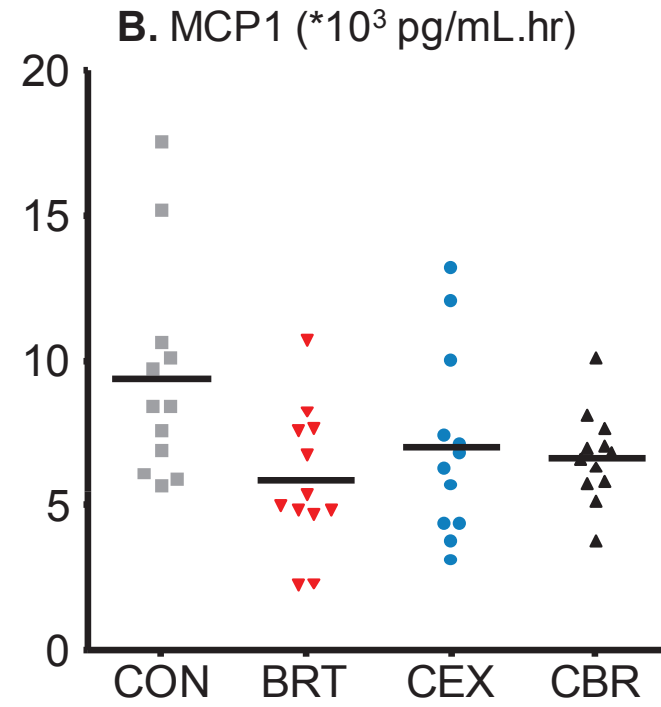
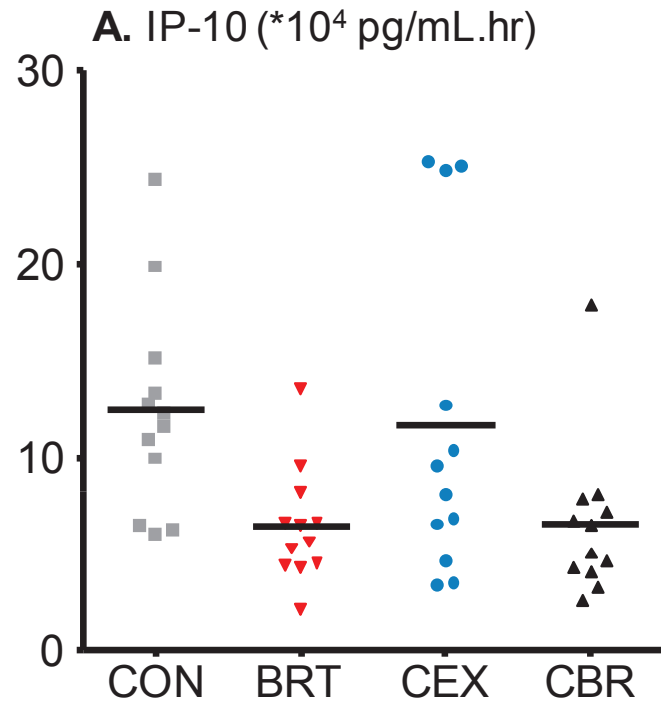


Figure S5.

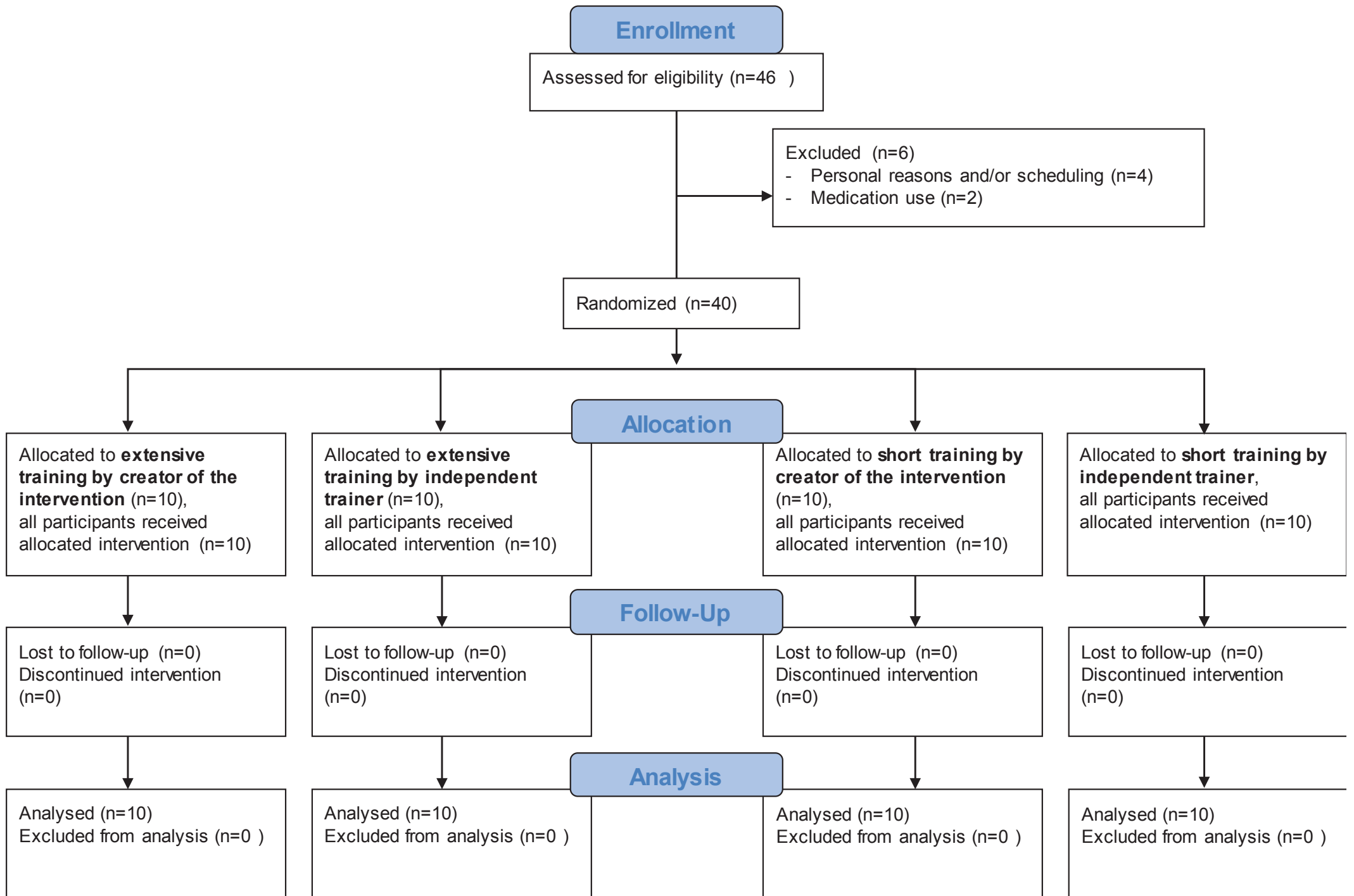






# CONSORT Diagram breathing exercises study

Figure S7.



# CONSORT Diagram endotoxemia study

Figure S8.

