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FULL RESEARCH PAPER
Air pollution and environmental science

Mitigation of aircraft noise-induced vascular dysfunction and oxidative stress by exercise, fasting, and pharmacological α1AMPK activation: molecular proof of a protective key role of endothelial α1AMPK against environmental noise exposure

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Aims Environmental stressors such as traffic noise represent a global threat, accounting for 1.6 million healthy life years lost annually in Western Europe. Therefore, the noise-associated health side effects must be effectively prevented or mitigated. Non-pharmacological interventions such as physical activity or a balanced healthy diet are effective due to the activation of the adenosine monophosphate-activated protein kinase (α1AMPK). Here, we investigated for the first time in a murine model of aircraft noise-induced vascular dysfunction the potential protective role of α1AMPK activated via exercise, intermittent fasting, and pharmacological treatment.

Methods and results Wild-type (B6.Cg-Tg(Cdh5-cre)7Mlia/J) mice were exposed to aircraft noise [maximum sound pressure level of 85 dB(A), average sound pressure level of 72 dB(A)] for the last 4 days. The α1AMPK was stimulated by different protocols, including 5-aminoimidazole-4-carboxamide riboside application, voluntary exercise, and intermittent fasting. Four days of aircraft

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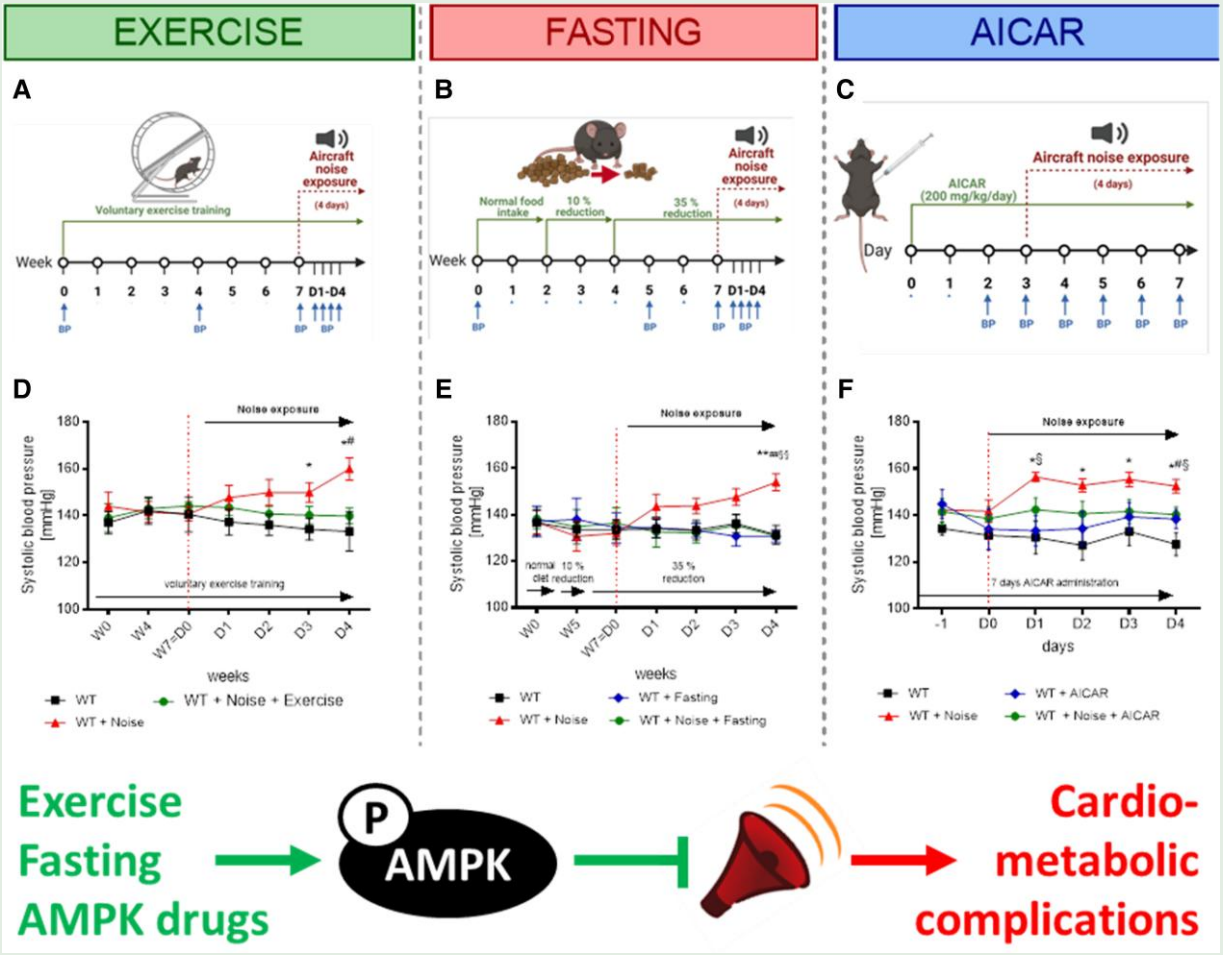
noise exposure produced significant endothelial dysfunction in wild-type mice aorta, mesenteric arteries, and retinal arterioles. This was associated with increased vascular oxidative stress and asymmetric dimethylarginine formation. The α1AMPK activation with all three approaches prevented endothelial dysfunction and vascular oxidative stress development, which was supported by RNA sequencing data. Endothelium-specific α1AMPK knockout markedly aggravated noise-induced vascular damage and caused a loss of mitigation effects by exercise or intermittent fasting.

Conclusion Our results demonstrate that endothelial-specific α1AMPK activation by pharmacological stimulation, exercise, and intermittent fasting effectively mitigates noise-induced cardiovascular damage. Future population-based studies need to clinically prove the concept of exercise/fasting-mediated mitigation of transportation noise-associated disease.

Lay summary Traffic noise, e.g. from aircraft, significantly contributes to an increased risk of cardiovascular or metabolic diseases in the general population by brain-dependent stress reactions leading to higher levels of circulating stress hormones and vasoconstrictors, all of which cause hypertension, oxidative stress, and inflammation. With the present experimental studies, we provide for the first time molecular mechanisms responsible for successful noise mitigation:

- Physical exercise, intermittent fasting, and pharmacological activation of the adenosine monophosphate-activated protein kinase (AMPK), a metabolic master regulator protein, prevent cardiovascular damage caused by noise exposure, such as hypertension, endothelial dysfunction, and reactive oxygen species formation (e.g. free radicals) and inflammation.
- These beneficial mitigation manoeuvers are secondary to an activation of the endothelial AMPK, thereby mimicking the antidiabetic drug metformin.

Graphical Abstract



Keywords Aircraft noise exposure • Noise mitigation • α1AMPK • Exercise • Fasting • AICAR • Reactive oxygen species • Endothelial dysfunction

Introduction

Environmental pollutants, such as noise exposure, increased substantially during the last decades due to modern lifestyle, industrialization, and urbanization. Epidemiological studies and the European Environment Agency and the World Health Organization (WHO) report that traffic noise is a rising environmental stressor with a substantial negative impact on the cardiovascular system and that more than one-third of the European population is exposed to noise levels exceeding the WHO guidelines [>55 dB(A) average over the entire day] (for review, see Munzel *et al.*[1–3]). It was shown that noise exposure, especially nocturnal aircraft noise, not only leads to annoyance and disturbed sleep[4,5] but also induces arterial hypertension, chronic coronary artery disease, heart failure, arrhythmia, and even triggers acute cardiovascular death,[2,3,6] mainly related to increased stress hormone levels as a consequence of neurohormonal activation of the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system.[7,8] Even short-term noise exposure for one night causes endothelial dysfunction, a biomarker for early atherosclerosis, diastolic cardiac dysfunction, and increased oxidative stress. It induces a prothrombotic inflammatory phenotype in healthy volunteers[9–11] and patients with coronary artery disease.[12]

In mice, noise-induced stress hormone release, activation of immune cells, and oxidative stress by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX-2) activation and circadian clock dysregulation were demonstrated to cause hypertension and endothelial dysfunction, an important biomarker for future cardiovascular events.[13–15] Thus, it is mandatory to identify noise-induced molecular pathomechanisms to establish practical therapeutic tools to lower or prevent noise-induced cardiovascular and cerebrovascular injury. Non-pharmacological interventions such as regular physical activity, a balanced healthy diet, and weight reduction have been demonstrated to represent effective ways to prevent or to treat cardiovascular disease (CVD) (e.g. atherosclerosis, hypertension, and diabetes).[16] Especially, exercise has a substantial impact on CVD prevention and is currently discussed as a mitigation strategy against air pollution-induced cardiovascular disease and mortality.[17,18]

In the past, adenosine monophosphate-activated protein kinase (AMPK) has been shown to play a central role in exercise-mediated vascular protection[19] as well as suppression of inflammatory conditions by caloric restriction[20,21] and beneficial health effects by intermittent fasting.[22] The AMPK acts as a fuel-sensing metabolic enzyme that phosphorylates several downstream targets like peroxisome proliferator-activated receptor gamma coactivator 1-alpha,[23] inhibiting energy consumption and promoting energy production to ensure cell survival. Especially, the α1AMPK subunit, predominantly expressed in the vasculature, has gained much attention since it regulates vascular homeostasis by increasing endothelial NO-synthase (eNOS) expression and improving antioxidant mechanisms to limit vascular reactive oxygen species (ROS) production.[24,25] Therefore, the present study aimed to test the hypothesis that noise-induced cardiovascular damage could be mitigated by lifestyle interventions based on caloric restriction, exercise training, and pharmacological activation of α1AMPK with 5-aminoimidazole-4-carboxamide riboside (AICAR). As a proof of concept, two approaches were also tested in endothelial-specific AMPK knock-out mice.

Materials and methods

Animal models

All animal experiments within the present study were performed in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the US National Institutes of Health and were approved by the ethics committee of the University Hospital Mainz (Az 23 177-07/G17-1-066). For the purpose of the study, we created mice with endothelial-specific deletion of α1AMPK (referred to as α1AMPK EC KO) by breeding B6.Cg-Tg(Cdh5-cre)7Mlia/J mice on a C57BL/6J background (hereinafter referred to as wild type; WT) with $\alpha1AMPK^{fl/fl}$ mice (*Prkaa1* mutant mice possess *loxP* sites flanking exon 4 and 5).[26] Mice (only male, 10–14 weeks old) were exposed to aircraft noise [maximum sound pressure level of 85 dB(A), average sound pressure level of 72 dB(A)] as described previously[13,14] for the last 4 days of the treatment. The α1AMPK EC KO and corresponding wild-type mice performed voluntary exercise for 7 weeks (start of the exercise at the age of 4–6 weeks) using cages with a running wheel as previously described,[19] which may be considered an aerobic exercise model. Our previous data revealed no significant difference in the running distance between wild-type and AMPK knockout mice (4336 vs. 4002 m/24 h). Intermittent fasting was induced by exact daily food administration (2 weeks: average food intake; 2 weeks: 10% reduction; and 3 weeks: 35% reduction of calories); the protocol was adapted from Shinmura *et al.*[27] As previously published, the pharmacological activation of AMPK was induced by AICAR, administered by daily subcutaneous injections (200 mg/kg/day) for 7 days.[28] For a detailed description of the treatment, see *Figure 1A–C*. At the end of the experiment, animals were sacrificed using ketamine anaesthesia + xylazine analgesia, followed by cervical dislocation and sample collection (aorta, heart, brain, mesenteric arteries, eyes, and plasma). In addition, blood samples were obtained by heart puncture using heparin and K_2EDTA.

Blood pressure measurement

Non-invasive measurement of blood pressure (BP) was performed by plethysmography technique using the CODA system (Kent Scientific Corporation, Torrington, CT); measurements were provided after previous repeated training to minimize stress.[13–15] Blood pressure was measured before, during, and at the end of the treatment; the time schedule of BP measurements is illustrated in *Figure 1A–C*.

Endothelial function

Endothelial function studies were performed by isometric tension studies in large conductance or small resistance arteries (Aorta and mesenteric arteries) using an organ bath and wire myography. Endothelium-dependent relaxation of intact mouse aortic rings (3 mm, perivascular fat, and connective tissues free) was tested with acetylcholine (ACh 10^{-9}–$10^{-5.5}$ M) upon pre-constriction with prostaglandin F2α[13,14] using Multi Wire Myograph system—620 M (Danish Myo Technology, Aarhus, Denmark) as described.[29] The isolated rings were pre-contracted with norepinephrine (10^{-5}μM, final concentration), followed by the cumulative addition of acetylcholine (ACh 10^{-9}–10^{-5} M).

Retinal microvascular function

Microvascular function was measured in first-order arterioles of isolated retinas by using video microscopy as previously described.[30,31] Following dissection, the eyes were transferred into ice-cold Krebs Henseleit buffer, and the retina was isolated with the optic nerve and the ophthalmic artery attached. Next, the ophthalmic artery was cannulated and sutured to a micropipette with 10.0 nylon suture material, and the retina was placed onto a transparent plastic platform. Subsequently, retinal arterioles were pressurized to 50 mmHg, visualized under brightfield conditions, and equilibrated for 30 min at 37°C. Subsequently, concentration–response curves for the thromboxane mimetic, U46619 (10^{-11} to 10^{-6} M; Cayman Chemical, Ann Arbor, MI, USA), were conducted. For measurement of vasodilation responses, vessels were pre-constricted to 50–70% of the initial luminal diameter by titration of U46619, and responses to the endothelium-dependent vasodilator, acetylcholine (10^{-9} to 10^{-4} M; Sigma-Aldrich, Taufkirchen, Germany), and the endothelium-independent nitric oxide donor, sodium nitroprusside (SNP, 10^{-9} to 10^{-4} M, Sigma-Aldrich), were determined.

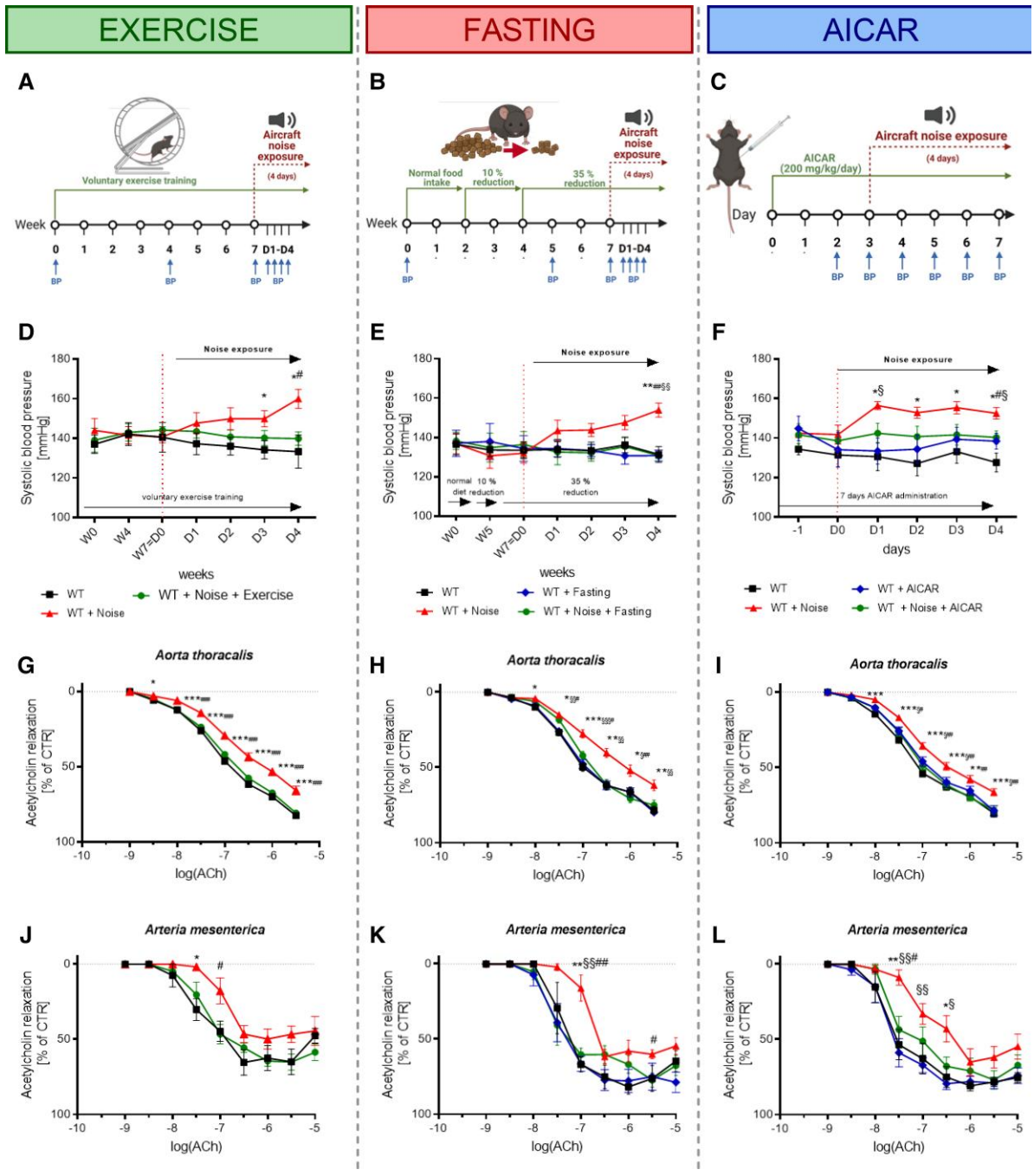


Figure 1 Exercise training, caloric restriction, and 5-aminoimidazole-4-carboxamide riboside treatment prevent noise-induced elevation of blood pressure and endothelial dysfunction. Schemes of the treatment protocols: voluntary exercise training (*A*), fasting (*B*), and 5-aminoimidazole-4-carboxamide riboside (*C*) administration. (*D–F*) Voluntary exercise training ($n = 6–20$), intermittent fasting ($n = 7–13$) and 5-aminoimidazole-4-carboxamide riboside treatment ($n = 7–16$) all restored noise exposure-induced blood pressure elevation to the level of unexposed control. Noise-triggered endothelial dysfunction in the aorta and mesenteric artery was prevented by exercise training [*G* ($n = 23–49$) and *J* ($n = 5–10$)], intermittent fasting [*H* ($n = 8–15$) and *K* ($n = 4–7$)] and 5-aminoimidazole-4-carboxamide riboside administration [*I* ($n = 17–33$) and *L* ($n = 6–11$)]. Data are presented as mean ± SEM. *P*-values <0.05 were considered significant (* = WT vs. Noise; #=WT + treatment vs. Noise; § = Noise vs. Noise + treatment; + = treatment vs. Noise + treatment. Treatment = exercise/fasting/ 5-aminoimidazole-4-carboxamide riboside). BP, blood pressure measurement; D1—D4, Day 1—Day 4 of noise exposure; AICAR, 5-aminoimidazole-4-carboxamide riboside.

RNA sequencing and bioinformatical analysis

RNA sequencing was carried out at Novogene Bioinformatics Technology Co., Ltd., in Cambridge (UK). According to the manufacturer's protocol, aortic mRNA was isolated using the RNeasy Mini kit (Qiagen, Hilden, Germany). RNA sample quality control, mRNA library preparation (poly A enrichment), NovaSeq PE150 sequencing (9 G raw data per sample), data quality control, and basic data analysis were performed by Novogene Bioinformatics Technology Co., Ltd., in Cambridge (UK). Volcano plots to envisage the number of changed genes by the three different therapeutic interventions as well as KEGG pathway analyses to envisage the biochemical processes that are changed by the three different therapeutic interventions were taken from the standard bioinformatical report provided by Novogene (see Supplementary material online, *Figure S4*). Cluster analysis was performed by Search Tool for the Retrieval of Interacting Genes (STRING) version 11.0.[32] To identify interactive relationships among identified target proteins, the protein list was mapped to STRING.

Vascular oxidative stress

The vascular production of ROS in aortic tissue was measured by an high-performance liquid chromatography (HPLC)-based dihydroethidium (DHE) assay as described previously.[14,33] In addition, the mitoSOX/HPLC method[34] was used to determine mitochondria-specific production of ROS. Mitochondria were isolated from cardiac tissues as described previously.

Protein and mRNA expression analysis

Western blot analysis was used to determine changes in protein expression. Aortic tissue was homogenized in cell lysis buffer [2- mM Tris-HCl; 250-mM saccharose; 3-mM EGTA; 20-mM EDTA; 0,5-mM PMSF; 1% Triton-X100; 0,5-mM Na-vanadate; 2,5-mM Na-fluoride; protease inhibitor cocktail % (P8340; Sigma-Aldrich, Taufkirchen, Germany); 1% phosphatase inhibitor cocktail (P2850; Sigma-Aldrich, Taufkirchen, Germany)]. Proteins (20 µg per lane) were separated using SDS-PAGE, followed by a blotting procedure onto nitrocellulose membranes. Immunostaining was performed as described previously.[28] Determination of asymmetric dimethylarginine (ADMA) levels in plasma was performed by dot blot system (Schleicher & Schuell, 10484138CP) using 50 µg of proteins per dot; analysis was performed as stated previously.[13–15] Polyclonal rabbit anti-α1AMPK (Merck-Millipore, Darmstadt, Germany; 1:1000), polyclonal rabbit anti-p-α1AMPK^{Thr172} (Cell Signaling, Boston, MA, USA; 1:500), monoclonal anti-α-Actinin (Sigma-Aldrich, Taufkirchen, Germany; 1:2000), and monoclonal rabbit anti-ADMA (Cell Signaling, Boston, MA, USA; 1:1000) were used for protein expression analysis. Gene expression changes were analysed by quantitative real-time PCR using the QuantiTect™ Probe RT-PCR kit (Qiagen) used according to the manufacturer's protocol as described previously.[35] For analysis, we used *TaqMan* real-time PCR assays: type 1 protein arginine N-methyltransferase (PRMT1) (Mm00480135_g1), dimethylarginine dimethylaminohydrolase 2 (DDAH2) (Mm00516769_g1), and Foxo3 (Mm01185722_m1); TATA box binding protein was used as the housekeeping gene for normalization (TBP; MM00446973_m1). Gene expression was analysed using the comparative ΔΔCt method and is expressed as the percentage of WT.

Statistical analysis

Prism for Windows, version 9, GraphPad Software Inc (GraphPad Software LLC, La Jolla, CA) was used for statistical analysis. Statistical comparisons were conducted using one-way ANOVA with Bonferroni correction to compare multiple means or two-way ANOVA with a repeated measurements approach with Tukey correction as appropriate. All presented results are expressed as the mean ± SEM, and the *n* for every independent experiment is indicated. Results where $P < 0.05$ were accepted as statistically significant results.

Results

Effects of aircraft noise up to 4 days on arterial blood pressure and endothelial function

All experimental animals were exposed to around-the-clock aircraft noise for 4 days; this led to a significant elevation of systolic BP in wild-type mice (*Figure 1D–F*). In addition, endothelial dysfunction was evident in the aorta (*Figure 1G–I*), mesenteric arteries (*Figure 1J–L*), and retinal arterioles (see Supplementary material online, *Figure S1*) of all noise-treated animals. The impairment of these functional parameters by noise was also supported by RNA sequencing data and advanced pathway analysis pointing towards impaired redox homeostasis, changes in circadian clock genes, dysregulated AMPK and eNOS/NO/cGMP signalling, activation of the oxidative and general stress response, as well as an altered inflammatory cascade (see Supplementary material online, *Figure S5* and *Table S1*).

Effects of exercise training, caloric restriction, and 5-aminoimidazole-4 -carboxamide riboside treatment on noise-induced hypertension and endothelial dysfunction

We used three different therapeutic interventions to prevent noise-induced cardiovascular damage, including exercise training, fasting, and AICAR-induced AMPK activation, as illustrated in *Figure 1A–C*. Voluntary exercise training, intermittent fasting, and AICAR treatment, all restored noise-induced hypertension to the level of unexposed control (*Figure 1D–F*). Fasting and AICAR treatment did not alter vascular function in wild-type controls, while all three interventions reversed endothelial dysfunction triggered by aircraft noise exposure. The beneficial effects of two therapeutic interventions on the noise-dependent impairment of these functional parameters were also supported by RNA sequencing data and advanced pathway analysis. The next-generation sequencing data revealed a striking improvement of antioxidant defence systems, activation of AMPK signalling and survival pathways, suppression of inflammation and largely improved eNOS/NO/cGMP signalling as well as a cellular response to oxidative stress in noise-exposed mice with fasting and AICAR interventions (see Supplementary material online, *Figures S7 and S8*; *Tables S3 and S4*). However, we could not establish such beneficial effects on the evaluated pathways for an exercise intervention in noise-exposed mice on the level of RNA sequencing data, despite the highly beneficial effects of exercise on the other functional and biochemical parameters (see Supplementary material online, *Figure S6* and *Table S2*) and despite more than 3000 genes showing a more than the log2-fold change in their expression, however without reaching significance (see Supplementary material online, *Figure S4*).

Effects of exercise training, caloric restriction, and 5-aminoimidazole-4 -carboxamide riboside treatment on noise-induced increases in asymmetric dimethylarginine plasma levels

We next investigated whether aircraft noise may increase plasma levels of ADMA, a potent endogenous competitive eNOS inhibitor shown to

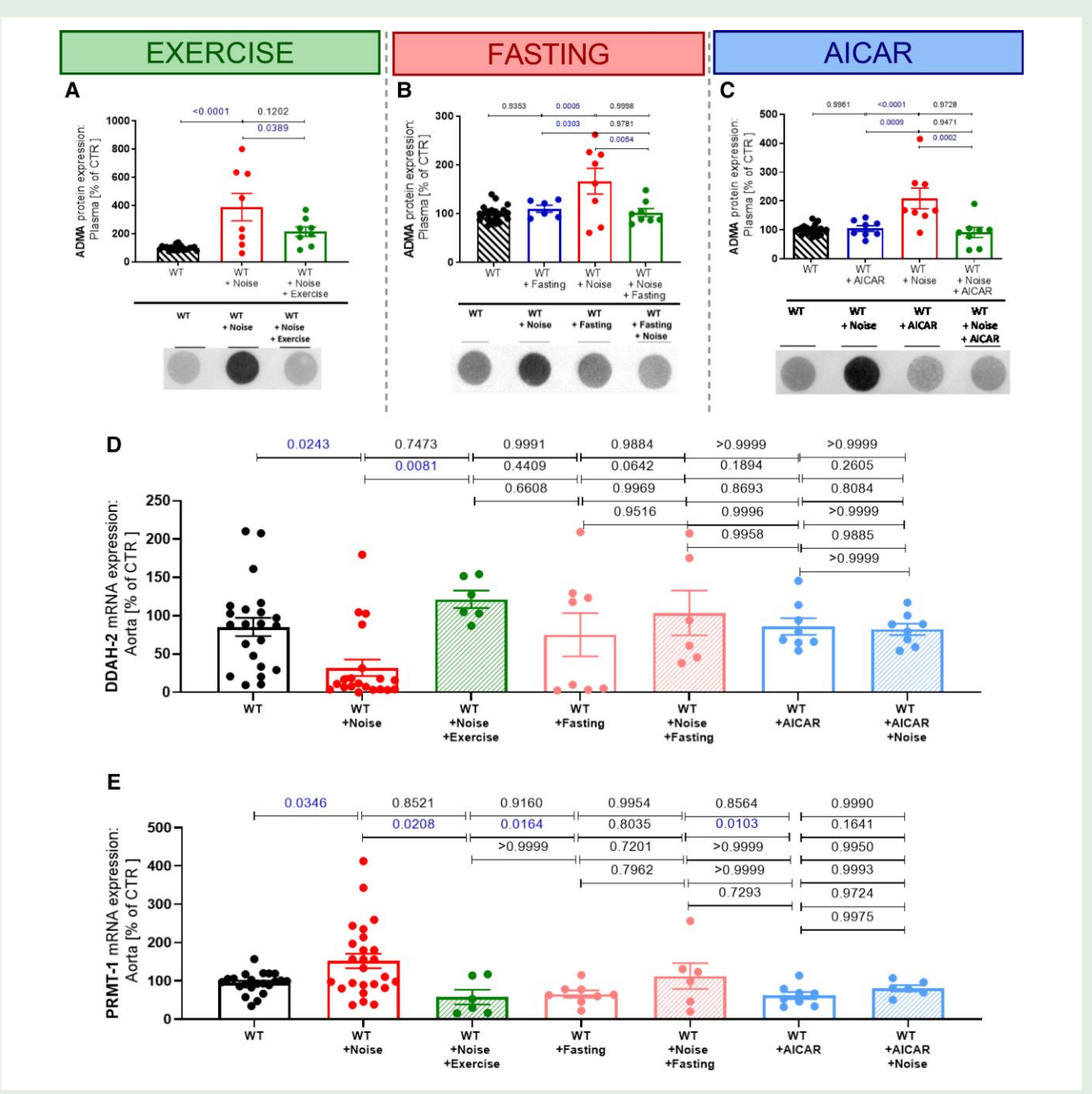


Figure 2 Exercise training, caloric restriction, and 5-aminoimidazole-4-carboxamide riboside treatment prevent a noise-dependent increase in asymmetric dimethylarginine levels and trigger endothelial NO-synthase expression. Asymmetric dimethylarginine plasma levels are upregulated by noise and down-regulated by voluntary exercise training (*A*), intermittent fasting (*B*) and 5-aminoimidazole-4-carboxamide riboside treatment (*C*). Noise-triggered adverse changes in mRNA expression of dimethylarginine dimethylaminohydrolase 2 (*D*) and type 1 protein arginine N-methyltransferase (*E*) were improved at least by trend by exercise training, intermittent fasting and 5-aminoimidazole-4-carboxamide riboside administration. Representative dot blot images are shown. Data are presented as mean ± SEM. Hatched groups in panels A, B, and C are the same. AICAR, 5-aminoimidazole-4-carboxamide riboside; ADMA, asymmetric dimethylarginine; eNOS, endothelial NO-synthase; DDAH-2, dimethylarginine dimethylaminohydrolase 2; PRMT-1, type 1 protein arginine N-methyltransferase; DDAH2, dimethylarginine dimethylaminohydrolase 2; PRMT-1, type 1 protein arginine N-methyltransferase.

uncouple eNOS and cause endothelial dysfunction.[36] Noise pollution increased plasma levels of ADMA, which was completely prevented by exercise, fasting, and AICAR treatment (*Figure 2A–C*). Asymmetric dimethylarginine is produced as a consequence of the methylation of L-arginine residues via the catalytic action of PRMT-1 and is degraded by DDAH-2.[37] The elevated plasma levels of ADMA in noise-exposed mice were associated with a decreased expression of DDAH-2 mRNA levels, encoding for the most essential ADMA degrading enzyme

(*Figure 2D*). They increased expression of PRMT-1 mRNA, encoding for a critical class of protein arginine N-methyltransferases that are responsible for ADMA formation (*Figure 2E*). The downregulated expression of DDAH-2 and upregulated expression of PRMT-1 was mostly reversed by exercise training, caloric restriction, and application of AICAR (at least by trend).

Effects of exercise training, caloric restriction and 5-aminoimidazole-4-carboxamide riboside administration on noise-induced oxidative stress in the cardiovascular system

The production of vascular superoxide formation was quantified using HPLC-based detection of oxidized DHE. Whereas noise exposure increased aortic superoxide levels, the mitigation measures exercise, fasting, and AICAR treatment normalized superoxide formation (*Figure 3A–C*). Those data were also mirrored by the normalization of mitochondria-specific superoxide levels determined by mitoSOX/HPLC in isolated heart mitochondria by all three manoeuvers (*Figure 3D–F*). Moreover, we can show that 4 days of aircraft noise exposure increases the production of cerebral superoxide (see Supplementary material online, *Figure S1*). Likewise, we observed a protective effect of exercise and fasting, resulting in reduced superoxide formation in response to noise. In addition, we observed the prevention of downregulation of the mRNA expression of cerebral Foxo3 (see Supplementary material online, *Figure S1*) with two of the manoeuvers, exercise, and fasting.

Effects of exercise training, caloric restriction, and 5-aminoimidazole-4-carboxamide riboside administration on adenosine monophosphate-activated protein kinase expression and activation

Noise exposure did not affect aortic α1AMPK protein expression nor its phosphorylation at threonine 172 (see Supplementary material online, *Figure S2*). In contrast, voluntary exercise training and fasting significantly increased the protein expression of aortic α1AMPK and p-α1AMPK^{Thr172} (AICAR administration at least by trend), an activation marker of AMPK, compared to wild-type controls (see Supplementary material online, *Figure S2*). Voluntary exercise training had the most substantial effect on α1AMPK protein expression and phosphorylation among the three investigated models.

Proof-of-concept studies by genetic deletion of endothelial-specific adenosine monophosphate-activated protein kinase show abrogation of the beneficial vascular effects of exercise and fasting in noise-exposed mice

To prove the functional relevance of α1AMPK in preventing noise-mediated vascular damage, we used endothelial-specific α1AMPK knockout (α1AMPK EC KO) mice. Endothelial-specific α1AMPK deletion resulted in a substantial reduction of α1AMPK and p-α1AMPK^{Thr172} expression, as shown at the level of aortic protein expression (see Supplementary material online, *Figure S2*). In addition, α1AMPK EC KO mice developed arterial hypertension in response to noise exposure that was not prevented but even aggravated by exercise and fasting (*Figure 4A and D*). Furthermore, α1AMPK EC KO mice developed endothelial dysfunction of the aorta in response to noise, which was not abolished by exercise and fasting (*Figure 4B and E*). Importantly, the unexposed α1AMPK EC KO mice showed no significant impairment of endothelial function in comparison with the wild-type mice in accordance to our previous report[25] (see also hatched lines in *Figure 4*). Endothelial dysfunction was also observed in resistance vessels of noise-exposed α1AMPK EC KO mice, as shown by the impaired vascular relaxation curve of mesenteric arteries (*Figure 4C and F*) as well as of retinal arterioles (see Supplementary material online, *Figure S3*), all of which was also not prevented by exercise and fasting in the absence of endothelial α1AMPK. A key role of AMPK for AICAR-mediated protective effects was previously demonstrated in septic mice with global AMPK knockout.[28]

Effects of genetic deletion of endothelial-specific adenosine monophosphate-activated protein kinase on plasma asymmetric dimethylarginine levels

We next examined the ADMA release in α1AMPK EC KO mice since ADMA plays an essential role in the impairment of NO production and subsequent endothelial dysfunction.[38,39] In contrast to our findings mentioned above in wild-type animals (*Figure 2*), plasmatic ADMA levels were increased in response to noise exposure in our knock-out mice and were not decreased by exercise training and fasting, although for the latter, a clear trend was noticed (*Figure 5A*). Furthermore, the expression of vascular DDAH-2 was almost absent in α1AMPK EC KO mice when compared to WT, which was also not improved by exercise or fasting (*Figure 5B*). Finally, aortic PRMT-1 expression displayed an opposite regulation, as it was dramatically increased in noise-exposed α1AMPK EC KO mice and rather further aggravated, at least by trend, by exercise, but was decreased mainly by fasting (*Figure 5C*). Endothelial α1AMPK deficiency did not change PRMT-1 expression when compared to WT. The loss of antioxidant and vascular protective effects of AICAR in global AMPK knockout mice was previously demonstrated.[28]

Effects of deletion of endothelial-specific adenosine monophosphate-activated protein kinase on antioxidant effects of exercise and fasting in noise-exposed mice

As measured by HPLC-based analysis in aortic tissue and cerebral cortex, we detected increased levels of superoxide in response to noise exposure in our knock-out animals, and mitigation by exercise and fasting failed in the knock-out mice (*Figure 6A and C*; see Supplementary material online, *Figure S3*). Similar results were obtained by mitoSOX/HPLC analysis of isolated cardiac mitochondria, where noise exposure increased mitochondrial superoxide formation. Exercise and fasting had no protective effect on the exacerbation of oxidative stress in the knock-out mice (*Figure 6B and D*).

Discussion

The knowledge of environmental risk factors such as noise exposure and air pollution leading to cardiovascular complications and disease development has increased substantially during the last decades.[1] The alarming data stated by the WHO points to the fact that every year, up to 23% (12,6 million) of global premature deaths can be attributed to environmental risk factors.[40] Here, the most significant contributors (5 million deaths per year) are cardiovascular diseases such as stroke,

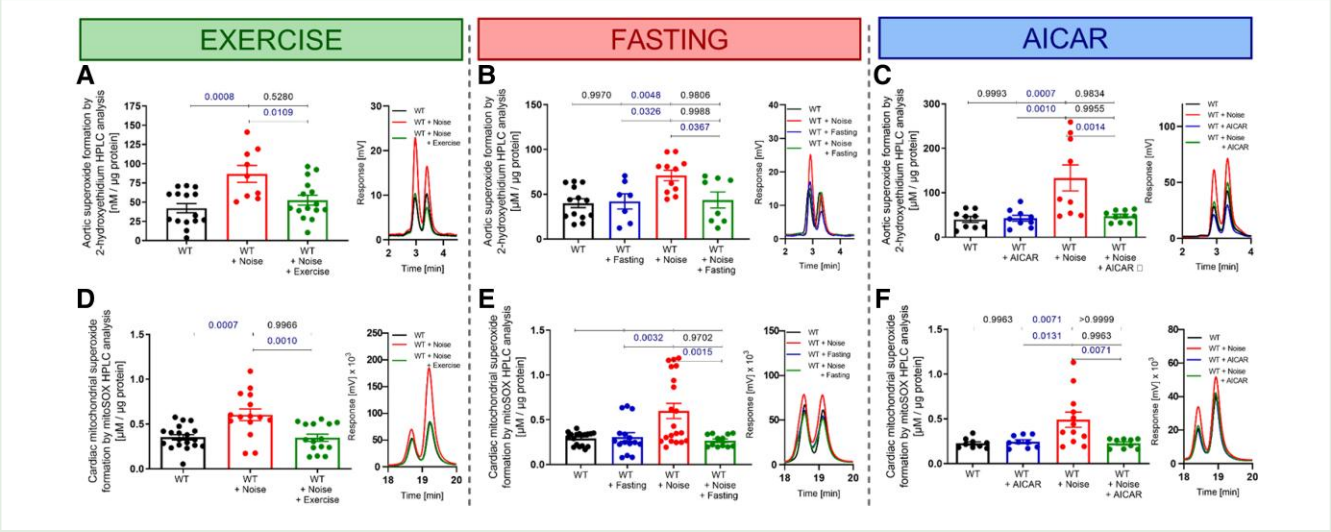


Figure 3 Exercise training, caloric restriction, and 5-aminoimidazole-4-carboxamide riboside administration abolish noise-induced oxidative stress in the aorta. Effect of voluntary exercise training (*A*), fasting (*B*) and 5-aminoimidazole-4-carboxamide riboside administration (*C*) on aortic superoxide production determined by high-performance liquid chromatography-based analysis. Aircraft noise exposure-increased production of mitochondria-specific superoxide production measured by mitoSOX/high-performance liquid chromatography method was prevented by exercise training (*D*), intermittent fasting (*E*), and 5-aminoimidazole-4-carboxamide riboside administration (*F*). Representative chromatograms are shown beside the quantification data. Data are presented as mean ± SEM. AICAR, AICAR, 5-aminoimidazole-4-carboxamide riboside; HPLC, high-performance liquid chromatography.

myocardial infarction, coronary artery disease, arrhythmia, and heart failure. It is undeniable that traffic noise represents one of the most pronounced environmental risk factors in urbanized countries.[41] A meta-analysis of the World Health Organization showed that road traffic noise elevates the incidence of coronary heart disease by 8% per 10-dB(A) increase starting at 50 dB(A).[42] The 10-dB(A) L_{den} increase of traffic noise is also raising the risk of myocardial infarction incidence by 12% [starting at 42 dB(A)][43] and risk of stroke by 14% [starting at 55 dB(A)],[44] concluded by a Danish cohort studies. These findings were also supported by a nationwide study on noise effects on cardiovascular disease in Switzerland.[45,46] Although we studied the detrimental health effects of aircraft noise, it should be noted that road traffic noise is the dominant source of noise, followed by railway noise, since much more people in the EU-28 are exposed to nocturnal noise >50 dB(A) originating from roads (76 million) than railways (17.6 million) or airports (1.3 million) as reported under the EU Environmental Noise Directive (2002/49/EC). Therefore, it is highly important to identify the primordial and primary prevention mechanisms promoting cardiovascular health endangered by environmental risk factors in general and traffic noise exposure in particular. One-third of cardiovascular deaths could be prevented by a healthy lifestyle, as stated by The American Heart Association.[47] Notably, up to 16% of the cardiovascular events and 9% of cardiovascular mortality could be reduced by caloric restriction and a healthy diet modification.[48]

It was shown that the prescription of physical activity represents a highly beneficial preventive/treatment manoeuver against cardiometabolic diseases[49–51] and significantly improves the quality of life measures.[52] An inverse relation was observed between all-cause mortality and increased physical activity.[53] Dose-response (linear) dependence was observed between physical activity and the reduction of BP dysregulation or endothelial dysfunction.[54,55] Moreover, exercise improves the quality of life and physical and mental health in post-stroke patients.[56] Another meta-analysis showed that regular physical activity positively affects mental health, especially in the elderly.[57] Likewise, intermittent fasting decreases cardiometabolic risk markers[58,59] and caloric restriction decreases BP and improves cardiovascular health.[60] These health benefits are based on the activation of cellular stress response elements, improved autophagy, modification of apoptosis, and alteration in hormonal balance.[61]

Several studies substantiated that the beneficial cardiovascular effects of caloric restriction and voluntary exercise depend on intact α1AMPK.[19,21,62] However, corresponding mechanisms have not been fully elucidated, contrary to the growing understanding of the protective effect of exercise and caloric restriction mediated by α1AMPK activation. This knowledge is even more emphasized in conditions of environmental risk factors, where these cardioprotective strategies could be used in the first place. Understanding the pathways influenced by α1AMPK might yield a new and attractive mitigation strategy to protect the general population from noise-induced cardiovascular damage. Especially, patients at high cardiovascular risk, with the pre-existing cardio-metabolic disease, or the elderly could benefit from supervised exercise training and/or caloric restriction to prevent additive cardio/cerebrovascular damage in response to transportation noise exposure.

With our previous study using the same aircraft noise exposure protocol, we were able to establish noise-mediated endothelial dysfunction, elevated BP, cardiovascular and systemic oxidative stress and inflammation, as well as substantial changes in gene networks.[13] In a subsequent study, we demonstrated a central role of the phagocytic NADPH oxidase (NOX-2) in the pathophysiological mechanisms of noise-mediated cardiovascular damage as NOX-2$^{-/-}$ mice were protected. They established cerebral oxidative stress and neuroinflammation as upstream mechanisms of cardiovascular complications.[14] Whereas most cardiovascular damage parameters became more pronounced during the exposure for 1, 2, or 4 days, endothelial dysfunction was already fully developed after 1 day of exposure,[13] in accordance with human data on impaired flow-mediated dilation after one night of aircraft noise exposure.[11] We also conducted long-term noise exposure of mice for up to 28 days, proving that the mice neither became

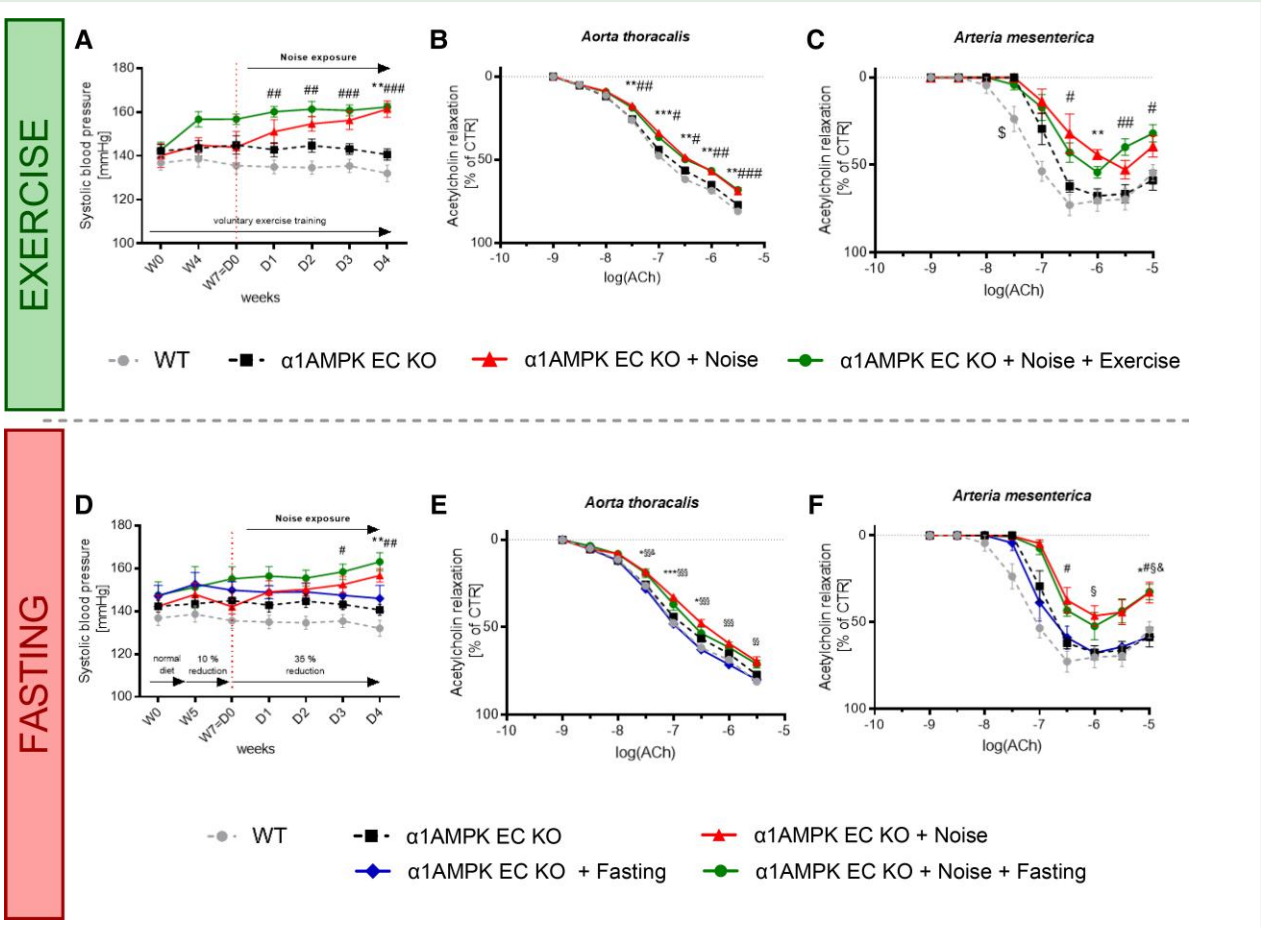


Figure 4 Proof-of-concept studies: genetic deletion of endothelial-specific adenosine monophosphate-activated protein kinase abrogates the beneficial vascular effects of exercise and fasting in noise-exposed mice. (*A*, *D*) Voluntary exercise training ($n = 4$–12) and intermittent fasting ($n = 5$–13) fail to prevent noise-induced blood pressure elevation in endothelial-specific adenosine monophosphate-activated protein kinase knock-out mice. Voluntary exercise training [*B* ($n = 24$–41) and *C* ($n = 6$–7)] and intermittent fasting [*E* ($n = 11$–29) and *F* ($n = 5$–7)] did not ameliorate endothelial dysfunction in the aorta and mesenteric artery of endothelial-specific adenosine monophosphate-activated protein kinase knock-out mice. Unexposed wild-type curves are shown for comparison and were reproduced from *Figure 1* as the sum of all wild-type data for each parameter. The traces of untreated adenosine monophosphate-activated protein kinase EC KO shown for exercise and fasting are the same. Data are presented as mean ± SEM. $P < 0.05$ were considered significant (* = AMPK EC KO vs. Noise; # = AMPK EC KO vs. Noise + Treatment; § = Treatment vs. Noise; & = Treatment vs. Noise + treatment; $ = WT vs. AMPK EC KO). α1AMPK, adenosine monophosphate-activated protein kinase; WT, wild type.

habituated to the noise stress nor developed hearing loss since the oxidative damage parameters, endothelial dysfunction, and high blood pressure remained stable over the entire exposure time.[63] Although animal studies are still pending, there is evidence from human studies that a short-term reduction in aircraft noise exposure levels due to the coronavirus disease 2019 lockdown may reverse the aircraft noise-induced negative effects on arterial stiffness and BP.[64,65]

Therefore, with the present study, we sought to determine α1AMPK-dependent mechanisms of fasting and exercise to evaluate cardiovascular protective pathways against aircraft noise exposure-induced injury. The AMPK is the 'master' energy sensor, activated by several physiological stimuli responding to small AMP/ATP ratio changes, leading to increased ATP consumption and a concomitant rise in cellular AMP.[24,66,67]

It is well-known that physical activity (voluntary exercise training) requires increased energy expenditure and *vice versa*; during fasting (caloric restriction), energy intake is reduced. Therefore, both interventions result in a relevant change in intracellular AMP/ATP ratio, resulting in AMPK activation.[23,68] Furthermore, *in vivo* studies showed that intact AMPK in skeletal muscle is essential for an adequate response to exercise.[69]

Similarly, AMPK could be activated in the cardiovascular system. Chen *et al.*[70] showed for the first time that AMPK could directly phosphorylate eNOS in cardiac capillary endothelial cells and monocytes *in vitro* and during cardiac ischaemia in rats. That is mediated mainly by the α1AMPK isoform, predominantly expressed in the vascular endothelium and smooth muscle cells.[71,72] Vascular α1AMPK is specifically responsible for maintaining vascular homeostasis by modulation of structure and function of endothelial, smooth muscle, and immune cells.[72] Vascular endothelium must constantly react to several physiological stimuli (shear stress) to modulate vascular tone and regulate tissue-specific metabolic demand. Laminar blood flow increases eNOS activation, phosphorylated by α1AMPK. Previously, we have shown that endothelium-specific α1AMPK deletion reduces expression and/or activation of eNOS (Ser1177 phosphorylation), resulting in increased oxidative stress.[19]

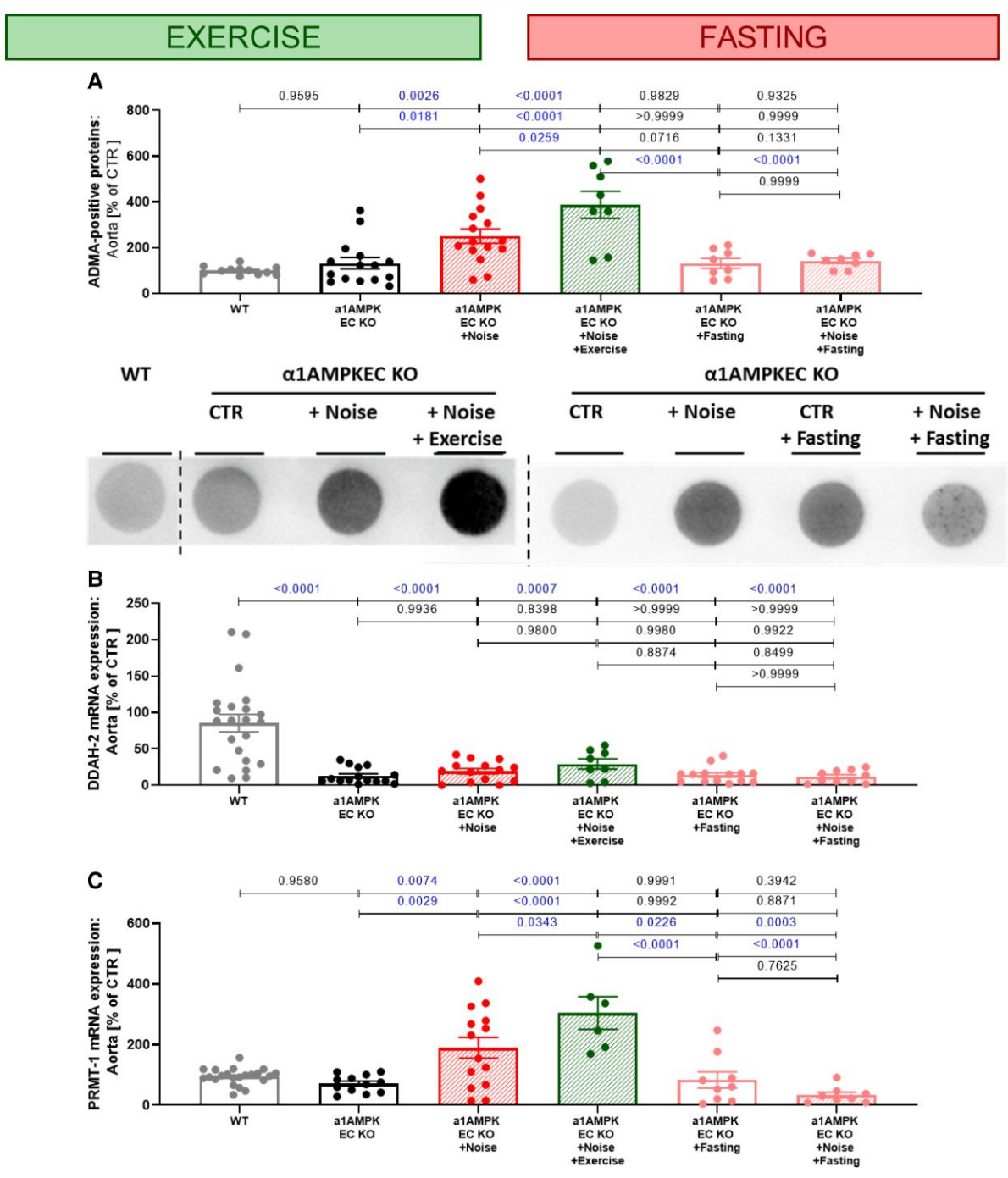


Figure 5 Proof-of-concept studies: genetic deletion of endothelial-specific adenosine monophosphate-activated protein kinase abrogates prevention of the noise-mediated increase in asymmetric dimethylarginine levels. Voluntary exercise training also failed to reduce higher asymmetric dimethylarginine levels in endothelial-specific adenosine monophosphate-activated protein kinase knock-out mice, whereas fasting showed a stable trend of reduction (*A*). Levels of dimethylarginine dimethylaminohydrolase 2 mRNA were not significantly changed after either noise exposure or combination of noise exposure and exercise or fasting treatments in aortic tissue of endothelium-specific adenosine monophosphate-activated protein kinase knock-out mice (*B*). Levels of type 1 protein arginine N–methyltransferase mRNA were elevated after noise exposure and returned to control values after intermittent fasting, but not after exercise treatment in aortic tissue of endothelium-specific adenosine monophosphate-activated protein kinase knock-out mice (*C*). Representative dot blot images are shown. Data are presented as mean ± SEM. α1AMPK, adenosine monophosphate-activated protein kinase; ADMA, asymmetric dimethylarginine; DDAH2, dimethylarginine dimethylaminohydrolase 2; PRMT-1, type 1 protein arginine N-methyltransferase.

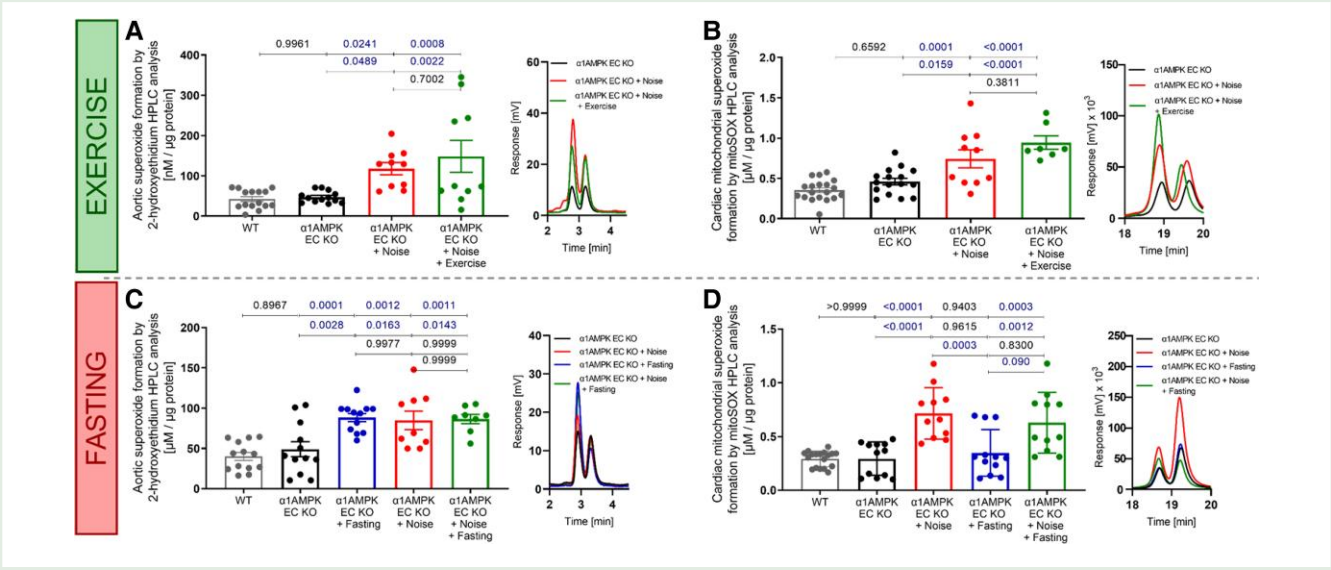


Figure 6 Proof-of-concept studies: genetic deletion of endothelial-specific adenosine monophosphate-activated protein kinase abrogates the beneficial antioxidant effects of exercise and fasting in noise-exposed mice. Voluntary exercise training failed to prevent noise-induced superoxide production (*A*) and mitochondrial superoxide formation (*B*). Also, intermittent fasting did not counteract noise-induced superoxide in the aorta (*C*) and mitochondria-specific production of superoxide (*D*) as well as in endothelial-specific adenosine monophosphate-activated protein kinase knock-out mice. Besides the quantification data, representative high-performance liquid chromatography chromatograms are shown. Data are presented as mean ± SEM. α1AMPK, adenosine monophosphate-activated protein kinase; HPLC, high-performance liquid chromatography.

Moreover, activating endothelial α1AMPK with AICAR is essential for attenuating oxidative endothelial injury caused by angiotensin II (AT-II) infusion.[28] We observed that endothelial α1AMPK plays a crucial role in vascular inflammation. The recruitment of inflammatory cells to the vascular wall is limited by endothelial α1AMPK expression and is closely related to the vascular redox status regulated by α1AMPK. During AT-II treatment, pro-inflammatory cytokines and oxidative stress lead to vascular dysfunction. Interestingly, this effect was aggravated in mice with endothelium-specific α1AMPK deletion compared to wild-type controls.[25] Likewise, we know that noise causes inflammation[13,14] by exacerbated infiltration of LysM-positive immune cells,[15] where immune cell activation, endothelial adhesion, and pro-inflammatory phenotype of the plasma proteome also play a central role in endothelial damage and microvascular dysfunction.[73] Recently, array experiments have demonstrated changes in inflammatory clusters of cytokines and chemokines by noise exposure of mice.[74] Therefore, the anti-inflammatory effects of exercise,[75] fasting,[76] and AICAR[77] may help to prevent noise-mediated cardiovascular damage as well as development and faster progression of atherosclerosis as driven by activated/infiltrated myelomonocytic cells.

Furthermore, we were able to highlight the vascular protective effects of α1AMPK in ROS-mediated vascular dysfunction and inflammation using tissue-specific knock-out models of α1AMPK. The deletion of α1AMPK resulted in aggravation of vascular ROS production at least by trend, which was the known protective and anti-inflammatory effects of α1AMPK.[25,78,79] The AMP analogue, 5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside (AICAR), was previously the most frequently used drug in animals to induce the pharmacological activation of α1AMPK. This results in reduced vascular oxidative stress and inflammation shown in an animal model of arterial hypertension.[28] Clinically, recent data suggest that metformin's antidiabetic drug mediates its beneficial cardioprotective effects, at least in part, via AMPK activation.[80] Thus, global, or cell-specific activation of α1AMPK could represent a novel preventive strategy against cardiovascular complications caused by environmental risk factors such as noise exposure. Although some of the ROS-related beneficial effects of α1AMPK activation on the cardiovascular system are known, the exact mechanisms and their role during noise exposure have not been addressed.

The protective/beneficial role of α1AMPK is undeniable and its importance is even more highlighted during vascular oxidative stress. That suggests that activation of α1AMPK physiologically (by exercise or fasting) or pharmacologically (AICAR, shown previously in global AMPK knockout mice[28]) could be used as a preventive strategy against aircraft noise-induced injury. The crucial role of endothelial-specific α1AMPK during voluntary exercise-mediated vascular protection was studied by our group as well. We demonstrated that the presence of the α1AMPK is essential during exercise, where its expression affects not only endothelial function (NO production, eNOS coupling) but also oxidative stress release. Exercise training increased the expression of NOX-2 in endothelial-specific α1AMPK knock-out mice, resulting in increased vascular superoxide production. On the other hand, Nrf-2-dependent antioxidant defence mechanisms were suppressed in mice lacking endothelial α1AMPK.[81] In this regard, translational studies with measurements of individual α1AMPK in endothelial precursor cells might help to understand the variation in existing stress and exercise tolerance among patients suffering from cardiovascular disease.

Our present data demonstrate that aircraft noise-mediated endothelial dysfunction and associated BP elevation could be normalized and/or preserved by α1AMPK activation. Moreover, the beneficial effect of voluntary exercise training and caloric restriction was endothelial α1AMPK-dependent. Undeniably, the endothelium's physiological function is closely linked to redox balance. Increased ROS production and/or insufficient antioxidant response impair endothelial function. Our previous study has shown that almost exclusively, nocturnal aircraft noise exposure triggers pathomechanisms (NO signalling disruption—eNOS uncoupling, oxidative stress, supersensitivity to vasoconstrictors, cardiovascular inflammation, etc.), causing vascular injury. Here, oxidative stress, predominantly driven by vascular NOX-2,

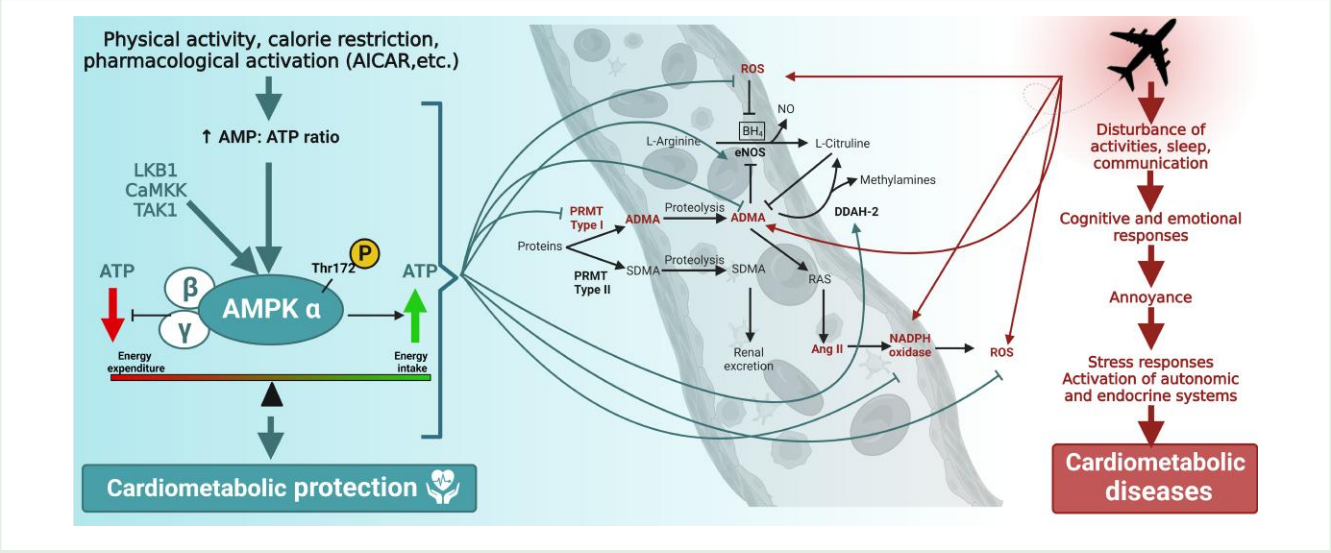


Figure 7 Mitigation of aircraft noise-induced vascular dysfunction and oxidative stress adenosine monophosphate-activated protein kinase activation—graphical summary. Aircraft noise exposure as an environmental risk factor disrupts cognitive and emotional responses resulting in activation of stress response mechanisms and activation of autonomic and endocrine systems as cardiovascular risk factor leads to cardiometabolic diseases development via increased production of reactive oxygen species, produced primarily by nicotinamide adenine dinucleotide phosphate oxidase, followed by endothelial nitric oxide synthase uncoupling, and reduction of nitric oxide. Noise affects the production of asymmetric dimethylarginine via its increased production mediated by arginine methylation catalyzed by protein arginine methyltransferase 1 and decreased degradation by dimethylaminohydrolase 2. The increased level of asymmetric dimethylarginine inhibits endothelial nitric oxide synthase and activates the renin-angiotensin system, resulting in nicotinamide adenine dinucleotide phosphate oxidase-produced reactive oxygen species levels elevation. Physical activity, caloric restriction, and pharmacological activation by 5-aminoimidazole-4-carboxamide ribonucleotide activate adenosine monophosphate-activated protein kinase. This protective enzyme mitigates aircraft noise-induced vascular dysfunction and oxidative stress via reactive oxygen species elimination by antioxidant mechanisms. Moreover, adenosine monophosphate-activated protein kinase decreases asymmetric dimethylarginine levels by type 1 protein arginine N–methyltransferase reduced expression and increased expression of dimethylaminohydrolase 2. ADMA, asymmetric dimethylarginine; ADP, adenosine diphosphate, AICAR, 5-aminoimidazole-4-carboxamide ribonucleotide (AMP analogue); AMP, adenosine monophosphate; AMPK, adenosine monophosphate-activated protein kinase; Ang II, angiotensin II; ATP, adenosine triphosphate; BH4, tetrahydrobiopterin; DDAH-2, dimethylaminohydrolase 2; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; PRMT, protein arginine methyltransferase; RAS, renin-angiotensin system; ROS, reactive oxygen species; SDMA, symmetric dimethylarginine; NADPH, nicotinamide adenine dinucleotide phosphate.

played a crucial role,[14] since the deletion of NOX-2 almost completely prevented the aircraft noise-induced cardiovascular and cerebral side effects. The vital role of oxidative stress has been proven by vitamin C administration, which was able to re-establish the physiological function of the endothelium and reduce vascular damage caused by aircraft noise exposure.[9,11,13] The present studies demonstrated that aircraft noise initiates oxidative stress by NOX-2-derived increased ROS production. The presented data of our study suggest that oxidative stress development can be rescued by α1AMPK activation (AICAR, voluntary exercise, and fasting). Whereas for exercise and fasting, we have shown in the present study that AMPK EC KO abrogates their beneficial effects on noise-mediated damage; the principle of action of AICAR via AMPK activation was previously demonstrated in a sepsis model with severe inflammation and oxidative stress.[28] Intact α1AMPK, pharmacologically or physiologically activated by these manoeuvers, decreased cardiovascular ROS production and reduced expression of NOX-2, resulting in more negligible oxidative protein damage/modification (3-NT).

ADMA inhibits eNOS activity by competitive inhibition along with modulated phosphorylation and/or modified gene expression.[38,39] Interestingly, the AMPK activator, metformin, has a similar structure to ADMA. It was proven that this structural analogue could reduce the ADMA level by DDAH-2-dependent degradation, resulting in increased NO bioavailability *in vivo*.[82] In this study, we noticed that increased ADMA accumulation caused by noise-induced PRMT-1-catalyzed arginine methylation could be decreased by these α1AMPK-dependent mechanisms. However, crosstalk between α1AMPK and ADMA production and/or degradation is not adequately investigated. Despite the knowledge about PRMTs and DDAHs in skeletal muscle, their interplay with α1AMPK activation in the vasculature is not very well known.

Although our mouse model uses an average sound pressure level of 72 dB(A), it is close enough to the exposure levels of the population living nearby railways [often up to 65 dB(A)] or main roads [often up to 60 dB(A)][83] and far below the sound pressure levels used by most of the animal studies on noise health effects [usually >100 dB(A)].[84] In addition, our mouse model of noise exposure is a short-term exposure protocol, which requires somewhat higher noise levels to see effects that in the population can accumulate over years. Also, a direct translation of the selected models into a human setting is not possible due to the complex interactions between noise exposure in the natural environment, which is often of chronic nature, and the characteristics of the subjects. Although there are studies showing that e.g. certain dietary patterns[85] and exercise[86] may be beneficial when it comes to the risk of noise-induced hearing loss, there are no manifest clinical guidelines quantifying the amount of caloric restriction or exercise. However, this is an important point that should be addressed in future human experimental and observational studies.

Conclusions

The present study demonstrates for the first time that exercise, fasting (caloric restriction), and pharmacological stimulation of the α1AMPK subunit effectively mitigate aircraft noise-induced cardiovascular adverse side effects (see *Figure 7* for summary). The detrimental health effects of noise and the protection against noise-mediated damage by fasting and AICAR therapy were also mirrored by RNA sequencing data and related bioinformatical analyses (for exercise, at least by trend). Moreover, genetic deletion of endothelial α1AMPK reversed these effects in certain treatment groups (exercise-mediated protection was almost completely lost, whereas fasting-dependent beneficial effects were partially lost), suggesting that the endothelial α1AMPK almost exclusively mediates these beneficial effects. Further, mainly prospective clinical trials need to prove now whether these strategies can effectively counteract the adverse cardiovascular and cerebral consequences of aircraft noise. According to previous data, the mode of activation of AMPK and subsequent signalling is comparable in human and experimental models. For example, activation of AMPK following exercise has been demonstrated in skeletal muscle from rodents and humans.[87,88] There have been also studies showing AMPK activation by various compounds in both human and animal cells, including endothelial cell lines.[28,89,90] In addition, in primary human and mouse hepatocytes, AMPK activation by direct and indirect activators promotes the phosphorylation of conserved downstream targets to a similar extent.[26,91] Of note, human translation of AMPK activation has been recently supported by phase 1b clinical trials[92] following promising results obtained in pathological mouse models.[93] We, therefore, conclude that our present findings can be extrapolated to humans exposed to traffic noise.

Authors' contributions

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Supplementary material

Supplementary material is available at *European Journal of Preventive Cardiology*.

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Conflict of interest: None declared.

Data availability

All data described in the manuscript are contained within this article and online supplement. Raw data are available from the corresponding author upon reasonable request.

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