

Exposure to aircraft noise exacerbates cardiovascular and oxidative damage in three mouse models of diabetes

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Aims

Epidemiology links noise to increased risk of metabolic diseases like diabetes and obesity. Translational studies in humans and experimental animals showed that noise causes reactive oxygen species (ROS)–mediated cardiovascular damage. The interaction between noise and diabetes, specifically potential additive adverse effects, remains to be determined.

Methods and results

C57BL/6 mice were treated with streptozotocin (i.p. injections, 50 mg/kg/day for 5 days) to induce type 1 diabetes mellitus, with S961 (subcutaneous osmotic mini-pumps, 0.57 mg/kg/day for 7 days) or fed a high-fat diet (HFD, 20 weeks) to induce type 2 diabetes mellitus. Control and diabetic mice were exposed to aircraft noise to an average sound pressure level of 72 dB(A) for 4 days. While body weight was unaffected, noise reduced insulin production in all diabetes models. The oral glucose tolerance test showed only an additive aggravation by noise in the HFD model. Noise increased blood pressure and aggravated diabetes-induced aortic, mesenteric, and cerebral arterioles' endothelial dysfunction. ROS formation in cerebral arterioles, the aorta, the heart, and isolated mitochondria was consistently increased by noise in all models of diabetes. Mitochondrial respiration was impaired by diabetes and noise, however without additive effects. Noise increased ROS and caused inflammation in adipose tissue in the HFD model. RNA-sequencing data and alteration of gene pathway clusters also supported additive damage by noise in the setting of diabetes.

Conclusion

In all three models of diabetes, aircraft noise exacerbates oxidative stress, inflammation, and endothelial dysfunction in mice with pre-existing diabetes. Thus, noise may potentiate the already increased cardiovascular risk in diabetic patients.

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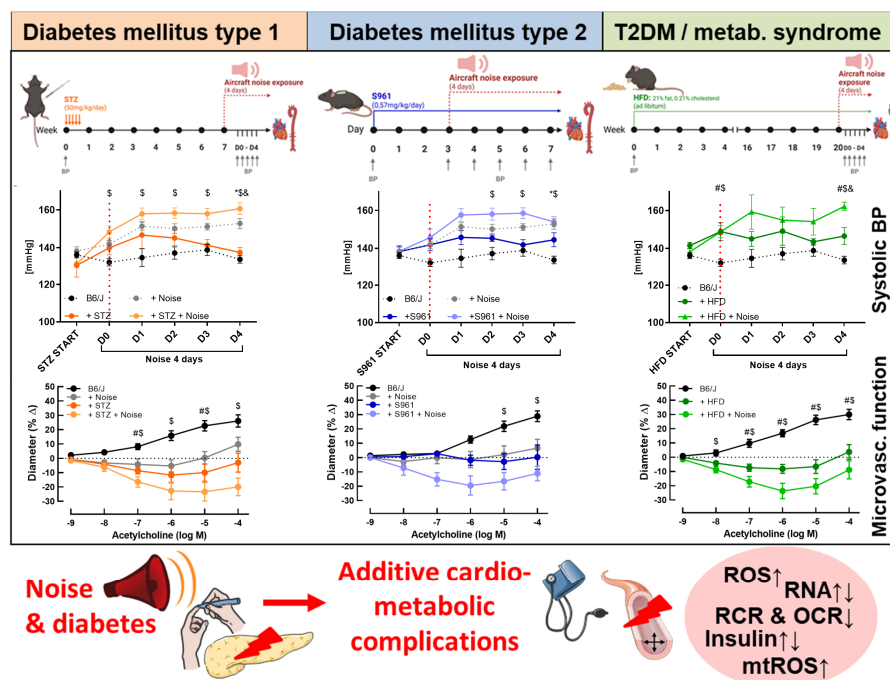
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Lay summary

Traffic noise significantly contributes to an increased risk of cardiometabolic diseases (including diabetes and obesity) in the general population via stress hormones, inflammation, and oxidative stress, all of which contribute to impaired vascular function and high blood pressure. However, the extent to which noise affects pre-existing diabetes is not sufficiently explained, which prompted us to investigate the molecular mechanisms responsible for noise-mediated exacerbation of cardiometabolic complications in three different animal models with diabetes mellitus:

- Noise exposure in diabetic mice caused further impairment of insulin signalling, increased blood pressure, and damage of small and large blood vessels as well as oxidative stress in the aorta, brain, and heart.
- Our functional observations were supported by gene analyses indicating combined effects of noise and diabetes on gene groups related to inflammation and metabolism, suggesting a need for further studies in humans to investigate how noise impacts cardiovascular risk in vulnerable groups such as patients with diabetes.

Graphical Abstract



Keywords

Noise exposure • Diabetes • Cardiovascular complications • Oxidative stress

Introduction

In recent years, transportation noise has been identified as a potential cardiovascular risk factor that is, according to the World Health Organization, associated with the loss of around 1.6 million healthy life-years in Europe annually.^{1,2} Transportation noise from roads, railways, and aircraft stimulates stress hormone release and increases blood pressure, especially when exposed at night.³ Like other cardiovascular risk factors, noise induces endothelial dysfunction and increases vascular oxidative stress and inflammation, especially in the cerebrovascular axis.^{4,5} Previously, we were able to identify the activation of phagocytic NADPH oxidase (NOX-2) and uncoupling of endothelial nitric oxide

synthase (eNOS) as key enzymes mediating aircraft noise-induced oxidative stress damage, especially in response to noise exposure during sleep phase.^{6,7} Oxidative stress and endothelial dysfunction in response to night-time noise exposure were also established in humans.^{8,9}

Analogous to type 2 diabetes mellitus (T2DM), reduced insulin sensitivity, impaired glucose regulation, dysregulation of appetite-regulating hormones, and inflammation processes have been reported to be the consequences of transportation noise exposure.¹⁰ Epidemiological studies demonstrated a link between long-term exposure to road, railway, and possibly aircraft traffic noise and T2DM.¹¹ This was also demonstrated in a meta-analysis, which showed that a 5 dB(A) increase in the intensity of traffic noise caused a 6% increased risk for diabetes.¹²

At the molecular level, mitochondrial dysfunction has been demonstrated as a key mechanism of cardiovascular damage for both aircraft noise and diabetes.^{13,14} We also demonstrated a relevant increase in blood glucose levels after 1 day of exposure to aircraft noise, resulting in a pre-diabetic phenotype.⁷ Importantly, co-exposure to different environmental stressors, e.g. road traffic noise, air pollution, and lack of green space, was reported to increase the risk of diabetes in a cumulative manner.¹⁵

Despite substantial overlaps, the cardiovascular risk factors, noise, and diabetes, have largely been examined separately, especially within basic research contexts. Based on this research gap, as well as previous experimental results and epidemiological studies, the present study aims to explore the hypothesis that noise exposure and diabetes share common signalling pathways and can cause additive damage to cardiovascular and metabolic function across various models of diabetes mellitus and metabolic syndrome.

Methods

Animal models

All animal experiments within this 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 and the Landesuntersuchungsamt Rheinland-Pfalz (Koblenz, Germany, permit number 23177-07/G 15-1-094 and G20-1-103). We used C57BL/6j mice ordered from Janvier (Le Genest-Saint-Isle, France) for the study. Animals (only male, 7–26 weeks old, depending on the diabetes model) were exposed to aircraft noise [maximum sound pressure level of 85 dB(A), average sound pressure level of 72 dB(A)] as described previously for the last 4 days of the treatment.⁶ Type 1 diabetes was induced by intraperitoneal injections of streptozotocin (STZ, Sigma S0130, 50 mg/kg/day in citrate buffer, 5 mM pH 4.5) for 5 days. The control animals were injected with the same volume of citrate buffer. To induce T2DM, we treated the animals with S961 (high-affinity and selective insulin receptor antagonizing peptide from Novo Nordisk, 0.57 mg/kg/d in 0.9% NaCl containing 10% DMSO), administered via subcutaneous osmotic mini-pumps (Alzet, 1007D, 0.5 μ L/h) for 7 days, adapted from a previous protocol.¹⁶ The control groups received osmotic mini-pumps containing the solvent (0.9% NaCl with 10% DMSO). Metabolic syndrome/T2DM was induced by feeding the animals a high-fat diet (HFD) [ssniff (#E15721-34), 42% energy from fat (21.1% of total food composition), 0.21% cholesterol, *ad libitum*] for 20 weeks.¹⁷ The control animals were fed standard rodent chow [ssniff (#V1126-000), 12% energy from fat (4.8% of total food composition), *ad libitum*] for the entire duration of the experiment. 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, spleen, mesenteric artery, epididymal white adipose tissue (eWAT)]. In addition, blood samples were obtained by heart puncture using heparin and K₂EDTA.

Details on the following methods can be found in the [Supplementary material](#): blood glucose and oral glucose tolerance test (OGTT), non-invasive blood pressure measurements, plasma insulin, isometric tension studies, microvascular function and oxidative stress measurement in cerebral arterioles, oxidative stress detection in the aorta and heart, mRNA expression analysis in eWAT, mitochondrial respiration measurement, RNA-sequencing, and bioinformatical analysis.

Statistical analysis

Statistical analysis used Prism for Windows, version 9, GraphPad Software Inc. (GraphPad Software LLC, La Jolla, CA). Normal distribution of data was estimated from QQ-plots. Statistical analysis was performed using one-way ANOVA for all data sets with three groups (the HFD model) and two-way ANOVA for all data sets with four groups and two independent factors (the STZ and S961 models). Three-way ANOVA test was used for all time or concentration (repeated measures) curves with four groups and two independent factors (the STZ and S961 models). In contrast, two-way ANOVA was used for all time or concentration (repeated measures) curves with three groups (the HFD model). *Post hoc* testing was performed using Tukey's correction to compare multiple means. All presented results are expressed as the mean \pm SEM, and the *n* for every independent experiment is indicated. Box plots are presented with the min/max values. *P* < 0.05 were considered significant.

Results

Noise exposure does not affect basic body and organ characteristics

Aircraft noise exposure did not affect the body weight of the animals, while their body weight was significantly decreased by STZ + noise and S961-alone treatment (see [Supplementary material online, Figure S1A and B](#)) and increased by the HFD (see [Supplementary material online, Figure S1C](#)). The lower body weight of STZ- and S961-treated mice is compatible with impaired glucose handling due to the absence of insulin secretion or inhibition of insulin receptors with subsequent fat burning in these models. No significant changes in liver weight were found for STZ and S961 treatment (see [Supplementary material online, Figure S1D and E](#)), but liver weight and size were increased in HFD-treated animals (see [Supplementary material online, Figure S1F](#)). The heart weight of the STZ-treated animals decreased significantly compared with control animals but was unchanged for the other models and noise exposure (see [Supplementary material online, Figure S2A–C](#)). Some minor changes in the weight of the spleen were observed in the S961 and HFD models, whereas noise exposure had no effect (see [Supplementary material online, Figure S2D–F](#)).

Exposure to aircraft noise interferes with insulin signalling and partially with glucose metabolism in diabetes mellitus

We observed no significant effect on insulin levels in control animals exposed to noise ([Figure 1D and E](#)). As expected, animals treated with STZ and without noise had significantly lower insulin levels than control ([Figure 1D](#)). The inhibition of insulin receptors by S961 caused a compensatory hyperinsulinemia which was slightly decreased upon noise exposure, which was also supported by the main effects analysis suggesting an interaction of noise and S961 ([Figure 1E](#)). High-fat diet treatment also initiated hyperinsulinemia that was significantly lowered in response to noise ([Figure 1F](#)). Noise alone did not alter the non-fasting blood glucose levels, while an increase in blood glucose was observed by induction of diabetes in all models, with an additive increase in HFD mice ([Figure 1G–I](#)). The OGTT measurement showed significantly elevated fasting glucose levels before administering glucose and a strongly elevated kinetic curve of blood glucose levels after administration in the STZ and S961 diabetes models but not in HFD mice ([Figures 1J–L](#)). Noise exposure had no effect on the glucose levels in STZ and S961 models ([Figures 1J,K](#)).

Diabetes mellitus type 1

Diabetes mellitus type 2

Diabetes mellitus type 2/
Metabolic syndrome

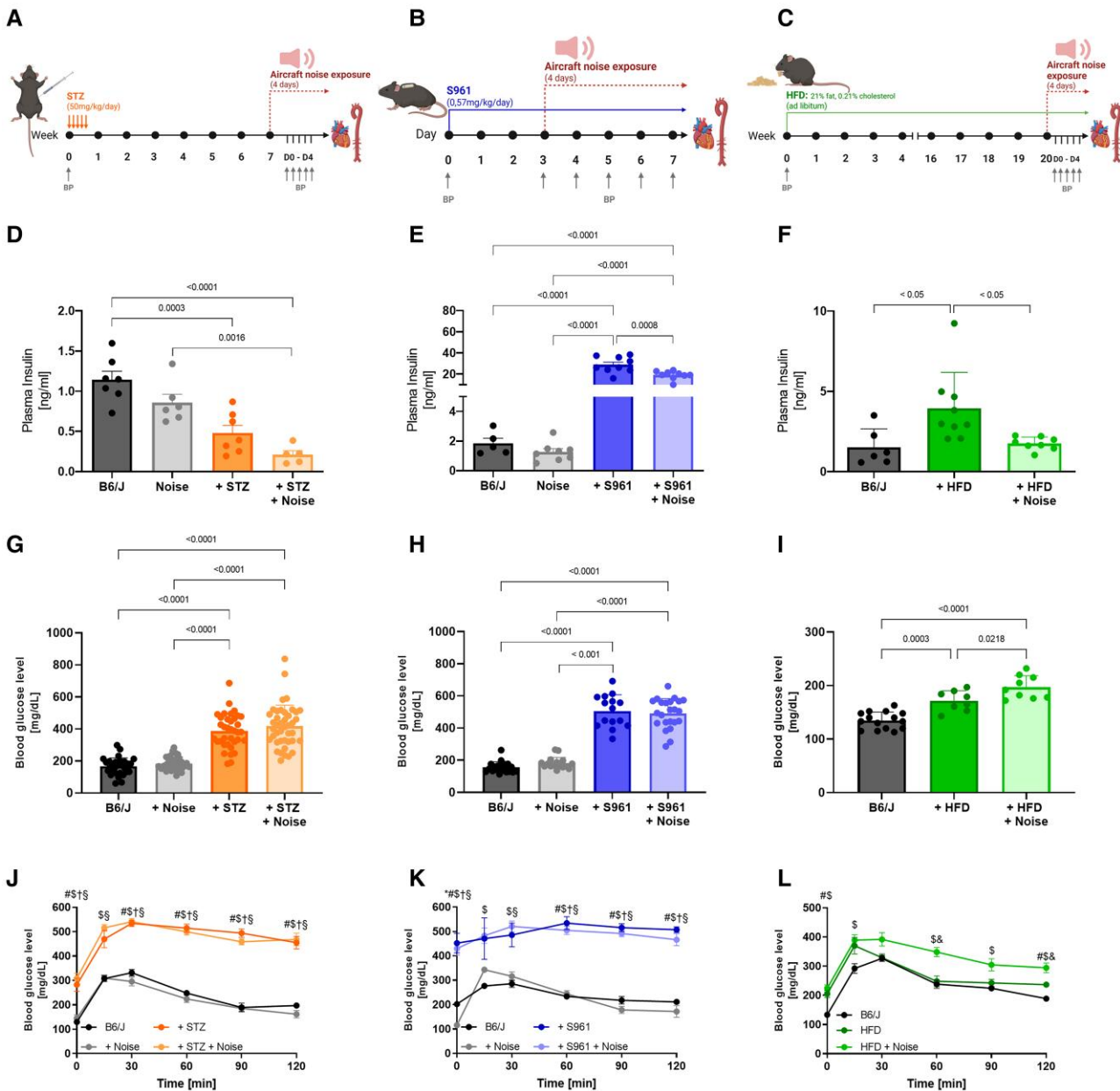


Figure 1 The effects of aircraft noise exposure on insulin signalling and glucose metabolism in diabetes. (A–C) Schemes of the treatment protocols and blood pressure measurements: streptozotocin (A), S961 (B), and high-fat diet (C) model. (D–F) Plasmatic insulin levels were determined using an ELISA Assay Kit. (G–I) Non-fasting blood glucose levels were measured by a glucometer from tail vein blood. (J–L) Oral glucose tolerance test shows glycaemia of fasting animals at basal level (before administration of glucose) and 30, 60, 90 and 120 min after administration of glucose. The quantification (area under the curve) is shown in [Supplementary material online, Figure S2](#). (A–C) Each data point represents a measurement from blood plasma pooled from three to six animals [D (n = 5–7), E (n = 5–9), F (n = 6–9)]. (G–I) Each data point represents an individual animal [G (n = 32–42), H (n = 15–23), I (n = 8–15)] and data in (J–L) are the mean of n = 6–18 (J), n = 3–15 (K) and n = 5–10 (L) independent measurements. For statistical analysis, we used two-way ANOVA for the bar graphs with four groups and two independent factors (D, E, G, H) and one-way ANOVA for the bar graphs with 3 groups (F, I). For the time curves with four groups and two independent factors, we used three-way ANOVA (J, K). For the time curves with three groups, we used two-way ANOVA (L). P < 0.05 were considered significant. (* = B6/J vs. Noise; # = B6/J vs. Diabetes; \$ = B6/J vs. Diabetes + Noise; † = Noise vs. Diabetes; ‡ = Noise vs. Diabetes + Noise; § = Diabetes vs. Diabetes + Noise; & = Diabetes vs. Diabetes + Noise; Diabetes = STZ/S961/HFD; OGTT = oral glucose tolerance test). Main effects: (D) noise *, STZ ****, (E) noise **, S961 ****, noise x S961 *, (G) STZ ****, (H) S961 ****, (J) time ****, STZ ****, time x STZ ****, (K) time ****, S961 ****, time x S961 **. *P < 0.05, **P < 0.01, ****P < 0.0001. The treatment schemes were generated with biorender.com.

In HFD animals, noise increased OGTT kinetics compatible with increased insulin resistance (Figure 1L). This effect is supported by the respective areas under the curve (AUC) (see [Supplementary material online, Figure S2G–I](#)).

Aircraft noise exposure has an additive effect on diabetes-induced development of hypertension and endothelial dysfunction of the aorta

Induction of diabetes by STZ or S961 alone did not modify blood pressure (BP) compared with wild-type animals; only in the HFD group a BP increase was observed. Noise exposure caused hypertension in control animals (Figure 2A and B). Aircraft noise exposure in diabetic animals caused a significant increase in BP compared with the diabetes-alone groups in the STZ and HFD models (Figure 2A and C). The main effects analysis suggested the effects of noise and diabetes on BP. Noise exposure and the condition of diabetes caused, as shown before,^{6,18} an impairment of the endothelial function, either indicated by group comparisons or by primary effects analysis (Figure 2D–F). Exposure of diabetic animals to aircraft noise exacerbated endothelial dysfunction in all diabetic models tested, although an additive effect was only supported by main effects analysis in the STZ model (Figure 2D–F). The EC₅₀ values showed a similar trend, however only reaching significant differences between the untreated control and the treatment groups in the STZ and S961 models.

Diabetes-induced endothelial dysfunction of small resistant vessels and microvasculature is further aggravated after exposure to aircraft noise

To assess the function of small resistant vessels and microvasculature, we measured the endothelial function of the mesenteric artery (Figure 2G–I) and cerebral arterioles (Figure 3A–C). Noise exposure and the condition of diabetes significantly worsened the relaxation of the mesenteric artery (Figure 2G and H) and cerebral arterioles (Figure 3A–C) compared with the control (Figures 2G–I and 3), as shown by group comparisons or main effects analysis. In all investigated models, microvascular function tended to deteriorate in mesenteric arteries as compared with noise- and diabetes-alone groups when diabetic mice were exposed to noise (Figure 2G–I). The EC₅₀ values showed a similar trend, however only reaching significant differences between the untreated control and the treatment groups in the STZ and S961 models. For cerebral arterioles, we established an even clearer trend of additive impairment of endothelial function by noise plus diabetes in all three models, supported by main effects analysis in the S961 model (Figure 3A–C). Endothelium-independent constriction by a thromboxane A₂ receptor agonist (U46619) and endothelium-independent but NO-dependent relaxation induced by sodium nitroprusside (SNP) were modified neither by noise nor by diabetes (see [Supplementary material online, Figure S3](#)). As a possible explanation of the pronounced exacerbation of microvascular dysfunction by noise in cerebral arterioles of diabetic mice, we found a correlating pattern of vascular ROS formation (Figure 3D–F).

Exposure to aircraft noise has an additive effect on increased superoxide formation caused by diabetes mellitus

Superoxide formation in aortic or cardiac tissue was increased by noise or diabetes, which was supported by main effects analysis. A further

additive increase by noise plus diabetes was observed in at least one of these tissues in the STZ and S961 mice (Figure 4A–H). In the HFD model, only the noise-exposed group with diabetes showed significantly increased superoxide levels (Figure 4I–L).

Adipose tissue dysfunction in diabetes is aggravated after aircraft noise exposure

There is evidence that adipose tissue inflammation and dysfunction (adverse adipokine signalling) leads to insulin resistance and T2DM.¹⁹ Our data demonstrate that HFD treatment causes increased levels of oxidative stress markers, NOX2 and p22phox (p67phox and p47phox only by trend), as well as inflammation markers, TNF α (PAI-1 only by trend) in the eWAT (Figure 4M). All of these markers were significantly increased in HFD mice plus noise compatible with an additive effect of aircraft noise on adipose tissue inflammation and oxidative stress pathways (Figure 4M). In contrast, no significant changes were established in adipose tissues of STZ or S961 mice exposed to noise (data not shown).

Elevated mitochondrial oxidative stress levels and mitochondrial dysfunction caused by diabetes are further impaired after aircraft noise exposure

We also measured mitochondria-specific superoxide formation in cardiac tissue (Figure 5A, C, and E). We could demonstrate a stepwise increase in mitochondrial superoxide production in noise-exposed and diabetic groups (STZ and HFD mice). Noise exposure plus diabetes significantly increased the mitochondrial superoxide levels compared to diabetic conditions alone, which was for STZ also supported by main effects analysis. Furthermore, by determining oxygen consumption rate (OCR) in a mitochondrial coupling assay, we analysed the degree of coupling between the electron transport chain (ETC), and the oxidative phosphorylation machinery (OXPHOS) concerning mitochondrial function/dysfunction in isolated cardiac tissue and calculated the respiratory control ratio (RCR). We found a markedly decreased OCR in diabetic animals in state III for the STZ model and all states of mitochondrial respiration for S961 and HFD models, however without a clear noise-alone effect (Figure 5B, D, and F; [Supplementary material online, Figure S2J–L](#)). No additive effect by noise was observed in any of the diabetes models. Of note, the RCR was decreased after noise exposure, as also reflected by the total RCR data including all controls and noise-exposed mice (see [Supplementary material online, Figure S2M–P](#)). However, we observed no additive noise effects on RCR in diabetic mice.

Dysregulation of inflammation markers, lipid biosynthesis and metabolism pathways, oxidative phosphorylation, and circadian rhythm after aircraft noise exposure revealed by RNA-sequencing

Analysing the RNA-seq data, we observe a consistent increase in inflammation-associated pathways such as the TNF, IL-17, AGE-RAGE, TGF- β , and cytokine–cytokine receptor signalling pathways (summarized in Figure 6; for a detailed analysis, see [Supplementary material online, Figure S4–S6](#)). We also established collective regulation of oxidative phosphorylation (Figure 6), where it was downregulated in mice that were diabetic and further dysregulated in mice that were subjected to diabetes and exposed to aircraft noise. Furthermore, we observe a dysregulation in

Diabetes mellitus type 1

Diabetes mellitus type 2

Diabetes mellitus type 2/
Metabolic syndrome

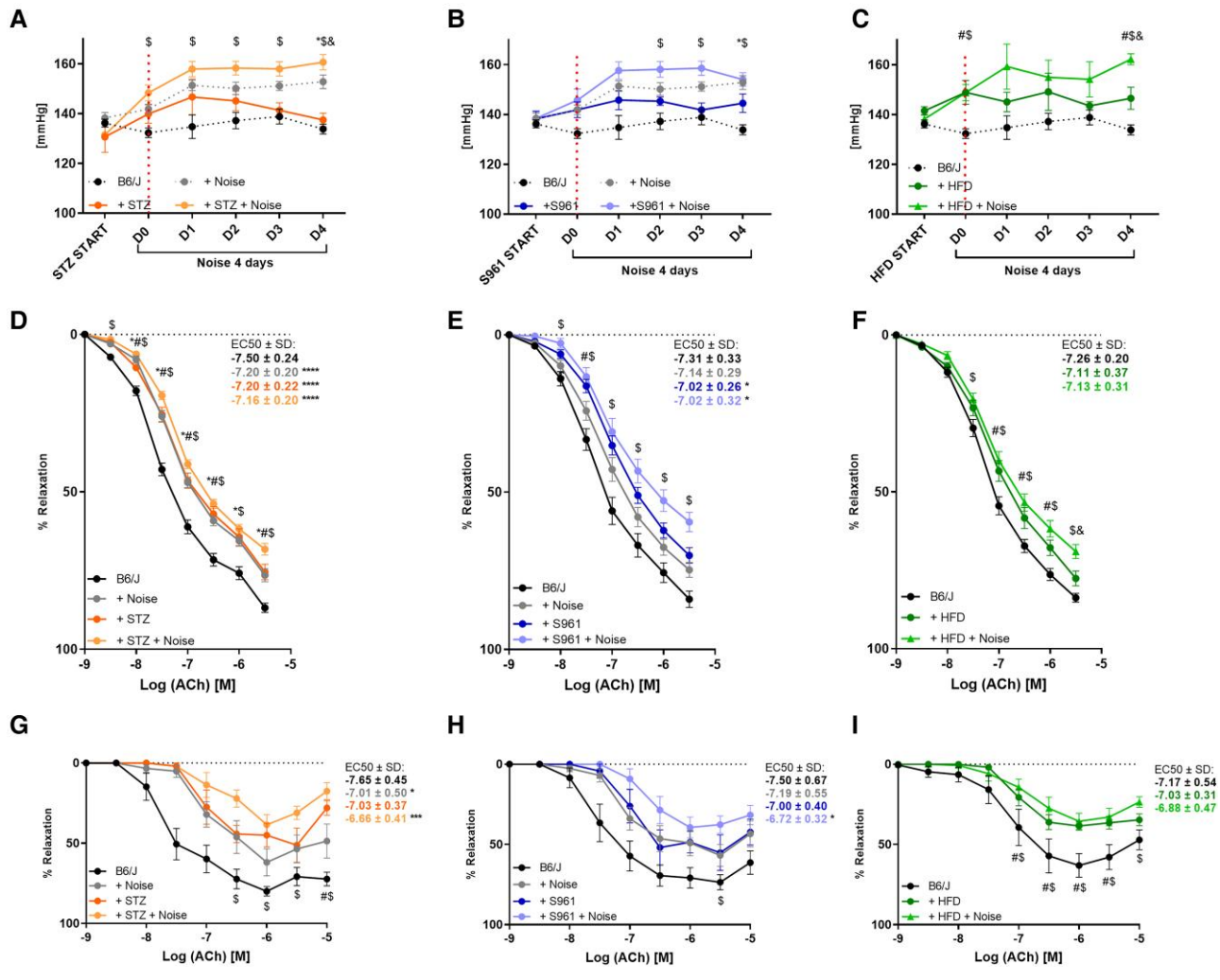


Figure 2 The effects of aircraft noise exposure on diabetes mellitus-induced hypertension and endothelial dysfunction. (A–C) Systolic blood pressure was measured using non-invasive plethysmography at the start of the treatment and D0–D4 of noise exposure. The control curves were merged from all three diabetes models and used in panels A–C; the noise-only curves were merged from the STZ and S961 models and used in (A) and (B). Merged curves are presented as dotted lines. (D–F) Endothelium-dependent (acetylcholine) relaxation of the aorta was measured using the isometric tension method. The results represent % relaxation of pre-constriction and EC₅₀ values are shown in each graph. (G–I) Endothelium-dependent (acetylcholine) relaxation of mesenteric arteries was measured using a myograph system. The results represent % relaxation of pre-constriction and EC₅₀ values are shown in each graph. (A–I) Data represent the mean of *n* = 5–20 (A–C), *n* = 14–41 (D–F), *n* = 6–14 (G–I) independent measurements. For the time (blood pressure)/concentration (acetylcholine) curves with four groups and two independent factors we used three-way ANOVA (A, B, D, E, G, H) for statistical analysis. For the time (blood pressure)/concentration (acetylcholine) curves with three groups, we used two-way ANOVA (C, F, I). *P* < 0.05 were considered significant. (* = B6/J vs. Noise; # = B6/J vs. Diabetes; \$ = B6/J vs. Diabetes + Noise; & = Diabetes vs. Diabetes + Noise; Diabetes = STZ/S961/HFD; For EC₅₀ values, **P* < 0.05, ****P* < 0.001, *****P* < 0.0001 vs. B6/J control. BP, blood pressure; D0–D4, Day 0–Day 4 of noise exposure; ACh, acetylcholine; EC₅₀, half maximal effective concentration). Main effects: (A) time ****, noise ****, STZ *, (B) time ****, noise ****, S961 ***, time x noise *, (D) concentration ****, noise ****, STZ ****, concentration x noise ****, concentration x STZ ****, noise x STZ *, concentration x noise x STZ * (EC₅₀: noise ****, STZ ****, noise x STZ ***); (E) concentration ****, noise **, S961 ****, concentration x noise ****, concentration x S961 **** (EC₅₀: S961 **); (G) concentration ****, noise **, STZ ****, concentration x noise **, concentration x STZ **** (EC₅₀: noise ****, STZ ****); (H) concentration ****, noise **, S961 **, concentration x noise **, concentration x S961 ** (EC₅₀: S961 **). **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001.

metabolism and biogenesis processes involving lipids such as glycolipids, glycerolipids, and glycosphingolipids in all the models on aircraft noise exposure. We also observed that proteins associated with the circadian

rhythm were downregulated in the STZ and HFD model, and they were further downregulated when the mice were exposed to aircraft noise (see [Supplementary material online, Figures S4 and S6](#)).

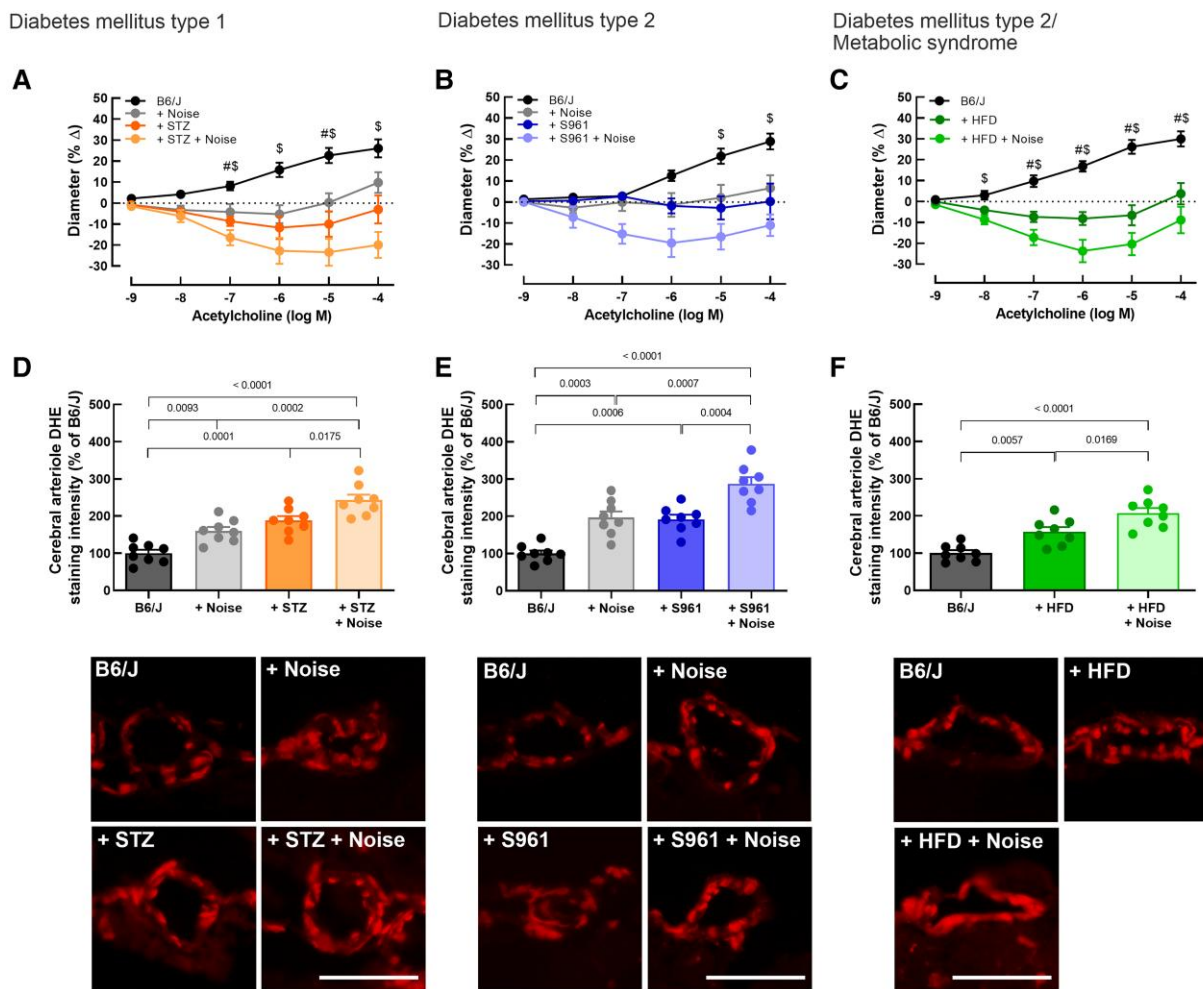


Figure 3 The effects of aircraft noise exposure on diabetes mellitus-induced endothelial dysfunction and on reactive oxygen species formation in cerebral arterioles. (A–C) Endothelium-dependent dilation of cerebral arterioles was measured by video microscopy. The response to a vasoconstrictor (U46619) and endothelium-independent vasodilator (sodium nitroprusside) is shown in [Supplementary material online, Figure S3](#). (D–F) The formation of reactive oxygen species was measured by *in situ* dihydroethidium fluorescence in cerebral arteriole cross-sections (Scale bar = 20 μ m). For statistical analysis, we used for the concentration (acetylcholine) curves with four groups and two independent factors three-way ANOVA (A, B; $n = 8$ per concentration and group) or for the concentration (acetylcholine) curves with three groups we used two-way ANOVA (C; $n = 8$ per concentration and group). For statistical analysis, we used two-way ANOVA for the bar graphs with four groups and two independent factors (D, E; $n = 8$ per group) and one-way ANOVA for the bar graphs with three groups (F; $n = 8$ per group). $P < 0.05$ were considered significant. (# = B6/J vs. Diabetes; \$ = B6/J vs. Diabetes + Noise; Diabetes = STZ/S961/HFD). Main effects: (A) concentration **, noise **, STZ ****, concentration \times noise ****, concentration \times STZ ****; (B) concentration **, noise **, S961 **, concentration \times noise ***, concentration \times S961 ****, concentration \times noise \times S961 *; (D) noise ****, STZ ****; (E) noise ****, S961 ****. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, **** = $P < 0.0001$.

Discussion

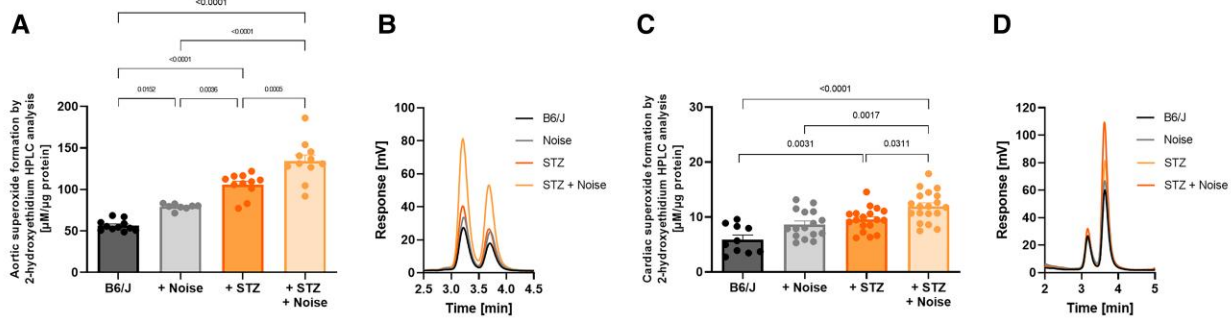
With the present studies, we explored the cardiovascular repercussions of aircraft noise exposure across three distinct animal models of diabetes mellitus, uncovering a range of synergistic harmful effects. Within the context of a HFD, indicative of metabolic syndrome, we observed an exacerbation of hyperglycaemia attributed to aircraft noise exposure. Furthermore, we noted a pronounced impairment in macro- and microvascular endothelial function, as evidenced *in vivo* by blood pressure alterations and *ex vivo* through isometric tension assessments across all diabetes models. This vascular dysfunction is attributable to an augmented generation of oxidative stress markers, particularly superoxide, within the aorta and cerebral arterioles. Additionally, we

detected compounded effects on superoxide levels within the aorta, cerebral arterioles, and cardiac tissue across the varied diabetes models when subjected to aircraft noise. Employing the mitoSOX/HPLC technique, we established that cardiomyocyte mitochondrial respiratory chain's functionality was predominantly compromised by diabetic conditions rather than noise exposure alone, except for a reduction in the RCR upon pooling data from all control and noise-exposed mice.

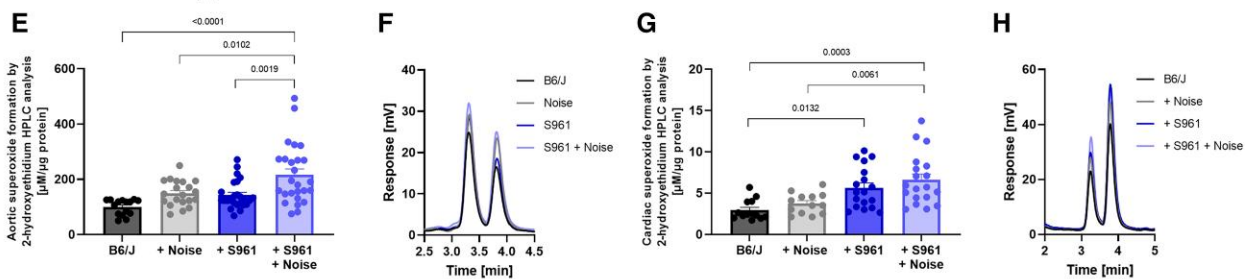
Noise is associated with diabetes and obesity: epidemiological evidence

Diabetes mellitus and noise pollution are intricately linked. Research posits a connection between diabetes mellitus and exposure to

Diabetes mellitus type 1



Diabetes mellitus type 2



Diabetes mellitus type 2/Metabolic syndrome

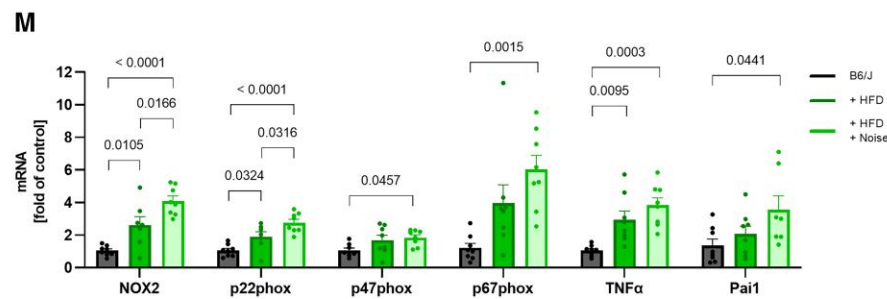
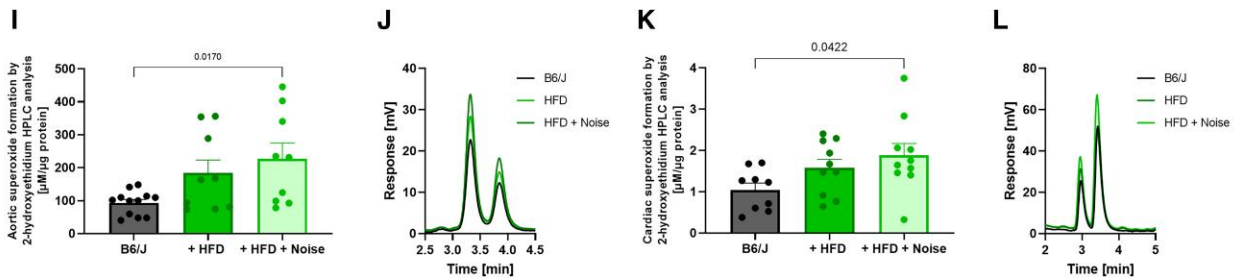
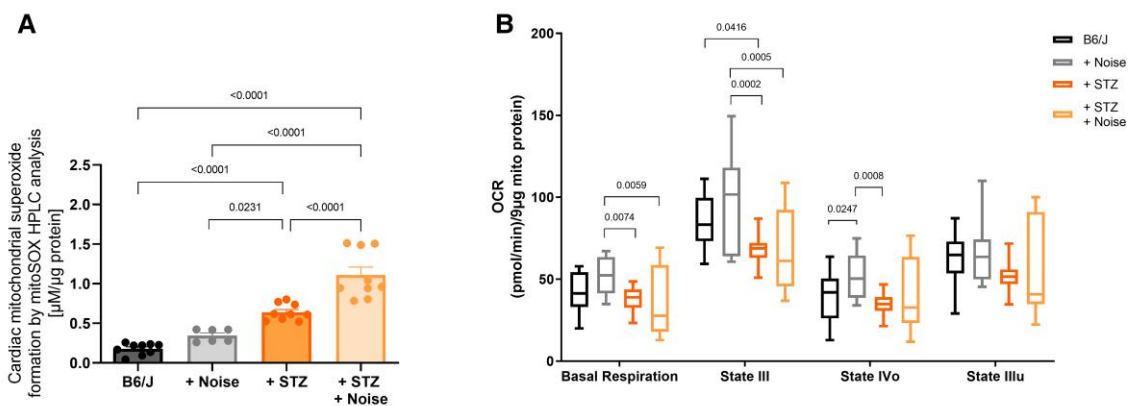
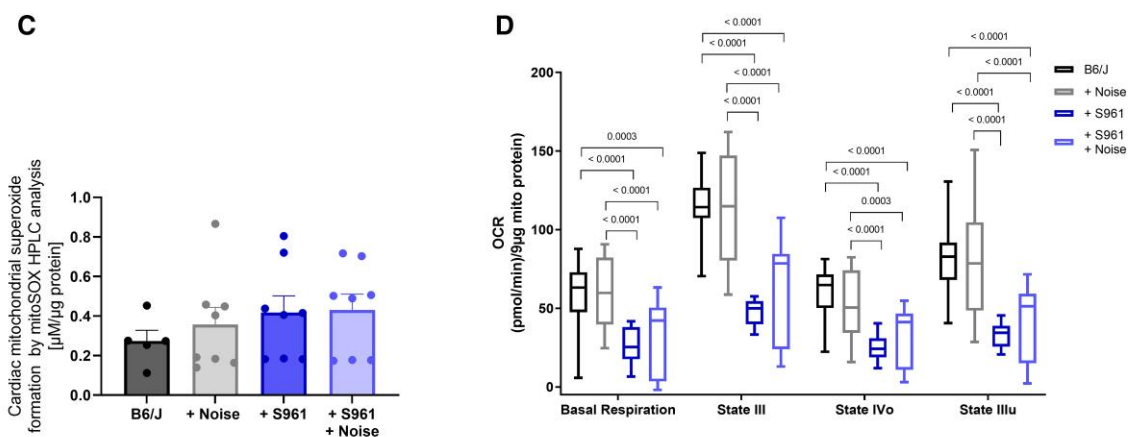


Figure 4 The effects of aircraft noise exposure on increased oxidative stress levels and inflammation caused by diabetes mellitus. (A, E, I) Aortic superoxide formation measured by 2-HE HPLC analysis. (C, G, K) Superoxide levels in cardiac tissue measured by 2-HE HPLC analysis. (B, D, F, H, J, L) Representative chromatograms of the respective quantifications of the superoxide measurements in both tissues. (M) mRNA expression of oxidative stress markers (NOX2, p22phox, p47phox, p67phox) and markers of inflammation (TNF- α and Pai1) measured in eWAT. (A, E, I) A pair of data points represents a sample pooled from three to six animals [A ($n = 5-6$), E ($n = 7-13$), I ($n = 4-5$)], and in (C, G, K) each data point represents an individual animal [C ($n = 10-19$), G ($n = 13-19$), K ($n = 9-10$)]. (M) Data points are measurements from tissues 7-8 animals. For statistical analysis, we used two-way ANOVA for the bar graphs with four groups and two independent factors (A, C, E, G) and one-way ANOVA for the bar graphs with three groups [I, K, M (each mRNA target was tested separately)]. $P < 0.05$ were considered significant. (2-HE, 2-hydroxyethidium; HPLC, high-performance liquid chromatography; NOX2, NADPH oxidase 2; p22phox, human neutrophil cytochrome b light chain; p47phox, neutrophil cytosolic factor 1; p67phox, neutrophil cytosolic factor 2; TNF α , tumour necrosis factor α ; PAI-1, plasminogen activator inhibitor-1; eWAT, epididymal white adipose tissue). Main effects: (A) noise ****, STZ ****, (C) noise ****, STZ ****, (E) noise ****, S961 ****, (G) S961 ****. **** $P < 0.0001$.

Diabetes mellitus type 1



Diabetes mellitus type 2



Diabetes mellitus type 2/Metabolic syndrome

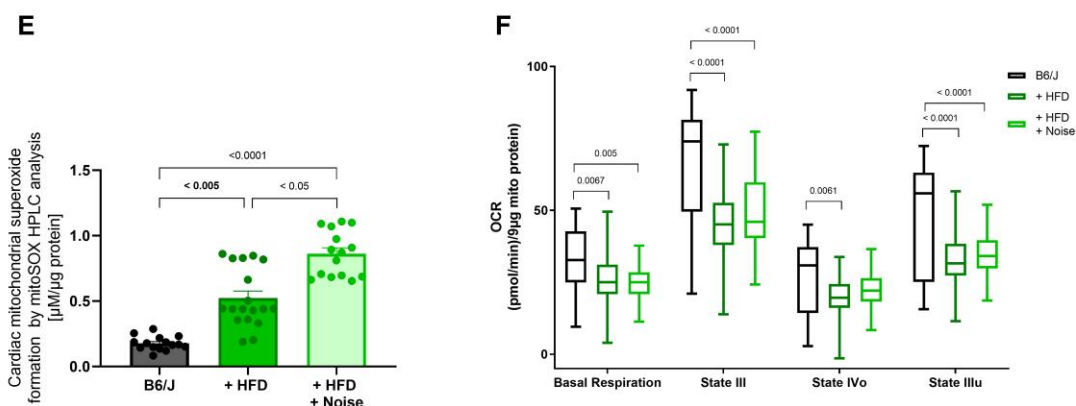


Figure 5 The effects of aircraft noise exposure on diabetes-induced mitochondrial dysfunction. (A, C, E) Cardiac mitochondrial superoxide formation measured by mitoSOX HPLC analysis. (B, D, F) Respiration states of isolated cardiac mitochondria in the presence of complex I substrates (glutamate, malate) baseline respiration, state III, IV_O, III_U after addition of ADP, oligomycin, FCCP and rotenone, respectively. Data were measured by XF96e Seahorse as oxygen consumption rates. Respective traces of coupling experiments are shown in [Supplementary material online, Figure S2](#). (A, C, E) A triplet of data points represents a sample obtained by pooling tissue from three to six animals [A ($n = 2-3$ pooled mitochondrial preparations), C ($n = 3-4$ pooled mitochondrial preparations), E ($n = 5-6$ pooled mitochondrial preparations)]. (B, D, F) The box plots are based on technical replicates from two independent experiments with samples pooled from three to four animals per group [B ($n = 2$), D ($n = 3$), F ($n = 2$)]. For statistical analysis, we used two-way ANOVA for the bar graphs with four groups and two independent factors [A, C and B, D (each respiratory component was tested separately)] and one-way ANOVA for the bar graphs with three groups (E, F). $P < 0.05$ were considered significant. (HPLC, high-performance liquid chromatography; OCR, oxygen consumption rate). Main effects: (A) noise ****, STZ ****, noise \times STZ *; (B) basal: STZ **, noise \times STZ *, III: STZ ****, IV_O: noise **, STZ *, III_U: STZ *; (C) nothing significant; (D) basal: S961 ****, III: S961 ****, IV_O: S961 ****, III_U: S961 ****. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

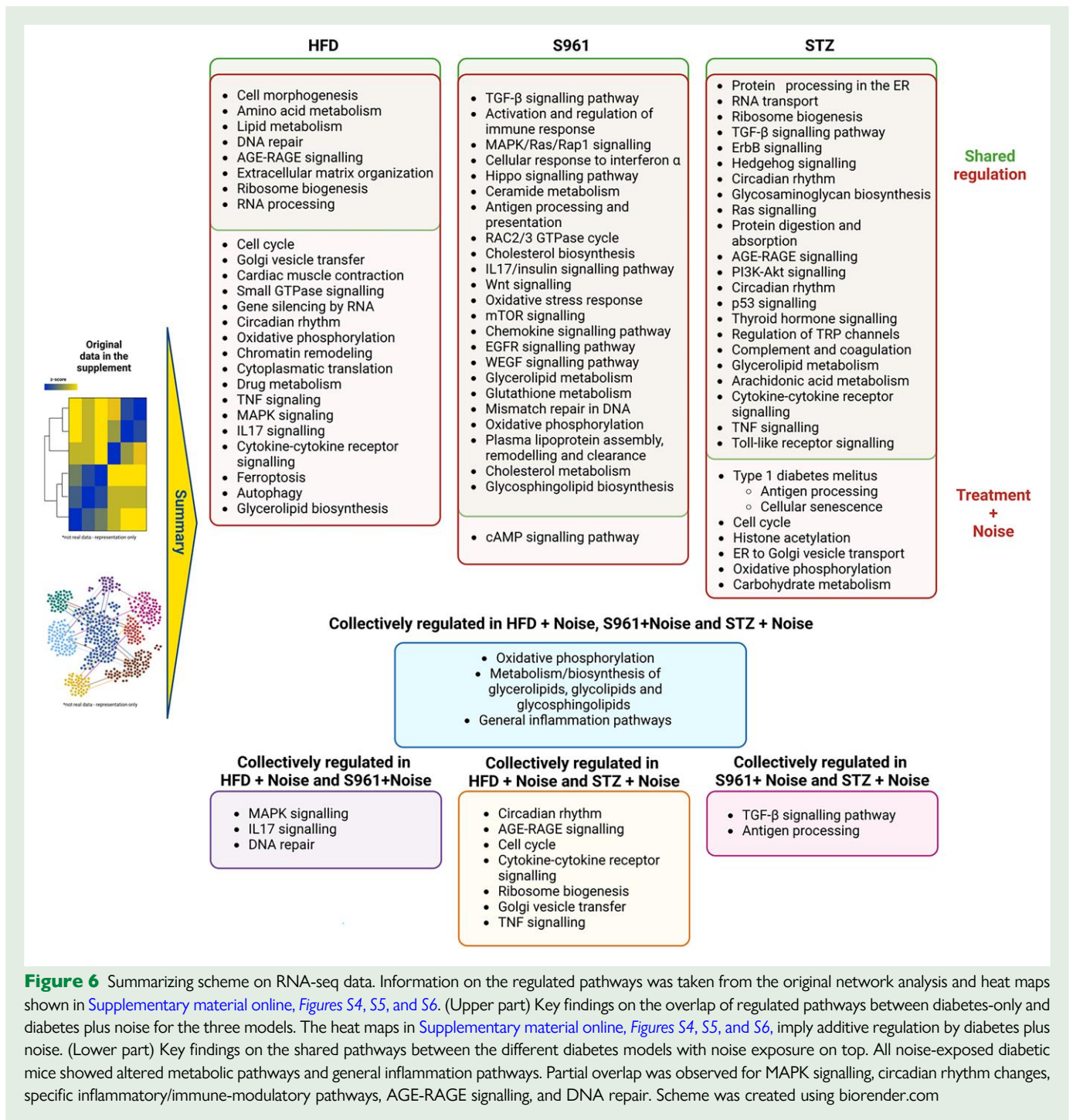


Figure 6 Summarizing scheme on RNA-seq data. Information on the regulated pathways was taken from the original network analysis and heat maps shown in [Supplementary material online, Figures S4, S5, and S6](#). (Upper part) Key findings on the overlap of regulated pathways between diabetes-only and diabetes plus noise for the three models. The heat maps in [Supplementary material online, Figures S4, S5, and S6](#), imply additive regulation by diabetes plus noise. (Lower part) Key findings on the shared pathways between the different diabetes models with noise exposure on top. All noise-exposed diabetic mice showed altered metabolic pathways and general inflammation pathways. Partial overlap was observed for MAPK signalling, circadian rhythm changes, specific inflammatory/immune-modulatory pathways, AGE-RAGE signalling, and DNA repair. Scheme was created using biorender.com

transportation noise.¹² Such noise triggers the hypothalamic–pituitary–adrenal (HPA) axis, elevating cortisol levels, which may precipitate metabolic diseases. Beyond disrupting sleep patterns through inadequate rest and frequent awakenings, noise exposure contributes to low-grade inflammation, diminished insulin sensitivity, and compromised glucose regulation.^{20,21} It may also disrupt appetite-regulating hormones, potentially leading to obesity, a recognized precursor to T2DM. Noise annoyance has been identified as a behavioural conduit through which noise influences cardiometabolic diseases, including diabetes.^{3,5} Furthermore, noise pollution directly escalates diabetes risk factors such as obesity and physical inactivity. While the World

Health Organization initially assessed the evidence linking T2DM development to noise exposure as ranging from very low to moderate in 2018, later prospective studies have underscored a significant correlation, particularly concerning road noise, even when adjusting for air pollution.^{22–24} Subsequent research indicated that prolonged exposure to noise from roads, railways, and possibly aircraft heightens the risk of T2DM in Danish populations, factoring in long-term air pollution exposure.¹¹ Exposure to multiple noise sources, especially combined road, and railway noise, has been deemed particularly detrimental.¹¹ A systematic review and meta-analysis by Sakhvidi et al.¹² showed that a 5 dB(A) increase in noise exposure correlates with a 6%

heightened diabetes risk, primarily due to air and road traffic noise. Vienneau *et al.*²⁵'s meta-analysis revealed relative risks for incident diabetes of 1.20 and 1.11 with every 10 dB(A) increase in aircraft and road noise exposure, respectively. Liu *et al.*²⁶ also documented a significant relationship between transportation noise exposure and T2DM. In light of this evidence, it is imperative to account for diabetes when evaluating the impact of traffic noise exposure on cardiometabolic disease. Nation-wide observational studies revealed an association of long-term traffic noise exposure with increased risk of obesity and higher body mass index (BMI)²⁷ and elevated markers of adiposity.²⁸

Noise, diabetes, and obesity: mechanistic insight from animal models

Concerning the induction of diabetes, obesity, and insulin resistance by noise, several mouse studies were published.^{29–31} Importantly, in all these studies, white noise (a continuous swoosh comprising a broad range of frequencies but no crescendo or decrescendo) and not transportation noise (containing irregular breaks and crescendo/decrescendo) have been employed as a noise stressor. This seems problematic since the cardiovascular and cerebral consequences of white noise differ greatly from the adverse effects of transportation noise. In our hands, white noise had no detrimental health effects when used at a mean sound pressure level of 72 dB(A) similar to aircraft noise.⁶ Liu *et al.* established that noise exposure of mice up to a sound pressure level of 95 dB caused insulin resistance, which was prolonged with longer noise exposure and was likely related to the observed blunted insulin signalling in skeletal muscle.²⁹ The same group demonstrated that chronic noise exposure at 85 dB sound pressure level (4 h/day, below the safety limit for occupational noise exposure) exaggerated multiple metabolic abnormalities induced by HFD in C57BL/6J male mice, including worsened glucose intolerance, insulin resistance, fasting hyperglycaemia, and dyslipidaemia.³⁰ In addition, it was demonstrated that white noise led to enhanced JNK phosphorylation and IRS1 serine phosphorylation as well as reduced Akt phosphorylation in skeletal muscles in response to exogenous insulin stimulation and that plasma levels of TNF- α , IL-6, and malondialdehyde concentrations in skeletal muscles were elevated after 20 days of noise exposure.³¹ These findings suggest that impaired insulin sensitivity in noise-exposed mice might be mediated by an enhancement of the JNK/IRS1 pathway and that inflammation and oxidative stress might contribute to insulin resistance after chronic noise exposure.

Our present study allows a comparison of noise effects in different models of metabolic dysregulation with high relevance for diabetes, which is important in light of differential insulin levels and effects—deficiency in STZ mice vs. impaired insulin action in S961 and HFD mice, which is characterized by an overproduction of insulin (hyperinsulinaemia). The impact of the different diabetes models becomes most obvious by the aggravated markers of inflammation and oxidative stress, which was observed in adipose tissue of HFD mice. Blood glucose levels were more dramatically increased in STZ and S961 mice compared with HFD mice, whereas mitochondrial function was mostly not affected in STZ mice (only state III respiration) but in S961 and HFD mice. The different metabolic features of the three models of diabetes and metabolic syndrome, including the wide variation from β -cell death and insulin deficiency (STZ mice) to impaired insulin signalling and resistance (S961 and HFD mice), probably affect all other measured parameters.

Noise aggravates the cardiovascular consequences of diabetes

So far, no study has investigated the metabolic adverse effects of a traffic noise stressor in different insulin-deficient and hyperinsulinaemic

diabetes models. The present data demonstrate significant adverse cardiometabolic effects by diabetes and by noise alone, including increased production of reactive oxygen species, endothelial dysfunction, and dysregulating metabolic processes. Importantly, these key pathomechanisms in the setting of diabetes were further substantially exacerbated in an additive manner with noise exposure—regardless of the diabetes model used.

In the HFD-induced obesity model, noise exposure aggravated adipose tissue inflammation. Adipose tissue expansion in obesity results in immune cell infiltration and adipose tissue inflammation. This is part of the chronic low-grade systemic inflammation leading to insulin resistance. There is evidence that obesity-associated systemic inflammation starts with adipose tissue inflammation.³² The upregulation of NADPH oxidase subunits (NOX2, p22phox, p47phox, and p67phox) in response to noise in HFD mice likely originates from macrophages accumulating in the inflamed adipose tissue. Also, the increased expression of TNF- α is mainly produced by the pro-inflammatory macrophages within the adipose tissue.³² Adipose tissue-derived TNF- α can promote insulin resistance by increasing free fatty acid release and by enhancing serine/threonine phosphorylation of insulin receptor substrate-1.³²

To gain deeper mechanistic insight, we performed next-generation sequencing. As expected, our RNA-seq data revealed an upregulation of pathways associated with inflammation in diabetic mice. Moreover, inflammatory pathways are further upregulated when diabetic mice are exposed to noise. Inflammation is a characteristic in both T1DM and T2DM, as the former is an autoimmune disorder and a side effect of insulin resistance in the latter.³³ In T1DM, inflammation is mediated by cytokines, TNF- α and interleukins, via increasing ROS production, thereby leading to β -cell death in the pancreas.³³ The substantial alteration of gene expression by diabetes and noise exposure is also reflected by the well-documented epigenetic changes in diabetic patients³⁴ and noise-exposed subjects.³⁵

In T2DM, inflammation is considered as important side effect of obesity where the accumulated white adipose tissue produces pro-inflammatory chemokines and cytokines that trigger inflammatory pathways.³³ In addition to this inflammatory process, we observe the consistent dysregulation of glycolipid homeostasis with its catabolism upregulated and biosynthesis downregulated. Disorders in glycolipid metabolism are a known cause of vascular injury in various organs such as kidneys, eyes, heart, and nerves, leading to diabetes-associated retinopathy, neuropathy, nephropathy, and polyneuropathy.³⁶

Another common dysregulation observed in our investigated diabetes models is the adverse effect on oxidative phosphorylation. Impaired oxidative phosphorylation and elevated oxidative stress are responsible for the main characteristics of diabetes, such as the dysfunction of β -cells and mitochondria and the impairment of glucose tolerance.³⁷ These adverse effects are mediated by an excessive release of ROS, disrupting the redox balance in these cells.³⁷ Hyperglycaemia also contributes to oxidative stress by altering the AGE-RAGE signalling pathway mainly by increasing the production of AGEs (advanced glycosylated end products).³⁷ The present data on additive damage by noise and diabetes are in good accordance with previous results demonstrating an aggravation of the cardiovascular side effects concerning the risk factor arterial hypertension by noise.³⁸ In these studies, noise caused a further exacerbation of blood pressure, endothelial dysfunction, cerebral and cardiovascular oxidative stress, and inflammatory pathways, with a key role in infiltrating inflammatory cells and phagocytic NADPH oxidase (NOX-2). Importantly, genetic ablation of myelomonocytic (LysM-positive) cells and deletion of the phagocytic NADPH oxidase prevented macro- and microvascular dysfunction and adhesion and infiltration of immune cells in response to noise exposure.^{39,40} Genetic deletion of the NOX-2 isoform protected mice

from noise-induced oxidative stress and cardiovascular damage.⁷ Prevention of noise health effects by antioxidant therapies related to NRF2 activation are discussed in the [Supplementary material](#).

Similar mechanisms were responsible for additive damage by noise exposure in mice subjected to myocardial infarction. Noise exposure alone caused an inflammatory 'priming' of the heart, which, in concert with MI, resulted in additive cardiac and vascular damage.⁴¹ The main drivers of the exacerbated I/R damage were dysfunctional mitochondria, activation of NOX-2, and enhanced mitochondrial and immune cell-derived reactive oxygen species formation.

Limitations

There are some limitations to the study. First, we just used male mice in order to limit potential hormonal influences on the results of our experiments. The use of both sexes would have been beneficial in unravelling sex-dependent effects in response to combined aircraft noise and diabetes mellitus effects. Upcoming experiments should address this question in e.g. translational approaches. However, concerning ethical considerations the use of both sexes would have doubled the number of animals.

The number of independent measurements varies between parameters, which is due to the fact that some tissues were only sufficient for one parameter—so animal numbers had to be split to assess different parameters. Also ethical approval for the different models allowed different animal numbers. An explicit limitation is the missing 'noise-only' group in the HFD model, which also prevented the proper use of two- and three-way ANOVA of the respective data.

For BP, measurements by the tail cuff method 5 mmHg variations (downwards or upwards) have been reported and also noticed by us. Therefore, changes of >10 mmHg are usually considered substantial. Although we have trained the mice before the measurement to adapt them to the restraint stress, minor stress reactions but also different operators may have caused some variations. To stabilize the results, we merged all BP control data and all noise-only data and included these merged curves in all three panels for comparison. Importantly, the tail cuff method has been proven in accuracy compared to radiotelemetric measurement by Feng et al.⁴²

The maximal endothelium-dependent relaxation observed in our myograph experiment for mesenteric artery relaxation reached only 65–78% of the preconstruction tone, which is a rather low effect (but still in the range of reported values) when compared to the literature. In addition, the paradoxical vasoconstriction at the highest acetylcholine concentrations may indicate a not fully intact endothelial response (e.g. due to some minor endothelial damage during preparation). However, we observed a clear endothelial dysfunction in all noise- and diabetes-alone groups and also a trend of further impairment by noise exposure for diabetic mice, which adds value to the study—especially since these data are supported by an alternative method based on pressure myograph with video microscopy and the observations on microvascular dysfunction of cerebral arterioles upon noise, diabetes, and noise plus diabetes.

To investigate the effects of aircraft noise on diabetes mellitus as comprehensively as possible, we used three different diabetes models. As we have used established protocols, the treatment and trial durations differ significantly. Type 2 diabetes mellitus was induced both pharmacologically by S961 (1 week) and by HFD (20 weeks). The STZ-induced type 1 diabetes mellitus model represents a completely different mode of action. The extent to which a transfer to other models or forms of diabetes mellitus is possible cannot be conclusively answered, and other animal models, e.g. HFD plus STZ, may be better suited to reflect the effects of noise in human diabetes.

Summary, conclusions, and clinical implications

Our study investigated the cardiovascular impacts of aircraft noise across three animal models of diabetes mellitus, revealing various synergistic detrimental effects. In models fed a HFD, aircraft noise significantly worsened hyperglycaemia. We also observed marked impairments in macro- and microvascular endothelial functions, demonstrated by blood pressure changes and isometric tension tests in all models. This vascular dysfunction was linked to increased oxidative stress, particularly higher superoxide production in the aorta and cerebral arterioles. Additionally, noise exposure intensified superoxide levels in the aorta, cerebral arterioles, and heart tissue. Using the mitoSOX/HPLC method, we found that mitochondrial dysfunction in cardiomyocytes was mainly due to diabetes, with a notable reduction in the RCR in combined data from all groups, suggesting a lesser impact from noise alone. The major pathomechanisms are summarized in [Supplementary material online, Figure S7](#).

These findings suggest that noise exposure is especially detrimental to vulnerable populations, such as individuals with pre-existing diabetes mellitus. A cumulative effect on risk for myocardial infarction, stroke, and diabetes by exposure to noise, air pollution, and lack of green space was recently published.^{15,43,44} Also, a higher aircraft noise-associated risk of recurrent cardiovascular events after acute coronary syndrome was demonstrated.⁴⁵ Accordingly, there is a strong need for political and societal interventions, given the fact that lower income individuals suffer the higher amount of noise exposure. These include implementing stricter noise regulations and enhancing urban planning to reduce noise pollution, which can significantly improve public health outcomes.⁴⁶ Additionally, there is a potential for mitigating noise-induced health effects through medical interventions that overlap with treatments for diabetes [e.g. AMP-activated protein kinase (AMPK) activation by metformin,⁴⁷] such as drugs that activate biochemical pathways or lifestyle modifications like physical exercise and intermittent fasting via stimulation of AMPK⁴⁸ (see [Supplementary material](#) for further details). Overall, addressing the impact of noise pollution involves a combined approach of policy changes, community planning, and medical research to protect and improve the health of those most at risk, such as individuals with diabetes. This is crucial for developing effective public health strategies and ensuring the well-being of all community members, particularly those in noise-polluted environments.

Further research is critical to establish definitive links and develop effective interventions that could leverage existing diabetes treatments to mitigate noise-induced health risks. Specifically, understanding of noise exacerbation of typical metabolic complications of diabetes, e.g. insulin deficiency, overproduction, and impaired efficacy, is of utmost importance. We have already published several studies on the effects of aircraft noise in humans.^{8,9} As a translational aspect of the present study, a similar investigation with patients suffering T1DM or T2DM could be conducted using similar parameters such as blood pressure, heart rate, flow-mediated dilatation, and inflammation markers such as CRP, cytokine release, insulin, and glucose levels.

Supplementary material

[Supplementary material](#) is available at *European Journal of Preventive Cardiology*.

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Author contribution

S.K.S., T.J., A.D., T.M., D.M., P.S., and M.Kv. contributed to the conception or design of the work. A.D., T.M., T.J., S.K.S., M.O., P.S., M.Kv., D.M., C.P., M.Ku., H.K., A.v.K., C.W., W.R., N.X., H.L., and A.G. contributed to the analysis or interpretation of data for the work. D.M., P.S., M.Kv., L.S., H.U., M.O., M.Ku., C.W., M.T.BJ., S.R., K.F., Q.T., Y.R., S.K., A.G., and N.X. contributed to the acquisition of data for the work. D.M., P.S., A.D., T.M., T.J., C.W., A.G., C.P., A.v.K., N.X., and H.L. drafted the manuscript. D.M., P.S., A.D., T.M., T.J., M.Kv., M.Ku., C.W., S.K., H.K., O.H., A.v.K., T.G., H.L., A.G., W.R., P.S.W., and P.L. critically revised the manuscript. All gave final approval and agreed to be accountable for all aspects of the work ensuring integrity and accuracy.

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Conflict of interest: The authors have no conflicts of interest associated with this work.

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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