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Research Article

Effect of a High-Fat Diet and Occupational Exposure in Different Rat Strains on Lung and Systemic Responses: Examination of the Exposome in an Animal Model

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ABSTRACT

The exposome is the measure of all exposures of an individual in a lifetime and how those exposures relate to health. The goal was to examine an experimental model integrating multiple aspects of the exposome by collecting biological samples during critical life stages of an exposed animal that are applicable to worker populations. Genetic contributions were assessed using strains of male rats with different genetic backgrounds (Fischer-344, Sprague Dawley, and Brown-Norway) maintained on a regular or high-fat diet for 24 weeks. At week 7 during diet maintenance, groups of rats from each strain were exposed to stainless steel welding fume (WF; 20 mg/m^3 × 3 h/d × 4 days/week × 5 weeks) or air until week 12, at which time some animals were euthanized. A separate set of rats from each strain were allowed to recover from WF exposure until the end of the 24-week period. Bronchoalveolar lavage fluid and serum were collected at 7, 12, and 24 weeks to assess general health indices. Depending on animal strain, WF exposure and high-fat diet together worsened kidney toxicity as well as altered different serum enzymes and proteins. Diet had minimal interaction with WF exposure for pulmonary toxicity endpoints. Experimental factors of diet, exposure, and strain were all important, depending on the health outcome measured. Exposure had the most significant influence related to pulmonary responses. Strain was the most significant contributor regarding the other health indices examined, indicating that genetic differences possibly drive the exposome effect in each strain.

Key words: exposome; animal model; inhalation exposure; welding fume; dietary effects.

The exposome is defined as the measure of all exposures of an individual in a lifetime from conception to death and how those exposures relate to health (Wild, 2012). Important components of the exposome include lifestyle factors and environmental and occupational exposures in conjunction with individual genetic predisposition. It is believed that successful "mapping" of the exposome could substantially improve the understanding of underlying causes of disease and potentially aid in the development of prevention strategies and possible cures for most diseases. Genome-wide association studies have indicated that the heritability of chronic disease is about 10% (Rappaport, 2011). Thus, "nongenetic" factors are believed to be responsible

for 90% of chronic disease risks, suggesting that from a quantitative assessment of an individual's environmental exposure history, it is possible to discover the major causes of diseases (Rappaport and Smith, 2010).

An individual's exposome is highly variable and dynamic throughout their lifetime (Center for Disease Control and Prevention, 2014). The impact of environmental exposures can vary with different stages of life as well as with different genetic predispositions or other susceptibility factors. In addition, endpoints of biological responses, particularly those that may predict chronic disease, are mostly unknown. Specific environmental and occupational exposures may prove difficult to determine because the exposure may be unknown or transient and only a short period of time exists for its direct measure (Center for Disease Control and Prevention, 2014).

Determining an exposome for an individual is an immense challenge because of the complexity of external environmental and occupational exposures from conception to adulthood as well as inherent genetic variations. Our study objectives were to (1) develop an exposure model that would collect longitudinal biological samples during critical "occupational" life stages of an exposed animal that are applicable to human populations and (2) measure health outcomes to assess multiple exposomal factors, such as lifestyle (eg, diet) and environmental (eg, occupational) exposure, which attempt to link a specific internal biological response/endpoint with a specific exposure. An animal model is particularly advantageous for studying the exposome because of the ability to control all external exposures and to measure potential adverse health outcomes of each animal over a significant period of time. Also, the influence of genetics can be assessed using multiple animal strains with varying susceptibilities to unique exposures to possibly model human susceptibility variations.

The 2 primary objectives of this project were, first, to determine whether exposure to a common but potentially toxic inhaled substance while maintained on an unhealthy diet increased the susceptibility to adverse health effects and, second, to determine what experimental factor (eg, diet, occupational exposure, or genetic [strain]) had the greatest influence on a specific health outcome. Three genetically distinct strains of rats were introduced to a high-fat (HF) diet at 6 weeks of age to mimic an unhealthy diet routine starting when young. Studies have shown that poor eating habits worldwide often begin at an early age in children (Hayter *et al.*, 2015; Laroche *et al.*, 2007; Sapkota and Neupane, 2017; Vilchis-Gil *et al.*, 2015; Waddingham *et al.*, 2015). Consumption of fast food (mostly high fat) is greatest among children, teens, and young adults (Paeratakul *et al.*, 2003), and total fat intake is greatest in teenagers (National Center for Health Statistics, 2005; Troiano *et al.*, 2000). The rats were maintained on the HF diet for 7 weeks before the start of a 5-week inhalation exposure to a complex metal particulate, stainless steel welding fume (WF), to model an occupational exposure beginning in early adulthood when one would expect to enter the job market. Pulmonary and extrapulmonary responses were measured at 7 weeks (baseline before WF exposure), 12 weeks (directly after WF exposure), and 24 weeks (after a 12-week recovery from WF exposure).

MATERIALS AND METHODS

Animals and diet. Male Sprague Dawley (Hla: SD CVF; Hilltop Lab Animals, Scottdale, Pennsylvania), Fischer-344 (F344/NHla CVF; Hilltop Lab Animals), and Brown-Norway (BN/RijHsd; Harlan Laboratories, Inc, Indianapolis, Indiana) rats were received at 5 weeks of age and were free of viral pathogens, parasites, mycoplasmas, *Helicobacter*, and *CAR Bacillus*. The rationale for the choice of the specific strain was based on the need to use strains with varying responses in pulmonary exposure studies. The BN inbred strain has been commonly used in allergic respiratory disease studies due to elevated IgE and Th2 dominant responses. The F344 inbred strain has been extensively used in lung toxicology studies due to susceptibility to pulmonary injury and inflammation. The SD rat is the most widely used outbred strain in animal research, and a large database exists for the SD strain in regards to lung toxicology studies.

The animal facilities are specific pathogen free, environmentally controlled, and accredited by AAALAC, International (Frederick, Maryland). All methods were performed in accordance with the relevant guidelines and regulations by CDC-NIOSH and AAALAC. Rats from each strain ($n = 120$/rat strain) were acclimated for a week after arrival and were provided tap water and irradiated Teklad 2918 regular 18% protein rodent diet (REG; Envigo Teklad Diets, Madison, Wisconsin) ad libitum. Nutritional composition of the Teklad 2918 regular diet was 18.6% protein, 44.2% carbohydrate, and 6.2% fat. After acclimation, sets of animals from each strain were continued on the REG Teklad 2918 diet ($n = 60$ rats/strain) or maintained on the Teklad Custom 45% Fat Kcal high-fat, western diet (HF; Envigo Teklad; $n = 60$/strain) ad libitum. The 45% Fat Kcal diet was designed with similarities to the western diet with the addition of 21% anhydrous milk fat and 34% sucrose. Soybean (2%) was included in the HF diet to supplement essential fatty acids. Nutritional composition of the HF diet was 14.8% protein, 40.6% carbohydrate, and 44.6% fat. Animals were maintained on the REG or HF diets until humanely euthanized with an intraperitoneal injection of sodium pentobarbital (> 100 mg/kg body weight; Fatal-Plus Solution, Vortech Pharmaceutical, Inc, Dearborn, Michigan) and then exsanguinated by severing the abdominal aorta at 7, 12, and 24 weeks. All animal procedures used during the study were reviewed and approved by the CDC-Morgantown Institutional Animal Care and Use Committee.

Experimental design and welding fume exposure. The experimental design of the study is described in Figure 1. At 6 weeks of age, the rats from the 3 strains with different genetic backgrounds (F344, SD, and BN) were started on a HF or REG diet. Animal body weight was measured throughout the 24-week regimen. At week 7 during diet maintenance, groups of rats from each strain were exposed by inhalation of stainless steel WF (target concentration of 20 mg/m^3 × 3 h/day × 4 days/week × 5 weeks) or filtered air (control) until week 12 at which time some animals from each strain were euthanized. A separate set of rats from each strain were allowed to recover from welding fume exposure until the end of the 24-week period. Whole blood, serum, selected organs, and bronchoalveolar lavage (BAL) fluid were collected at 7 weeks (baseline before welding fume exposure), 12 weeks, and 24 weeks to assess common health indices. The time points were chosen to assess responses directly after the WF exposure (12 weeks) to mimic a worker being actively exposed to WF as well as after a recovery period (24 weeks) to model a worker who has been removed from the exposure.

The rationale for the welding fume concentration and duration used in the study was to model a typical welder exposure. To relate the pulmonary exposure dosing paradigm in the current study to a workplace exposure of welders, we used a mathematical calculation (Erdely *et al.*, 2011) to determine the daily lung burden of a welder during an 8-h work schedule. Incorporating factors such as fume concentration (5 mg/m^3, previous threshold limit value [TLV] for welding fumes), human

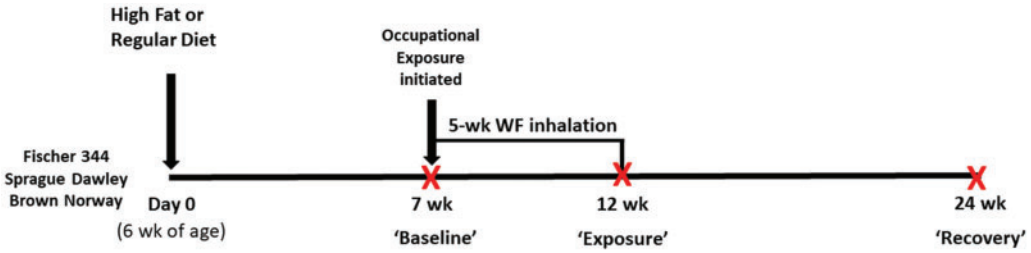

Figure 1. Experimental design and exposure regimen. The 3 strains of male rats were maintained on a high-fat (HF) or regular (REG) diet for 24 weeks. At week 7 during diet maintenance, groups of rats from each strain were exposed by inhalation of stainless steel welding fume (WF) (20 mg/m^3 × 3 h/day × 4 days/week × 5 weeks) or filtered air (control) until week 12 at which time some animals from each strain were euthanized. A separate set of rats from each strain were allowed to recover from welding fume exposure until the end of the 24-week period. Biological samples were collected at 7 weeks (baseline before welding fume exposure), 12 weeks, and 24 weeks.

minute ventilation volume (20 000 ml/min × 10^{-6} m^3/ml), exposure duration (8 h/day × 60 min/h), and deposition efficiency (predicted as 16%, ICRP, 1994), it was determined that the daily lung burden of a welder is approximately 7.7 mg. It was observed in a previous rodent study by our group that a stainless steel welding fume exposure regimen of 40 mg/m^3 × 3 h/day × 10 days led to an equivalent daily lung deposition of approximately 2–4 times greater than a worker welding at 5 mg/m^3 for 8 h (Erdely *et al.*, 2011). A similar dosing regimen was used in the current study in which the total exposure (concentration × time; 20 mg/m^3 × 3 h/day × 20 days = 1200 mg/m^3 × h) would be equivalent to the total exposure dose in the previous study (40 mg/m^3 × 3 h/day × 10 days = 1200 mg/m^3 × h). Therefore, the WF dose in the current study was approximately 2–4 times that of the TLV for a welder working an 8-h shift, assuming the worker is exposed to welding fumes for 60 min/h for 8 h. Given the intermittent nature of welding as a job task (ie, meaning welders rarely weld for 8 consecutive hours), our depositions were intended to reflect a cumulative worker exposure.

The design and construction of the welding fume aerosol generator and the characterization of the fume were previously described (Antonini *et al.*, 2006, 2011). Briefly, welding fume composition was determined by inductively coupled plasma-atomic emission spectroscopy according to NIOSH method 7300 (NIOSH, 1994) and was composed of the following metals (weight %): Fe (53%), Cr (17%), Mn (24%), Ni (6%), Cu (0.4%), and (0.2%). Particle size distribution was determined in the exposure chamber in the breathing zone of the rats by using a Micro-Orifice Uniform Deposit Impactor (MOUDI, MSP Model 110, MSP Corporation, Shoreview, Minnesota) for general purpose aerosol sampling, and a Nano-MOUDI (MSP Model 115) that is specifically designed for sampling aerosols in the size range down to 0.010 μm. The mass median aerodynamic diameter was 0.26 μm with a geometric standard deviation of 1.4 on a series of random collected samples. The actual animal chamber concentrations (mean ± standard deviation) for the exposures were 18.6 mg/m^3 ± 7.2 for the F344 strain exposure, 20.3 mg/m^3 ± 6.4 for the SD strain exposure, and 19.4 mg/m^3 ± 7.1 for the BN strain exposure.

Lung injury and inflammation. At 7 weeks (baseline before WF exposure), 12 weeks, and 24 weeks, BAL was performed to assess lung injury and inflammation. The lungs were first lavaged with a 1 ml/100 g body weight aliquot of calcium- and magnesium-free PBS, pH 7.4. The first fraction of recovered BAL fluid was centrifuged at 500 × g for 10 min, and the resultant cell-free supernatant was analyzed for lactate dehydrogenase (LDH) as a marker for lung cell damage. The lungs were further lavaged with 6-ml aliquots of PBS until 30 ml were collected. These samples also were centrifuged for 10 min at 500 × g and the cell-free BAL fluid discarded. The cell pellets from all washes for each rat were combined, washed, and resuspended in 1 ml of PBS buffer and counted and differentiated. Total cell numbers recovered by BAL were determined as a marker for lung inflammation with a Coulter Multisizer II and AccuComp software (Coulter Electronics, Hialeah, Florida). Using the acellular first fraction of BAL fluid, LDH activity was determined by measuring the oxidation of lactate to pyruvate coupled with the formation of NADH at 340 nm as described by Wroblewski and LaDue (1955). Measurements were performed with a COBAS MIRA autoanalyzer (Roche Diagnostic Systems, Montclair, New Jersey) and expressed as units/liter (U/l).

Serum and liver analysis. At 7 weeks (baseline before WF exposure), 12 weeks, and 24 weeks, whole blood was collected via the abdominal vena cava using an 18-gauge needle and delivered to BD Vacutainer tubes (Becton, Dickinson, and Co, Franklin Lakes, New Jersey). A panel of different lipids (triglycerides), enzymes (aspartate transaminase [AST], alanine transaminase [ALT], and alkaline phosphatase [ALKP]), proteins (total protein [TP], albumin [ALB], and globulin [GLB]), and other markers (blood-urea-nitrogen [BUN] and creatinine [CREAT]) was measured in serum isolated from whole blood using the IDEXX BioResearch platform (North Grafton, Massachusetts). In addition, portions of excised livers were collected from each animal, cryo-preserved with Tissue Plus OCT Compound (Scigen Scientific, Gardena, California), and stored at −80°C until analysis. Representative fresh frozen liver tissue was cut into 5-μm sections with a cryo-stat and stained with Oil Red O for 10 min at 60°C to assess lipid accumulation. After incubation with Oil Red O, the slides were rinsed with distilled water and stained with Gill's hematoxylin solution.

Statistical analysis. All analyses were performed using JMP version 13 and SAS version 9.4 for Windows (SAS Institute, Cary, North Carolina). Analyses of variance (ANOVAs) were performed within strain and time points for all variables. Several measures were modeled using a natural log transformation (serum ALB, ALT, AST, BUN, and triglyceride) prior to analysis due to heterogeneous variance and to meet the assumptions of the ANOVA. The 7-week time point utilized a one-way layout (Diet, tests have df = 1, 11), and the 12- and 24-week time points utilized a factorial two-way layout (Exposure by Diet) within each strain. For the 12- and 24-week analyses, post hoc pairwise comparisons among treatment groups were performed using the Fishers least significant difference test (df = 1, 20) (Figures

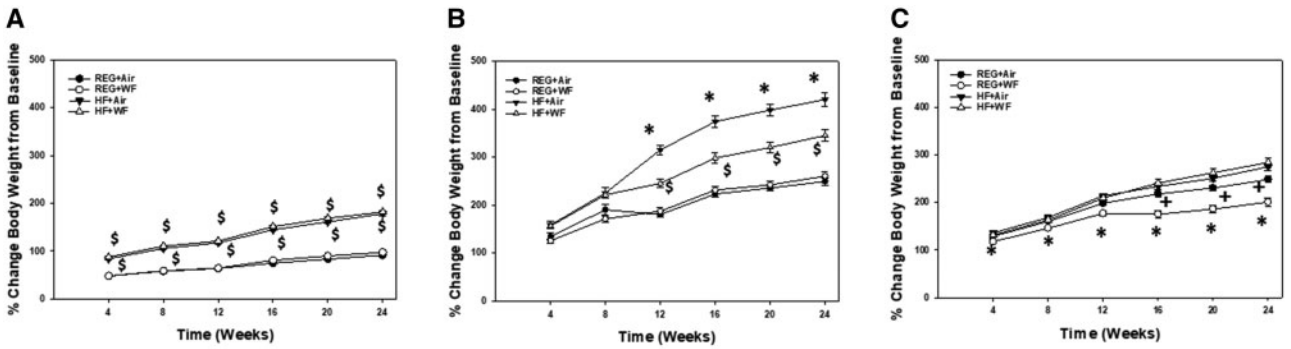

Figure 2. Percent change in body weight from corresponding baseline comparing HF and REG diets for (A) Brown-Norway (BN), (B) Fischer-344 (F344), and (C) Sprague Dawley (SD) rat strains during the 24-week period. At week 7 during diet maintenance, groups of rats from each strain were exposed to stainless steel WF (20 mg/m^3 × 3 h/day × 4 days/week × 5 weeks) or filtered air (control) until week 12 at which time some animals from each strain were euthanized. A separate set of rats from each strain were allowed to recover from welding fume exposure until the end of the 24-week period. Values are means ± standard error ($n = 12$–24/group); *, different from other groups within a time point; **$**, different from REG + Air and REG + WF groups within a time point; **+**, different from HF + Air and HF + WF groups within a time point ($p < .05$). A portion of the data in this were modified and used (Skovmand *et al.*, 2020))

3–5 and Tables 1–3). For all analyses, a p value of <.05 was set as the criterion for statistical significance.

Three-way ANOVAs were performed on the variables at each time point accounting for strain, exposure, diet, and all interactions to determine the proportion of variance attributed to each source of variation in the model (Table 4). These analyses were based on the sum of squares (variation) for each source divided by the total sum of squares. These particular estimates are known as type III sums of squares, and partition the variance from each source while all other sources were included in the model. Thus, if an interaction between exposure and diet takes up 25% of the variance, it means that there is 25% of the variance that cannot be accounted for by examining the main effects of exposure and diet by themselves. These analyses were performed on natural log-transformed data, as the data were generally distributed lognormally with a tail on the right, and thus heterogeneous variance. Only the sources of variance that were statistically significant were presented.

In addition, Principal Component Analysis (PCA) plots were used to simplify representation of multidimensional data and create weighted summation points for ease of visualization. PCA was performed on the variables (Figure 7) and generated clustering for the primary variables and the principal components. The analyses were done with all the data combined, as well as stratified by time and diet. Each point represents an individual animal's body weight, a specific BALF parameter (eg, LDH, BAL cell influx), and each individual serum measurement (eg, triglycerides, CREAT, BUN, BUN/CREA ratio, TP, GLB, alanine aminotransferase, ALKP, and aspartate aminotransferase). Using a linear mathematical algorithm, a new set of variables (principal components) were derived that allowed us to highlight and visualize the differences between test group animals and variance among the animals in a group.

RESULTS

Body Weight and Serum Triglycerides

The HF diet in both the WF and air groups caused a greater increase in the % change in body weight from baseline compared with both REG diet groups for all 3 strains over the 24-week period (Figure 2). This increase was most pronounced in the BN and F344 strains (Figs. 2A and B), the 2 inbred, leaner models, compared with the outbred SD strain (Figure 2C). Interestingly, the WF exposure suppressed the increase in the % change body weight when comparing the HF + WF and HF + Air groups for the F344 strain (Figure 2B) and the REG + WF and REG + Air groups for the SD strain (Figure 2C). The differences in body weight response indicate that there was likely different susceptibilities among the strains regarding exposure to welding fume and maintenance on a specific diet.

It was anticipated that the HF diet would increase serum triglycerides compared with the REG diet in the different strains. At 7 weeks of maintenance on each diet, the HF diet significantly increased serum triglycerides in both the BN and F344 inbred strains at baseline before WF exposure (Figs. 3A and B), whereas the HF diet had no effect at baseline in the SD outbred strain (Figure 3C). At 12 weeks, the triglycerides remained significantly elevated in HF diet exposed to WF in the BN and F344 strain compared with the other groups (Figs. 3A and B). The response was sustained at 24 weeks for HF diet groups in the BN strain only. The triglyceride response was more variable in the outbred SD strain, and in some cases, the serum triglycerides were unexpectedly elevated in the REG diet groups compared with the HF diet groups at 12 and 24 weeks (Figure 3C).

Pulmonary Responses

In assessing lung injury (LDH activity) and inflammation (total BAL cells recovered), it was not surprising that the groups exposed to WF demonstrated significant increases in both parameters for all 3 strains immediately after the exposure at week 12 (Figs. 4 and 5). For LDH, the response was sustained at week 24 after the WF exposure recovery period for the BN strain (Figure 4A) but not the F344 strain (Figure 4B). Interestingly, the only group that had a sustained LDH response at 24 week in the SD strain was the WF-exposed group that had been maintained on the HF diet (Figure 4C). For total BAL cells recovered, the response was sustained at week 24 after the WF exposure recovery period in both the inbred BN and F344 strains (Figs. 5A and B) but not the outbred SD strain (Figure 5C).

Systemic Responses

In the serum, a variety of enzymes (Table 1), proteins (Table 2), and markers of kidney function (Table 3) were measured to assess the effect of WF exposure and diet on systemic responses. Generally, if a statistically significant difference was observed

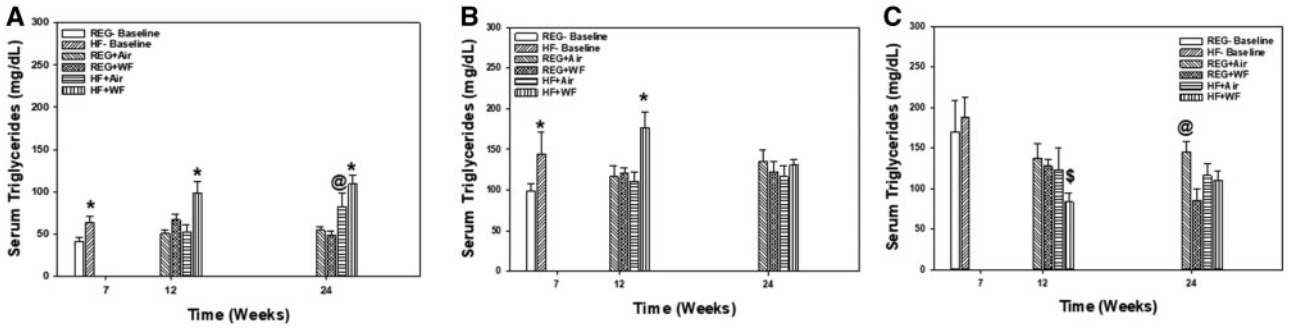

Figure 3. Serum triglyceride values comparing high-fat and regular diets over a 24-week period for (A) BN, (B) F344, and (C) SD rat strains. At week 7 during diet maintenance, groups of rats from each strain were exposed to stainless steel WF (20 mg/m^3 × 3 h/day × 4 days/week × 5 weeks) or filtered air (control) until week 12 at which time some animals from each strain were euthanized. A separate set of rats from each strain were allowed to recover from welding fume exposure until the end of the 24-week period. Values are means ± standard error ($n = 6$/group); *, different from other groups within a time point; **$**, different from REG + Air and REG + WF groups within a time point; @, different from REG + WF group within a time point ($p < .05$).

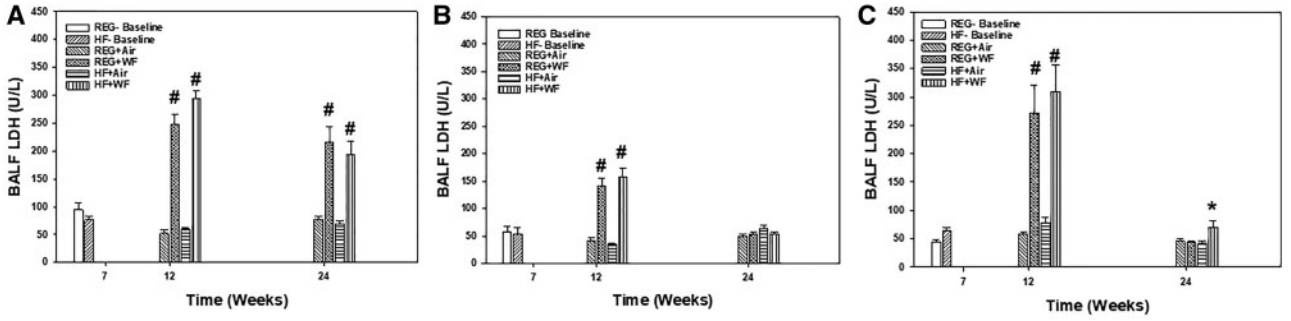

Figure 4. BALF LDH values comparing high-fat and regular diets over a 24-week period for (A) BN, (B) F344, and (C) SD rat strains. At week 7 during diet maintenance, groups of rats from each strain were exposed to stainless steel WF (20 mg/m^3 × 3 h/day × 4 days/week × 5 weeks) or filtered air (control) until week 12 at which time some animals from each strain were euthanized. A separate set of rats from each strain were allowed to recover from welding fume exposure until the end of the 24-week period. Values are means ± standard error ($n = 6$/group); #, different from REG + Air and HF + Air groups within a time point; *, different from other groups within a time point ($p < .05$). A portion of the data in this was modified and used (Skovmand *et al.*, 2020).

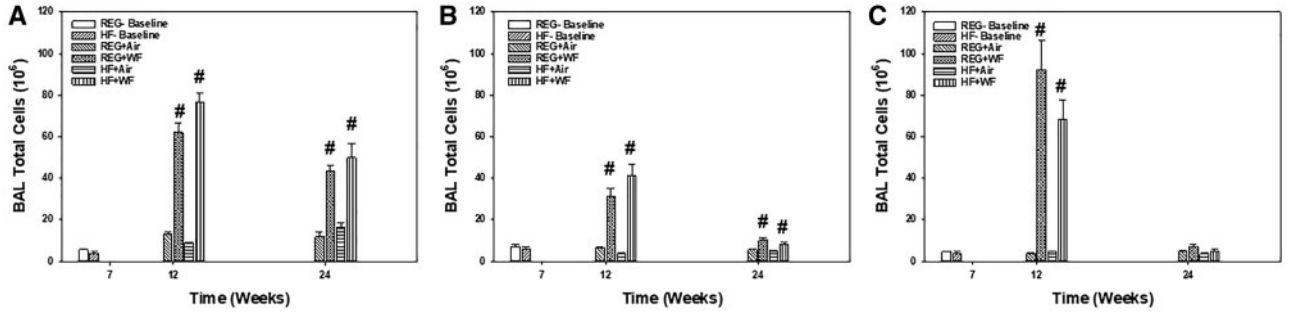

Figure 5. Recovered total BAL cells comparing high-fat and regular diets over a 24-week period for (A) BN, (B) F344, and (C) SD rat strains. At week 7 during diet maintenance, groups of rats from each strain were exposed to stainless steel WF (20 mg/m^3 × 3 h/day × 4 days/week × 5 weeks) or filtered air (control) until week 12 at which time some animals from each strain were euthanized. A separate set of rats from each strain were allowed to recover from welding fume exposure until the end of the 24-week period. Values are means ± standard error ($n = 6$/group); *, different from other groups within a time point; #, different from REG + Air and HF + Air groups within a time point ($p < .05$). A portion of the data in this was modified and used (Skovmand *et al.*, 2020).

in any of parameters, it occurred when comparing the groups maintained on a HF diet to the REG diet groups within a time point. The difference appeared to be most pronounced in the HF + WF group, particularly at 24 weeks.

In assessing whether the HF diet predisposed the WF-exposed animals to increases in liver enzymes, the HF + WF group had significant elevations in ALT (BN strain at 12 and 24 weeks), AST (BN strain at 12 and 24 weeks), and ALKP (F344 strain at 12 and 24 weeks; SD strain at 24 weeks) compared with the REG + AIR group within a time point (Table 1). Interestingly, ALKP at 12 weeks for the HF + WF group was significantly increased when compared with the REG + AIR group (Table 1). Histopathological analysis of liver sections stained with Oil Red O indicated a substantial increase in lipid staining in all strains maintained on the HF diet at 12 weeks (Figure 6) that also was still observed at 24 weeks (images not shown). Exposure to WF

Table 1. Serum ALT, AST, and ALKP Enzymes Comparing the Different Strains at 7 Weeks (Baseline), 12 Weeks, and 24 Weeks

Groups	BN ALT (U/l)	BN AST (U/l)	BN ALKP (U/l)	F344 ALT (U/l)	F344 AST (U/l)	F344 ALKP (U/l)	SD ALT (U/l)	SD AST (U/l)	SD ALKP (U/l)
REG-baseline	54.8 ± 2.1	128 ± 4.2	167 ± 10	72.8 ± 5.6	117 ± 9.3	321 ± 11	71.2 ± 1.8	139 ± 11	190 ± 9.2
HF-baseline	25.3 ± 2.4[a]	108 ± 5.4	190 ± 11	62.3 ± 8.0	119 ± 21	405 ± 18[a]	59.7 ± 5.1	151 ± 15	225 ± 18
REG + Air-12 weeks	89.0 ± 19	176 ± 28	150 ± 7.1	66.0 ± 3.4	95.8 ± 4.0	283 ± 7.9	108 ± 15	187 ± 21	172 ± 18
REG + WF-12 weeks	158 ± 23	249 ± 31[b]	165 ± 8.0	59.8 ± 2.4	86.8 ± 1.6	280 ± 4.8	118 ± 13	212 ± 12	165 ± 9.3
HF + Air-12 weeks	82.0 ± 16[c]	183 ± 22	173 ± 7.0	50.0 ± 2.5	75.3 ± 2.3	340 ± 12[d]	49.0 ± 2.4[d]	132 ± 11[a]	206 ± 12[d]
HF + WF-12 weeks	**139 ± 37**[e]	**281 ± 55**[e]	178 ± 12	60.7 ± 8.4	97.0 ± 8.1	**379 ± 8.6**[a]	**71.5 ± 11**[d]	208 ± 25[f]	191 ± 15[d]
REG + Air-24 weeks	104 ± 18	241 ± 47	161 ± 7.2	89.2 ± 7.4	122 ± 10	223 ± 17	100 ± 7.5	165 ± 13	132 ± 14
REG + WF-24 weeks	76.0 ± 8.4	177 ± 14	157 ± 5.3	89.7 ± 6.6	126 ± 5.9	219 ± 15	99.3 ± 15	162 ± 12	161 + 8.3
HF + Air-24 weeks	139 ± 17[c]	274 ± 27[c]	147 ± 6.5	83.7 ± 8.8	120 ± 11	313 ± 21[d]	54.8 ± 7.4[d]	123 ± 9.2	174 ± 11[b]
HF + WF-24 weeks	**196 ± 16**[a]	**374 ± 47**[d]	162 ± 8.4	101 ± 14	154 ± 14	**310 ± 20**[d]	80.5 ± 13[c]	194 ± 21[f]	**179 ± 13**[b]

Values are means ± standard error ($n = 6$). $p < .05$. Bold values indicate significant differences comparing the HF + WF group with the REG + AIR control within a time point.
Abbreviations: REG, regular diet; HF, high-fat diet; WF, welding fume.
[a]Significantly different from other groups within a time point.
[b]Significantly different from the REG + Air group within a time point.
[c]Significantly different from the REG + WF group within a time point.
[d]Significantly different from the REG + Air and REG + WF groups within a time point.
[e]Significantly different from the REG + Air and HF + Air groups within a time point.
[f]Significantly different from the HF + Air group within a time point.

Table 2. Serum TP, ALB, and GLB Proteins Comparing the Different Strains at 7 Weeks (Baseline), 12 Weeks, and 24 Weeks

Groups	BN TP (g/dl)	BN ALB (g/dl)	BN GLB (g/dl)	F344 TP (g/dl)	F344 ALB (g/dl)	F344 GLB (g/dl)	SD TP (g/dl)	SD ALB (g/dl)	SD GLB (g/dl)
REG-baseline	5.65 ± 0.12	2.72 ± 0.03	2.93 ± 0.10	5.86 ± 0.07	3.18 ± 0.10	2.72 ± 0.07	5.48 ± 0.07	3.17 ± 0.08	2.77 ± 0.09
HF-baseline	5.33 ± 0.04[a]	2.57 ± 0.04	2.78 ± 0.03	6.15 ± 0.08[a]	3.43 ± 0.11	2.68 ± 0.04	6.32 ± 0.11[a]	2.72 ± 0.04	3.15 ± 0.06[a]
REG + Air-12 weeks	6.12 ± 0.14	2.92 ± 0.10	3.20 ± 0.05	5.83 ± 0.11	3.15 ± 0.10	2.68 ± 0.05	5.93 ± 0.21	2.75 ± 0.15	3.18 ± 0.07
REG + WF-12 weeks	6.08 ± 0.11	2.88 ± 0.05	3.20 ± 0.07	5.77 ± 0.05	3.03 ± 0.04	2.73 ± 0.04	6.08 ± 0.09	2.93 ± 0.07	3.15 ± 0.11
HF + Air-12 weeks	5.92 ± 0.10	2.75 ± 0.06	3.17 ± 0.05	5.63 ± 0.10	3.13 ± 0.04	2.50 ± 0.06[b]	5.80 ± 0.14	2.78 ± 0.04	3.02 ± 0.12
HF + WF-12 weeks	5.95 ± 0.15	2.85 ± 0.07	3.10 ± 0.08	6.08 ± 0.12[d]	3.37 ± 0.13[b]	2.72 ± 0.08[c]	5.73 ± 0.05	2.80 ± 0.06	**2.93 ± 0.04**[d]
REG + Air-24 weeks	6.13 ± 0.46	3.17 ± 0.08	3.47 ± 0.05	6.31 ± 0.33	3.35 ± 0.19	2.97 ± 0.15	6.14 ± 0.11	2.86 ± 0.05	3.28 ± 0.08
REG + WF-24 weeks	6.43 ± 0.04	2.92 ± 0.02	3.48 ± 0.05	6.41 ± 0.32	3.38 ± 0.22	3.03 ± 0.22	5.98 ± 0.24	2.77 ± 0.13	3.22 ± 0.13
HF + Air-24 weeks	6.28 ± 0.13	3.00 ± 0.10	3.28 ± 0.07	7.35 ± 0.29[d]	4.00 ± 0.24	3.35 ± 0.12[d]	5.67 ± 0.10	2.60 ± 0.04	3.07 ± 0.06
HF + WF-24 weeks	6.35 ± 0.14	3.10 ± 0.10[b]	3.25 ± 0.06	**7.63 ± 0.37**[d]	**4.25 ± 0.24**[d]	**3.38 ± 0.15**[d]	5.73 ± 0.11	2.75 ± 0.08	**2.98 ± 0.06**[e]

Values are means ± standard error ($n = 6$). $p < .05$. Bold values indicate significant differences comparing the HF + WF group with the REG + AIR control within a time point.
Abbreviations: REG, regular diet; HF, high-fat diet; WF, welding fume.
[a]Significantly different from other groups within a time point.
[b]Significantly different from the REG + WF group within a time point.
[c]Significantly different from the HF + Air group within a time point.
[d]Significantly different from the REG + Air and REG + WF groups within a time point.
[e]Significantly different from the REG + Air group within a time point.

appeared to have no effect on lipid accumulation as determined by Oil Red O staining when comparing the corresponding Air groups for each strain.

In measuring different serum proteins (TP, ALB, and GLB), there were varied responses among the strains (Table 2). The HF diet and WF exposure increased all 3 protein measures in the F344 strain compared with the REG + AIR group at 24 weeks (Table 2). However, serum GLB was significantly decreased in the HF + WF group compared with the REG + AIR control at 12 and 24 weeks (Table 2) in the SD strain.

In the assessment of several kidney function comparing the HF + WF and REG + AIR groups, a variable response also was observed depending on the strain (Table 3). No differences were observed in the kidney function parameters comparing the HF + WF and REG + AIR groups in the BN strain at any time point. However, BUN and CREAT were significantly elevated in the HF + WF group compared with the REG + AIR control at 12 and 24 weeks for the F344 strain and at 24 weeks for the SD strain (Table 3). The BUN/CREAT ratio was significantly decreased in both the F344 and SD strains at 24 weeks when comparing the HF + WF and REG + AIR groups (Table 3).

Proportion of Variance and PCA

A proportion of variance was determined by assessing 3-way ANOVAs on the variables at each time point accounting for strain, exposure, diet and all interactions among the 3 factors to make conclusions as to which experimental factor had the most significant contribution on a particular health index (Table 4). In regards to body weight and serum triglyceride response, animal strain overwhelmingly had the greatest influence at both time

Table 3. Serum BUN, CREAT, and BUN/CREAT Ratio Comparing the Different Strains at 7 Weeks (Baseline), 12 Weeks, and 24 Weeks

Groups	BN BUN (mg/dl)	BN CREAT (mg/dl)	BN BUN/CREAT Ratio	F344 BUN (mg/dl)	F344 CREAT (mg/dl)	F344B UN/CREAT Ratio	SD BUN (mg/dl)	SD CREAT (mg/dl)	SD BUN/CREAT Ratio
REG-baseline	18.0 ± 0.89	0.27 ± 0.02	70.2 ± 8.0	20.0 ± 0.07	0.26 ± 0.02	79.2 ± 6.8	17.7 ± 0.61	0.23 ± 0.02	78.3 ± 6.5
HF-baseline	17.3 ± 0.33	0.28 ± 0.02	62.5 ± 4.6	24.0 ± 1.2[a]	0.28 ± 0.02	86.5 ± 7.0	19.3 ± 0.61[a]	0.33 ± 0.02[a]	58.5 ± 2.1[a]
REG + Air-12 weeks	18.7 ± 0.49	0.26 ± 0.02	70.8 ± 8.0	18.8 ± 0.31	0.28 ± 0.02	68.2 ± 6.4	18.0 ± 0.58	0.30 ± 0.03	62.5 ± 6.3
REG + WF-12 weeks	18.0 ± 0.37	0.28 ± 0.02	65.3 ± 6.0	18.3 ± 1.2	0.28 ± 0.02	67.8 ± 5.9	19.0 ± 0.86	0.30 ± 0.00	63.3 ± 2.9
HF + Air-12 weeks	18.2 ± 0.60	0.35 ± 0.02[b]	53.3 ± 4.0	21.5 ± 0.85[c]	0.32 ± 0.02	68.5 ± 3.9	20.8 ± 0.40[d]	0.30 ± 0.00	69.5 ± 1.2
HF + WF-12 weeks	20.3 ± 1.2	0.35 ± 0.02[b]	58.3 ± 0.84	**23.2 ± 1.3[c]**	**0.37 ± 0.02[a]**	65.3 ± 7.2	19.3 ± 0.33	0.28 ± 0.02	69.5 ± 4.2
REG + Air-24 weeks	19.2 ± 0.48	0.32 ± 0.02	61.6 ± 3.8	22.8 ± 1.2	0.30 ± 0.04	80.0 ± 6.7	17.2 ± 0.79	0.24 ± 0.02	73.6 ± 5.4
REG + WF-24 weeks	18.5 ± 0.72	0.32 ± 0.02	59.0 ± 4.6	21.0 ± 1.0	0.30 ± 0.03	71.7 ± 4.6	16.2 ± 0.66	0.24 ± 0.01	69.4 ± 4.8
HF + Air-24 weeks	21.8 ± 0.95	0.45 ± 0.03[a]	50.5 ± 2.2[d]	24.7 ± 1.3[c]	0.40 ± 0.03[c]	62.2 ± 1.6[d]	19.5 ± 1.1	0.32 ± 0.02	61.8 ± 3.0
HF + WF-24 weeks	19.7 ± 0.49	0.33 ± 0.02	59.8 ± 3.0	**27.0 ± 1.7[a]**	**0.53 ± 0.06[a]**	**53.0 ± 4.7[c]**	**20.3 ± 0.99[d]**	**0.35 ± 0.02[c]**	**59.2 ± 3.7[d]**

Values are means ± standard error ($n = 6$). $p < .05$. Bold values indicate significant differences comparing the HF + WF group with the REG + AIR control within a time point.

Abbreviations: REG, regular diet; HF, high-fat diet; WF, welding fume.

[a]Significantly different from other groups within a time point.

[b]Significantly different from the REG + WF group within a time point.

[c]Significantly different from the REG + Air and REG + WF groups within a time point.

[d]Significantly different from the REG + Air group within a time point.

points. Not surprisingly, WF exposure significantly influenced lung toxicity and inflammation at 12 weeks directly after the exposure ended. Importantly, the contribution of WF exposure lessened after the recovery phase at 24 weeks as the influence of rat strain became greater. At 12 weeks, animal strain had the greatest effect on the 2 enzymes, ALT and AST, but by 24 weeks, the interaction of strain and diet had the most significant influence. Interestingly, WF exposure and the interaction of diet and exposure were significant contributors for the AST response at 12 and 24 weeks, respectively. For the ALKP response, diet had the only significant interaction at 12 weeks, but at 24 weeks, animal strain, diet, and the interaction of strain and diet significantly contributed to the response. Diet, and to a lesser extent rat strain, had the most significant impact on kidney function (eg, BUN, CREAT) at 12 weeks, whereas diet had the only significant interaction for these parameters at 24 weeks. In the assessment of the different serum proteins, diet had the only significant interaction at 12 weeks in the measurement of GLB, whereas animal strain along with the interaction of strain and diet had the greatest influence on the TP, GLB, and ALB response at 24 weeks after the recovery phase.

PCA plots were generated to evaluate the influence of rat strain in more detail in the current study (Figure 7). For the control REG + AIR group, the data clustered together for the 3 strains as there was an overlap of the profiles at both 12 and 24 weeks (Figure 7A). When the animals maintained on the REG diet were exposed to WF, a distinct separation of the data for the different strains developed at 12 weeks after exposure. By 24 weeks after the recovery period, some overlap of the data profiles was observed for the F344 and SD strains but not the BN strain (Figure 7B), which reflected the sustained pulmonary inflammatory response. Unlike the animals maintained on the REG diet, the data profiles for the 3 strains of rats maintained on the HF diet and exposed to air were completely separated by 24 weeks (Figure 7C). This separation was maintained in the HF + WF group for all 3 strains at 24 weeks as well, indicating the significant influence of animal strain on the diet- and exposure-induced effects alone and in combination (Figure 7D).

DISCUSSION

Few studies have examined the pulmonary effects after exposure to welding fume in animals maintained on a HF diet. Hemmati *et al.* (2018) demonstrated that mice fed a HF diet and exposed to arsenic for 20 weeks had a significant increase in oxidant-induced lung toxicity compared with arsenic-exposed animals maintained on a standard diet as evidenced by histopathological and biochemical measurements of fibrosis. But unlike the current study, the animals were exposed to arsenic through drinking water and not by inhalation. In a study using a design that more closely resembled the one used currently, Tilton *et al.* (2013) observed that high-fat diet induced obese mice exposed to mainstream cigarette smoke for 5 h/day for 2 weeks developed a significant increase in the influx of lung inflammatory cells (eg, macrophages and neutrophils) compared with similarly exposed nonobese, regular weight mice. However, Yanagisawa *et al.* (2014) demonstrated that obese mice exposed to diesel exhaust particles for 2 weeks were more resistant to inflammation, as determined by BAL cell profile, and histopathological changes in the lung compared with lean mice. Based on these findings, it is possible that an unhealthy diet may change the susceptibility of pulmonary responses in a worker exposed to specific inhaled particulate matter.

Table 4. Influence of Exposure, Diet, and Strain on Each Parameter After Data Partitioning

Organ/Tissue	Parameter	Source	12 Weeks	24 Weeks
General	Body weight	—	Strain 94% Diet 2% Strain × Diet 1% Strain × Exposure 1% Diet × Exposure 1%	Strain 82% Diet 13% Strain × Diet 3% Strain × Diet × Exposure 1%
Lung	Toxicity (LDH)	BALF	Exposure 97% Strain 2%	Strain × Exposure 37% Exposure 32% Strain 22% Strain × Diet × Exposure 5%
	Inflammation (BAL cells)	BAL	Exposure 85% Strain 10% Strain × Exposure 4% Strain × Diet × Exposure 1%	Exposure 61% Strain 19% Strain × Exposure 12% Diet 5%
Lipid	Triglycerides	Serum	Strain 41% Strain × Exposure 21% Strain × Diet 18% Exposure 10%	Strain 47% Strain × Diet 21% Diet × Exposure 12% Diet 9% Strain × Exposure 8%
Liver	ALT	Serum	Strain 47% Diet 16% Exposure 14% Strain × Diet 13%	Strain × Diet 55% Strain 26% Diet × Exposure 12%
	AST	Serum	Strain 45% Exposure 29%	Strain × Diet 32% Diet × Exposure 27% Diet 12% Exposure 11%
	ALKP	Serum	Diet 66%	Strain 36% Diet 29% Strain × Diet 25%
Kidney	BUN	Serum	Diet 41% Strain 28%	Diet 75%
	CREAT	Serum	Diet 31% Strain 28% Strain × Diet 27%	Diet 70%
Proteins	TP	Serum		Strain 49% Strain × Diet 39%
	ALB	Serum		Strain 50% Strain × Diet 36% Diet 7%
	GLB	Serum	Diet 44%	Strain 55% Strain × Diet 43%

A proportion of variance from a 3-way ANOVA was performed at 12 and 24 weeks, accounting for strain, exposure, diet, and all interactions among the 3 factors. The analysis was performed on log-transformed data as the data were generally distributed lognormally with a tail on the right, and thus had heterogeneous variance. The colors were used as a way to highlight each of the specific exposomal factors- red = strain; green = diet; blue = exposure.

In regards to the pulmonary responses in the assessment of the current study's first objective, the HF diet had minimal influence on lung injury and inflammation at 12 and 24 weeks based on the diet and exposure paradigm used, except in one case by which BAL fluid LDH activity was significantly elevated in the SD strain at 24 weeks in the HF + WF group only. This observation was confirmed from a proportion of variance analysis using a 3-way ANOVA as exposure had overwhelmingly influenced lung responses compared with strain and diet at 12 weeks when the exposure ended. However, the contribution of WF exposure lessened to a small degree after the recovery phase at 24 weeks, indicating that resolution of inflammation had a dependency on strain as well as highlighting the importance of selection of a particular strain in animal inhalation toxicity and recovery studies. Previous studies by other laboratories assessing the pulmonary responses to inhaled toxicants (eg, ozone, silver nanoparticles) in multiple strains of mice also observed variations in lung injury and inflammation that were dependent on strain, suggesting the important role that genetics play in the sensitivity to inhaled substances (Scoville *et al.*, 2017; Vancza *et al.*, 2009). Based on the findings of Scoville *et al.* (2017) in the examination of lung inflammation after exposure to silver nanoparticles in multiple strains of mice, specific susceptibility genes, such as *Nedd41*, *Ano6*, and *Rnf220*, were identified by genome-wide association (GWA) mapping as possible candidates related to the varied inflammatory response in the lung among strains. Importantly, *Nedd41* and *Rnf220* have been linked with pulmonary responses in human GWA mapping studies; in addition, *Ano6* has been associated with the inflammatory mediator C-

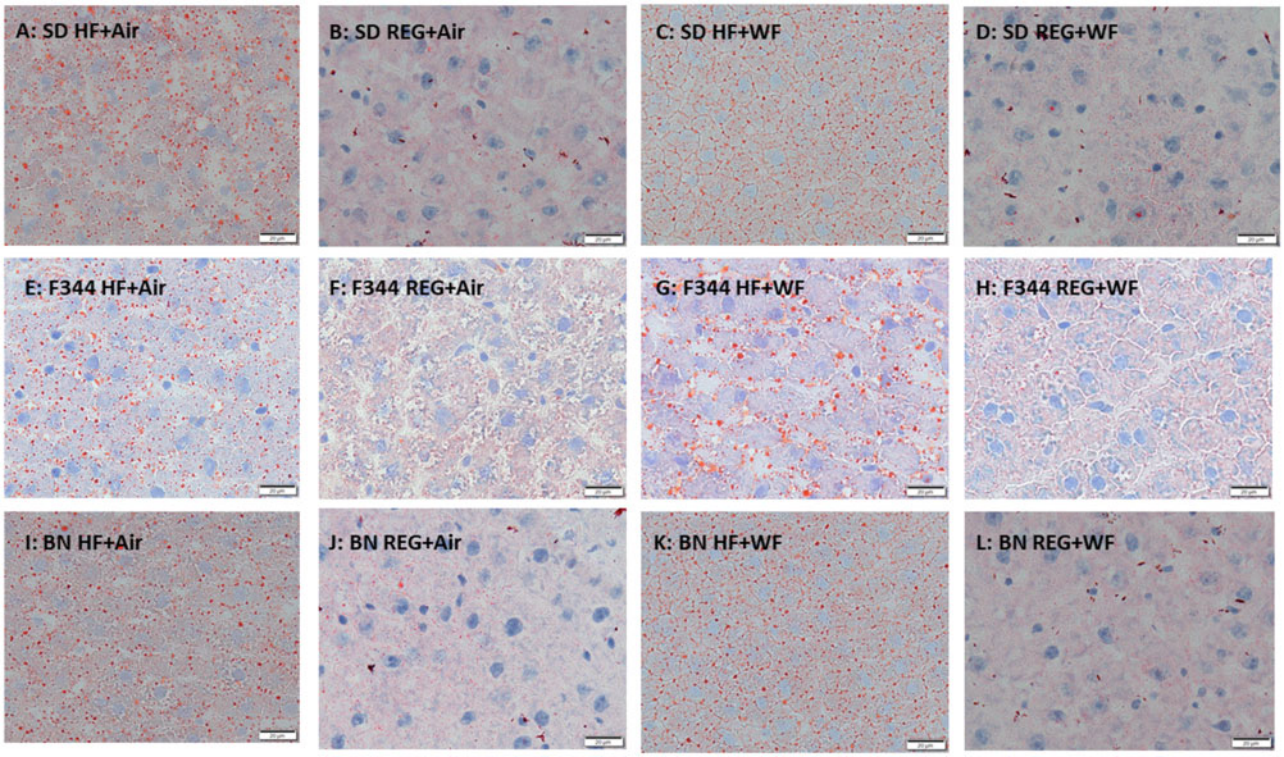

Figure 6. Representative cryo-preserved liver sections stained with Oil O Red from the (A–D) SD, (E–H) F344, and (I–L) BN rat strains at 12 weeks for HF + Air, REG + Air; HF + WF, and REG + WF exposure groups. Lipid was stained red, and nuclei were stained purple; ×100 magnification with oil; bar = 20 μm.

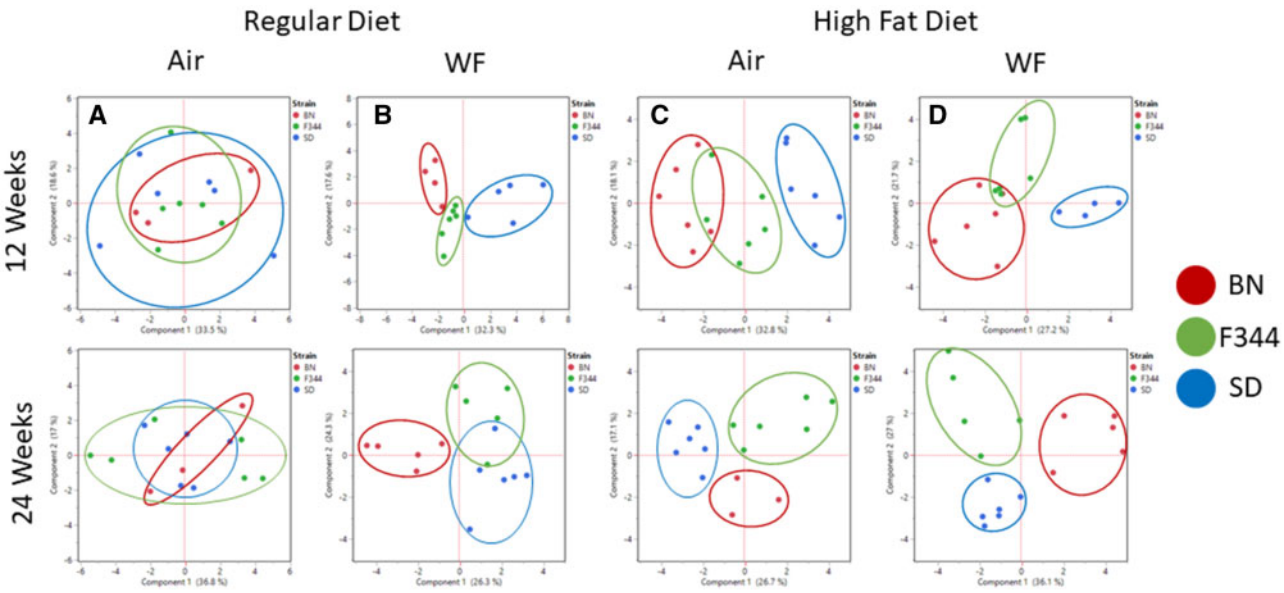

Figure 7. Principal Component Analysis (PCA) plot scores of health effect indices comparing the (A) REG + Air, (B) REG + WF, (C) HF + AIR, and (D) HF + WF groups at 12 and 24 weeks for BN (red symbols), F344 (green symbols), and SD (blue symbols) rat strains.

reactive protein (Pepys and Hirschfield, 2003; Ramos *et al.*, 2014). It is possible these susceptibility candidate genes may influence the resolution of the lung inflammation that was observed to be different among the different rat strains in the current investigation.

Compared with the pulmonary responses, the systemic responses were more varied and highly dependent on animal strain. Compared with a regular diet, maintenance of the WF and air groups on a HF diet caused a significantly greater increase in the % change in body weight from baseline for all 3 strains over the 24-week period. The body weight response in the leaner, inbred strains (F344 and BN) was pronounced compared with the more obese, outbred SD strain. An even more varied response was observed when examining the effects of

diet with exposure to WF on the levels of triglyceride in the serum, a marker of hyperlipidemia, over 24 weeks. No clear pattern of triglyceride response was observed related to diet, exposure, or time in comparing the different strains. These findings were not surprising as the causes of weight gain and hyperlipidemia have been shown to be multifactorial with both nongenetic (eg, composition of diet, eating and nutritional habits, physical activity, lifestyle, and chronic stress) and genetic (eg, leptin gene mutation) factors, likely due to an complex interaction of genetic, epigenetic, and environmental influences (Kucera and Cervinkova, 2014; Rohde *et al.*, 2019). Also, resting metabolic rate and fat-free mass, important factors known to be highly variable among different individuals based on age, sex, and ethnicity, have been observed to influence weight gain and increased body fat and are determined by both hereditability and nongenetic causes, such as physical activity (Oussaada *et al.*, 2019). Interestingly, both body weight and serum triglycerides responses in the current study were driven more by strain than diet at both 12 and 24 weeks based on a proportion of variance analysis, emphasizing the important influence of genetics in the exposome.

Three enzymes (AST, ALT, and ALKP) that are indicators of changes in liver function were measured in the serum. These enzymes may be present in other organs (eg, kidney, muscles, heart, and lungs) but are found at the highest concentrations in the liver and are used to detect the presence of liver injury. When the different treatment groups were compared with the REG + AIR control group, significant elevations in the enzymes were most often associated with HF + WF group at both 12 and 24 weeks. It has been clearly demonstrated that a diet high in fat content can lead to liver abnormalities (Klaunig *et al.*, 2018). Histopathological changes were observed in the animals maintained on the HF diet in the current study as evidenced by a significant accumulation of fat in all 3 strains at each time point. Also, metals associated with WF have been previously observed to accumulate in the liver after exposure (Antonini *et al.*, 2010; Sriram *et al.*, 2012), and inhalation of different types of metal particulates has been shown to induce liver toxicity (Husain *et al.*, 2015; Liu and Meng, 2005; Sung *et al.*, 2009; Vranic *et al.*, 2017). Interestingly, WF exposure had no effect on lipid accumulation as determined by Oil Red O staining when compared with the corresponding Air groups for each strain. In a related study, Qiu *et al.* (2017) observed that fine particulate matter stimulated hepatic autophagy in mice maintained on a HF diet, thus attenuating lipid accumulation in the liver.

When a proportion of variance was determined, strain and/or diet had the greatest influence on the liver enzyme responses in the current animal model at the 12-week time point. During the recovery phase, the interaction of strain and diet as well as exposure and diet became more prominent influencers of the hepatic effects. The data indicate that the BN strain is quite susceptible to liver effects and possibly could be driving the influence of strain and strain × diet on the liver enzyme response observed. It has been previously shown that the BN rat strain was more susceptible to *Pseudomonas* Exotoxin A-induced hepatotoxicity compared with F344 and Wistar strains as evidenced by significantly elevated AST and ALT levels and massive necrosis and hemorrhage that was not seen in the other 2 strains (Chiu *et al.*, 2017). Together with the findings of the current study, this observation further highlights the importance that the genetic background of different rat strains has on toxicological responses.

With obesity and altered lipid profiles being more prevalent, lipid accumulation in the kidney is relevant for the development of chronic kidney disease (Gai *et al.*, 2019). In addition, potentially toxic metals, such as manganese and chromium, have both been shown to deposit in the kidney after WF exposure (Antonini *et al.*, 2010). A variety of kidney damage markers (eg, proteinuria, alterations in BUN and CREAT) were measured in the serum to assess the effects of the HF diet and WF exposure on renal function in the current study. No differences were observed in TP, ALB, GLB, BUN, and CREAT comparing the HF + WF and REG + AIR groups in the BN strain. However, elevations in multiple kidney function parameters were observed for the HF + WF group compared with the REG + AIR control at 12 and 24 weeks for the F344 and SD strains. Interestingly, the BUN/CREAT ratio was significantly decreased in the F344 and SD strains at 24 weeks when comparing the HF + WF control group that may be a result of reduced urea formation and malnutrition, altered urea cycle in the liver, or liver injury, indicating poor kidney health. Based on a proportion of variance analysis, diet had the greatest influence for both BUN and CREAT at both 12 and 24 weeks, whereas strain was the most significant contributor to the protein response, particularly at 24 weeks.

In summary, an experimental model of the exposome was examined by which different strains of rat were exposed to an occupational toxicant while maintained on a HF diet. Pulmonary exposure to WF during maintenance on a HF diet caused specific adverse health outcomes directly after exposure as well as after a 12-week recovery phase. Depending on the animal strain, there was evidence that WF exposure and HF diet together worsened kidney toxicity as well as altered different serum enzymes and proteins. Diet had minimal interaction with WF exposure for pulmonary toxicity endpoints. The factors of diet, exposure, and strain were all important, depending on the health outcome measured. Exposure had the most significant influence on the pulmonary responses (eg, the target organ). Other than lung inflammation, animal strain had the most influence on most other health outcome indices comparing the different experimental factors, indicating that genetic differences likely drive the exposome effect in each strain. PCA further confirmed the influence of strain on the responses measured, also highlighting the importance genetic predisposition in the study. The lack of consistency across the different strains for all endpoints illustrated the complexity in developing experimental models to study the exposome. Thus, the translational aspect to humans was that genetic background (eg, strain differences) may predispose certain susceptibilities to various concomitant exposures. In general, our results suggest difficulty in establishing a predictive experimental model for the human exposome.

DECLARATION OF CONFLICTING INTERESTS

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