Older women exhibit greater airway 8-isoprostane responses to strenuous exercise compared to older men and younger controls

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Older women exhibit greater airway 8-isoprostane responses to strenuous exercise compared to older men and younger controls

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Abstract:

**INTRODUCTION:** Development of late-onset respiratory diseases is associated with elevated 8-isoprostane, a marker of oxidative stress, in the airways. However, sex differences exist in development of these diseases. Using an exhaustive exercise bout as a physiological stressor may elucidate whether there is a sex difference with aging in pre- to post-exercise airway 8-isoprostane generation. **PURPOSE:** To determine whether older women exhibit a greater airway 8-isoprostane response to exhaustive exercise compared to older men and younger controls.

**METHODS:** Thirty-six individuals completed the study (12 post-menopausal older women (OW) and 12 age-matched older men (OM) 65±4 years of age, and 12 younger controls (YC) 21±2 years of age). Baseline measurements included exhaled breath condensate (EBC) for assessment of airway 8-isoprostane and standard pulmonary function testing (PFTs) to assess forced expiratory volume in 1-second (FEV₁), forced vital capacity (FVC), FEV₁/FVC, and forced expiratory flow at 25-75% of FVC (FEF₂₅₋₇₅%). Subjects then performed a VO₂peak test to exhaustion on a cycle ergometer. Immediately post-exercise, PFTs and EBC were performed.

**RESULTS:** The generation of airway 8-isoprostane from pre- to post-exercise was greater in OW compared to OM and YC (p<0.01), increasing ~74±77% in OW, while decreasing in OM (~12±50%) and YC (~20.9±30%). **CONCLUSIONS:** The OW exhibited a greater airway 8-isoprostane response to exhaustive exercise compared to OM and YC, which may suggest that sex differences in oxidative stress generation following exhaustive exercise may provide a mechanistic rationale for sex differences in late-onset respiratory diseases.

Keywords: pulmonary physiology, sex differences, aging, exhaled breath condensate, exercise physiology
Introduction:
The prevalence of late-onset asthma diagnoses in individuals over 60 years of age is increasing, with a greater percentage of women diagnosed between 65 and 75 years of age as compared to age-matched males (Gibson et al. 2010). Oxidative stress and inflammation increase with age (De Martinis et al. 2005), which may contribute to airway remodeling, changes in airway structure, and changes in pulmonary function (Kurti et al. 2015). Recent investigations have also confirmed that lipid peroxidation, which is damaging to the airways, increases with age (Cruz et al. 2009). Specifically, one particular marker of lipid peroxidation, 8-isoprostane, is elevated in asthmatics (Montuschi et al. 1999) as well as individuals with chronic obstructive pulmonary disease (Montuschi et al. 2000). High levels of airway 8-isoprostane are associated with decrements in lung function (Samitas et al. 2009). Additionally, the generation of 8-isoprostane in the airways is associated with airway hyperresponsiveness, a hallmark characteristic of asthma that has been reported in asthmatics (Barreto et al. 2009) and more recently in non-asthmatic subjects (Morisette et al. 2016). There is some dispute regarding the contribution of oxidative stress to the aging process (Rikans and Hornbrook, 1997), yet several studies have confirmed that both aging and development of respiratory diseases are associated with higher airway 8-isoprostane (Bloomer and Fisher-Wellman 2008; Practico et al. 1998; Vassalle et al. 2003).

Older adults have a varying health-span (amount of years they are healthy in their lifespan), and lipid peroxidation and oxidative stress may play a role in the aging process. Additionally, several studies have reported sex differences in oxidative stress in older adults (Wang et al. 2006), which are associated with virtually all pathologies explored. The prevalence
of respiratory diseases differs between older men and post-menopausal women, and previous investigations have shown that post-menopausal women are at higher risk for negative respiratory system health outcomes as compared to older men (de Nijs et al. 2013). Older adults (age 60 and over) may experience complications as a result of acute strenuous physical exertion, which causes a transient increase in reactive oxygen species generation (Nikolaidis et al. 2011; Quindry et al. 2003). Therefore, it is important to determine whether OM and OW respond differently to various physiological stressors that elicit oxidative stress, and whether the 8-isoprostane response is affected by both sex and age.

There are no existing studies, to our knowledge, that have investigated whether sex differences in aging impact the airway 8-isoprostane responses to exhaustive exercise. In addition, no previous research has elucidated whether changes in airway oxidative stress are associated with changes in lung function from pre- to post-exercise in older women (OW), older men (OM) and younger control (YC) subjects. The primary purpose of the present investigation was to determine whether there were sex differences with aging that impacted airway 8-isoprostane generation following a bout of exhaustive exercise. A further exploratory analysis was conducted to determine whether changes in airway 8-isoprostane generation were correlated with changes in lung function. We hypothesized that OW would have elevated airway 8-isoprostane responses from pre- to post-exercise compared to OM and YC.

Materials and Methods

Subject characteristics

Thirty-six individuals were recruited by age to participate in the study (12 OW, 12 OM, and 12 YC). The OW were post-menopausal and matched for age with the OM and for habitual physical activity level with the OM and YC. All subjects were recreationally active and not...
exercise-trained. Subjects visited the laboratory after 24 hours of no exercise and a 2-hour fast. They were also asked to refrain from antioxidants, vitamins or other supplementation that was not a part of their normal food intake for at least 3 days prior to their testing session. When subjects first came to the laboratory, they were briefed on the study protocol, signed an informed consent document, and then completed a medical history questionnaire. The study protocol was approved by the Institutional Review Board at Kansas State University and conformed to the principles set forth in the Declaration of Helsinki.

Experimental Design

After completion of the required questionnaires and paperwork, a trained investigator completed the physiological assessment. Subjects laid down in the supine position for 15 minutes and blood pressure was measured a total of three times with five minutes in between each measurement (Pickering et al. 2005). The blood pressures were then averaged for analysis. Next, subject height was determined using on a portable stadiometer to the nearest 0.1 cm, and weight was recorded to the nearest 0.1 kg by an investigator using a standard physician’s scale. Subjects then underwent a Dual X-ray Absorptiometry scan (Prodigy software version 5.6, GE Lunar, Milwaukee, WI) to determine body composition. After the initial measurements, subjects underwent exhaled breath condensate (EBC) testing, followed by baseline pulmonary function testing (PFTs). After PFTs, subjects performed the incremental exercise to exhaustion, followed by PFTs within two minutes after completion/termination of the exercise test on a cycle ergometer. Subjects then underwent EBC, followed by the last round of PFTs at 20 minutes post-exercise. The experimental measures are elaborated in the section below.

Experimental Measurements

Questionnaires
The medical history questionnaire was used as a health-screening tool and also to ensure that all OW participating were post-menopausal. To ensure that subjects were ready to engage in vigorous physical activity, they were required to complete the physical activity readiness-questionnaire (PAR-Q) (ACSM 2013). Subjects were not hypertensive (Systolic BP<142; Diastolic BP<92) per the PAR-Q guidelines. If a subject had greater than two risk factors for cardiovascular disease, but did not have any signs and/or symptoms of disease, they were still able to participate under the recommendation of their primary care physician.

**Pulmonary function testing**

Pulmonary function was assessed using standard PFTs and according to the American Thoracic Society/European Respiratory Society guidelines (Miller et al. 2005). The maximum flow volume loop was used to assess pulmonary function, measuring forced vital capacity (FVC), forced expiratory volume in 1-second (FEV₁), forced expiratory flow between 25% and 75% of FVC (FEF₂₅₋₇₅%), and peak expiratory flow (PEF) (SensorMedics 229 Metabolic Cart, SensorMedics Corp., Yorba Linda, CA). Subjects performed PFTs until three measurements were within 10% of one another, and averaged for analysis. This procedure is more stringent than ATS guidelines, and has been used many times previously in our laboratory (Kurti et al. 2015a, b, Smith et al. 2015). Percent of predicted lung function was calculated using reference values (Knudson et al. 1983).

**Incremental exercise test to exhaustion**

Subjects performed an incremental cycle ergometer (SensorMedics 800, SensorMedics Corp., Yorba Linda, Calif., USA) test to exhaustion to determine peak oxygen consumption (VO₂peak) according to similar methods published in our laboratory (Emerson et al. 2015). During the entire test, subjects were required to maintain a cadence of 60-80 revolutions per minute.
(rpm). The incremental test to exhaustion began with three minutes of resting data collection where metabolic and ventilatory responses were recorded. Next, subjects began the test with a 3-minute unloaded warm-up where they cycled at 60-80 rpm. After the warm-up, the incremental test began at a load of 25 watts and increased by 25 watts per minute until either the subject reached volitional fatigue, or investigators’ terminated the test due to the subject not maintaining a cadence of at least 50 rpm for at least 5 revolutions. The final 10 seconds of every minute, heart rate and breath-by-breath data for ventilation were recorded. Carbon dioxide production CO_{2} and oxygen consumption were recorded through the entire test. Specific criteria were used to determine whether subjects reached VO_{2}peak; achieving a respiratory exchange ratio of greater than 1.15 and a heart rate max (HR_{max}) within 10 beats per minute of an age-predicted HR_{max}, as well as that subjects reached volitional fatigue.

**Assessment of Airway 8-isoprostane**

Subjects performed tidal breathing for 10 minutes into an RTube (Respiratory Research, Austin, TX), collecting ~1.0-1.5 mL of exhaled breath condensate sample. The samples were then plunged from the RTubes, aliquoted into microcentrifuge tubes for freezing, and immediately frozen in a -60 degree Celsius freezer. Samples were analyzed within six months of data collection. Samples from EBC were concentrated by C-18 solid phase extraction prior to analysis and analyzed using a commercially available ELISA kit (Cayman Chemicals, Ann Arbor, MI, USA, Kit #516351). Samples were analyzed in triplicate with an intra-assay coefficient of variation of 5.2%. The average of the three values was used for analysis.

**Statistical Analyses**

Data were analyzed using SPSS Statistical Software v.24 (IBM, Armonk, NY, USA). The data are expressed as mean±SD. Data were checked for normality using the Shapiro-Wilk test
and to verify that parametric assumptions were met prior to the analysis. All data were normally
distributed except for airway 8-isoprostane pre-exercise in OW; therefore all pre- and post-
exercise airway 8-isoprostane values were log10-transformed. Data were normally distributed
after log 10-transformation. Two-way analysis of variance (ANOVA) was used where the
within-subjects effect was time (pre- and post-exercise) for airway 8-isoprostane generation and
time (pre-, post-, and 20-minutes post-exercise) for lung function. The between-subjects factor
was group (OM, OW, YC). A one-way repeated measures ANOVA was subsequently used to
determine time point differences in lung function within each group. Correlations were assessed
between changes in airway 8-isoprostane and lung function with the Pearson Product moment
correlation coefficient. Bonferroni adjustments were made to reflect multiple comparisons. For
all analyses, significance was set to $p<0.05$.

Results

Subject characteristics

Baseline subject characteristics and exercise responses are displayed in Table 1. The OW
and OM were not different in age from each other ($p=0.23$). The OW had greater body fat
percentage and were shorter compared to OM, and OM had greater body fat percentage
compared to YC ($p<0.01$). The PA levels were not different from each other between the OW,
OM, and YC ($p>0.05$).

Data from the incremental exercise test to exhaustion are also displayed in Table 1. The
OW had lower absolute VO$_{2\text{peak}}$, relative VO$_{2\text{peak}}$, and peak power at VO$_{2\text{peak}}$ compared to OM
and YC ($ps<0.01$). The OM had lower absolute and relative VO$_{2\text{peak}}$ compared to the YC,
however peak power ($p=0.06$), respiratory exchange ratio ($p=0.43$) and peak ventilation ($p=0.22$)
were not different compared to the YC. Women had a higher RER at VO$_{2\text{peak}}$ compared to OM.
(\(p=0.03\)), and a lower peak ventilation (\(p<0.01\)). The maximum heart rate achieved at \(\text{VO}_{2\text{peak}}\) was not different between OW and OM (\(p=0.96\)), however was higher in the YC (\(ps<0.05\)).

### 3.2 Generation of Airway 8-isoprostane

There were no differences in airway 8-isoprostane pre-exercise between the OW (11.3±7.9 pg/mL), OM (11.3±3.8 pg/mL) and YC (11.1±2.9 pg/mL) (\(p=0.98\)). There were also no differences in 8-isoprostane generation from pre- to post-exercise in the six younger men and six women (\(p=0.56\)), so the results are collapsed in the YC group for all subsequent analyses.

Considering the airway 8-isoprostane pre-exercise values were not normally distributed in OW, results are based on the log10-transformed data pre- to post-exercise. Figures have been left in absolute values to better illustrate the variability in individual airway 8-isoprostane values and responses. Figure 1 displays the mean airway 8-isoprostane responses from pre- to post-exercise in OW, OM and YC. There was a significant interaction between time and group (\(p<0.01\)), where OW had a greater airway 8-isoprostane response from pre- to post-exercise compared to the OM and YC. There was not a significant difference between the OM and YC (\(p=0.67\)), however there was a less generation of airway 8-isoprostane post-exercise in YC and OM when compared to the OW (\(p<0.01\)). Figure 2a displays the individual responses of the OW, Figure 2b displays the responses of the OM and Figure 2c displays the YC responses. As can be seen in the individual absolute data, 9/12 OW exhibited an increase in 8-isoprostane while the other three OW remained relatively unchanged, or showed a small decrease in the generation of airway 8-isoprotane. In contrast, 9/12 OM showed decreased generation of 8-isoprostane in response to the maximal bout of exercise, while only 3/12 showed increased 8-isoprostane. In the YC, 3/12 exhibited an increased generation of 8-isoprostane while 9/12 showed decreased generation of 8-isoprostane in response to the strenuous bout of exercise. In OW alone, 8-isoprostane generation
significantly increased by ~74% \((p<0.01)\), while in OM it did not change significantly (~11%, \(p=0.20)\). However, the YC exhibited a decreased airway 8-isoprostane generation from pre- to post-exercise by ~21% \((p=0.04)\).

**Lung function**

Absolute lung function data are displayed in Table 2. When analyzing group differences in percent of predicted lung function, there were no significant differences in main outcome measures of lung function between OW, OM and YC in FVC (OW: 102±9%; OM: 110±18%; YC: 108±14%), FEV\(_1\) (OW: 95±10%; OM: 100±18%; YC: 99±9%), and FEV\(_1\)/FVC (OW: 91±6%; OM: 91±7%; YC: 93±8%) \((p_s>0.05\), respectively). Also, FEF\(_{25-75}\)% and PEF did not differ in percent of predicted between OW, OM and YC \((p_s>0.05)\). However YC had greater absolute values for PEF, FVC, FEV\(_1\)/FVC and FEF\(_{25-75}\)% of FVC at baseline \((p_s>0.05)\). In all groups, there were significant increases in percent of predicted lung function from pre- to 20-minutes post-exercise as a main effect of time in FEV\(_1\) \((p<0.01)\), FEV\(_1\)/FVC \((p<0.01)\) and FEF\(_{25-75}\)% \((p<0.01)\). Also, changes in lung function between OW, OM and YC were different across time \((\text{time}* \text{sex interaction})\) from pre-to 20-minutes post-exercise in FEV\(_1\)/FVC \((p<0.01)\) and FEF\(_{25-75}\)% \((p=0.03)\). When further examining pair-wise comparisons within each group, there was a significant increase in FEV\(_1\) from baseline to post-exercise \((p=0.02)\) that returned to baseline by 20 minutes post-exercise \((p>0.90)\) in OW. There were no significant changes in FVC, FEV\(_1\)/FVC, FEF\(_{25-75}\)% or PEF from baseline, post- or 20-minutes post-exercise \((\text{all } p\text{-values } >0.10)\) in OW. In OM, there was no change in FVC from baseline to immediately post-exercise \((p>0.90)\) or baseline to 20 minutes post-exercise \((p=0.91)\). However, there was a significant increase in FEV\(_1\) post-exercise \((p=0.03)\) that returned back to baseline 20 minutes post \((p=0.25)\). There was also an increase in FEV\(_1\)/FVC post-exercise \((p=0.01)\) and 20 minutes post \((p<0.01)\).
FEF<sub>25-75</sub>% increased post-exercise ($p=0.03$) and remained higher at 20 minutes after the exercise bout ($p<0.01$) compared to baseline. There were no changes observed in PEF immediately or 20-minutes post-exercise ($p>0.90$). In YC, there was an increase in FEV<sub>1</sub>/FVC ($p<0.01$), which was driven by a small decrease in FVC, although it was not statistically significant ($p=0.053$). Also, FEF<sub>25-75</sub>% was greater from pre- to post-exercise in YC ($p<0.01$).

**Associations between variables**

There were no significant correlations between airway 8-isoprostane generation from pre- to post-exercise and changes in any indices of lung function in OW, OM or YC combined (all $p$-values $>0.05$). In OW, OM and YC alone, there were no associations between absolute or percentage changes in 8-isoprostane and lung function ($ps>0.05$).

**Discussion**

**Major Findings**

The primary purpose of this study was to assess the airway 8-isoprostane responses following an exhaustive bout of exercise, and determine whether airway 8-isoprostane generation was impacted by sex differences in older adults compared to younger subjects from pre- to post-exercise. We further explored whether the changes in airway 8-isoprostane were correlated with changes lung function. The results from the current study supported our hypothesis that airway 8-isoprostane generation was greater from pre- to post-exercise in OW compared to OM and YC. These results show age-related changes in OW compared to OM and YC, which may suggest 8-isoprostane generation from pre- to post-exercise is a possible mechanism to explore with specific interest in its possible role in producing structural changes in the airways that are associated with late-onset respiratory disease development in women. However, our exploratory
analysis showed that changes in 8-isoprostane were not correlated with changes in lung function, which will be discussed further.

**Airway 8-isoprostane generation**

Airway 8-isoprostane increased following exhaustive exercise in the OW and not in the OM or YC, which is a novel finding in the present study that provides more support for the hypothesis that estrogen loss through aging may contribute to respiratory disease development in older women. The generation of 8-isoprostane in the airways is a derivative of arachidonic acid metabolism (Stafforini et al. 2006), resulting primarily from free radical-induced peroxidation. The most abundant of the F$_2$-isoprostanes is 8-isoprostane, which is a validated marker of lipid peroxidation and oxidative stress (Morrow and Roberts 1996). While our results show that OW would have a higher airway oxidative stress response following exhaustive exercise compared to age-matched OM and YC, an interesting step would be to determine the mechanisms in aging that contribute to the elevated response in the OW when compared to the OM and YC. Post-menopausal women have age-related immune changes, which may be due to the loss of estrogen, that make them especially susceptible to oxidative stress generation (Townsend et al. 2012; di Nijs et al. 2013). In fact, Kos-Kudla et al. (2000) showed that serum estrogen was lower in postmenopausal asthmatics compared to non-asthmatic post-menopausal women. However when post-menopausal asthmatics were given hormone replacement therapy (HRT), asthma symptoms improved. The associations reported between HRT and lung function reported by Kos-Kudla are in agreement with Carlson et al. (2001) who reported that post-menopausal women using HRT exhibited a higher FEV$_1$ compared to post-menopausal women not using HRT. Therefore future research should examine the influence of sex hormones on airway oxidative stress. In addition, Huh (1994) and colleagues reported that young male rats have higher lipid peroxidation...
compared with young female rats, however the response was attributed to higher estradiol
concentration in females. Since the younger women in our YC group showed a similar response
to the exhaustive exercise as OM and younger men, it is probable that the loss of estrogen
through menopause coupled with age-related changes in immune cells may provide a
mechanistic explanation for the present findings, although more research needs to be conducted
to test these hypotheses.

Changes in airway 8-isoprostane and lung function

Previous research has shown that exhaled 8-isoprostane, which is indicative of
concentrations found in the airway, has negative impacts on the respiratory system and
associated with clinical severity of asthma (Samitas et al. 2009). More recent literature has
reported that 8-isoprostane generation after a strenuous bout of exercise was significantly
associated with the degree of hyper-responsiveness in non-asthmatics (Morisette at al. 2016).
Considering that research has shown that 8-isoprostane is detrimental to the respiratory system,
and lipid peroxidation increases with both age and acute exercise, there is a need to elucidate the
8-isoprostane responses in the airways after exhaustive exercise. In the current study, 8-
isoprostane generation was not correlated with any index of lung function, which was a
surprising result that makes us question whether repeated exposures to increased airway 8-
isoprostane may produce structural damage in the airways. Supporting this query are existing
data from Wong-ekkabut et al. (2007) that confirm increased lipid peroxidation was related to
membrane damage induced by the oxidized lipids, subsequently producing changes in the
structural properties of the lipid bilayer. Therefore it is possible that repeated stimuli that
produce 8-isoprostane could alter airway structure and lead to the airway hyper-responsiveness
that was present in the previous literature.
In the present study, all subjects increased FEV$_1$ from pre-to post-exercise, although the magnitude of the response was larger in OM compared to OW, yet OW still had an elevated 8-isoprostane response as compared to age-matched males and YC. The previous research has shown that bronchodilation occurs after deep inspirations (i.e. post-exercise) in healthy, non-asthmatics (Kapsali et al. 2000), yet this bronchodilatory effect of deep inspirations (such as during exercise) diminishes with age (Scichilone at al. 2004). In the current study, post-exercise bronchodilation was more pronounced and lasted until 20 minutes post-exercise in OM, while in OW it returned back to baseline values by 20 minutes post-exercise. The OW still exhibited an increase in FEV$_1$, but it did not last as long as OM or YC. These sex differences in lung function following exercise in older adults should be explored further to determine whether the onset of changes in airway structure could be a contributing factor to our results.

Variability in airway 8-isoprostane responses

While the mean airway 8-isoprostane pre-exercise was not significantly different between groups, there was considerable variability in exhaled 8-isoprostane values within each group in the present study. Borill and colleagues (2007) have suggested there may be methodological considerations when assessing 8-isoprostane, and have recommended running all of the samples within the same assay on the same day (as performed in the present study). Researchers also found that 8-isoprostane variability within a single day was lower than measuring 8-isoprostane production across multiple days. All of our subjects only completed one strenuous exercise test with pre- and post-exercise exhaled 8-isoprostane collections on a single day. Recent data from our laboratory show that there can be a large range in exhaled 8-isoprostane when subjects visit the laboratory on two separate trials (Kurti et al. 2017), which is consistent with day-to-day variability published by Borill et al. (2007). While many studies have confirmed the negative
respiratory consequences of elevated exhaled 8-isoprostane levels using ELISA analyses, there is still more methodological work that needs to be conducted. Currently there is published evidence that both supports and does not support the reliability of ELISAs compared to other laboratory methods such as gas-chromatography/mass-spectrometry, which could explain the discrepancies in the current literature (Berdeaux et al. 2006).

While it is possible the variability in responses was methodological, there may also be physiological factors that contribute to the variability in the individual airway 8-isoprostane responses at baseline and from pre- to post-exercise. While subjects were asked to refrain from anti-oxidants prior to testing, we were not able to collect food logs from all participants enrolled in the study to account for fruit and vegetable consumption. Considering researchers have reported that increasing fruit and vegetable consumption lowers urinary 8-isoprostane (Thompson et al. 2005), the subjects’ dietary antioxidants could also impact pre- to post-exercise responses.

**Limitations**

While we have done our best to present the most relevant data, there are many studies referenced previously that show conflicting results with regard to changes in 8-isoprostane generation. Many issues may contribute to the conflicting findings in the current literature, and we were not able to account for several of those variables. Antioxidant status and intake of fruit and vegetables may impact the generation of 8-isoprostanes, however previous results are inconsistent and some studies show no changes after antioxidant supplementation, while others show an attenuation of 8-isoprostane based on antioxidant status (Pansarasa et al. 1999; Thompson et al. 2005). While we asked our subjects refrain from supplementation or vitamins for 3 days, we do not have a measure of total antioxidant status in our subjects. Finally, previous
studies have suggested that habitual physical activity level (Morisette et al. 2016) as well as body fat percentage (Komakula et al. 2007) may impact 8-isoprostane production. While we were not able to match OM and OW for body fat percentage and VO$_{2_{\text{peak}}}$, neither variable was correlated with changes in airway 8-isoprostane from pre- to post-exercise. Further, to verify there was not an interaction between body fat percentage or peak oxygen consumption and changes in airway 8-isoprostane, we included both as covariates in an ANCOVA (results not presented). Neither body fat percentage ($p=0.91$) nor absolute VO$_{2_{\text{peak}}}$ ($p=0.54$) impacted 8-isoprostane generation in this model. Therefore, we are confident that the changes in airway 8-isoprostane from pre- to post-exercise were due to sex and age differences alone, and were not impacted significantly by body fat percentage or peak oxygen consumption. Further, it would be interesting to assess oxygen uptake kinetics and whether changes in VCO$_2$ and VO$_2$ could impact the generation of 8-isoprostane from pre- to post-exercise, which could be an avenue for future mechanistic research.

**Conclusions**

In summary, this study suggests that sex differences exist in airway 8-isoprostane responses following an exhaustive bout of exercise in older adults. In our study, OW had a greater airway 8-isoprostane response following exhaustive exercise compared to age-matched OM and YC. Post-exercise bronchodilation also did not occur to the same magnitude in OW compared to OM and YC. While the changes in 8-isoprostane from pre- to post-exercise were not associated with changes in lung function, changes in oxidative stress may be apparent prior to decrements in lung function. Given these novel findings regarding oxidative stress responses in the airways of older adults, further research should explore the mechanisms behind these sex differences as well as the possibility of modification of these responses through lifestyle intervention.
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Competing Interest: All authors declare no competing interests.

Conflicts of Interest: None
References


Table 1. Subject Characteristics for older women (OW), older men (OM), and younger controls (YC)

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<td>41.5 ± 11.5*</td>
<td>25.8 ± 9.9</td>
<td>11.8 ± 5.8^</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>128.1 ± 11.3</td>
<td>132.9 ± 9.0</td>
<td>116 ± 4.0^</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>80.1 ± 4.4</td>
<td>81.6 ± 4.8</td>
<td>72.0 ± 2.8^</td>
</tr>
</tbody>
</table>

Peak Exercise Data

<table>
<thead>
<tr>
<th></th>
<th>OW (n=12)</th>
<th>OM (n=12)</th>
<th>YC (6M/6F)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Value</td>
<td>SD</td>
<td>Value</td>
</tr>
<tr>
<td>VO_2peak (L/min)</td>
<td>1.5 ± 0.3*</td>
<td>2.5 ± 0.6</td>
<td>3.1 ± 0.7^</td>
</tr>
<tr>
<td>Relative VO_2peak (mL/kg/min)</td>
<td>20.6 ± 6.7*</td>
<td>29.7 ± 9.39</td>
<td>46.3 ± 5.3^</td>
</tr>
<tr>
<td>RER</td>
<td>1.4 ± 0.2*</td>
<td>1.2 ± 0.1</td>
<td>1.2 ± 0.1¥</td>
</tr>
<tr>
<td>Ventilation (L/min)</td>
<td>67.3 ± 11.2*</td>
<td>102.8 ± 33.8</td>
<td>119.9 ± 32.3¥</td>
</tr>
<tr>
<td>Power (watts)</td>
<td>129.2 ± 23.4*</td>
<td>216.7 ± 63.4</td>
<td>260.4 ± 41.9¥</td>
</tr>
<tr>
<td>HRmax (bpm)</td>
<td>147.4 ± 8.8</td>
<td>146.9 ± 30.3</td>
<td>179.3 ± 8.7^</td>
</tr>
</tbody>
</table>

BP, blood pressure, mmHg, millimeters of mercury; VO_2, absolute oxygen consumption at VO_2peak; VO_2peak, relative oxygen consumption at VO_2peak; RER, respiratory exchange ratio; HRmax, maximum heart rate

*Significantly different compared to OM
^Significant different compared to OM and OW
¥Significant different compared to OW but not OM
Table 2. Pulmonary Function Data before exercise, immediately post-exercise, and 20 minutes post-exercise

<table>
<thead>
<tr>
<th></th>
<th>OW</th>
<th></th>
<th>OM</th>
<th></th>
<th>YC</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-Exercise</td>
<td>Post-Exercise</td>
<td>20 minutes post</td>
<td>Pre-Exercise</td>
<td>Post-Exercise</td>
<td>20 minutes post</td>
</tr>
<tr>
<td></td>
<td>Value</td>
<td>SD</td>
<td>Value</td>
<td>SD</td>
<td>Value</td>
<td>SD</td>
</tr>
<tr>
<td>PEF (L/s)</td>
<td>5.60±0.67</td>
<td>5.63±0.78</td>
<td>5.75±0.63</td>
<td>8.95±1.71</td>
<td>8.80±1.72</td>
<td>9.05±1.76</td>
</tr>
<tr>
<td>FVC (L)</td>
<td>2.87±0.31</td>
<td>2.93±0.29</td>
<td>2.86±0.32</td>
<td>4.51±0.96</td>
<td>4.54±0.94</td>
<td>4.46±0.92</td>
</tr>
<tr>
<td>FEV$_1$ (L)</td>
<td>2.15±0.22</td>
<td>2.21±0.20*</td>
<td>2.21±0.36^</td>
<td>3.25±0.72</td>
<td>3.39±0.73*</td>
<td>3.32±0.69</td>
</tr>
<tr>
<td>FEV$_1$/FVC (%)</td>
<td>75.08±4.76</td>
<td>75.67±4.72</td>
<td>75.61±4.54^</td>
<td>72.22±5.56</td>
<td>74.68±5.78*</td>
<td>74.75±5.05*</td>
</tr>
<tr>
<td>FEF$_{25-75}$ (L/s)</td>
<td>1.78±0.54</td>
<td>1.87±0.55</td>
<td>1.81±0.50*</td>
<td>2.34±0.89</td>
<td>2.72±1.06*</td>
<td>2.68±0.86*</td>
</tr>
</tbody>
</table>

PEF, peak expiratory flow; FVC, forced vital capacity; FEV$_1$, forced expiratory volume in 1-second
FEF$_{25-75}$, forced expiratory flow between 25 and 75% of FVC
*Significantly different change when analyzing pairwise comparisons within groups (p<0.05)
^Significantly different compared to OM and YC across time (p<0.05)
Figure Captions

**Fig 1** Data are displayed as mean with standard error bars. The mean airway 8-isoprostane generation in OM (●) and OW (○) and YC (▼) is shown. Airway 8-isoprostane significantly increased in older women, and decreased in OM and YC (*ps<0.05)

**Fig 2a** Individual airway 8-isoprostane responses are shown in OW. The mean data is displayed with a filled in black circle. 9/12 women exhibited an increase in the generation of exhaled 8-isoprostane from pre- to post-exercise

**Fig 2b** Individual airway 8-isoprostane responses are shown in OM. The mean data is displayed with an open circle. 9/12 men exhibited a decrease in the generation of exhaled 8-isoprostane from pre- to post-exercise

**Fig 2c** Individual airway 8-isoprostane responses are shown in YC. The mean data is displayed with an open circle. 9/12 younger subjects showed a decrease in the generation of exhaled 8-isoprostane from pre- to post-exercise
Fig 1

Exhaled 8-isoprostane (pg/mL)

OM
OW
YC

Exercise Bout
Pre
Post

https://mc06.manuscriptcentral.com/apnm-pubs
Fig 2a OW

Exhaled 8-isoprostane (pg/mL)

Pre Post

Exercise Bout

Fig 2b OM

Exhaled 8-isoprostane (pg/mL)

Pre Post

Exercise Bout

Fig 2c YC

Exhaled 8-isoprostane (pg/mL)

Pre Post

Exercise Test