Applied Physiology, Nutrition, and Metabolism
 Physiologie appliquée, nutrition et métabolisme

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

Journal:	<i>Applied Physiology, Nutrition, and Metabolism</i>
Manuscript ID	apnm-2017-0565.R1
Manuscript Type:	Article
Date Submitted by the Author:	08-Nov-2017
Complete List of Authors:	Kurti, Stephanie; Kansas State University, Kinesiology; James Madison University, Kinesiology Emerson, Sam; Kansas State University, Food, Nutrition, Dietetics and Health; Oklahoma State University, Nutritional Sciences Smith, Joshua; Kansas State University, Kinesiology; Mayo Clinic, Division of Cardiovascular Medicine Rosenkranz, Sara; Kansas State University, Food, Nutrition, Dietetics and Health Alexander, Samantha; Kansas State University, Kinesiology Lovoy, Garrett; Kansas State University, Kinesiology Harms, Craig; Kansas State University,
Keyword:	pulmonary physiology < physiology, sex differences, aging, exhaled breath condensate, exercise physiology < exercise
Is the invited manuscript for consideration in a Special Issue? :	

SCHOLARONE™
 Manuscripts

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

Kurti, Stephanie P.^{1,3,4}, Emerson, Sam R.^{2,3,5}, Smith, Joshua R.^{1, 6}, Rosenkranz, Sara K.^{2,3}, Alexander, Samantha A.¹, Lovoy, Garrett M.¹, Harms, Craig A.^{1,3}

Corresponding author: Stephanie P. Kurti
kurtisp@jmu.edu
Department of Kinesiology, James Madison University
314 Godwin Hall, 261 Bluestone Drive
Phone: 540-568-3947

¹Department of Kinesiology, Kansas State University
Manhattan, KS, USA

²Department of Food, Nutrition, Dietetics and Health, Kansas State University,
Manhattan, KS, USA

³Physical Activity and Nutrition-Clinical Research Consortium, Kansas State University,
Manhattan, KS, USA

⁴Department of Kinesiology, James Madison University
Harrisonburg, VA, USA

⁵Departmental of Nutritional Sciences, Oklahoma State University,
Stillwater, OK, USA

⁶Mayo Clinic-Division of Cardiovascular Medicine
Rochester, MN, USA

ORCID ID: 0000-0003-4803-9054

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 (FEV_1), forced vital capacity (FVC), FEV_1/FVC , and forced expiratory flow at 25-75% of FVC ($FEF_{25-75\%}$). Subjects then performed a VO_{2peak} 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 $\sim 74 \pm 77\%$ in OW, while decreasing in OM ($\sim 12 \pm 50\%$) and YC ($\sim 20.9 \pm 30\%$). **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

59

60 **Introduction:**

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

78 Older adults have a varying health-span (amount of years they are healthy in their
79 lifespan), and lipid peroxidation and oxidative stress may play a role in the aging process.
80 Additionally, several studies have reported sex differences in oxidative stress in older adults
81 (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 (FEF_{25-75%}), 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 (VO_{2peak}) 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 $\text{VO}_{2\text{peak}}$; 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 $\pm$ 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 $\text{VO}_{2\text{peak}}$, relative $\text{VO}_{2\text{peak}}$, and peak power at $\text{VO}_{2\text{peak}}$ compared to OM and YC ($p < 0.01$). The OM had lower absolute and relative $\text{VO}_{2\text{peak}}$ 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 $\text{VO}_{2\text{peak}}$ 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 ($p<0.05$).

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-isoprostane. 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: $102\pm9\%$; OM: $110\pm18\%$; YC: $108\pm14\%$), FEV₁ (OW: $95\pm10\%$; OM: $100\pm18\%$; YC: $99\pm9\%$), and FEV₁/FVC (OW: $91\pm6\%$; OM: $91\pm7\%$; YC: $93\pm8\%$) ($p>0.05$, respectively). Also, FEF_{25-75%} and PEF did not differ in percent of predicted between OW, OM and YC ($p>0.05$). However YC had greater absolute values for PEF, FVC, FEV₁/FVC and FEF_{25-75%} of FVC at baseline ($p>0.05$). 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 FEV₁ ($p<0.01$), FEV₁/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 (time* sex interaction) from pre-to 20-minutes post-exercise in FEV₁/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 FEV₁ 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, FEV₁/FVC, FEF_{25-75%}, or PEF from baseline, post- or 20-minutes post-exercise (all p -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 FEV₁ post-exercise ($p=0.03$) that returned back to baseline 20 minutes post ($p=0.25$). There was also an increase in FEV₁/FVC post-exercise ($p=0.01$) and 20 minutes post ($p<0.01$).

FEF_{25-75%} 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 FEV₁/FVC ($p<0.01$), which was driven by a small decrease in FVC, although it was not statistically significant ($p=0.053$). Also, FEF_{25-75%} 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 F₂-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 FEV₁ 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 (Morissette et 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 FEV₁ 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 et 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 FEV₁, 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 (Morissette 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 $\text{VO}_{2\text{peak}}$, 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 $\text{VO}_{2\text{peak}}$ ($p=0.54$) 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.

380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419

Acknowledgements: S.P Kurti received funding from an AHSS small grant program as well as the College of Human Ecology at Kansas State University for a Doctoral Dissertation Award.

Competing Interest: All authors declare no competing interests.

Conflicts of Interest: None

Draft

References

- American College of Sports Medicine. 2013. ACSM's guidelines for exercise testing and prescription. Lippincott Williams & Wilkins.
- Barreto, M., Villa, M.P., Olita, C., Martella, S., Ciabattini, G., and Montuschi, P. 2009. 8-Isoprostane in exhaled breath condensate and exercise-induced bronchoconstriction in asthmatic children and adolescents. *CHEST J.* 135(1): 66-73. doi: 10.1378/chest.08-0722. PMID: 18753466
- Berdeaux, O., Scruel, O., Cracowski, J.L., and Durand, T. 2006. F2-Isoprostanes: review of analytical methods. *Current. Pharmaceutical. Analysis*, 2(1), pp.69-78.
- Bloomer, R.J., and Fisher-Wellman, K.H. 2008. Blood oxidative stress biomarkers: influence of sex, exercise training status, and dietary intake. *Gender. Med.* b(3): 218-228. doi: 10.1016/j.genm.2008.07.002. PMID: 18727988.
- Borill, Z.L., Starkey, R.C. and Singh, S.D., 2007. Variability of exhaled breath condensate leukotriene B4 and 8-isoprostane in COPD patients. *Int. J. Chronic Obstructive Pulmonary Dis.* 2(1), p.71. PMID: 18044068.
- Carlson, C.L., Cushman, M., Enright, P.L., Cauley, J.A., and Newman, A.B. 2001. Hormone replacement therapy is associated with higher FEV₁ in elderly women. *Amer. J. Respir. Crit. Care. Med.* **163**(2): 423-428. DOI:10.1164/ajrccm.163.2.2003040. PMID: 11179117.

444 Cruz, M.J., Sánchez-Vidaurre, S., Romero, P.V., Morell, F., and Munoz, X. 2009. Impact of age
445 on pH, 8-isoprostane, and nitrogen oxides in exhaled breath condensate. *CHEST J.* **135**(2): 462-
446 467. DOI: 10.1378/chest.08-1007. PMID: 19017879.

447
448 De Martinis, M., Franceschi, C., Monti, D., and Ginaldi, L. 2005. Inflamm-aging and lifelong
449 antigenic load as major determinants of ageing rate and longevity. *FEBS letters*, **579**(10): 2035-
450 2039. DOI: 10.1016/j.febslet.2005.02.055. PMID: 15811314.

451
452 de Nijs, S.B., Venekamp, L.N., and Bel, E.H. 2013. Adult-onset asthma: is it really different?
453 *Eur. Respir. Rev.* **22**(127): 44-52. DOI: 10.1183/09059180.00007112. PMID: 23457164.

454
455 Emerson, S.R., Kurti, S.P., Rosenkranz, S.K., Smith, J.R., and Harms, C.A. 2015. Decreased
456 Prevalence of Exercise Expiratory Flow Limitation from Pre-to Postpuberty. *Med. Sci. Sports.*
457 *Exerc.* **47**(7): 1503-1511. DOI: 10.1249/MSS.0000000000000566. PMID: 25380473.

458
459 Gibson, P.G., McDonald, V.M., and Marks, G.B. 2010. Asthma in older adults. *Lancet*, **376**:
460 803-813. doi: 10.1016/S0140-6736(10)61087-2. PMID: 20816547.

461
462 Huh, K., Shin, U.S., Choi, J.W., and Lee, S.I. 1994. Effect of sex hormones on lipid peroxidation
463 in rat liver. *Archives. Pharmacal. Research*, **17**(2): 1009-1014. PMID: 10319141.

464

465

- 466 Kapsali, T., Permutt, S., Laube, B., Scichilone, N., and Togias, A. 2000. Potent
467 bronchoprotective effect of deep inspiration and its absence in asthma. *J. Appl. Physiol.* **89**(2):
468 711-720. PMID: 10926658.
- 469
- 470 Knudson, R.J., Lebowitz, M.D., Holberg, C.J., and Burrows, B. 1983. Changes in the Normal
471 Maximal Expiratory Flow-Volume Curve with Growth and Aging 1–3. *Amer. Rev. Respir. Dis.*
472 **127**(6): 725-34. DOI: 10.1164/arrd.1983.127.6.725. PMID: 6859656.
- 473
- 474 Komakula, S., Khatri, S., Mermis, J., Savill, S., Haque, S., Rojas, M., et al. 2007. Body mass
475 index is associated with reduced exhaled nitric oxide and higher exhaled 8-isoprostanes in
476 asthmatics. *Respir. Res.* 8(1): 1. DOI: 10.1186/1465-9921-8-32. PMID: 17437645.
- 477
- 478 Kos-Kudła, B., Ostrowska, Z., Marek B, Ciesielska-Kopacz, N., Sieminska, L., Kajdaniuk, D., et
479 al. 2000. Hormone replacement therapy in postmenopausal asthmatic women. *J. Clin. Pharm.*
480 *Therapeut.* **25**(6): 461-466. PMID: 11123500.
- 481
- 482 Kurti, S.P., Emerson, S.R., Rosenkranz, S.K., Teeman, C.S., Emerson, E.M., Cull, B.J., et al.
483 2017. Post-prandial systemic 8-isoprostane increases after consumption of moderate and high-fat
484 meals in insufficiently active males. *Nutr. Res.* 39, pp.61-68. DOI: 10.1016/j.nutres.2017.02.003.
485 PMID: 28385290.
- 486
- 487 Kurti, S.P., Smith, J.R., Emerson, S.R., Castinado, K.M., and Harms, C.A. 2015a. Absence of
488 respiratory muscle fatigue in high-intensity continuous or interval cycling exercise. *J. Strength.*
489 *Cond. Res.* 29(11): 3171-3176. DOI: 10.1519/JSC.0000000000000974. PMID: 25932987.

- 490 Kurti, S.P., Murphy, J.D., Ferguson, C.S., Brown, K.R., Smith, J.R., and Harms, C.A. 2015b.
491 Improved lung function following dietary antioxidant supplementation in exercise-induced
492 asthmatics. *Respir. Phys. Neurobiol.* 220: 95-101. DOI: 10.1016/j.resp.2015.09.012. PMID:
493 26453914.
- 494
- 495 Miller, M.R., Hankinson, J.A.T.S., Brusasco, V., et al. 2015. Standardisation of spirometry. *Eur.*
496 *Respir. J.* **26**(2): 319-338. DOI: 10.1183/09031936.05.00034805.
- 497
- 498 Montuschi, P., Collins, J.V., Ciabattoni, G., Lazzeri, N., Corradi, M., Kharitonov, S.A., et al.
499 2000. Exhaled 8-isoprostane as an in vivo biomarker of lung oxidative stress in patients with
500 COPD and healthy smokers. *Amer. J. Respir. Crit. Care. Med.* **162**(3): 1175-1177. DOI:
501 10.1164/ajrccm.162.3.2001063. PMID: 10988150.
- 502
- 503 Montuschi, P., Corradi, M., Ciabattoni, G., Nightingale, J., Kharitonov, S.A., and Barnes, P.J.
504 1999. Increased 8-isoprostane, a marker of oxidative stress, in exhaled condensate of asthma
505 patients. *Amer. J. Respir. Crit. Care. Med.* **160**(1): 216-220. DOI:
506 10.1164/ajrccm.160.1.9809140. PMID: 10390403.
- 507
- 508 Morisette, M.C., Murray, N., Turmel, J., Milot, J., Boulet, L.P., and Bougault, V. 2016.
509 Increased exhaled breath condensate 8-isoprostane after a swimming session in competitive
510 swimmers. *Eur. J. Sport. Sci.* **16**(5): 569-576. DOI: 10.1080/17461391.2015.1063702. PMID:
511 26279287.
- 512
- 513

- 514 Morrow, J.D., and Roberts, L.J. 1996. The isoprostanes: current knowledge and directions for
515 future research. *Biochem. Pharmacol.* **51**(1):1-9. PMID: 8534261.
- 516
- 517 Nikolaidis, M.G., Kyparos, A., and Vrabas, I.S. 2011. F2-isoprostane formation, measurement
518 and interpretation: the role of exercise. *Progress. Lipid. Res.* 50(1): 89-103. DOI:
519 10.1016/j.plipres.2010.10.002. PMID: 20951733.
- 520
- 521 Pansarasa, O., Bertorelli, L., Vecchiet, J., Felzani, G., and Marzatico, F. 1999. Age-dependent
522 changes of antioxidant activities and markers of free radical damage in human skeletal muscle.
523 *Free. Rad. Biol. Med.* **27**(5): 617-622. PMID: 10490283.
- 524
- 525 Pickering, T.G., Hall, J.E., Appel LJ, Falkner, B.E., Graves, J., Hill, M.N., et al. 2005.
526 Recommendations for blood pressure measurement in humans and experimental animals part 1:
527 blood pressure measurement in humans: a statement for professionals from the Subcommittee of
528 Professional and Public Education of the American Heart Association Council on High Blood
529 Pressure Research. *Hypertension*, 45(1): 142-161. DOI: 10.1161/01.CIR.0000154900.76284.F6.
530 PMID: 15699287.
- 531
- 532 Praticò, D., Basili, S., Vieri, M., Cordova, C., Violi, F., and Fitzgerald, G.A. 1998. Chronic
533 obstructive pulmonary disease is associated with an increase in urinary levels of isoprostane F2
534 α -III, an index of oxidant stress. *Amer. J. Respir. Crit. Care. Med.* **158**(6): 1709-1714. DOI:
535 10.1164/ajrccm.158.6.9709066. PMID: 9847257.
- 536

- 537 Quindry, J.C., Stone, W.L., King, J., and Broeder, C.E. 2003. The effects of acute exercise on
538 neutrophils and plasma oxidative stress. *Med. Sci. Sports. Exerc.* **35**(7): 1139-1145. DOI:
539 10.1249/01.MSS.0000074568.82597.0B. PMID: 12840634.
- 540
- 541 Rikans, L.E., and Hornbrook, K.R. 1997. Lipid peroxidation, antioxidant protection and aging.
542 *Biochimica et Biophysica Acta (BBA)-Molecr. Basis. Disease*, **1362**(2): 116-127. PMID:
543 9540842.
- 544
- 545 Samitas, K., Chorianopoulos D, Vittorakis S, Zervas, E., Economidou, E., Papatheodorou, G., et
546 al. 2009. Exhaled cysteinyl-leukotrienes and 8-isoprostane in patients with asthma and their
547 relation to clinical severity. *Respir. Med.* **103**(5): 750-756. DOI: 10.1016/j.rmed.2008.11.009.
548 PMID:19110408.
- 549
- 550 Scichilone, N., Marchese, R., Catalano, F., Togias, A., Vignola, A.M., and Bellia, V. 2004. The
551 bronchodilatory effect of deep inspiration diminishes with aging. *Respir. Med.* **98**(9): 838-843.
552 PMID: 15338795.
- 553
- 554 Smith, J.R., Kurti, S.P., Johnson, A.M., Kolmer, S.A., and Harms, C. 2015. Impact of varying
555 physical activity levels on airway sensitivity and bronchodilation in healthy humans. *Appl.*
556 *Physiol. Nutr. Metab.* **40**(12): 1287-1293. DOI: 10.1139/apnm-2015-0185. PMID: 26575101.
- 557

558 Stafforini, D.M., Sheller, J.R., Blackwell, T.S., Sapirstein, A., Yull, F.E., McIntyre, T.M., et al.
559 2006. Release of free F2-isoprostanes from esterified phospholipids is catalyzed by intracellular
560 and plasma platelet-activating factor acetylhydrolases. *J. Biol. Chem.* **281**(8): 4616-4623. DOI:
561 10.1074/jbc.M507340200. PMID: 16371369.

562

563 Thompson, H.J., Heimendinger, J., Sedlacek, S., Haegele, A., Diker, A., O'Neill, C., et al. 2005.
564 8-Isoprostane F2 α excretion is reduced in women by increased vegetable and fruit intake. *Amer.*
565 *J. Clin. Nutr.* **82**(4):768-776. PMID: 16210705.

566

567 Townsend, E. A., Miller, V.M., and Prakash, Y.S. 2012. Sex differences and sex steroids in lung
568 health and disease. *Endocrine. Rev.* **33**(1):1-47. DOI: 10.1210/er.2010-0031. PMID: 22240244.

569

570 Vassalle, C., Botto, N., Andreassi, M.G., Berti, S., and Biagini, A. 2003. Evidence for enhanced
571 8-isoprostane plasma levels, as index of oxidative stress in vivo, in patients with coronary artery
572 disease. *Coronary. Artery. Dis.* **14**(3): 213-218. DOI: 10.1097/01.mca.0000063504.13456.c3.
573 PMID: 12702924.

574

575 Wang, B., Pan, J., Wang, L., Zhu, H., Yu, R., and Zou, Y. 2006. Associations of plasma 8-
576 isoprostane levels with the presence and extent of coronary stenosis in patients with coronary
577 artery disease. *Atherosclerosis*, **184**(2): 425-430. DOI: 10.1016/j.atherosclerosis.2005.05.008.
578 PMID: 15996671.

579

580 Wong-Ekkabut, J., Xu, Z., Triampo, W., Tang, I.M., Tieleman, D.P. and Monticelli, L. 2007.
581 Effect of lipid peroxidation on the properties of lipid bilayers: a molecular dynamics
582 study. *Biophysical J.* **93**(12): 4225-4236. DOI: 10.1529/biophysj.107.112565. PMID:
583 17766354.
584

Draft

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

	OW (n=12)		OM (n=12)		YC (6M/6F)	
	Value	SD	Value	SD	Value	SD
Age (years)	64.1	± 4.1	66.2	± 4.1	21.5	± 2.3 [^]
Height (cm)	160.1	± 5.6*	174.6	± 7.2	172.2	± 8.1
Weight (kg)	76.7	± 20.7	85.6	± 16.4	66.5	± 11.4*
Body mass index (BMI) (kg/m ²)	30.2	± 9.2	27.6	± 4.3	22.3	± 2.8 [^]
Body Fat (%)	41.5	± 11.5*	25.8	± 9.9	11.8	± 5.8 [^]
Systolic BP (mmHg)	128.1	± 11.3	132.9	± 9.0	116	± 4.0 [^]
Diastolic BP (mmHg)	80.1	± 4.4	81.6	± 4.8	72.0	± 2.8 [^]
Peak Exercise Data						
VO _{2peak} (L/min)	1.5	± 0.3*	2.5	± 0.6	3.1	± 0.7 [^]
Relative VO _{2peak} (mL/kg/min)	20.6	± 6.7*	29.7	± 9.39	46.3	± 5.3 [^]
RER	1.4	± 0.2*	1.2	± 0.1	1.2	± 0.1 [¥]
Ventilation (L/min)	67.3	± 11.2*	102.8	± 33.8	119.9	± 32.3 [¥]
Power (watts)	129.2	± 23.4*	216.7	± 63.4	260.4	± 41.9 [¥]
HRmax (bpm)	147.4	± 8.8	146.9	± 30.3	179.3	± 8.7 [^]

586
587 BP, blood pressure, mmHg, millimeters of mercury; VO₂, absolute oxygen consumption at VO_{2peak}; VO_{2peak}, relative oxygen
588 consumption at VO_{2peak}; RER, respiratory exchange ratio; HRmax, maximum heart rate

589 *Significantly different compared to OM

590 [^]Significant different compared to OM and OW

591 [¥]Significant different compared to OW but not OM

592

593

594

595 Table 2. Pulmonary Function Data before exercise, immediately post-exercise, and 20 minutes post-exercise
596

	OW						OM						YC					
	Pre-Exercise		Post-Exercise		20 minutes post		Pre-Exercise		Post-Exercise		20 minutes post		Pre-Exercise		Post-Exercise		20 minutes post	
	Value	SD	Value	SD	Value	SD	Value	SD	Value	SD	Value	SD	Value	SD	Value	SD	Value	SD
n=	12		12		12		12		12		12		12		12		12	
PEF (L/s)	5.60	± 0.67	5.63	± 0.78	5.75	± 0.63	8.95	± 1.71	8.80	± 1.72	9.05	± 1.76	8.33	± 2.02	8.32	± 1.77	8.34	± 2.01
FVC (L)	2.87	± 0.31	2.93	± 0.29	2.86	± 0.32	4.51	± 0.96	4.54	± 0.94	4.46	± 0.92	4.85	± 1.06	4.69	± 0.96	4.80	± 1.00
FEV ₁ (L)	2.15	± 0.22	2.21	± 0.20*	2.21	± 0.36^	3.25	± 0.72	3.39	± 0.73*	3.32	± 0.69	3.87	± 0.86	3.93	± 0.83	3.92	± 0.81
FEV ₁ /FVC (%)	75.08	± 4.76	75.67	± 4.72	75.61	± 4.54^	72.22	± 5.56	74.68	± 5.78*	74.75	± 5.05*	79.72	± 6.81	83.97	± 7.52*	81.94	± 6.26
FEF _{25-75%} (L/s)	1.78	± 0.54	1.87	± 0.55	1.81	± 0.50^	2.34	± 0.89	2.72	± 1.06*	2.68	± 0.86*	3.70	± 1.15	4.12	± 1.28*	3.89	± 1.10

597
598 PEF, peak expiratory flow; FVC, forced vital capacity; FEV₁, forced expiratory volume in 1-second
599 FEF_{25-75%}, forced expiratory flow between 25 and 75% of FVC
600 *Significantly different change when analyzing pairwise comparisons with-in groups (p<0.05)
601 ^Significantly different compared to OM and YC across time (p<0.05)
602
603

Figure Captions

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

Fig 2a OW **Fig 2b OM** **Fig 2c YC**