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Transcriptional differences between smokers and non-smokers and variance by obesity as a risk factor for human sensitivity to environmental exposures

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Abstract

Background—Obesity has been shown to alter response to air pollution and smoking but underlying biological mechanisms are largely unknown and few studies have explored mechanisms by which obesity increases human sensitivity to environmental exposures.

Objective—Overall study goals were to investigate whole blood gene expression in smokers and non-smokers to examine associations between cigarette smoke and changes in gene expression by obesity status and test for effect modification.

Methods—Relative fold-change in mRNA expression levels of 84 genes were analyzed using a Toxicity and Stress PCR array among 50 21–54 year old adults. Data on smoking status was confirmed using urinary cotinine levels. Adjusted models included age, gender, white blood cell count and body-mass index.

Results—Models comparing gene expression of smokers vs. non-smokers identified six differentially expressed genes associated with smoking after adjustments for covariates. Obesity was associated with 29 genes differentially expressed compared to non-obese. We also identified 9 genes with significant smoking/obesity interactions influencing mRNA levels in adjusted models comparing expression between smokers vs non-smokers for four DNA damage related genes (*GADD45A*, *DDB2*, *RAD51* and *P53*), two oxidative stress genes (*FTH1*, *TXN*), two hypoxia response genes (*BNIP3L*, *ARNT*), and one gene associated with unfolded protein response (*ATF6B*).

Conclusions—Findings suggest that obesity alters human sensitivity to smoke exposures through several biological pathways by modifying gene expression. Additional studies are needed

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to fully understand the clinical impact of these effects, but risk assessments should consider underlying phenotypes, such as obesity, that may modulate sensitivity of vulnerable populations to environmental exposures.

1. INTRODUCTION

More than one half of the global population is overweight or obese [1, 2]. Obesity contributes significantly to the overall burden of complex chronic disease morbidity and mortality including cancer, diabetes and cardiovascular disease [3]. Despite the increasing prevalence of obesity coupled with growing health and economic consequences [4], few studies have examined mechanisms by which obesity may alter human sensitivity to environmental exposures. One such environmental exposure is smoking, which is another leading cause of preventable death and disability worldwide. Approximately 33% of all deaths from tobacco smoke are related to cancer, followed by cardiovascular (29%) and respiratory (29%) diseases [5]. Although there is a sizable volume of research documenting health impacts of cigarette smoke at the population level, the variability in responses at the individual molecular and cellular level remains largely uncharacterized. Smoking thus provides a valuable model for studying human sensitivity and response to environmental exposures. Further understanding of the biologic mechanisms that determine individual responses to smoking or other environmental factors are essential for identifying vulnerable and sensitive populations.

Recent advances in genomic and epigenomic research provide novel insights into the complexity of the toxic effects of smoking, yet many questions remain regarding cellular and molecular mechanisms through which smoking exerts its effects. Further, few studies have examined how obesity may modify smoking related gene expression via different biological pathways. Genetic factors play a significant role in susceptibility to both smoking behavior and sensitivity to the toxic effects of tobacco smoke and several genes determining increased risk for lung cancer among smokers were recently identified [6]. Epigenetic studies examining differential DNA methylation associated with cigarette smoking provide further insights into biological mechanisms of toxicity [7–10] and have shown that some but not all epigenetic changes associated with cigarette smoke persist over time. Whole blood DNA methylation studies revealed up to 2653 CpG sites annotated to 1405 genes that were differentially methylated (either hyper- and hypo-methylated) in current smokers compared to non-smokers [11–13]. In addition to DNA methylation, altered gene expression in whole blood in smokers has also been observed [14–16]. However, due to the limited number of studies examining transcription, we are just beginning to understand inter-related and independent effects that genetics and epigenetics have on gene expression [17–20]. A whole blood transcriptome meta-analysis of more than 10,000 participants identified 1270 genes with altered expression in smokers, with 12 genes that remained elevated after 30 years of cessation [14]. Since leukocytes are the main source of DNA and RNA in whole blood samples, these and other studies suggest that smoking has profound effects on gene expression particularly with respect to immune responses including inflammation [21]. Most studies to date have explored smoking related diseases with genetic and epigenetic signatures as mediators of response but often overlook how an existing disease state such as obesity may also alter response to toxicants found in cigarette smoke.

Obesity itself is a phenotype that leads to epigenetic changes and altered gene expression and may modify biological responses to smoking [22–26]. While the molecular mechanisms underlying these associations are still largely unknown, it is hypothesized that oxidative stress in the lungs after smoking or exposure to air pollution may lead to systemic inflammation, increasing cardiovascular and other chronic disease risks [27]. Obesity related diseases have similarly been linked to a state of chronic systemic inflammation and compromised oxidative defense, thus, obesity itself may increase susceptibility to environmental exposures [27–30]. Obese individuals may be more prone to exposures based on increased inhalation, however, it is also likely that differences at the molecular level change cell signaling in response to environmental threats. Obesity is traditionally defined and measured in clinical or epidemiologic studies based on a measured body-mass index (BMI) >30, [2]; however, it is largely the excess adiposity and its role in inflammation, oxidative stress, and hormone disruption in the body that is of greatest concern with potential to influence human sensitivity to environmental stressors [31–33].

Smoking and obesity have independently been found to influence gene expression along common biological pathways in whole blood but few have analyzed results for potential synergistic effects [34]. Recent genome-wide meta-analysis identified nine loci implicated in gene-smoking interactions with obesity suggesting that smoking may alter susceptibility to adiposity and fat distribution [35]. Another large-scale gene expression analysis of human adipose cells identified inflammatory genes as major targets of smoking-induced aryl-hydrocarbon receptor activation; suggesting that environmental toxicants may contribute to low-grade inflammation associated with obesity [36]. Oxidative stress is another common consequence of both cigarette smoke and obesity with profound effects on cell physiology [20, 25]. Smoking was also shown to exaggerate non-alcoholic fatty liver disease, a common trait associated with obesity related metabolic dysregulation [37]. These studies suggest that smoking contributes to metabolic differences through multiple pathways, however, few have studied how the human response to environmental toxicants is modified by being obese; and no studies have examined how obesity alters gene expression among smokers and non-smokers.

In this study, we hypothesize that obese individuals will have differential smoking-associated gene expression compared to non-obese due to adverse effects of adiposity on host immune and inflammatory response. As a first step in understanding this differential sensitivity to smoking exposure among individuals with an obese phenotype, we examined transcriptional markers of toxicity and stress pathways including oxidative stress, hypoxia, autophagy, unfolded protein response, apoptosis, and inflammation in whole blood among 50 adults, ages 21–54 from the Survey of the Health of Wisconsin (SHOW). Observed differences in differential gene expression in smokers and associations with obesity will provide further insight into potential molecular mechanisms underlying human sensitivity to environmental exposures such as tobacco smoke.

2. METHODS

2.1. Study participants

Participants included 25 current smoking and 25 non-smoking adults selected from the 2014–2015 SHOW survey. The SHOW is an ongoing annual examination survey described in detail elsewhere [38]. In brief, participants are randomly selected using a 3-stage sampling method to obtain a representative sample of Wisconsin residents. In-home survey and self-administered questionnaires are used to collect information about health, demographics, behaviors and lifestyle; interviews are followed by an in-home physical exam measuring anthropometrics, blood pressure and lung function. Participants are then invited to a study site for collection of blood and urine samples. Selection criteria for this study included self-reported smoking status, between 21–54 years of age and completion of the physical exam and biosample collection. Smokers (15 non-obese and 10 obese) fulfilling the above criteria were then randomly selected from the available cohort sample. Non-smokers were selected to match smokers on sex, age, BMI, and poverty level. The study was approved by the Health Sciences Institutional Review Board of the University of Wisconsin.

2.2. Sample collection and RNA extraction

Biosample collection included urine and blood collected by trained phlebotomists during SHOW exams. Participants were asked to fast 8–12 hours prior to the exam. Immediately after sample collection, a portion of blood samples were processed and shipped to Marshfield Laboratories for analysis of total cholesterol, triglycerides, hemoglobin A1c, and white blood cell counts (WBC). Serum, plasma, and urine were aliquoted and frozen at -80°C for long term storage in the SHOW biorepository. Additional whole blood samples were collected into a PAXgene tube (PreAnalytic) and stored at -80°C . Total RNA was extracted using PAXgene Blood RNA kit (PreAnalytic) according to manufacturer's instructions yielding 3–18 μg RNA/sample. Purity of RNA was determined by a 260/280 ratio that ranged from 1.8 to 2.1.

2.3. Gene Expression

Stress and Toxicity RT² Profiler PCR Array (Qiagen) was selected to examine an expression profile of 84 genes. The specific pathways and genes included in the Stress and Toxicity array are listed in Table 1. The Stress and Toxicity RT² Profiler PCR Array gene set was selected because it included oxidative stress, apoptosis and inflammatory pathway genes relevant to the study hypothesis. Several of the genes were also included in previous genome wide association and whole blood DNA methylation studies of smoking and whole blood [14–16]. cDNA synthesis was performed using the RT² First Strand kit using 400 ng of total RNA according to manufacturer's protocol (Qiagen). The PCR array was run on QuantStudio 7 Flex. Gene level data were normalized to average Ct of five housekeeping genes: ACTB, B2M, GAPDH, HPRT1 and RPLP0. All samples passed QC tests incorporated into PCR array plate, namely: no genomic DNA contamination detected and Cts for reverse transcription controls and positive PCR controls were within limits recommended by the manufacturer. Relative fold change was calculated using the delta-delta Ct method with non-obese, non-smoking males as controls which allowed for comparisons between different strata based on sex, BMI and smoking status [39].

2.4 Smoking Status, Exposure Verification, Obesity and Co-variates

Smoking status (current, never) was assessed using a self-administered questionnaire and validated using measured urinary cotinine. Urine was stored at -80°C until analyzed using liquid chromatography-tandem mass spectrometry [40, 41]. Cotinine levels in non-smokers ranged between 0.4 – 2.1 ng/ml and in smokers between 373 – 12,000 ng/ml, confirming that all 50 participants reported true smoking status.

The physical exam included standardized protocols for measuring objective height (meters) and weight (kilograms), waist to hip ratio, and blood pressure. Body-mass index (BMI) was derived as the weight in kilograms (kg) divided by height in meters (m) squared. Individuals were classified as obese based on standard World Health Organization cut-points for a measured BMI of ≥ 30 (kg/m^2) [2]. Self-reported data on age, gender, median household income, medication used and health history of cardiovascular disease and diabetes along with many other covariates were collected via personal interview.

2.5 Statistical analysis

Group differences in unadjusted relative gene expression were compared using standardized *t*-tests. Due to exploratory nature of the analyses, the small sample size and targeted hypothesis testing standard *p*-values within significance $p < 0.05$ were used to identify statistically significant differences in gene expression between groups. The Benjamini-Hochberg procedure was also employed to control for potential false discovery rate (FDR, set to 0.1) associated with multiple comparison testing [42]. Due to a small sample size, the FDR analyses were grouped according to genes within each biological pathways (e.g. oxidative stress and hypoxia were analyzed separately). Outcomes were provided for both standardized *p*-values with significance of $p\text{-value} < .05$ and genes that remained significantly different even after FDR correction were also noted in bold.

To further account for confounding factors, generalized linear regression models were used to examine differential expression of each of the 84 genes adjusting for age, gender and WBC. Analysis of differential expression comparing smokers and non-smokers was adjusted for obesity and analysis of differential expression comparing obese vs. non-obese was adjusted for smoking status. Obesity and smoking interactions were examined using interaction terms in fully adjusted regression models comparing expression in smokers vs. non-smokers. All statistical analyses were performed using SAS v9.3 software (SAS Institute, Cary, NC).

3. RESULTS

Demographic differences between smokers and non-smokers were minimal with equal numbers of male and female participants in each group (Table 2). The majority of participants had more than a high school education and approximately 40% had mean family income less than 200% of the federal poverty level. While average BMI did not differ between smokers and non-smokers, a slightly higher proportion of smokers were diagnosed with hyperlipidemia, however, fewer smokers were taking medication compared to non-

smokers. Similarly, a greater number of non-smokers were taking prescription medications for high blood pressure compared to smokers but differences were non-significant.

Overall serum markers of metabolic and cardiovascular disease including lipids and hemoglobin A1c did not significantly differ between smokers or non-smokers. Smokers had higher white blood cell counts and total cholesterol confirming previous observations in the literature [43–46]. However, further analysis showed that total cholesterol was significantly elevated only in obese smokers (231.1 ± 16.4 mg/dl) but not in non-obese smokers (199.4 ± 11.7 mg/dl). In addition, obese smokers had significantly decreased levels of high-density lipoprotein (HDL) (37.9 ± 2.2 mg/dl), whereas non-obese smokers did not (54.7 ± 3.1 mg/dl) suggesting that obese smokers have higher risk for cardiovascular disease than non-obese smokers or obese non-smokers.

Of the 84 genes analyzed, 6 genes were undetectable in whole blood: *CA9*, *EPO*, *LDHA*, *AQP2*, *AQP4*, *CFTR*. Unadjusted analyses identified nine genes with increased relative fold change among smokers compared to non-smokers and four genes with significantly lower expression (Figure 1). Table 1 presents the list of genes by functional group analyzed on the PCR array. Genes with increased expression fell into inflammatory response (*CCL2*, *IL-6*), DNA damage (*XPC*), cell cycle (*DDIT2*, *HUS1*), apoptosis (*TRAIL-R*), osmotic stress (*HSPA4L*) and oxidative stress (*GSTP1*, *NQO1*) pathways. Genes with decreased expression could be placed in functional groups associated with oxidative stress (*HMOX1*, *SQSTM1*), hypoxia signaling (*SERPINE1*) and cell cycle arrest (*CDKN1A*, *WAF1*). In models comparing individual gene expression of smokers vs. non-smokers and adjusted for age, gender, white blood cell count and obesity, the expression of three genes (*HSPA4L*, *TRAILR*, *GSTP1*) remained positively associated with smoking and one gene (*SERPINE1*) was negatively associated with smoking (Table 3). In addition, two genes (*IL-8*, *ULK1*) that were downregulated, although insignificantly, in smokers in unadjusted analysis were found to be negatively associated with smoking in adjusted models.

Obesity itself (independent of smoking, age, sex and WBC) also appeared to have a strong effect on gene expression across all response pathways. We found 29 genes differentially expressed in obese compared to non-obese, whereas smoking was associated with only 6 differentially expressed genes. There was little overlap in differential gene expression profiles between smokers vs non-smokers and obese vs. non-obese with the exception of *ULK1* in the autophagy pathway.

Generalized linear models testing for significant interaction between smoking and obesity status revealed that obesity did modify the effects of smoking on the expression of 9 genes (Table 4). Smoking and obesity interaction terms were significant in adjusted models comparing expression between smokers vs non-smokers for four DNA damage related genes (*GADD45A*, *DDB2*, *RAD51* and *P53*), two oxidative stress genes (*FTH1*, *TXN*), two hypoxia response genes (*BNIP3L*, *ARNT*), and one gene associated with unfolded protein response (*ATF6B*).

To further explore interactions, we conducted stratified analyses comparing relative fold change in obese vs. non-obese smokers (Fig 2). The largest difference between obese and

non-obese smokers was found for *GADD45A* (role in DNA damage repair). *GADD45A* was significantly upregulated in non-obese (by 1.67 fold) but significantly downregulated in obese smokers (by 40%) (Fig 2). *DDB2* and *TP53* were significantly decreased in non-obese smokers whereas unchanged in obese smokers. On the other hand, the expression of several genes was significantly decreased in obese smokers (*TXN*, *FTH1*, *BNIP3L*, *ARNT*) but unaffected in non-obese smokers. These data further support the idea that significant differences in sensitivity to environmental factors exist within populations.

4. DISCUSSION

Both environmental exposures (such as smoking) and obesity can modify risk for many complex chronic conditions including cardiovascular diseases and cancer. Obesity is associated with inflammation, hypoxia, oxidative stress and DNA damage with profound effects on cellular and organ physiology with many pathophysiological consequences [47–53]. We, therefore, hypothesized that obesity would modify exposure-effect associations and increase susceptibility of the obese individuals to the toxic effects of smoking. Common biological pathways affected by both smoking and increased adiposity include systemic effects on immune, inflammatory and oxidative stress pathways [27–30]. Cigarette smoke contains thousands of potentially toxic chemicals such as oxidizing agents that can induce direct and indirect cellular toxicity and damage in lungs. Oxidative stress can cause endoplasmic reticulum (ER) stress, hypoxia and DNA damage activating many biological pathways that remove these toxins and ameliorate their effects [54–56]. To gain insight into potential interactions of smoking with obesity within these common biological responses we analyzed the expression of 84 predetermined stress and toxicity PCR array genes in whole blood to explore oxidative stress, DNA damage and repair, unfolded protein response, autophagy, hypoxia and inflammation.

We identified 9 genes that were significantly upregulated and 4 genes that were significantly downregulated in smokers in unadjusted analyses. After adjusting for sex, age, WBC and BMI only four genes remained significantly associated with smoking and in addition another two genes were identified that did not appear in the unadjusted analysis. Smoking, adjusted for age, sex, BMI and WBC, was positively associated with *GSTP1* (oxidative stress), *HSPA4L* (osmotic stress) and *TRAILR* (apoptosis) whereas *SERPINE1* (hypoxia), *IL-8* (inflammation) and *ULK1* (autophagy) were negatively associated. A recent whole blood transcriptome meta-analysis found 1270 differentially expressed genes between current smokers and never smokers and based on *p*-value they identified top 25 smoking-associated genes [14]. The PCR array we used in this study did not contain any of the top 25 genes; but 6 genes analyzed in our study were among the 1270 genes identified [14]. We found similar smoking-associated expression for *GSTP1* and *TRAILR* (upregulated in both studies) and *HMOX1* (downregulated in both studies; although in the current study *HMOX01* was only in unadjusted analysis). We did not find statistically significant changes for *CD40LG*, *PRDX1* and *TXN* between smokers and non-smokers that were also observed in Huan's study, but the directional changes (down- or up-regulation) were the same. However, there are significant differences between these two studies in terms of adjustment for co-variables, population characteristics, and sample size. In addition, although genome- wide, epigenome-wide or transcriptome-wide analysis are invaluable in discovering new targets, there are

limitations most notably due to stringent significance thresholds [35]. Thus, genes below the threshold but with potential biological impact may be missed.

We identified 9 genes with significant smoking/obesity interactions influencing mRNA levels. We found that the most differentially affected biological pathways were DNA damage/repair responses (4 genes), oxidative stress (2 genes), hypoxia responses (2 genes) and unfolded protein response (1 gene). The opposite effects of cigarette smoke on the expression of these genes in obese and non-obese further supports the idea that responses to environmental toxins vary in different populations. We found that obesity itself, independent of age, sex and smoking, has a profound effect on expression of key genes in stress and toxicity responsive pathways suggesting that sensitivity/vulnerability of obese populations to environmental exposures is different than in non-obese populations.

Oxidative stress

Oxidative stress is a hallmark of both smoking and obesity and a major pathophysiological mechanism underlying many adverse effects of these conditions [54, 57–61]. Oxidative stress can result in chronic inflammation, which is well documented in both smoking and obesity [59]. Moreover, it was reported that smoking could weaken anti-oxidant defense systems resulting in decreased levels of anti-oxidants in blood [62, 63]. In the present study, both smoking (only in unadjusted analysis) and obesity decreased levels of HMOX1 mRNA in whole blood. Smoking-induced downregulation of HMOX1 was also found in a whole blood wide-transcriptome meta-analysis of six cohorts of more than 10,000 participants [14]. HMOX1 is a cytoprotective enzyme playing a role in defense against oxidative stress and is highly expressed in the immune system, lungs and gastrointestinal tract. *Hmox1* $-/-$ cells are hypersensitive to cytotoxicity of oxidative stress [64]. Besides its role as an anti-oxidant, HMOX1 has anti-inflammatory properties that are mediated via up-regulation of IL-10 and IL1RA [65]. Therefore, downregulation of *HMOX1* by both obesity and smoking suggests increased sensitivity to damage caused by oxidative stress among obese. Two additional genes with a role in anti-oxidant defense, *TXN* and *FHT1*, were downregulated only in obese smokers further supporting the idea of decreased anti-oxidant capacity in obese compared to non-obese smokers.

DNA damage and repair

DNA damage and repair response is critical in chronic disease etiology, particularly cancer. *GADD45A* plays a significant role in DNA damage repair induced by environmental stress or by DNA-damaging agents, many of which are present in cigarette smoke [66, 67]. The significance of *GADD45A* in tumorigenesis was recently demonstrated by findings that *GADD45A* (1506T>C) polymorphism significantly increases risk of ovarian cancer [68]. Therefore, downregulation of *GADD45A* in obese smokers compared to non-obese smokers observed in our study suggests impaired DNA damage/repair responses that may increase susceptibility of the obese population to cancer; findings supported by several studies reporting that reduced DNA repair capacity is associated with a significantly increased risk for smoking-related cancers [69, 70]. *GADD45A* downregulation found in lung cancer and glioblastoma is associated with poorer prognosis [71, 72] while overexpression of this gene is associated with a favorable prognosis among breast cancer survivors [73]. In the present

study *GADD45A* mRNA was upregulated 1.67 times in non-obese smokers but was significantly downregulated by 40% in obese smokers, the most striking difference between obese and non-obese smokers found. Interestingly, the significant upregulation of *GADD45A* and *XPC* in non-obese smokers suggests they may have better protection against DNA damage compared to obese smokers. At the same time, *DDB2*, *p53* and *RAD51*, three important DNA repair genes, were selectively downregulated by 10–20% in non-obese smokers making the interpretation of these data complex and an area of inquiry that warrants further investigation.

Unfolded protein response

Unfolded protein response (UPR) is a highly conserved compensatory mechanism to reverse an accumulation of misfolded proteins in the endoplasmic reticulum (ER) [74]. Multiple lines of evidence show that smoking induces ER stress and accumulation of protein aggregates [75–77]. Interestingly, we found that several genes in the UPR pathway were downregulated in obese participants independent of smoking suggesting that responses to ER stress may be insufficient among individuals with higher levels of adiposity.

Autophagy

Autophagy is important for normal functioning of the innate immune cells and T cells [78, 79]. Abnormal autophagy (down- or up-regulated) is associated with many pathologies including cancer [80, 81]. Autophagy is a protective mechanism responsible for elimination of defective or damaged organelles, protein aggregates and pathogens but is also induced by insufficient nutrient supply [82–84]. A low level of basal autophagy is necessary to sustain cell homeostasis and is closely connected with unfolded protein responses and apoptosis. We found that 4 out of 6 genes involved in autophagy assessed in the stress and toxicity array were downregulated in obese individuals. Independent of obesity, smoking also reduced *ULK1* expression. Our results suggest that autophagy may be impaired to a greater extent in obese individuals, which may significantly affect immune responses to pathogens or cancer progression.

Hypoxia

Tobacco smoke contains high levels of carbon monoxide resulting in decreased blood oxygen levels that leads to hypoxia. *BNIP3L* was one of genes with the most significant smoking/obesity interaction resulting in 50% decreased expression in obese smokers but not in non-obese smokers. *BNIP3L* belongs to a pro-apoptotic subfamily of Bcl2-2 proteins and plays an important role in tumor suppression. Thus, our data further point to an increased susceptibility of obese smokers to cancer compared to non-obese smokers.

Inflammation

Despite evidence that inflammation is an important and common mechanism impacted by both smoking and obesity we found that only two of the inflammatory genes on the array were upregulated by smoking, *CCL2* and *IL-6*, but after adjustment for age, gender, WBC count and obesity there was no significant association with smoking. On the other hand, we found that *IL-8* was negatively associated with smoking in adjusted models. These findings

are consistent with previous studies as none of the genes on the inflammatory panel was identified among 1270 genes significantly altered by smoking in whole-blood transcriptome meta-analysis [14]. Our observations are supported by another study that did not find any changes in the inflammatory genes that we studied in human monocyte-derived macrophages exposed to cigarette smoke extract *in vitro* [85]. One potential explanation is that whole blood gene expression may not capture cell specific differences in inflammatory gene expression suggesting further investigation including cell sub-type analyses are warranted.

Limitations in data interpretation—This study is one of the first to examine human sensitivity to environmental exposures that may in part be a result of obesity, however, some limitations exist. Besides a small sample size, several other factors need to be considered for data interpretation. First, this is an exploratory analysis that focused on a target set of genes using a pre-set array. The strengths of this approach are that the study can be easily replicated, on the other hand, the pre-set array limits the number of genes examined which means other genes in each pathway may also be significantly affected but are not captured in the current array. At the same time, the array provided an efficient and replicable method for testing the primary study hypotheses. Further, we acknowledge that whole blood mRNA expression may introduce some potential unmeasured bias. Whole blood is a mixture of several cell types, and the size of specific cell populations may be affected by both smoking and obesity but also by other factors. Indeed, we found, in agreement with other studies, that smoking increases white blood cell count, but it is unclear what type of leukocytes were responsible for this increase. We did attempt to adjust for this variation by including white blood cell count in the analysis, while not ideal, it did account for relative variation in cell count. Future investigations should explore cell specific variation in response and more detailed assessment of cell composition not possible in the current study. In addition, we might not be able to detect changes in gene expression that occur in a relatively small population of cells like monocytes that comprise only up to 8% of all leukocytes. At the same time, whole blood is a relatively available matrix and is sensitive to other metabolic changes. Other factors to consider are duration of smoking, dose of smoking (light/heavy smokers) and timing of last cigarettes prior to blood collection. All of these factors could influence gene expression in ways that are not well established. It is quite possible that immediate responses to cigarette smoke will differ comparing to chronic exposures.

Despite these limitations, study findings have several strengths and implications for examining human sensitivity to environmental exposures beyond studies of smoking. While cell mixture is important to consider, particularly for immune response functions, the analysis of whole blood has several advantages. For example, whole blood is easily accessible across multiple cohorts making reproducibility of study findings feasible. It is also metabolically active allowing for measurement of the metabolome, and whole blood plays an important role in the adverse effects that adiposity plays in promoting cardiovascular disease, aberrant immune function and increased inflammation [86, 87]. Previous studies have shown that like cigarette smoke [14–16], exposure to fine particle mixtures found in ambient air pollution can enter lungs and increase circulating measures of oxidative stress, and systemic inflammation. Cantone et al., 2017 found differential effects

of smoking on DNA methylation of inflammatory markers in obese vs. non-obese individuals. At the same time, the molecular mechanisms underlying these observations are largely unknown. Obesity itself has also been hypothesized to increase the amount of exposure to air pollution as obese individuals are more likely to inhale a greater volume of air [27]. Finally, obesity is a physiological state that includes increased adiposity, which leads one to also consider the role of adipocyte signaling of hormonal and inflammatory markers as another potential mechanism by which obese individuals may be more sensitive to environmental exposures.

5. Conclusion

The bulk of research to date focuses on understanding environmental factors driving the growing obesity epidemic with less focus on mechanisms by which being obese may alter sensitivity to environmental exposures. There are data emerging to support this claim, but few studies examining potential functional pathways of response exist. This preliminary investigation is one of the first documented to examine differential expression of genes associated with smoking by obese phenotype and their interaction. The interpretation of these results is complex, yet we see evidence of effects within pathways such as oxidative stress that are consistent with previous findings from larger scale transcriptome-wide analysis and to some extent epigenomic and GWAS analyses. Despite limitations, our data show that there is an interaction between smoking and obesity affecting key biological pathways. Results suggest that some protective responses aimed at ameliorating the hypoxic, oxidative stress and DNA damage induced by smoking may be impaired in obese individuals which may have profound consequences for the etiology of many disorders including cancer and cardiovascular disease. This research offers a first exploratory step towards biomarker development [88] and understanding how obesity modulates human sensitivity and variability in response to environmental exposures. Additional research is needed to investigate and how these interactions translate into clinical outcomes [89]. Our findings suggest that when assessing health risks of environmental exposures, it is important to consider factors, such as obesity, that may modulate sensitivity of vulnerable populations to toxic effects of environmental factors.

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Highlights

- Gene expression is different among obese smokers compared to non-obese smokers
- Obesity by smoking interactions influenced the expression of 9 genes in whole blood
- Obesity modified DNA damage, oxidative stress and hypoxia pathways in smokers
- Findings suggest that obesity modulates sensitivity to toxic effects of smoking

Figure 1. Gene expression [fold change smokers vs nonsmokers]

Unadjusted analysis of gene expression of 78 genes on PCR array. Genes that were undetected are not included. Data are shown as a fold change in relative gene expression between smokers and nonsmokers. Genes marked with “*” are differentially expressed between smokers and non-smokers with p-value < 0.05 or smaller.

Figure 2. Gene expression in smokers [fold change comparing to nonsmokers]

Gene expression between smokers and non-smokers stratified by obesity status. Data are shown as a fold change in relative gene expression between smokers and nonsmokers. Genes marked with “*” are differentially expressed between smokers and non-smokers with p-value < 0.05 or smaller.

Table 1

Stress and toxicity PCR array: biological pathways and gene list*

Oxidative stress	Hypoxia signaling	Osmotic stress	Apoptosis	Autophagy	Necrosis	Inflammatory response	Cell Cycle	DNA damage responses	Unfolded protein Response (UPR)
<i>FTH1</i>	<i>ADM</i>	<i>AKR1B1</i>	<i>CASP1</i>	<i>ATG5</i>	<i>FAS</i>	<i>CCL2</i>	<i>CDKN1A</i>	<i>ATM</i>	<i>ATF4</i>
<i>GCLC</i>	<i>ARNT</i>	<i>AQP1</i>	<i>FAS</i>	<i>ATG7</i>	<i>GRB2</i>	<i>CD40LG</i>	<i>CHECK1</i>	<i>ATR</i>	<i>ATF6</i>
<i>GCLM</i>	<i>BNIP3L</i>	<i>AQP2</i> †	<i>MCL1</i>	<i>ATG12</i>	<i>PARP1</i>	<i>CRP</i> †	<i>CHECK2</i>	<i>DDB2</i>	<i>ATF6B</i>
<i>GSR</i>	<i>CA9</i> †	<i>AQP4</i> †	<i>TRAIL-R</i>	<i>BECN1</i>	<i>PVR</i>	<i>IFNG</i>	<i>DDIT3</i>	<i>GADD45A</i>	<i>BBC3</i>
<i>GSTP1</i>	<i>EPO</i> †	<i>CFTR</i> †	<i>DR5</i>	<i>FAS</i>	<i>RIPK1</i>	<i>IL1A</i>	<i>HLS1</i>	<i>GADD45G</i>	<i>BID</i>
<i>HMOX1</i>	<i>HMOX1</i>	<i>EDN1</i>	<i>TNFR1</i>	<i>ULK1</i>	<i>TRAIL-R</i>	<i>IL1B</i>	<i>MRE11A</i>	<i>RAD51</i>	<i>CALR</i>
<i>NQO1</i>	<i>LDHA</i> †	<i>HSPA4L</i>			<i>TNFR1</i>	<i>IL6</i>	<i>NBN</i>	<i>TP53</i>	<i>DDIT3</i>
<i>PRDX1</i>	<i>MMP9</i>	<i>NFAT5</i>			<i>TXNL4B</i>	<i>CXCL8</i>	<i>RAD17</i>	<i>XPC</i>	<i>DNAJC3</i>
<i>SQSTM1</i>	<i>SERPINE1</i>	<i>SLC5A3</i>				<i>TLR4</i>	<i>RAD9A</i>		<i>HSP90AA1</i>
<i>TXN</i>	<i>SLC2A1</i>					<i>TNF</i>			<i>HSP90B1</i>
<i>TXNRPD1</i>	<i>VEGFA</i>								<i>HSP70</i>
									<i>HSPA5</i>

* From Qiagen (<http://www.sabiosciences.com/pcrarrayprotocolguide.php>)

† undetectable in whole blood

Table 2

Study Population Characteristics

	Nonsmokers (n=25)	Smokers (n=25)	P- value
Demographics			
Gender: Males	13 (52%)	13 (52%)	1.0000
Females	12 (48%)	12 (48%)	
Age, years	37.4 ± 1.6	37.3 ± 1.7	0.9595
Education: < High school	2	3	0.3181
High school	4	8	
> High school	19	14	
Poverty <200	10 (40 %)	10 (40 %)	0.9055
BMI	29.9 ± 1.6	28.9 ± 1.1	0.6311
Self-Report Health History			
Diagnosis of hyperlipidemia	3 (12%)	6 (24%)	0.2695
Prescription medicine for hyperlipidemia	3 (12%)	1 (4%)	0.2971
Diagnosis of high blood pressure	5 (20%)	3 (12%)	0.4404
Prescription medicine for high blood pressure	4 (16%)	2 (8%)	0.3841
Diagnosis of diabetes	6 (24%)	2 (8%)	0.1228
Cardio-metabolic Traits			
WBC count (x10 ³ /μl)	6.1 ± 0.4	8.4 ± 0.4	0.0001
HbA1c (%)	5.4 ± 0.2	5.4 ± 0.1	0.9002
Total cholesterol (mg/dl)	177.7 ± 6.7	211.3 ± 9.9	0.0070
HDL (mg/dl)	51.4 ± 3.8	48.4 ± 2.7	0.5300
Triglycerides (mg/dl)	167.2 ± 36.5	230.6 ± 44.9	0.2782

Table 3

Generalized linear model analysis of whole blood gene expression. P-values are bolded when the difference is significant after FDR correction.

	Smokers vs non- smokers*		Obese vs non-obese**	
	β	p-value	β	p-value
Oxidative stress				
GCLC			1.56 $\pm$ 0.34	0.0025
GSTP1	0.40 $\pm$ 0.13	0.0039		
HMOX1			-0.19 $\pm$ 0.05	0.0008
TXN			0.62 $\pm$ 0.19	0.0025
TXNRD1			-0.21 $\pm$ 0.09	0.0299
Hypoxia				
BNIP3L			2.06 $\pm$ 0.58	0.0010
SERPINE1	-0.55 $\pm$ 0.25	0.0334		
Osmotic stress				
AKR1B1			-0.19 $\pm$ 0.08	0.0312
AQP1			2.09 $\pm$ 0.69	0.0047
HSPA4L	0.37 $\pm$ 0.17	0.0315		
NFTA5			0.23 $\pm$ 0.11	0.0389
Inflammation				
IL-8	-0.45 $\pm$ 0.16	0.0097		
TLR4			-0.34 $\pm$ 0.11	0.0031
Apoptosis				
DR5			-0.24 $\pm$ 0.10	0.0194
MCL1			-0.24 $\pm$ 0.06	0.0004
TNFR1			-0.36 $\pm$ 0.07	<0.0001
TRAILR	0.31 $\pm$ 0.11	0.0119		
Necrosis				
GRB2			-0.29 $\pm$ 0.07	0.0003
PVR			-0.23 $\pm$ 0.07	0.0040
RIPK1			-0.21 $\pm$ 0.09	0.0282
TNFR1			-0.34 $\pm$ 0.09	0.0012
Autophagy				
ATG5			-0.14 $\pm$ 0.05	0.0089
ATG7			-0.30 $\pm$ 0.07	0.0001
ATG12			-0.14 $\pm$ 0.05	0.0106
ULK1	-0.17 $\pm$ 0.07	0.0300	-0.31 $\pm$.01	0.0002
UPR				
ATF6B			-0.28 $\pm$ 0.07	0.0002
BBC3			-0.46 $\pm$ 0.11	<0.0001
CALR			0.23 $\pm$ 0.08	0.0103
DNA damage				

	Smokers vs non- smokers [*]		Obese vs non-obese ^{**}	
	β	p-value	β	p-value
GADD45A			0.71 ± 0.27	0.0139
XPC			-0.51 ± 0.15	0.0014
TP53			-0.19 ± 0.09	0.0101
Cell cycle				
CHECK1			0.35 ± 0.13	0.0134
CHECK2			0.51 ± 0.21	0.0185
NBN			-0.17 ± 0.07	0.0216

^{*} model adjusted for age, sex, white blood cell count (WBC), obesity;

^{**} model adjusted for age, sex, white blood cell count (WBC), smoking.

Table 4

Genes with significant smoking* obesity interaction in generalized linear model analysis of whole blood gene expression. P-values are bolded when the difference is significant after FDR correction.

Pathway	Gene	p-value*
DNA damage	GADD45A	0.0019
	DDB2	0.0058
	RAD51	0.0291
	TP53	0.0368
Oxidative stress	FTH1	0.0126
	TXN	0.0053
Hypoxia signaling	BNIP3L	0.0148
	ARNT	0.0498
Unfolded Protein (UPR)	ATF6B	0.0362

*
p-value for interaction term using GLM model