Workshop Report: Understanding the Combined Effects of Environmental Chemical and Non-Chemical Stressors: Atherosclerosis as a Model

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Workshop Report Authors: Danielle J. Carlin, Michelle Olive, and Catherine M. Stoney

Affiliations of all authors (department, institution, city, state/province, and country):

Danielle J. Carlin, PhD
Division of Extramural Research & Training
Hazardous Substances Research Branch
Superfund Research Program
National Institute of Environmental Health Sciences (NIEHS)
Keystone Bldg., 530 Davis Dr.
P.O. Box 12233, Maildrop K3-04
Research Triangle Park, NC 27560, USA
Telephone: 984-287-3244
Email: danielle.carlin@nih.gov

Michelle Olive, PhD
Division of Cardiovascular Sciences
Atherothrombosis and Coronary Artery Disease Branch
National Heart Lung and Blood Institute (NHLBI)
6701 Rockledge Drive
Bethesda, MD 20892-7940, USA
Telephone: 301.443.7933
Email: olivem@mail.nih.gov

Catherine M. Stoney, PhD
Division of Cardiovascular Sciences
Program in Prevention and Population Sciences
Clinical Applications and Prevention Branch
National Heart, Lung and Blood Institute (NHLBI)
6701 Rockledge Drive
Bethesda, MD 20892-7940, USA
Telephone: 301-435-6670
Email: stoneyc@nhlbi.nih.gov

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Abstract

Background
Atherosclerosis is a primary disease associated with exposure to both environmental chemicals and non-chemical stressors, but few studies have been conducted to address the cumulative effects of these stressors. To further explore this research area, the National Institute of Environmental Health Sciences (NIEHS) and National Heart Lung and Blood Institute (NHLBI) held a workshop on April 3-4, 2018, where experts discussed the state-of-the-science pertaining to underlying biological pathways associated with the combined effects of these stressors in relation to atherosclerosis.

Objectives
This workshop report provides an overview of scientific evidence presented by the experts at the workshop, and it is not meant to be an exhaustive summary of the literature or comprehensive description chemical/non-chemical stressors or the mechanisms involved in atherosclerosis. In addition, research gaps and potential future directions that were discussed at the workshop are also included to address this complex research area.

Discussion
Environmental chemical and non-chemical stressors play a role in atherosclerosis, and the evidence for interaction between these stressors are just now beginning to be explored. In addition, we identified knowledge gaps, technologies, data, and the use of the Adverse Outcome Pathway (AOP) framework to study the interaction of these combined stressors.

Conclusion
Studying the health effects posed by the combination of chemical and non-chemical stressors is highly complex, even for a disease (i.e., atherosclerosis) where significant research has been conducted. The discussion from the workshop participants indicated that there is no one specific pathway or pathways by which these stressors may interact with one another. In addition, pathways mapped using the AOP framework may be useful in developing hypotheses about the mechanisms by which chemical and non-chemical stressors could interact and could be evaluated in future studies. AOPs will also be highly useful once more basic research on connecting the mechanisms of chemical and non-chemical stressors has been accomplished. Modeling these interactions and their mechanistic pathways (i.e., using AOPs) will require highly collaborative and creative research teams. Furthermore, these studies will require the use of established animal models and epidemiological studies with well-characterized exposures to these stressors, which has been lacking in the field.

Executive Summary

Description

The National Institute of Environmental Health Sciences (NIEHS) and National Heart Lung and Blood Institute (NHLBI) convened a workshop on April 3-4, 2018, at NIEHS Main Campus, Research Triangle Park, NC, utilizing atherosclerosis as a case-study to explore the interactions between chemical and non-chemical stressors. in Bethesda, MD. The workshop, which was open to the public, gathered leading experts in the field to discuss the current knowledge and identify scientific gaps and challenges related to research on atherosclerosis, environmental chemicals, and non-chemical stressors.

Background

The biological mechanisms, interactions, and health effects from both environmental chemicals (e.g., air pollution, arsenic, polychlorinated biphenyls) and non-chemical stressors (e.g., psychosocial and behavioral factors, socioeconomic status, poor nutrition, infectious agents, and noise) over time are not completely known. How these stressors interact biologically is a critical research area because several diseases have been associated with these stressors (e.g., cardiovascular disease, hypertension, and cancer). Moreover, humans are exposed to these stressors in varying amounts/degrees over the lifetime, with vulnerable human populations exposed to them more often, in combination, and at vulnerable times, putting them at greater risk for disease. In addition, a better understanding of these relationships is key for elucidating the underlying causes of atherosclerosis in those populations that are disproportionately affected by this disease.

Workshop Discussion

Experts were brought together at the workshop to discuss the state-of-the-science pertaining to underlying biological pathways associated with the combined effects of chemical and non-chemical stressors in relation to atherosclerosis. The primary goals of the workshop were to determine 1) which components of these combined exposures may contribute to the initiation and/or progression of atherosclerosis, 2) if these stressors share common mechanistic pathways, and 3) how researchers can study the biological mechanisms and interactions of these stressors. In addition, the experts discussed how these stressors affect susceptible populations and the development of prevention/intervention strategies to mitigate disease. Participants were also asked to describe the types of scientific data (e.g., mechanistic, epidemiological), new technologies, and innovative research approaches that could be leveraged to address underlying knowledge gaps. The workshop utilized the Adverse Outcome Pathway (AOP) framework, which is an approach for combining information from studies to elucidate knowledge of complex biological pathways.

The first day of the workshop consisted of four sessions with scientific presentations in each session: 1) Environmental Chemical Stressors and Atherosclerosis; 2) Non-Chemical Stressors and Atherosclerosis; 3) Modifying Factors of Atherosclerosis; and 4) Chemical and Non-Chemical Stressors in Atherosclerosis: Can They Be Studied Together?

The second day of the workshop all attendees participated in one of four breakout sessions to address the following questions: 1) Which environmental chemicals are known to affect key biological mechanisms/pathways leading to atherosclerosis, and which key biological mechanisms/pathways are affected by these chemicals? 2) Which non-chemical stressors are known to affect key biological mechanisms/pathways leading to atherosclerosis, and which key biological mechanisms/pathways are affected by these non-chemical stressors? 3) Which key biological mechanisms/pathways of atherosclerosis are known to be affected by the combined exposures of chemical and non-chemical

stressors? 4) What are the qualitative and quantitative (i.e., dose-response) impacts of exposure to the combination of chemical and non-chemical stressors and technical challenges in quantitative assessment of these exposures or impacts? 5) What are the types of scientific data (e.g., mechanistic, epidemiological) needed to address underlying knowledge gaps of chemical and non-chemical stressors leading to atherosclerosis? 6) What are the new technologies and innovative research approaches that could be leveraged to address these underlying knowledge gaps?

Workshop Recommendations

The workshop demonstrated the significant complexity of studying the combination of chemical and non-chemical stressor interactions resulting in atherosclerosis. However, further investigation into how to formulate and test hypotheses of the joint action of chemical and non-chemical stressors on atherosclerosis is required. The workshop included the AOP framework approach, which may be useful in organizing existing information on mechanistic pathways and determining research knowledge gaps, especially when more basic research on connecting the mechanisms of chemical and non-chemical stressors has been accomplished.

In addition, the workshop recognized several scientific areas, challenges, and opportunities for further investigation: 1) the importance of multidisciplinary collaboration to address the interaction of these stressors, especially to corroborate epidemiological findings and cumulative risk assessment with mechanistic studies; 2) the development of studies that are specifically geared towards the study of chemical and non-chemical stressor interactions; 3) the need for well-characterized cohorts (with respect to exposure and health outcomes), standard animal models of stress, and methods for studying the combination of chemical and non-chemical stressor interactions resulting in atherosclerosis; 4) further understanding if exposures to chemical and non-chemical stressors initiate or just accelerate disease; 5) how diet, exercise, sleep, and meditation may mitigate/prevent diseases; 6) how resilience plays a role in prevention of disease; 7) how genetic and epigenetic factors may initiate the progression of disease; 8) the development of biomarkers of acute and/or chronic stress response such as allostatic load, cortisol levels, etc.; 9) and better understanding fundamental drivers of geographic and demographic disparities as well as temporal shifts in incidence and prevalence of this disease, particularly if we are to better formulate effective intervention and prevention strategies. While the field of environmental health research is moving in the direction of understanding complex interactions, it continues to be challenging and will require a phased approach so that progress and successes can be documented. Moreover, this workshop may serve as a model to determine the role that chemical and non-chemical stressors may play in other diseases.

Publication Plans
A white paper outlining the recommendations that arose from the deliberations of the workshop is in preparation.

Workshop Organizers
Danielle Carlin, PhD, NIEHS
Michelle Olive, PhD, NHLBI
Catherine Stoney, PhD, NHLBI

Workshop Planning Committee Members
Janice Allen, PhD, NIEHS
Wayne Cascio, MD, USEPA
Yuxia Cui, PhD, NIEHS
Steve Edwards, PhD, RTI
Barbara Gittleman, MA, NIEHS
Michelle Heacock, PhD, NIEHS
Heather Henry, PhD, NIEHS
Ron Hines, PhD, USEPA
Mike Humble, PhD, NIEHS
Bonnie Joubert, PhD, NIEHS
Moiz Mumtaz, PhD, ATSDR
Wendy O'Brien, DVM, PhD, USEPA
Youngsuk Oh, PhD, NHLBI
Hana Pohl, PhD, ATSDR
Glenn Rice, PhD, USEPA
Cynthia Rider, PhD, NTP/NIEHS
Andrew Rooney, PhD, NTP/NIEHS
John Rogers, PhD, USEPA
Thad Schug, PhD, NIEHS
William Suk, PhD, NIEHS

Workshop Participants
Janice Allen, PhD, NIEHS
Jesus Araujo, MD, PhD, University of California, Los Angeles
Reto Asmis, PhD, Wake Forest School of Medicine
Aruni Bhatnagar, PhD, University of Louisville
Danielle Carlin, PhD, NIEHS
Wayne Cascio, MD, USEPA
Michele Casper, PhD, CDC
Deborah Cohen, MD, RAND Corporation
Daniel Conklin, PhD, University of Louisville
Yuxia Cui, PhD, NIEHS
Karina Davidson, PhD, Columbia University
Stephen Edwards, PhD, RTI
Zahi Fayad, PhD, Icahn School of Medicine at Mount Sinai
Robert Furberg, MBA, PhD, RTI
Chiara Giannarelli, MD, PhD, Icahn School of Medicine at Mount Sinai
Young-Mi Go, PhD, Emory University

David Goff, MD, PhD, NHLBI
Brooks Gump, PhD, Syracuse University
Michelle Heacock, PhD, NIEHS
Anjum Hajat, PhD, University of Washington
Jaime Hart, ScD, Harvard T.H. Chan School of Public Health
Heather Henry, PhD, NIEHS
Ron Hines, PhD, USEPA
Dean Jones, PhD, Emory University
Joel Kaufman, MD, University of Washington
Nick Leeper, MD, Stanford University
Koren Mann, PhD, McGill University
Jaymie Meliker, PhD, Stoneybrook Medicine
Andrew Morris, PhD, University of Kentucky
Matthias Nahrendorf, MD, PhD, Harvard Medical School
Ana Navas-Acien, MD, PhD, Columbia University
Michelle Olive, PhD, NHLBI
Rakesh Patel, PhD, University of Alabama at Birmingham
Michael Petriello, PhD, University of Kentucky
Arshed Quyyumi, MD, Emory University
Glenn Rice, PhD, EPA
Carol Shively, PhD, Wake Forest University
Sanjay Srivastava, PhD, University of Louisville
Thomas Register, PhD, Wake Forest School of Medicine
Andrew Rooney, PhD, NTP/NIEHS
Cynthia Rider, PhD, NTP/NIEHS
Catherine Stoney, PhD, NHLBI
William Suk, PhD, NIEHS
Filip Swirski, PhD, Massachusetts General Hospital
Cavin Ward-Caviness, PhD, USEPA
Changcheng Zhou, PhD, University of Kentucky

Introduction

The biological mechanisms, interactions, and health effects from both environmental chemicals (e.g., air pollution, arsenic, polychlorinated biphenyls) and non-chemical stressors (e.g., psychosocial and behavioral factors, socioeconomic status, poor nutrition, infectious agents, and noise) over time are not completely known. How these stressors interact biologically is a critical research area because several diseases have been associated with these stressors (e.g., cardiovascular disease, hypertension, and cancer) (Kivimäki and Steptoe 2018; Liu et al. 2017; Shin et al. 2016). Moreover, humans are exposed to these stressors in varying amounts/degrees over the lifetime, with vulnerable human populations exposed to them more often, in combination, and at vulnerable times, putting them at greater risk for disease. In addition, a better understanding of these relationships is key for elucidating the underlying causes of atherosclerosis in those populations that are disproportionately affected by this disease.

To explore this research area, the National Institute of Environmental Health Sciences (NIEHS) and National Heart Lung and Blood Institute (NHLBI) convened a workshop on April 3-4, 2018, utilizing atherosclerosis as a case-study to explore the interactions between chemical and non-chemical stressors. Atherosclerosis was selected as a case-study because there are established links between this disease and both environmental chemical and non-chemical stressors. Much is also known about the morbidity and mortality of this condition which is a leading cause of death in the US, and it is associated with significant health care and economic burdens (Bloom et al. 2011; CDC 2016; Roth et al. 2017). Experts were brought together at the workshop to discuss the state-of-the-science pertaining to underlying biological pathways associated with the combined effects of chemical and non-chemical stressors in relation to atherosclerosis. The primary goals of the workshop were to determine 1) which components of these combined exposures may contribute to the initiation and/or progression of atherosclerosis, 2) if these stressors share common mechanistic pathways, and 3) how researchers can study the biological mechanisms and interactions of these stressors. In addition, the experts discussed how these stressors affect susceptible populations and the development of prevention/intervention strategies to mitigate disease. Participants were also asked to describe the types of scientific data (e.g., mechanistic, epidemiological), new technologies, and innovative research approaches that could be leveraged to address underlying knowledge gaps. The workshop utilized the Adverse Outcome Pathway (AOP) framework, which is an approach for combining information from studies to elucidate knowledge of complex biological pathways (Ankley et al. 2010; LaLone et al. 2017; Yauk et al. 2015).

This workshop report provides an overview of scientific evidence presented by the experts at the workshop, and it is not meant to be an exhaustive summary of the literature or comprehensive description of chemical/non-chemical stressors or the mechanisms involved in atherosclerosis.

Discussion

Factors that potentially increase the risk of atherosclerosis include age, high blood pressure, high cholesterol, diabetes, obesity, tobacco use, family history, lack of exercise, and an unhealthy diet. These factors are also influenced by environmental chemical and non-chemical stressors. In addition, the biological mechanisms that lead to atherosclerosis are highly complex because they have the potential to interact at multiple stages of the disease. What is known is that atherosclerosis is a disease of middle and large size arteries; it is a progressive process that begins early in life; and mechanisms of development have been extensively studied (Hansson et al. 2015; Kasikara et al. 2018). The clinical syndromes resulting from atherosclerosis (e.g., myocardial infarction, unstable angina, stroke) are also dependent on the vessel location, what organ it is supplying, and the characteristics of atherosclerotic

plaque. In addition, the heritability of this disease, which can be evaluated from a genome-wide perspective, has shown that approximately 60 genomic loci are associated with atherosclerosis (Khera and Kathiresan 2017), although less than 50% of the known coronary artery disease can be explained by heritability (Nikpay et al. 2015), suggesting that there are factors such as environmental chemical and/or non-chemical stressors that may play a role in the disease. This workshop addressed the evidence suggesting that these factors may be contributors to the disease.

The workshop also included discussion of the persistent long-standing geographic disparities in mortality for cardiovascular disease that also exist (Roth et al. 2017). For example, studies of U.S. spatiotemporal data have shown that the geographic pattern of heart disease mortality has changed over time (Casper et al. 2016), where the concentration has dramatically shifted down from the northeastern US to southern states, where declines are also slower than in other parts of the country. This is also accompanied by racial and geographic disparities in heart disease. Although there is a link to increasing rates of diabetes and obesity (Ford et al. 2017; Lloyd-Jones 2016), there may be additional factors (e.g., chemical and non-chemical stressors) playing a role in these disparities. However, there is a lack of robust county level data with biomedical and behavioral risk factors that make this research difficult to do on a large scale.

Environmental Chemical Stressors

While many environmental chemicals are associated with cardiovascular disease (Cosselman et al. 2015), the workshop focused on three chemical classes for which substantial data are available: air pollution, arsenic (and it's many forms), and polychlorinated biphenyls (PCBs). Other chemicals mentioned at the workshop included lead, cadmium, and endocrine disrupting chemicals, but few studies have evaluated their mechanisms leading to atherosclerosis.

Air pollution is recognized for being associated with atherosclerosis (Brunefreef and Hoffman 2016; Hartiala et al. 2016; Kaufman et al. 2016) and genetic variants may increase the susceptibility to disease from air pollution exposure (Ward Caviness et al. 2016). In addition to epidemiological studies, in vivo and in vitro studies have furthered the understanding of air pollution and its mechanisms leading to atherosclerosis (Araujo et al. 2008; Sun et al. 2005; Suwa et al. 2002). These studies have indicated that particulate matter (PM) induces endothelial dysfunction, systemic vascular inflammation, oxidative stress, and lipid oxidation (Nurkiewicz et al. 2006; Sun et al. 2005; Yin et al. 2013). More recently, there has been interest in the role that oral exposure to particulate matter in air pollution may play on atherosclerosis (Li et al. 2015, 2017).

Although many studies exist, the exact progression of key events leading from air pollution exposure to development of atherosclerosis remains elusive. For example, a linear pathway is possible, where air pollution leads to increased reactive oxygen species (ROS) and inflammation, thus leading to atherosclerosis. However, there are several examples indicating that this pathway is not linear and may involve additional signaling factors (Araujo and Bhetraratana 2018; Araujo and Rosenfield 2015). A second knowledge gap is how air pollutants and their effects translocate from the lungs to the systemic tissues. Thirdly, it is unknown which chemical constituent(s) of the air pollution mixture may be responsible for vascular toxicity. There may be constituents of air pollution that lead to some effects (e.g., inflammatory) and while others demonstrate other effects (e.g., ROS production). Finally, there may be a temporal relationship to these pathways, where timing of the exposures (regarding both stage and duration of exposure) influences observed effects.

Arsenic is another contaminant known to play a role in cardiovascular disease (Cosselman et al. 2015; James et al. 2014; Moon, et al. 2013, 2017; Rosenberg 1974; Yuan Y et al. 2007). Arsenic induces atherosclerotic plaque formation and changes plaque components to make the plaque more vulnerable to rupture (Lemaire et al. 2011). As with air pollution, there are several studies evaluating the effects of arsenic, but there are still many knowledge gaps. For example, those discussed at the workshop included: can an animal model be used for detection of changes in the atherosclerotic plaque at very low concentrations of arsenic (<200 mcg/L)?; does arsenic target specific cell types resident in the plaque?; is arsenic-3-methyltransferase (i.e., the sole enzyme known to methylate arsenic) an important mediator in atherosclerosis (Negro Silva et al. 2017)?; why do in utero and post-natal arsenic exposures appear to have different proatherogenic mechanisms?; why is the dose-response for arsenic and coronary heart disease incidence non-linear (Moon et al. 2013, 2017)?; and how does arsenic interact with other metals to exacerbate atherosclerosis (Pang et al. 2016)? In addition, studies are needed on potential intervention strategies to inhibit arsenic's effects.

Researchers have also shown that dioxin-like pollutants (e.g., PCBs) are involved in atherosclerosis (Lind and Lind, 2012). Mechanisms related to PCBs and the development and progression of atherosclerosis include oxidative stress, chronic inflammation, and vascular endothelial cell dysfunction, as well as increased proatherogenic adhesion molecules, monocyte attraction, enhanced vascular permeability, and activation and modulation of immune cells (Han et al. 2010; Majkova et al. 2009; Petriello et al. 2014; Wang et al. 2015). Some of the research presented at the workshop indicated that when mice were exposed to dioxin-like PCB 126 and then gavaged with phosphatidylcholine, there was an elevation in flavin containing monooxygenase (FMO3), along with circulating Trimethylamine N-oxide (TMAO), an emerging biomarker of cardiovascular diseases (CVD) (Petriello et al. 2016; Tang et al. 2017). This phenomenon (i.e., increased TMAO) has also been identified in the Anniston, Alabama, community, a highly exposed population with increased body burden of dioxin-like pollutants (Petriello et al. 2018).

Non-chemical Stressors

Non-chemical stressors are associated with cardiovascular disease risk and events both longitudinally and cross-sectionally (Rosengren et al. 2004; Steptoe and Kivimaki 2013). The stressors and stress responses discussed that the workshop included those as a result of natural disasters (e.g., earthquakes) and other acute emotional triggers (e.g., sporting events), precipitate acute cardiac events such as myocardial infarction and cardiac death (Leor et al. 1996; Itoh et al. 2014; Strike and Steptoe 2005; Wilbert-Lampen et al. 2008). More chronic stressors, such as those associated with work, interpersonal conflict, and social burdens, also increase risk for future cardiovascular events (Gallo et al. 2013). Other non-chemical stressors, such as depression and anxiety disorders are also associated with coronary heart disease risk and events (Davidson et al. 2018). Relatedly, individuals with posttraumatic stress disorder (PTSD) due to military conflict and other traumas are at elevated risk for cardiovascular events (Ahmadi et al. 2011).

Unlike many of the chemical stressors discussed above, the mechanisms of non-chemical stressors leading to atherosclerosis are not specific (i.e., there is no one pathway by which depression, anxiety, or acute depressive reaction is operating), making the study of mechanism/pathways of these stressors highly challenging. The workshop included one growing area of mechanistic research is the evaluation of the potential role that stress may play on the sympathetic nervous system (i.e., amygdala activity) and bone marrow activation, leading to a complex process of leukocytosis, hematopoiesis, macrophage production/stimulation, and ultimately to atherosclerosis (Emami et al. 2015; Nahrendorf and Swirski

2015; Tawakol et al. 2017). For example, imaging methodology studies have shown that individuals with high amygdala activity have more subclinical atherosclerosis (Fox et al. 2012; Gianaros et al. 2009; Oler et al. 2010; Tawakol et al., 2017).

The major knowledge gaps mentioned at the workshop is a lack of investigation to explain why some patients exposed to non-chemical stressors are at risk for excess myocardial infarction recurrence and mortality. In addition, some of the research questions that need to be further explored include the following: 1) how to assess predispositions to high stress reactivity and underlying vulnerabilities; 2) how can we develop better measurement tools to understand individual differences in resilience; 3) how can we understand how non-chemical stressors affect hematopoiesis, macrophage differentiation, and atherosclerosis formation using well-established models of stress; 4) how do beneficial effects of exercise and sufficient sleep influence these mechanisms and how persistent are these effects? 5) what are the different levels at which people experience non-chemical stressors—for example, neighborhood-level poverty, lack of social cohesion, social capital, etc. versus individual-level stressors including difficulty paying for basics, food insecurity, job stress, etc. and how these things connect to each other and may affect risk of atherosclerosis.

The joint action of Chemical and non-chemical stressors in atherosclerosis

The workshop also included discussion of the joint action of chemical and non-chemical stressors contributing to or leading to atherosclerosis. For example, neighborhood socioeconomic status (SES) has a strong association with air pollution and has a strong effect modification on CVD mortality compared to individual SES (Hajat 2013; Chi et al., 2016). Studies such as the Multi-Ethnic Study of Atherosclerosis (MESA) and the ancillary MESA Neighborhood Study have extensive data to evaluate both chemical and non-chemical stressors and cardiovascular disease outcomes. Studies are currently underway that are stratifying neighborhood factors (e.g., neighborhood SES, social cohesion, neighborhood problems) and measurements of atherosclerosis.

In addition to studies in adults, there has been an attempt to address how early childhood may interact with environmental contaminants (e.g., lead) to impact cardiovascular disease. For example, studies have indicated that low level lead (Pb) exposure may increase children's cardiovascular responses to acute psychological stress (Gump et al. 2005, 2011).

There were several limitations discussed when assessing the effects of combined chemical and non-chemical exposures. For epidemiological studies, there is a lack of data on well-measured, spatially defined exposures of chemical and non-chemical stressors, their dose response, timing of exposure, and various types of life histories. For example, exposures are inconsistently measured in cohort studies. Another challenge is the outcome that is being measured. Population studies that include chemical and non-chemical stressors need to be representative of the population (e.g., variability of exposures). For example, NHANES, although not a longitudinal dataset, may be more representative of the broader US population, but not of specific populations. This may require community-based participatory research (CBPR) studies and community engaged work on specific populations. In addition, resilience and other health promoting resources should be explored for how they may prevent long term CVD. It is also well understood that both chemical and non-chemical exposures are not randomly distributed, and there is need to determine what contributions they are making to health disparities.

Mechanistic studies are also needed to inform the epidemiologic studies. For example, genetic and epigenetic susceptibility factors may play an important role in chemical and nonchemical stressors on

atherosclerosis. Indeed, a recent study has shown that chemical and non-chemical exposures affect both gene expression and other phenotypic traits in a genotype-dependent manner (Favé et al., 2018). A combination of in vitro and in vivo studies where direct modifications of genetic and epigenetic backgrounds can be made (e.g., Crisper modification; knockout), followed by exposure to chemical and non-chemical stressors are needed. Integration of genetics, epigenetics, and phenotypic data with -omics data may also help identify key pathways and biological processes to further understanding inter-individual variability and response to various exposures. In addition, studies of acute effects to both acute pollutant exposure and acute stress are needed such as those in a controlled exposure facility to evaluate underlying mechanisms involved, such as biomarkers, coagulation, inflammation, and stress hormones as well as heart rate and heart rate variability.

Prevention/intervention to chemical and non-chemical stressors

Diet and dietary supplements can mitigate the effects of environmental chemical and non-chemical stressors (Petriello et al., 2014; Hennig et al., 2018). At the workshop, there was discussion that a typical Western diet (i.e., high in saturated fat and sodium and low in and low in monounsaturated and omega-3 fatty acids) may exaggerate the psychological stress response which in turn increases risk for cardiovascular disease, and consumption of a healthier diet (e.g., Mediterranean diet) may attenuate the physiological stress responses. For example, when fed a Western diet, cynomolgus monkeys develop atherosclerotic plaques in their coronary arteries, similar to humans (Shively et al., 2009), and social stress increases atherosclerosis in monkeys independently of plasma cholesterol levels (Shively et al., 2008). The autonomic nervous system and the hypothalamic pituitary adrenal axis are potential mechanisms involved in these effects (Michopoulos et al., 2012; Shively et al., 2009). In addition, studies are underway to test the hypothesis that a Mediterranean diet is protective against stress responses compared to a Western diet.

Another aspect of prevention/intervention is the built environment which refers to "the human-made space in which people live, work, and recreate on a day-to-day basis" and is often associated with physical activity. Furthermore, the built environment may help mitigate the responses to psychosocial stress and subsequently the development of atherosclerosis. A first-time study of reactional parks documented the impact parks have on physical activity (Park et al., 2018). The results indicate that, in general, there are significant gender, age, and socioeconomic disparities in neighborhood park use. Factors associated with higher park use and duration of use were availability of a walking loop, large acreage size, high population density, low poverty, availability of facilities and amenities, more playground elements, supervised activities, programming, and marketing. Studies such as these suggest that increasing these factors in parks could increase physical activity.

Cumulative Risk Assessment

Most environmental chemical risk assessments consist of evaluation of a single chemical stressor (National Research Council, 1983), but the field of cumulative risk assessment (CRA) is emerging to combine risks from exposures to multiple stressors (deFur et al. 2007; Evans et al. 2007 USEPA, 2003). Two examples of cumulative risk assessment were presented at the workshop. The first was air pollution with Type 2 diabetes as a susceptibility factor (Zanobetti and Schwartz, 2002), and the second example included air pollution with socioeconomic status, which is associated with increased carotid intima-media thickness and increase carotid plaque (Thurston et al., 2014). As sufficient evidence becomes available, cumulative risk assessments may provide information for setting exposure standards. In addition, cumulative risk assessment is an opportunity to systematically evaluate

multifactorial diseases such as atherosclerosis. Although most of the evidence for these assessments is obtained from epidemiologic studies, the challenge will be to integrate mechanistic information from in vivo, in vitro, and in silico studies. More effort is also needed to understand the joint pathways of chemical and non-chemical stressors to support cumulative risk assessment.

Adverse Outcome Pathways (AOPs) and Atherosclerosis

The Adverse Outcome Pathway (AOP) model is a conceptual framework that portrays a linkage between a molecular initiating event and key event relationships that lead to a perturbation in an organism's biology (i.e. adverse outcome) (Ankley et al. 2010; LaLone et al. 2017; Yauk et al. 2015). The framework uses various levels of biological information and different types of data at these levels (e.g., in vivo, in vitro, epidemiological, mechanistic, genetic/epigenetic data, etc.). As AOP networks are assembled, modifying factors (such as non-chemical stressors, genetic/epigenetic susceptibility, etc.) can be incorporated (Nelms et al., 2017; Edwards and Preston, 2008). In addition, AOPs can also be used to illustrate complex potential interaction(s) and may evolve as methods for observing biology are improved (i.e., key events are measured with greater precision or accuracy). More importantly, AOPs can aid in a strategic approach to identify knowledge and evidence gaps that can be prioritized experimentally. For the workshop, the AOP framework was used to evaluate whether environmental chemical and nonchemical stressors interact at a mechanistic level resulting in atherosclerosis. To do so, workshop participants provided input to the following questions:

1) Which key biological mechanisms/pathways of atherosclerosis are known to be affected by the combined exposures of chemical and non-chemical stressors?

Potential pathways for the interaction of chemical and non-chemical stressors were identified for distinct components of atherosclerosis, including plaque formation, plaque vulnerability to rupture, and repair/regenerative capacity. These include blood monocyte reprogramming, oxidative stress, endothelial activation, tissue regeneration, HPA axis activation, inflammasome activation, IL1-B production, IL-6 production, fibrogenin formation, C-reactive protein production, lipoprotein metabolism/peroxidation, TMAO formation, and sympathethic and parasympathetic upregulation. Modifying factors (e.g., age, sex, genetic/epigenetic background, race, and SES) may play a role for each.

2) What are the types of scientific data (e.g., mechanistic, epidemiological) needed to address underlying knowledge gaps of chemical and non-chemical stressors leading to atherosclerosis?

Types of data discussed included high-resolution, quantitative mass spectrometry (MS) exposome data from well-phenotyped cohorts (i.e. multi -omics approaches); cohort studies with detailed exposure, lifestyle, medications, outcomes, and biomarkers over the entire life course; digital phenotyping using consumer wearable devices; well-characterized and validated biomarkers/functional biomarkers; data from animal models at the interface of non-chemical/chemical stressors; better measurement tools; and data from clinical trials. In addition, there is the accompanying need for novel statistical approaches that will be required to robustly integrate and analyze these diverse datasets, including machine learning techniques and agnostic analytical methods, etc.

3) What are the new technologies and innovative research approaches that could be leveraged to address these underlying knowledge gaps?

New technologies and innovative research approaches included: novel statistical packages/tools for processing large data sets needed to study chemical and non-chemical stressors in the same study (for both human and animal studies); developing animal models in ways that are truly reflective of the kinds of stressors experienced by humans and use of these animal models of non-chemical stressors (e.g., restraint, swim, sleep deprivation, circadian disruption) into environmental chemical exposure studies and; phones or telemetry devices to better temporally resolve measures of exposure and atherosclerotic events; imaging techniques that allow for multi-organ level imaging; measures of exposure (e.g., from satellites, passive sampling, teeth for early life exposures) and layering with health data using geocoding or other technologies (e.g., Cal EPA EnviroScreen); leveraging novel cohorts and initiatives (e.g., All Of Us, Environmental influences on Child Health Outcomes (ECHO), NHLBI Trans-Omics for Precision Medicine (TOPMed), Children's Health Exposure Analysis Resource (CHEAR), Human Health Exposure Analysis Resource (HHEAR), National Health and Nutrition Examination Survey (NHANES); utilizing data scientists and their ability to locate available applicable databases; utilizing machine learning; and integrating multi-omic approaches. In addition, determining how scientists can leverage existing cohorts and data to begin asking more novel and upstream questions about fundamental causes of atherosclerosis in terms of the link to chemical and non-chemical stressors.

While there was extensive discussion of the topics listed above and a clear sense that integrating an AOP framework may help to characterize chemical and non-chemical stressors and their links to atherosclerosis, the workshop participants indicated that there is still a need to more clearly delineate the potential linkages between these stressors and their mediators along diverse causal pathways that increase risk of this disease. In addition, the AOP framework approach, which may be useful in organizing existing information on mechanistic pathways and determining research knowledge gaps, will be better applied when more basic research on connecting the mechanisms of chemical and non-chemical stressors has been accomplished. A future endeavor could include the development of a conceptual AOP framework to incorporate the points listed above and the different factors that may be associated with atherosclerosis.

Conclusions

In conclusion, the workshop demonstrated the significant complexity of studying the combination of chemical and non-chemical stressor interactions resulting in atherosclerosis. However, further investigation into how to formulate and test hypotheses of the joint action of chemical and non-chemical stressors on atherosclerosis is required. The workshop included the AOP framework approach, which may be useful in organizing existing information on mechanistic pathways and determining research knowledge gaps, especially when more basic research on connecting the mechanisms of chemical and non-chemical stressors has been accomplished.

In addition, the workshop recognized several scientific areas, challenges, and opportunities for further investigation: 1) the importance of multidisciplinary collaboration to address the interaction of these stressors, especially to corroborate epidemiological findings and cumulative risk assessment with mechanistic studies; 2) the development of studies that are specifically geared towards the study of chemical and non-chemical stressor interactions; 3) the need for well-characterized cohorts (with respect to exposure and health outcomes), standard animal models of stress, and methods for studying the combination of chemical and non-chemical stressor interactions resulting in atherosclerosis; 4) further understanding if exposures to chemical and non-chemical stressors initiate or just accelerate disease; 5) how diet, exercise, sleep, and meditation may mitigate/prevent diseases; 6) how resilience plays a role in prevention of disease; 7) how genetic and epigenetic factors may initiate the progression of disease; 8) the development of biomarkers of acute and/or chronic stress response such as allostatic

load, cortisol levels, etc.; 9) and better understanding fundamental drivers of geographic and demographic disparities as well as temporal shifts in incidence and prevalence of this disease, particularly if we are to better formulate effective intervention and prevention strategies. While the field of environmental health research is moving in the direction of understanding complex interactions, it continues to be challenging and will require a phased approach so that progress and successes can be documented. Moreover, this workshop may serve as a model to determine the role that chemical and non-chemical stressors may play in other diseases.

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