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Environmental vegetation : a possible association with cardiovascular disease risk.

Ray Anthony Yeager II
University of Louisville

ENVIRONMENTAL VEGETATION: A POSSIBLE ASSOCIATION WITH CARDIOVASCULAR DISEASE RISK

By

Ray Anthony Yeager II
B.S., University of Louisville, 2008
M.P.H., University of Louisville, 2010

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by the following Dissertation Committee

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Dissertation Committee Chair, David Tollerud

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Research Mentor, Aruni Bhatnagar

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Robert Jacobs

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Kathy Baumgartner

_____

Gilbert Liu

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Jeffrey Wilson

DEDICATION

This dissertation is dedicated to my mother, Linda – a shining example of the power of love and kindness. Thanks for everything.

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have made this work possible knows no bounds. They have enabled and inspired me to pursue a life of meaning and purpose in serving others.

ABSTRACT

ENVIRONMENTAL VEGETATION: A POSSIBLE ASSOCIATION WITH CARDIOVASCULAR DISEASE RISK

Ray Anthony Yeager II

April 12, 2016

The built environment affects numerous aspects of human health and wellbeing, including risk of cardiovascular disease (CVD), the leading cause of morbidity and mortality in both the U.S. and worldwide. The amount and quality of environmental vegetation, is an important aspect of the built environment that affects known CVD risk factors, including psychosocial stress, health behaviors, and exposure to air pollutants. Nevertheless, little evidence on the role of potential mechanisms and geographic scale in these relationships exists. Of previous studies that describe associations between vegetation and CVD outcomes, limited investigation has been performed to assess these observed relationships in differing urban environments. It also remains unclear how environmental vegetation may affect biological processes that affect CVD risk, prevalence, and outcomes. This dissertation investigates relationships between environmental vegetation and possible associations with aspects of CVD risk among residents.

The first chapter describes CVD risks, vegetation, and relationships therein. The second chapter describes the association between environmental vegetation and CVD

hospital admissions at the zip code level. The third chapter assesses relationships between residential area vegetation and metabolites of harmful VOCs, benzene and acrolein, among participants in the Louisville Healthy Heart Study. The fourth chapter examines relationships between residential vegetation and circulating angiogenic cells among participants in the Louisville Healthy Heart Study. The fifth and final chapter reviews findings of previous chapters and describes potential future investigations into links between vegetation and cardiovascular health. Results of this work contribute to existing literature on the relationships between vegetation and human health, which may be useful in the development of future studies.

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INTRODUCTION

The environment is a well-recognized determinant of risk for a wide range of diseases that include cardiovascular disease (CVD), the leading cause of death in both the United States and globally. [1-9] Impacts of natural, built, and social environments on health are known to be moderated by a multitude of characteristics of urban environments, as well as individual choices and interactions.[1-7, 10-12] CVD is a non-infectious disease of the circulatory system.[13] It has been shown to be particularly sensitive to a wide variety of environmental influences on health.[4, 14-17] Numerous recent studies have found that people who live in areas of higher "greenness", or amount and quality of environmental vegetation, report a significantly better perception of their health and have a lower incidence of CVD.[18-22] Exposure to greenspace has been shown to be associated with numerous important CVD risk factors including lower stress levels, increased exercise, lower rates of obesity, and improved social cohesion.[19, 23-27] Vegetation has also been shown to reduce levels of air pollution, which is both a chronic and acute risk factor of CVD risk and outcomes.[28-34] Emerging evidence also suggests an association between metrics of vegetation and greenspace and decreased CVD in ecologic analyses.[18, 21, 35] However, it remains unclear how urban greenspaces may affect biological processes that determine CVD risk, prevalence, and outcomes. The overall objective of this dissertation is to determine whether residential area greenness is associated with decreased CVD risk factors. To test this hypothesis, I will address the following specific aims:

Specific Aim 1: Assess the relationship between neighborhood area vegetation and CVD hospital admissions utilizing an ecologic approach.

Rationale: Previous studies have found links between various environmental factors and CVD outcomes. However, little work has been performed to assess the role of environmental greenness in influencing CVD hospitalizations at the neighborhood level, or specific types and severity of CVD admissions. I will evaluate the relationship between zip code area vegetation and CVD hospital admission rates and length of hospital stay in an ecologic analysis. Hypothesis: Neighborhood greenness is negatively associated with rate of CVD hospitalizations and length of hospital stay from CVD admissions.

Specific Aim 2: Evaluate the relationship between residence level vegetation and biomarkers of volatile organic compound exposure using individual data from the Healthy Heart Study.

Rationale: Exposure to many types of volatile organic compounds (VOCs), are a known risk factor for the development of CVD. Existing literature examining links between vegetation in the outdoor environment, air pollution, and facets of human health supports the hypothesis that the ability of vegetation to mitigate air pollution is an important mechanism in the relationship between vegetation and health. VOCs and associated exposures are potentially key factors in this relationship. Therefore, I will evaluate the relationship between residential level vegetation and metabolites of VOCs, indicative of exposure, found in the urine of study participants.

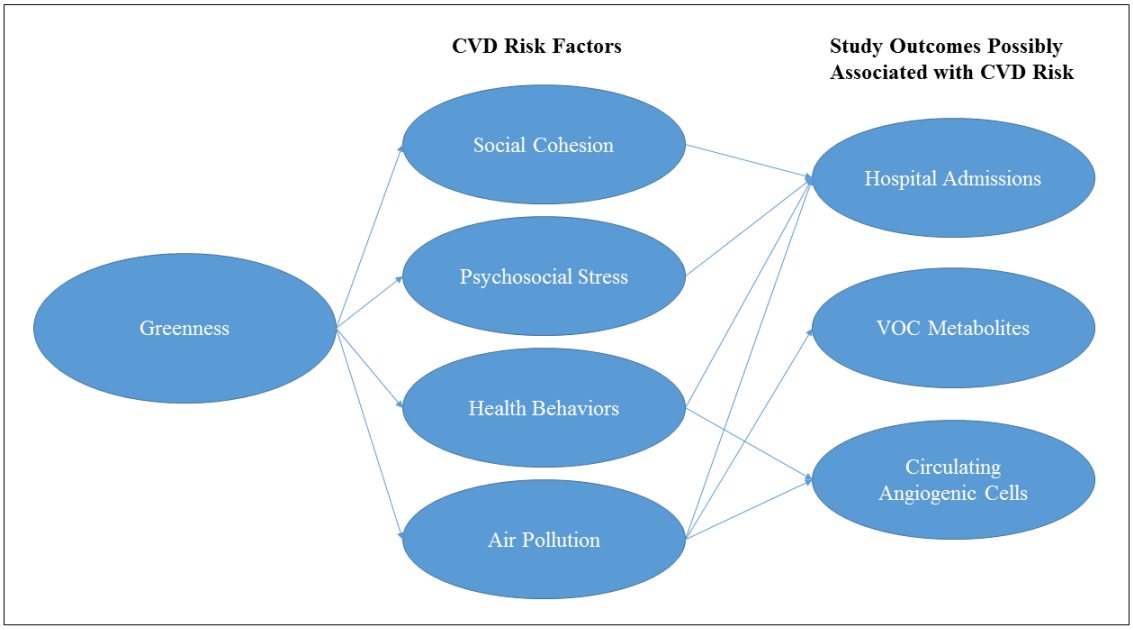
Hypothesis: Residential vegetation is negatively associated with levels of metabolites of VOCs.

Specific Aim 3: Examine the relationship between residence level vegetation and circulating angiogenic cells using individual data from the Healthy Heart Study.

Rationale: Previous studies have reported associations between circulating angiogenic cells and environmental exposures as well as proximity to roadways. These cells are thought to be important in the maintenance of vascular health and repair of vascular damage. Therefore, I will evaluate the relationship between residential level vegetation and subtypes of circulating angiogenic cells. Hypothesis: Residential area vegetation is associated with increased levels of circulating angiogenic cells.

Taken together, these aims will address important gaps in knowledge pertaining to the relationships between vegetation and CVD. This dissertation assesses ways in which environmental vegetation, quantified by satellite-derived metrics of vegetation, may affect cardiovascular risk (Figure 1.). Results of this study may provide important insights to inform future studies of relationships between vegetation and health, and may provide support for the development of vegetation-based public health policy.

Figure 1. Pathways through which outcomes of aims may be related to cardiovascular risk.



CHAPTER I: BACKGROUND

Cardiovascular Disease (CVD) is the leading cause of mortality both in the U.S. and globally, accounting for over 17.5 million yearly deaths worldwide and 800,000 yearly deaths in the U.S.[8, 9] CVD is classified as a non-infectious disease of the circulatory system responsible for various conditions adversely affecting these systems.[13] Conditions stemming from CVD primarily include stroke and heart diseases, such as myocardial infarction, peripheral artery disease, and sudden cardiac arrest.[36] These conditions account for approximately 30.8% of total mortality in the U.S.[8] Other CVD types include heart failure, arterial diseases, rheumatic heart disease, and cardiomyopathy.[37] CVD is also a leading cause of morbidity, with over 85.6 million, or 1 in 3 people in the U.S. currently affected.[8] The condition primarily associated with this morbidity is high blood pressure (hypertension), which affects over 80 million Americans, while the condition associated with the highest CVD mortality in the U.S. is coronary heart disease (CHD), accounting for 49.9% of all CVD deaths.[8, 37]

CVD is a largely preventable disease, albeit with many risk factors.[38] Over three quarters of all CVD deaths worldwide take place in low or middle income countries, mainly due to lack of detection and adequate treatment options.[9] In the U.S., the highest rates of heart disease are found in the Southern and Appalachian regions of the country.[39] This trend largely mirrors geographic trends of increased rates of smoking and obesity.[39] From 2001 to 2011, the death rate from heart disease fell by about 39%, largely due to reduced rates of smoking and increased standards of care.[8] During this time, the number of

cardiovascular surgeries and procedures increased by 28%.[8] As the primary cause of preventable mortality in the U.S., CVD is an important public health concern. Risk factors for the development of CVD include smoking, poor diet, obesity, age, sex, race, , stress, physical inactivity, cholesterol, high blood pressure, diabetes, and exposure to air pollution. [8, 14, 40, 41]

Demographic Risk Factors

There are several non-modifiable risk factors, including age, race, and sex. Age is a key risk factor, with Americans aged 20-39 having a CVD prevalence of 11% while those aged 80+ have a prevalence of 85%.[42] Race/ethnicity has been observed as an independent risk-factor for CVD, with persons of African ancestry at highest risk and those of Asian ancestry with the lowest risk, although socioeconomic status (SES) among these populations is an important consideration.[43, 44] Women account for roughly 60% of stroke deaths, while rates of most other CVD subgroups are highest in men.[45]

Behavioral Risk Factors

Most CVD risk factors can be prevented by behavioral changes.[9, 46] Both smoking and exposure to second-hand tobacco smoke are leading risk factors for CVD.[47] Much of the decline in heart disease rates in past decades has been attributed to declines in smoking, with similar trends observed in other countries.[47] Inhalation of tobacco smoke causes acute exposures to harmful chemicals including particulate matter, oxidant chemicals, carbon monoxide, nicotine, and other classes of chemicals.[47] These exposures lead to large increases in oxidative stress and inflammation, leading to both onset and

exacerbation of poor endothelial function, atherosclerosis, and prothrombotic effects.[47] Diet, physical activity, and obesity represent other key behavioral risk factors for CVD.[48] Dietary fat intake affects blood lipid levels, in turn affecting risk for coronary artery disease and heart attacks.[41] Additionally, saturated fats and trans fats have been shown to increase blood cholesterol levels, and increase progression of atherosclerosis.[41] Sodium intake is another major dietary concern, with excess sodium associated with hypertension.[41]

While it is difficult to precisely quantify physical activity of a population over long periods of time, longitudinal epidemiological studies have consistently shown strong associations between physical activity and cardiovascular health.[16] Conversely, sedentary behaviors have been strongly associated with adverse cardiovascular outcomes. [16] Exercise has been shown to improve cardiovascular health by leading to reductions in blood pressure, reductions in low-density lipoprotein (LDL) and total cholesterol, increases in high density lipoprotein (HDL) cholesterol, and increased insulin sensitivity.[49] Poor diet and lack of physical activity lead to excessive caloric intake and contribute to obesity, a metabolic disorder characterized by excessive adiposity.[11] The prevalence of obesity has grown substantially in the U.S. during recent decades, largely due to increased caloric intake and high dietary fat and reduced physical activity.[11] Long-term longitudinal studies indicate that obesity is an independent predictor of certain subtypes of CVD such as coronary heart disease and atherosclerosis.[11]

Psychosocial Risk Factors

Another major category of CVD risk is psychosocial stress, related to social environment, personality, and negative personal affect.[50] Social environment is largely characterized by socioeconomic status, adverse life events, family stress, job stress, and low social support.[50] Most individuals who develop heart disease have at least one psychosocial risk factor.[51] High levels of social support have been shown to mitigate other psychosocial stressors and appear to be cardioprotective, while low levels exacerbate existing CVD.[52] Mental stress related to occupation has been shown to be consistently predictive of CVD outcome in men, but less so for women.[52] A smaller body of evidence points to acute stressors, such as major adverse life events or natural disasters, as being strongly correlated with sudden cardiac deaths.[52] The mechanisms with which psychosocial stress affects CVD risk are largely accounted for by autonomic nervous system (ANS) dysfunction, serotonergic dysfunction, secretion of proinflammatory cytokines, and platelet activation.[52]

Psychosocial factors have also been shown to contribute to depression, which is consistently identified as a risk factor for CVD.[53] Ongoing depression, depressive symptoms, and history of depression have all been associated with increased CVD morbidity and mortality.[52] Higher severity of depression has also been associated with earlier and more severe cardiac events.[53] CVD patients suffering from depression have displayed significantly higher levels of biomarkers predictive of cardiac events, such as reduced heart rate variability, increased plasma platelet activation, impaired vascular function, and markers suggesting an increased innate inflammatory response.[53]

Built Environment Risk Factors

The built environment includes all physical aspects of where people live and work, including buildings, homes, streets, open spaces, and various infrastructure.[54] The built environment is known to play a large role in influencing CVD risk factors such as physical activity, obesity, social interaction, transportation, access, stress, mental health, and pollution exposure.[1-7] These effects appear to play a role in both acute and chronic health conditions including CVD, cancers, respiratory diseases, and unintentional injury.[3, 55] Attributes of the built environment influencing CVD and obesity are of key concern.[56] These attributes, including sidewalks, perceptions of safety, and features inducing or reducing stress, have consistently been shown to have significant influences on obesity, social factors, physical activity, and other behaviors that are known risk factors for CVD.[56] Environmental exposures to toxins such as pollen, ambient pollutants, and indoor pollutants associated with CVD risk are also influenced by the built environment.[55]

Air Pollution Risk

Air pollution, including ambient and indoor pollutants, is the second leading risk factor for the overall global burden of disease.[15] The primary cause of mortality from exposure to air pollutants is CVD.[10] To a much lesser extent, lung cancers, pulmonary disorders, and respiratory infections make up other causes of mortality attributed to air pollution.[10] Air pollutants are typically released into the environment through a wide range of natural and anthropogenic sources, often related to fuel combustion and transportation.[57]

Exposure to air pollutants, especially particulates, has been identified as a uniquely important risk factor for CVD, largely due to the ubiquitous and involuntary exposure of large populations.[58] Among common air pollutants, particulate matter is generally thought to have the greatest adverse impact to overall human health, and cardiovascular health in particular.[58] Short-term exposure to fine particulate matter, on the scale of hours to weeks, has been shown to trigger fatal and nonfatal cardiovascular events.[58] Long term exposure to fine particulate matter has demonstrated an even greater risk, with the potential to reduce life expectancy by over a year.[58] Particulates are often divided into classifications of coarse particulate matter (PM_{10}), fine particulate matter ($PM_{2.5}$), and ultrafine particulates (UFP), based on particle diameter.[59] Most existing literature and the regulatory framework for particulates centers on exposure to PM_{10} and $PM_{2.5}$, due to the large and consistent body of evidence regarding the health effects of exposure.[60] However, UFPs are also thought to pose an important risk due to a relatively high particle number, surface area, and levels of organic carbon.[60]

A common source of residential exposure to air pollutants in the U.S. is vehicle emissions, which have been observed to elevate pollutant concentrations up to 400m away from roadways.[61] Concentrations of pollutants, including particulates, VOCs, aldehydes, and NO_x near roadways are often highly correlated.[62-64] Measurement of many pollutants is often complicated by highly complex interactions that take place after emission into environments.[65] Indoor concentrations of pollutants also tend to be highly correlated with outdoor concentrations.[63, 66] However, infiltration of pollutants into

homes and buildings can vary greatly by season, building characteristics, and occupant behaviors.[66]

The cardiovascular system is particularly sensitive to air pollution, with increases in concentrations directly linked to CVD risk, hospitalizations, and mortality.[67] Exposure to high levels of air pollution is associated with inflammation, progression of subclinical CVD, and atherogenesis, all of which contribute to adverse CVD outcomes.[58] While many regulatory strategies have addressed emissions of pollutants into the environment, vegetation is one of the few ways to actually remove pollutants from outdoor air.[30, 33, 68, 69] However, no studies to-date have established the amount and attributes of vegetation necessary to significantly affect ambient pollutant concentrations. Specific air pollutants shown to be removed by vegetation include ozone (O_3), PM_{10}, $PM_{2.5}$, nitrogen dioxide (NO_2), Sulfur dioxide (SO_2), VOCs, and Carbon Monoxide (CO).[30, 33, 68] Despite links between greenness, health risks, and CVD, little evidence exists to identify specific biological mechanisms of the effects of greenness on CVD.

Benzene

VOCs, including benzene, are ubiquitous and harmful pollutants that are associated with motor vehicle emissions, combustion, and various other industrial processes.[70, 71] Benzene is a common industrial chemical found widely in certain environments.[72] It is released into the environmental from industrial processes, burning of fossil fuels, tobacco smoke, evaporation from gasoline stations, and motor vehicle exhaust.[73] In the U.S., gasoline can contain up to 2% benzene and up to 5% in certain other countries.[72] After

release into the air, benzene can react with other chemicals and tends to break down within a few days.[73] All humans are exposed to some amount of benzene on a daily basis.[73] About half of benzene exposure in the U.S. is from exposure to tobacco smoke, while 40% of the non-tobacco related exposure is from industrial processes or roadway emissions.[73] Food, drinking water, and other beverages can also contain benzene.[73] After entering the bloodstream, benzene travels throughout the body and is eventually converted into various metabolites, some of which are also known to be harmful.[73] Found in the urine, trans,trans-muconic acid (TTMA) is a benzene-specific metabolite useful in the measurement of exposure to low concentrations of benzene.[74]

No safe level of exposure to benzene has been identified.[75] Due to the abundance and potency of benzene, it has been linked to a variety of adverse health effects.[75] Systematic effects, including cardiovascular, have been shown to result from benzene exposure.[73] Few results have been reported on the cardiovascular effects of chronic benzene exposure among humans, however, acute exposure through inhalation has shown to induce ventricular fibrillation in humans.[76, 77] Acute exposure has also been shown to induce ventricular dysfunction in animal studies.[78] More recently, exposure to increased ambient benzene concentrations has been observed to be associated with the onset of myocardial infarction.[79]

Acrolein

Aldehydes, particularly acrolein, are an abundant, highly reactive, and toxic roadway pollutant.[80] Exposure to acrolein has been associated with increased CVD risk.[81] Acrolein

accounts for roughly 80-85% of the non-cancer risk from smoking, most of which is cardiovascular in nature.[82] In addition to being a byproduct of combustion, acrolein has been utilized in various industries, as a pesticide, and as a chemical weapon.[83] Ambient concentrations have been found to be strongly associated with concentrations of other common traffic-related pollutants including NO, NO_2, NOx, O_3, and $PM_{2.5}$.[84] After release into the air, the environmental half-life of acrolein can vary greatly depending on atmospheric conditions, but is generally around 1 day.[85, 86]

Sources of human exposure to acrolein, other than air pollution, include endogenous processes and various foods - particularly fried and heavily cooked foods and alcohol.[83] Foods have been observed to contain as much as 0.59 mg/kg of acrolein, though most foods contain much less.[87] Given the difficulties in measuring acrolein in dietary sources, variability of intake, and other exposure sources, total human dietary consumption estimates cannot be precisely quantified.[87] Dietary intake also varies substantially based on dietary behaviors, particularly alcohol consumption.[87] A total dietary intake of 7.5μg per kg body weight of acrolein per day is considered to have negligible health risks.[68] Typical human exposure, estimated from metabolite concentration, from all sources, is about half of this.[87, 88] Assuming a total air intake of $20m^3$ per day and a concentration of acrolein in U.S. cities of 14.3 $\mu g/m^3$, there is a high, respiratory intake of acrolein of about 286 μg per day.[87]

Compared to dietary intake and ambient air pollution, exposure to acrolein through smoking tobacco is very high, with urinary metabolites of acrolein in smokers being roughly 4 to 7 times higher than that of non-smokers.[89, 90] While acrolein is produced endogenously, sufficient evidence does not yet exist to estimate the relative contribution of endogenous production to overall exposure.[87] The importance of route of exposure and intracellular variations in acrolein concentrations also remains unknown.[88]

After entering the blood stream, acrolein is metabolized into 15 different detectable metabolites.[91] Of these, about 30% are excreted through feces and 70% through urine. Hydroxypropylmercapturic acid (HPMA) is the most abundant and is an acrolein-specific metabolite.[92] Both acute and chronic exposure to acrolein have been associated with significant adverse health effects.[93] Acrolein affects various aspects of health through many mechanisms including DNA adduction, mitochondrial dysfunction, inflammation, oxidative stress, protein adduction, membrane disruption, and endoplasmic reticulum stress.[88] Among other health effects, acrolein is known to adversely affect the cardiovascular system and increase risk for CVD.[81] CVD risks from acrolein exposure include atherogenesis, cholesterol transport impairment, dyslipidemia, hypertension, inhibition of blood flow, plaque ruptures, and thrombosis.[94-96] Exposure to acrolein has been associated with endothelial cell migration and circulating angiogenic cell levels.[81, 97] Much of the cardiovascular toxicity of acrolein is thought to be mediated through oxidative stress.[98, 99]

Association of CVD Risk Factors with Circulating Angiogenic Cells

Circulating angiogenic cells are a class of cells that are believed to aid in vascular healing and regeneration and endothelial repair, upkeep and functionality.[100-103] These cells are a class of stem cells that are also known as circulating angiogenic progenitors, peripheral blood mononuclear cells, vascular progenitor cells, endothelial progenitor cells, or vascular progenitors. They are mobilized into the circulation from the bone marrow through signaling pathways such as cytokines, hormones, and growth factors, often resulting from exercise, injury, ischemia, statin use, and tumors.[103] Once recruited into the bloodstream, these cells often adhere to the vascular endothelium, a cellular monolayer largely responsible for vascular tone, coagulation, vasodilation, thromboresistance, angiogenesis, and inflammation.[104] While levels of circulating angiogenic cells are highly variable by type, relatively low levels have been associated with endothelial dysfunction, a key risk factor for myocardial infarction and development of atherosclerosis.[105-114], as well as CVD risk, severity, progression, and mortality. However, some studies have found conflicting results, with circulating angiogenic cells being significantly and positively associated with risk or inversely associated with some risk factors and not associated with others.[115, 116] Despite a quickly developing body of literature regarding circulating angiogenic cells, much remains to be understood about cell release, homing, and mechanisms involved.[117] In particular, there is a relative lack of understanding about overlap in cell type functionality, unique markers for identification, and the ability to identify and quantify rare cell populations.[118]

Previous work has shown that circulating angiogenic cell levels are associated with exposure to air pollutants, cigarette smoke, obesity, and a variety of adverse cardiovascular conditions.[119, 120] Air pollution effects on circulating angiogenic cells could represent another important mechanism in which exposure to pollutants could affect vascular function.[121] Repeated episodic exposure to high levels of $PM_{2.5}$ has been associated with lower levels of circulating angiogenic cells.[122] This observation may relate to other work showing that human exposure to combustion-related air pollutants is associated with vasoconstriction.[17] Levels of these cells have also been associated with exposure to acrolein.[123] Higher cell levels have been associated with chronic exposure to roadways, perhaps reflecting repair of ongoing vascular damage from exposure to associated traffic related air pollutants.[124]

Association of CVD Risk with Vegetation

Vegetation is a key attribute of global landcover, critical to food systems, atmospheric regulation, and habitats necessary to ecosystems supporting animal life.[125-127] Environmental vegetation, commonly referred to as “greenness”, in urban areas, affects human health through a variety of means that include promoting physical activity, stress reduction, increased social cohesion, noise mitigation, heat and humidity regulation, and air pollution filtration.[19] Combinations of these effects may impact a range of health outcomes, including obesity, psychiatric morbidity, birth outcomes, all-cause mortality, and CVD.[19]

Much of the effect of greenness on health outcomes may be due to higher rates of healthy behaviors found among residents of high greenness areas.[25, 128] Greenspace, especially parks, serves as a potential venue to enable the physical activity requirements of a healthy lifestyle.[129] Park features such as access, aesthetics, safety, policies, and perceived condition help to encourage or deter park use for physical activity.[129] Vegetation and greenspace may also encourage healthy behaviors through increasing accessibility to walkable areas, fostering social support, and reducing related stress.[130] Nearby greenspace is strongly associated with increased levels of physical activity.[19, 128] In addition to increased overall physical activity, proximity to greenspace, particularly forests, has been associated with decreased sedentary behavior. Overall neighborhood vegetation, has been associated with neighborhood walkability and body mass index (BMI).[131, 132]

Vegetation and greenspace may promote mental health through direct influences on mental stress, by promoting physical activity, or by improving social cohesion.[19] A variety of study designs have demonstrated links between measures of vegetation and mental stress.[19] Much of this investigation into the relationship between vegetation and mental stress has utilized a cross-sectional design, often with self-reported measures of mental health.[19] However, some studies have utilized objective outcome measures such as hospital records or physiologic indicators and reported inverse associations between vegetation and stress.[133-135]

Vegetation and Air Pollution

Air pollution has particularly direct physiological impacts on health, with established outcomes of exposure to air pollution including CVD, cancers, asthma, and adverse pregnancy outcomes.[136] However, there is a paucity of evidence linking the effects of vegetation on health to air pollution, mainly due to a lack of high quality exposure assessments. CVD is particularly sensitive to air pollution, with changes in concentrations directly linked to CVD risk, hospitalizations, and mortality.[67] Despite the links between greenness, health risks, and CVD, little evidence exists to identify specific biological mechanisms of the effects of greenness on CVD.

Trees and other vegetation have been observed to reduce air pollutant concentrations by capturing pollutants making contact with leaf surfaces, with the amount removed being proportional to total surface area.[69] Vegetation has been shown to remove specific air pollutants such as O_3, $PM_{2.5}$, PM_{10}, NO_2, SO_2, CO, and VOCs from the environment, which appear to be correlated with adverse health outcomes.[30, 33, 68, 137] Several types of tree species have been shown to capture large quantities of small particulates in the size ranges known to affect human health.[138] However, coniferous species have been observed to capture substantially more particulates than broad-leaf species, likely an effect of a higher leaf/needle surface area.[138] Trees situated in high particulate exposure areas were shown to capture a comparable amount of small particulates to trees in more rural sites.[138] However, the amount of large particulates captured in high exposure areas was significantly greater.[138] Leaves most effective at capturing pollutants tend to exhibit relatively larger surface area, hair, and wax cover.[139] Optimal air speed velocity for

particle deposition onto leaf surface has also been shown to vary by species and leaf characteristics.[140] While much of the research on pollutant removal has focused on woody tree species, herbaceous plants have also been shown to remove pollutants at comparable rates.[31] Similar to woody tree species, pollutant removal by herbaceous plants varies greatly between species and is largely dependent on leaf characteristics such as surface area and hairiness.[31]

A potential negative effect of vegetation on air pollution is the emission of considerable amounts of biologic VOCs (BVOCs), which may subsequently react with urban air pollution to form O_3 and other secondary aerosols in the atmosphere.[141-143] Many types of BVOCs are emitted from plants, varying widely by plant type and season, and are thought to primarily be a signaling and communication mechanism between plants.[143] About half of the total plant BVOC emissions are made up of isoprene.[144] Despite this release of BVOCs and secondary formation of O_3, vegetation has been shown to remove both O_3 and VOCs from ambient air.[143, 145] In indoor environments, plants have been shown to lower overall VOC concentrations.[146, 147]

Personal monitoring has associated higher surrounding greenness with lower personal, indoor home, and outdoor home levels of $PM_{2.5}$.[148] Pollutant modeling using validated land use regression models suggests that, in densely populated high-emission urban areas, tree canopy has a weak but significant effect on removing ambient pollutants at the neighborhood level.[149] Vegetation can also act as a physical barrier between roadway

pollutant emissions and human populations.[28] Both wide area vegetation barriers with high leaf density and vegetation combined with solid or sound barriers have been shown to significantly reduce downwind particle combinations.[28] The physical reductions of downwind air pollution tend to be regulated by both dispersion of pollutants and deposition on surfaces.[32, 150] However, many types of physical barriers, while providing large reductions in downwind pollutant concentrations, tend to result in much higher on-road pollutant concentrations.[32]

Vegetation and CVD

Vegetation is thought to affect CVD risk through known risk factors such as physical activity, mental health, and pollution exposure.[19] A review of the health benefits of greenness has reported consistent associations between vegetation and CVD risk factors, but few studies have examined CVD outcomes.[19] CVD has been found to be associated with both vegetation density and level of vegetation variability within areas.[151] Other studies have found associations between vegetation and mortality of various causes, including CVD.[152-154] Some studies examining differences in vegetation between cities found that cities with higher greenness have no difference in CVD mortality after adjustment for covariates[155, 156] This may be the result of more influential factors of between-city variation on CVD rates such as urban sprawl, car-dependency, health behaviors, and many other factors influencing risk.[155]

A small randomized study of coronary artery disease patients observed that patients adopting a walking regimen in green spaces had better improvements in multiple measures of cardiac function than those walking in urbanized spaces.[20] Other studies have employed an experimental design to explore links between vegetation and CVD risk factors. In one study, participants exposed to images of greenspace while running on a treadmill demonstrated larger improvements in both blood pressure and self-esteem than those exposed to no images or images of low-vegetation areas.[157] A similar study in children reported that participants viewing a film of a forest setting demonstrated significantly lower systolic blood pressure than a control group with no imagery.[158] Importantly, these results demonstrate an effect of exposure to greenness on cognition, independent of physical characteristics affected by vegetation, such as noise, walkability, and air pollution. Similarly, in an outdoor exercise study, higher perceived exposure to greenness during exercise was significantly associated with larger reductions in anxiety.[159] A separate study reported that exposure to a more "natural" view from home windows after a change in place of residence was associated with better cognitive function in children.[160]

A quickly growing body of evidence points to positive effects of greenness on CVD and overall health, as summarized by a recent review article by laden et al.[19] For example, In the United States, spread of the emerald ash borer, a tree killing insect, has been associated with increased incidence of CVD among participants in the women's health initiative, suggesting a possible association between tree loss and CVD.[21] However, the relative contribution of the three main hypothesized mechanistic categories, improved

social cohesion, decreased stress, and air pollution reduction, remain far from understood.[19] The associations between vegetation and other attributes of the built environment such as open space, crime, pollution, etc. make assessing the role of vegetation exceedingly difficult. As an added complication, many of these built environment characteristics are highly correlated with socioeconomic status, known to have substantial influences on human health. Importantly, a recent study found both greenness and NO_2, a marker of traffic pollution, exposure independently associated with insulin resistance, a risk factor for diabetes and CVD.[29] However, when these variables were included into the same model, only NO_2 remained significantly associated, suggesting that the association between insulin resistance, and CVD by extension, and greenness may be confounded by exposure to traffic pollutants.[29]

Measuring Vegetation

There are numerous objective and subjective measures of localized and neighborhood-level vegetation, including canopy quantification, leaf area index, subjective perception, distance to green spaces, and satellite-derived vegetation indices.[132, 161, 162] These data may be utilized to examine residential-level vegetation, or aggregated to assess vegetation within buffer areas, neighborhoods, or other geographic units. While these measures are usually highly correlated, they likely capture different aspects of greenness.[161, 163] Of these measures, satellite derived vegetation indices are a readily accessible and provide an objective estimate of total vegetative cover and health in a given area.[163, 164]

Previous studies have demonstrated the ability of indices and ratios of reflected sunlight in the red and near infrared portions of the electromagnetic spectrum to be highly indicative of photosynthetically active biomass.[165] The Normalized Difference Vegetation Index (NDVI) is a commonly utilized satellite derived ratio of these spectra that is highly indicative of healthy and actively photosynthetic biomass.[166] While commonly utilized for environmental assessments, past studies have also utilized NDVI to study relationships between blood pressure, physical activity, obesity, and cardiovascular hospitalization.[25, 128, 167, 168] A commonly used, retrospective, and publically available source of these data are the Landsat series of satellites, with 30x30m spatial resolution and available worldwide.

Thirty-meter resolution Landsat imagery to calculate NDVI is available from 1997 through 2011 from Landsat 4-5, 2003 through the present from Landsat 7, and 2013 through the present from Landsat 8. The Landsat series of satellites are a joint initiative by the National Aeronautics and Space Agency (NASA) and the United States Geological Survey (USGS). Landsat satellites orbit the earth 14 times per day and provide complete global imagery cover every 16 days.[169] While global imagery is provided every 16 days, many of these days cannot be utilized to calculate NDVI over a large area due to cloud cover. As a result, in the Louisville Area, approximately 25% of images may be utilized for NDVI quantification. These gaps in data collection, sometimes lasting for months, represent an important limitation in the use of Landsat-derived NDVI. Other limitations of NDVI data include the inability to quantify specific plants, species types, vegetation below tree canopies, total biomass, or total leaf area.

Satellite imagery used to calculate NDVI is publicly available from the USGS, via the USGS EarthExplorer website, allowing no cost search and download functionality for all prepared and publically available Landsat imagery.[170] When images are taken, they are divided into spectral bands based on wavelength. For Landsat 8, these bands are categorized as coastal aerosols, blue, green, red, near infrared, short-wave infrared, panchromatic, cirrus clouds, and 2 separate thermal bands.[171] NDVI derived from these imagery bands is a ratio calculation of red and infrared light indicating chlorophyll cover in the given image.[172]

Louisville

The City of Louisville, in Jefferson County, Kentucky, is a predominantly urbanized area of about 700,000 residents in the East-Central U.S.[173] Similar to many U.S. cities, Louisville has a wide range of landcover and population groups.[174, 175] Land cover in Louisville primarily consists of residential, business, transportation-related, and park or unoccupied areas.[174] Louisville also suffers from poor air quality, often exceeding regulatory standards.[176]

Louisville has an urban layout typical of many U.S. cities, with a core central business district and steadily declining density of residences and businesses based on distance from the urban core. Suburban areas of the city typically consist of open space, residential space with large lots, and space-intensive industry and businesses. Also like

many U.S. cities, there are large disparities in socioeconomic status, largely following racial boundaries.[175] In Louisville, these differences strongly vary by location, with populations residing in eastern suburbs exhibiting the highest socio-economic status[175] Western areas of Louisville, particularly between the Ohio River and central business district, exhibit the lowest socioeconomic status and a high percentage of minority residents.[175] As with trends across the U.S., Louisville residents in lower socio-economic groups have a high prevalence of health risk factors and adverse outcomes.[175] As a result, geographic trends in socioeconomic status are reflected in incidence and prevalence of disease.[175] This low socioeconomic status is often mirrored by a multitude of factors in the built environment that may adversely influence health, such as lack of access to healthy food and health services, high crime rates, poor air quality, and environmental features not conducive to physical activity.[12, 175]

Louisville/Jefferson County, Kentucky has both is a wide range of vegetation, and patterns in disparities of CVD outcomes.[174, 175] An aging tree canopy, encroachment of invasive insects harmful to vegetation, and weather events have diminished the Louisville tree canopy by 15% across the city since 2004 and by much more in certain localized areas.[174] Current projections suggest a total canopy loss of 47% by 2052.[174] Given the range of vegetation cover, existing knowledge of vegetation, and existing cohort with detailed participant data from residents across the city, Louisville is an ideal study site to examine the relationships between vegetation in the environment, exposures, and human health.

Summary

The current study seeks to add to the knowledge base on the potential links between neighborhood greenness and cardiovascular risk. Despite evidence of associations between vegetation and health, there is a paucity of literature that addresses this complex and multifaceted relationship. Detailed evaluation of vegetation, environmental exposures, and biomarkers of cardiovascular injury may suggest insights into the contribution of environmental factors, particularly vegetation, to the risk of CVD among residents.

This project utilizes a multidisciplinary approach to examine the influence of neighborhood vegetation on CVD risk. Chapter 2 utilizes retrospective data on CVD hospital admissions to examine associations between CVD and vegetation at the neighborhood scale. Chapter 3 evaluates the association between urinary metabolites of VOCs and vegetation found at residences and surrounding areas of participants in the Louisville Healthy Heart Study. Chapter 4 explores relationships between residential vegetation and circulating angiogenic cells in Healthy Heart Study participants. Chapter 5 synthesizes results of these analyses in the context of existing literature on vegetation and CVD, describes potential future investigations, and discusses the potential public health implications.

CHAPTER II: SPECIFIC AIM 1

Introduction

CVD is the most common, serious, and deadly disease in the United States, accounting for over 30% of all deaths.[8, 9] Key risk factors include obesity, diabetes, lifestyle, hypertension, and age.[38] Environmental factors have been shown to modify important risk factors, affecting established risks such as exercise, stress, mental health, and accessibility.[1-7] The built environment has also been shown to directly affect health through increased concentrations of harmful air pollutants such as particulates, ozone, and VOCs – all of which are known to adversely affect cardiovascular health.[10, 58, 67, 70, 84] Among the many effects of the built environment on cardiovascular health, recent evidence has linked CVD with various metrics of proximate greenness in diverse locations.[151-154] Little evidence currently exists on the relationship between vegetation and specific CVD outcomes.

Greenness, or the amount and quality of environmental vegetation, is a potentially important aspect of the built environment.[19] There are a number of approaches to quantify greenness and vegetation including environmental perception, distance to parks and other greenspaces, percent tree canopy cover, and satellite derived metrics.[132, 161, 162] A common satellite derived metric is the Normalized Difference Vegetation Index (NDVI), a measure of the normalized ratio of red and infrared light spectral bands.[166] Because it is highly indicative of photosynthetic activity, this ratio correlates well with vegetation density and overall plant health.[165] NDVI is easily and retrospectively available through

satellite image archives. It is a common metric of vegetation utilized in environmental, agricultural, and health studies.

Greenness may affect various facets of the built environment, including air pollution, aesthetics, accessibility, and noise.[19] Influences on mental health and stress have also been observed, which may consequently affect human physical health, including risk for cardiovascular disease (CVD).[19] These effects are potentially observable at the population level, but influences, modifiers, and covariates of the relationship between greenness, the built environment, and CVD are complex and multifaceted.

The present study assesses CVD hospital admission rates, types, and severity in relation to neighborhood level greenness in Louisville, Kentucky. Louisville is a city of about 700,00 residents, and has similar characteristics to many mid-sized American cities.[173] There is a wide range of land cover types, primarily consisting of single family housing, business, open space, various impermeable surfaces, and a wide range of socioeconomic background among residents, which is highly correlated with health outcomes. [174, 175] Due to this correlation, both SES and outcomes follow similar geographic trends.[175] Because of the array of potential impacts of greenness on both the built environment and CVD risk, further study is warranted to assess the role of greenness in the maintenance and promotion of cardiovascular health. Based on the many influences of greenness on CVD risk factors, I hypothesize that neighborhood greenness is negatively associated with rate of CVD hospitalizations and length of hospital stay from CVD admissions.

Materials and Methods

Study population

Information and records on the population assessed in this study was collected from the Kentucky Cabinet for Health and Family Services. Before release, the population data set utilized was de-identified by removal of any potentially identifiable information, such as name, date of birth, precise residential location, social security number, etc. To further de-identify data, patient residential locations were aggregated to the ZIP code level, age was classified into 5-year age groups, and date of admissions was compiled into 3-month yearly quarters. The study population includes residents of Jefferson County, Kentucky meeting both location and hospital admission criteria. Location criteria include residents of a zip code falling predominantly within Jefferson County and with a total number of residents greater than 2000. Admission criteria include those admitted to a hospital in Kentucky with the primary cause of admission reported as CVD from 2005 through 2014. ICD-9 codes 400 through 460 were used to define CVD. Subsequent analyses were stratified by specific subtypes of CVD and thus included only patients admitted for the specified subtype.

Exposure to Greenness

Greenness was primarily quantified using NDVI, a satellite derived metric of overall vegetation. NDVI takes into account trees, shrubs, grass, and other photosynthetically active plant material with an unobstructed view from space.[166] This metric is quantified by examining the ratio of red and near infrared reflectance, providing a metric which is

highly indicative of photosynthetic activity, vegetation health, and density.[165] All geographic analyses were performed in ArcMap10.3 geographic information systems (GIS) software. The data used to calculate NDVI are publically available at 30x30m resolution and collected worldwide every 16 days. However, due to cloud cover, summer NDVI data without interference is typically available from 1 to 4 dates through the course of the summer growing season (May-September) in Louisville.

For comparability of imagery, the NDVI calculation taken from each year included in the analysis was determined by selecting the imagery collected nearest to the expected peak yearly NDVI date, approximately mid-June. Due to changes in satellite sensors used to collect NDVI over this time span, NDVI based on surface reflectance was utilized, which normalizes the results across different sensors. Once calculated at 30x30m resolution, NDVI was then aggregated into zip codes, from which average zip code NDVI for each year, 2005 through 2014, was calculated. Due to the anomalous effect of open water on NDVI values, satellite data covering the Ohio River was excluded from the analysis; this did not affect mean NDVI in zip codes overlapping the river.

Quantification of CVD Rates

CVD hospital admission data were obtained from the Kentucky Cabinet for Health and Family Services. These data include all inpatient hospitalizations in Kentucky from years 2005 through 2014. To maintain patient confidentiality these data were de-identified by reporting at the zip code level, by quarter of admission, and with age of patient

aggregated into 5-year age groups. Records include the reason (by ICD code) for hospitalization, cost of admission, length of stay in the hospital, gender, and race for each hospitalization record. Records of admissions for residents of Jefferson County were then selected for the present analysis. This analysis also excludes admissions and records of patients under the age of 35, due to the substantially higher prevalence of congenital heart diseases and other forms of CVD not related to traditional or environmental risk factors in younger populations. To calculate yearly CVD admissions rate, total CVD admissions was first quantified by taking the sum of admissions from residents of each zip code in Jefferson County. CVD admissions were identified by ICD-9 codes 400 through 460. The number of admissions meeting criteria for inclusion into the analysis in each individual zip code was then divided by the total population over the age of 35 in that zip code on a yearly basis. Total zip code population above age 35 was collected from the U.S. Census Bureau. CVD rates were then further stratified by CVD type using the same method with ICD-9 codes for stroke and coronary heart disease (CHD) separately.

Covariates

Heart disease is affected by a multitude of risk factors. Therefore, several potential covariates were included into the analysis. However, due to the de-identified nature of the hospital admissions data available, many common individually linked demographic covariates could not be included. Among covariate data provided with admissions data, 5-year age group of patients, year of admission, and quarter of admission were included into the analysis. Racial group of participants was also included. Individual-level indicators of socioeconomic status were not available. To account for socioeconomic

status, several socioeconomic indicators were collected from the U.S. Census Bureau at the zip code level and included as potential covariates. These indicators include median household income, percent of residents in poverty, rate of unemployment, and high school education or equivalent. Population density, mean age, mean age of individuals over 35, percent male, and percent of a racial minority at the zip code level collected from the U.S Census Bureau were also included as potential covariates.

Many environmental characteristics have been previously shown to affect both urban vegetation and cardiovascular risk.[19] To account for these characteristics, variables indicative of the zip code scale built environment were calculated and included into the analysis. To account for roadway cover and traffic density on those roadways, total distance of daily vehicle travel on roadways falling within the zip codes was calculated. Data including roadway segment length and vehicles per day on those segments were collected from the Kentucky Transportation Cabinet. Vehicles per day on roadway segments was then multiplied by segment length and subsequently aggregated into zip code areas in order to obtain total vehicle distance travelled. Percent residential area in zip codes was calculated with data obtained from the Office of the Jefferson County Property Valuation Administrator. GIS layers of all property parcels in the county were obtained, from which single family residential parcels were selected. Area of these single family residential parcels was quantified, aggregated into overlapping zip codes, and then divided by total zip code area to obtain percent residential area. Areas of high intensity land use were collected from the U.S. Geological Survey National Land Cover Data Set. Areas of high intensity land use overlapping industrial areas, as defined by Jefferson

County property records, were selected as high intensity industrial areas. These areas were aggregated into zip code polygons and used to calculate percent of high intensity industrial landcover in each zip code, which was subsequently included as a potential covariate.

Statistical Analysis

All statistical analyses were performed using SAS, version 9.4, software (SAS Institute, Inc., Cary, North Carolina). Characteristics of zip codes are expressed as mean(SD) for continuous variables. Two sample t-tests were used to test for significant differences in mean of covariates in the highest versus lowest NDVI zip codes. To take into account repeated observations over time, zip code level hospital admissions rates for CVD were analyzed using a repeated measures mixed modeling procedure. This procedure accounts for the temporal correlation in yearly observations of both NDVI and admissions rates by zip code. All years from 2005 through 2014 were included in the analyses. However, due to the lack of valid satellite data available for times near peak NDVI in 2012, due to cloud cover and sensor faults, NDVI values were estimated by taking the mean of values collected for 2011 and 2013. To select covariates in the final model, a stepwise procedure was utilized, excluding the least associated covariate at each step until all remaining variables were significantly associated at the $p<.05$ level. When no metrics of socioeconomic status were significantly associated using the stepwise procedure, median household income was included a priori into the final model. While spatial autocorrelation was not directly taken into account, adjustment for demographic covariates largely accounts for this, as these factors generally follow the same spatial

patterns as CVD.[177] In addition to the primary outcome variable of CVD hospitalization rate, length of hospital stay, rate of emergency CVD admissions, and specific CVD types of stroke and CHD were also assessed with NDVI at the zip code level.

Results

Vegetation

Vegetation in Jefferson County largely followed patterns typical of U.S. cities with similar size and climate. As landcover varies widely within the county, so does NDVI, with observed ranges at the $30m^2$ scale falling between -0.1 and 0.8 NDVI units. Very low vegetation was found in the urban core and central business district, industrial areas, and transportation related areas. Moderate vegetation was observed in residential areas. High vegetation was observed in urban parks, forests, and undeveloped space. In predominantly low vegetation areas, mean NDVI ranged from 0.08 to 0.25. The lowest zip code NDVI average of 0.08 was observed in the Louisville central business district. Moderate vegetation and residential zip codes typically ranged from 0.4 to 0.5 NDVI, largely varying based on average residential parcel size. High vegetation zip codes ranged from 0.55 to 0.69 NDVI, primarily based on amount of residential space.

Patient Characteristics and Demographics

After exclusion of zip codes with a total residential population of less than 2000 and those not falling mostly within Jefferson County, 31 zip codes were included in the analysis. In total, 6,297,359 hospital admissions records were retrieved for the period

between 2005 through 2014. Of these, 146,333 met the inclusion criteria of the patient residing within one of the selected zip codes and with CVD as the primary reason for admission. However, the number of unique patients included cannot be discerned from the available data due to potential repeat admissions of the same individual. Subsequent analysis for CHD, stroke, and emergency CVD admissions included 37,194, 11,099, and 95,092 hospitalizations, respectively. Rates of CVD admissions by zip code ranged from 259 to 942 admissions of persons over the age of 35 per 10,000 residents over the age of 35.

Significant differences in demographic characteristics were observed between neighborhoods. (Table 1) Low greenness zip codes had a higher proportion of minority residents, rates of poverty, unemployment, and lower household income than high greenness areas. Large differences in the built environment also were also observed with low greenness areas having significantly higher population density, vehicle traffic, % high intensity industrial area, and lower family residential area.

Associations between Greenness and CVD Admissions

There were highly significant unadjusted associations between high and low greenness areas and CVD hospital admissions of all types, as well as length of stay. (Table 2.) Mean rate of all admissions among residents of low vegetation zip codes was 413 per 10,000, compared with 307 per 10,000 in high vegetation areas. Respectively, mean rate per 10,000 in for CHD, stroke, and emergency admissions among residents of low vegetation

zip codes was 118, 34, and 326, compared with 97, 27, and 221 in high vegetation areas. Average length of hospital stay for admissions of residents from low greenness areas was 4.97 days, compared with 4.7 days among residents of high vegetation areas. The zip code displaying the highest rate of admissions included the central business district. (Figure 2.) Geographic distribution of admissions rates by zip code largely followed demographic patterns across the county. Low rates were found in high income areas in the Eastern areas of the county and high rates in low income areas situated in the Western areas.

Using stepwise regression procedures for overall CVD rate and each CVD subtype, results were similar to unadjusted associations. (Table 3.) Rates of all admissions, emergency admissions, CHD, and stroke remained highly significant. Average length of stay was also significantly associated. The final model for rate of add admissions included NDVI, percent employed, vehicle density, and percent male. The final model for emergency admission rate included NDVI, percent minority, percent male, and education. The final model for stroke admission rate included NDVI and median household income. The final model for CHD included NDVI, percent minority, age above 35, education, and income. The final model for length of stay included NDVI percent male, percent employed, and vehicle density.

Discussion

The results of this study are consistent with the hypothesis that neighborhood greenness is inversely associated with CVD hospitalization rate. Greenness is associated with stroke, CHD, and emergency admission rate. It was also significantly associated with length of hospital stay. Unadjusted associations between greenness and CVD admissions may be driven by the large demographic differences between high and low greenness zip codes. After adjustment for available relevant covariates, these associations remained significant. These results differ from a past study showing limited associations between self-reported CVD and no association with hospital admissions.[35] Results from the present study are consistent, but not directly comparable, with previous analyses reporting lower rates of CVD mortality among residents of high greenness areas.[18]

An important strength of this analysis is the ability to examine neighborhood scale greenness with not only CVD hospitalization rate, but also specific CVD subgroups and length of stay. Comparison of effect size of these different CVD categories may add potentially useful perspective to these results, with a larger effect size observed between CHD and emergency rates than stroke or overall admissions. The mixed repeated measures model utilized in this analysis takes into account repeated observations of both hospitalizations and NDVI from 2005 through 2014. This analysis not only utilizes 10 years of data, but also takes temporal autocorrelation and changes in relationships over time into account. Another strength is the large and inclusive population assessed through hospital admissions records.

There are numerous limitations to this ecologic analysis. Individual level socioeconomic status data on income, education, and occupation were not available. Data on patient residence was not available at a geographic scale smaller than zip codes. Zip codes are large areas lacking standardization of structure or size, generalizing large continuous areas. In many cases, within zip code variability of greenness is higher than between zip code variability. This analysis also cannot account for time spent outside of the residential zip code and vegetation at those locations. There is also a large degree of variability in socioeconomics and characteristics of the built environment within zip codes that cannot be accounted for. As a crossectional analysis, the observed link between CVD admissions and vegetation in this study is limited to association.

Significance

Results of this analysis suggest that rates of overall CVD hospital admissions, specific CVD admission subtypes, and length of stay of CVD admissions are inversely associated with neighborhood vegetation. These results remained statistically significant after adjustment for relevant available covariates. These results may indicate that neighborhood vegetation affects cardiovascular risk, as indicated by hospital admissions rates, or that there are other factors driving this observed relationship that remain unaccounted for. Further study of these relationships is needed to better evaluate these relationships.

Figures and Tables

Figure 2. Vegetation by zip code.

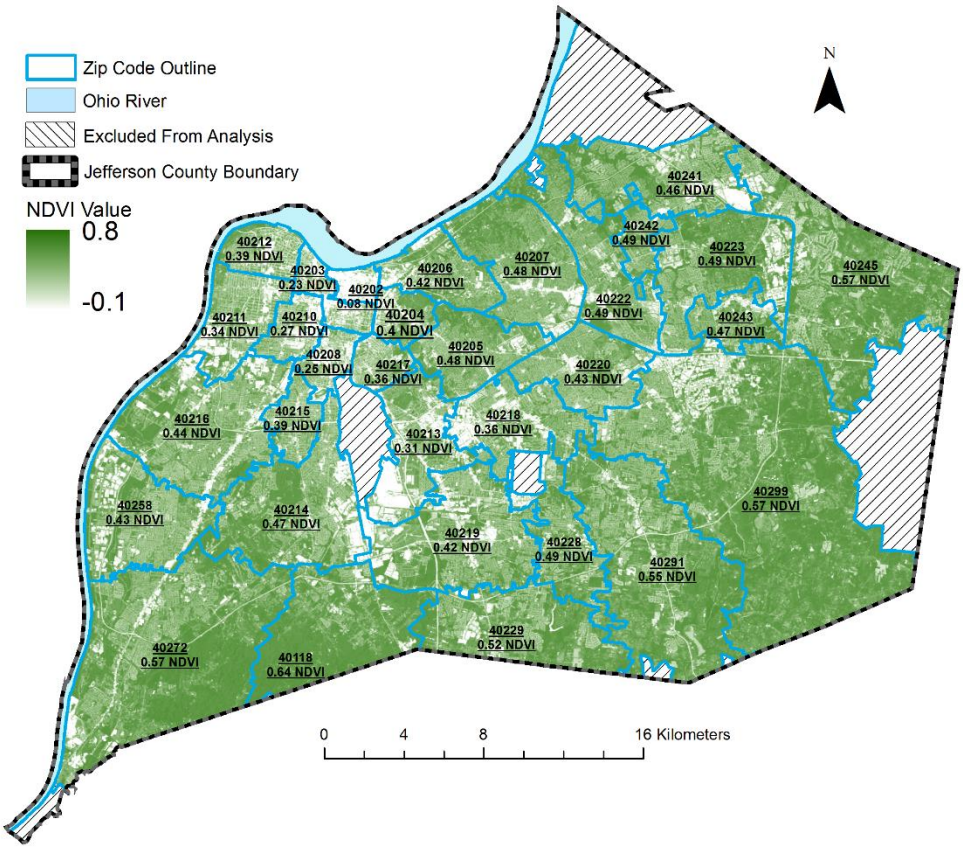
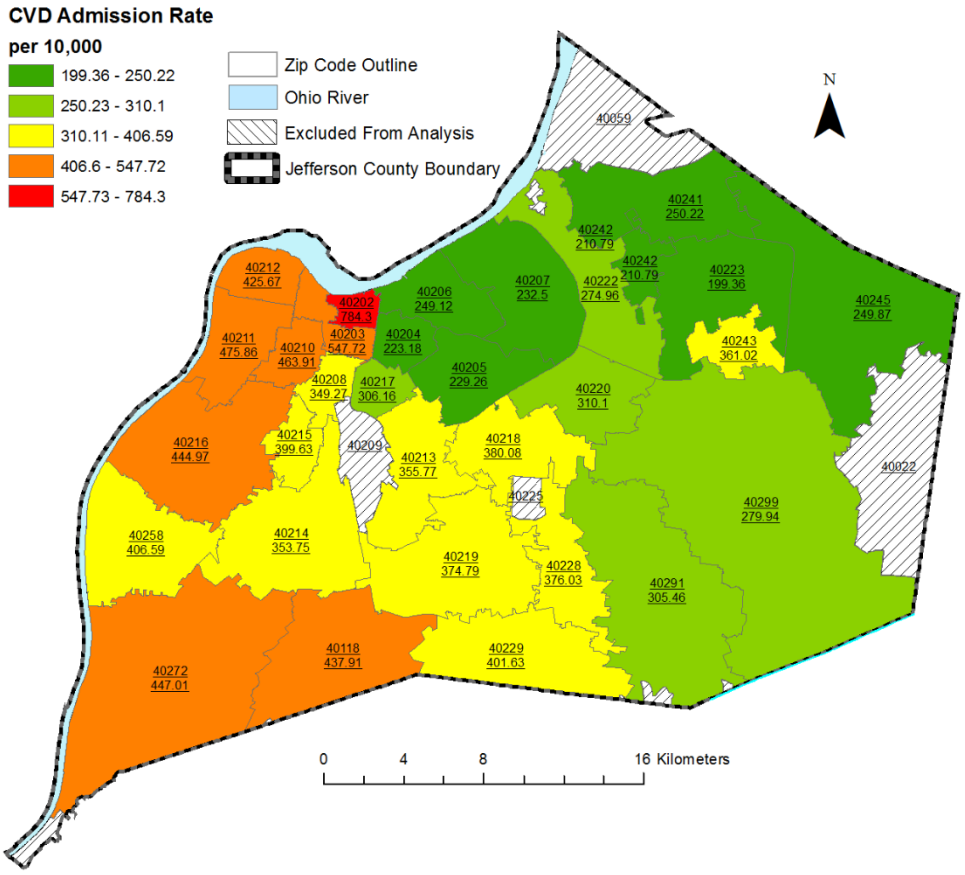


Figure 3. Hospital admission rate by zip code.



	Low NDVI (n=15)	High NDVI (n=16)	
	Mean(SD)	Mean(SD)	p-value
Area (m)	15.91(11.35)	41.65(11.35)	<.0001
% Males, of Population >35 years	47.48(0.06)	46.23(1.05)	0.4157
Mean Age, Residents >35 years	54.68(1.27)	53.82(1.82)	0.1978
Median Household Income	$33,299($12,283)	$60,580($12,283)	<.0001
% Minority	39.65(29.49)	9.63(29.49)	<.0001
% Poverty	28.4(13.3)	9.75(13.3)	<.0001
% Unemployed	15.2(6.98)	7.43(3.37)	0.0004
% no HS Education	20.26(10.71)	13.75(5.99)	0.0438
% Canopy	24.16(8.16)	40.27(8.16)	<.0001
Vehicle Density (km/km^2)	58399(44021)	25151(44021)	0.0041
Average NDVI	0.49(0.1)	0.65(0.1)	<.0001
Population Density per km^2	1637(552.5)	789.2(341.5)	<.0001
% Residential Area	28.35(12.39)	40.49(5.94)	0.0015
%High Intensity Industry	14.06(9.59)	5.23(5.95)	0.0042

Table 1. Zip code demographics and vegetation.

	Low NDVI (n=15)	High NDVI (n=16)	
	Mean(SD)	Mean(SD)	p-value
All Admissions Rate	413.313(167.58)	306.70(1675.77)	<.0001
CHD Rate	118(47)	96.9(46.2)	0.0006
Stroke Rate	33.71(12.1)	26.98(6.86)	<.0001
CVD Emergency Admission Rate	326(125)	221(60.7)	<.0001
Average Length of Stay	4.96(0.36)	4.7(0.34)	<.0001

Table 2. Unadjusted admissions rates and vegetation
All rates are per 10,000

	β	p-value
All Admissions Rate	-2.21	<.0001
CHD rate	-6.9	<.0001
Stroke Rate	-2.25	<.0001
Emergency Visit Rate	-4.89	<.0001
All admissions Length	-0.09	0.0043

Table 3. Association between vegetation and admissions in fully adjusted models.

β -value for 0.1 increase in NDVI.

CHAPTER III: SPECIFIC AIM 2

Introduction

CVD is an established health outcome associated with exposure to air pollution.[178] VOCs, including benzene, are a ubiquitous and highly harmful pollutant type associated with motor vehicle emissions. [70, 71] Benzene, for which no safe level of exposure has been established, is a particularly abundant and harmful, and strongly linked to a variety of adverse health effects.[75] Aldehydes, particularly acrolein, are another abundant and especially toxic roadway pollutant.[80] Acrolein has been associated with substantially increased CVD risk, imparting roughly 80-90% of the non-cancer risk from smoking.[81, 82] A metabolite specific to acrolein is 3-hydroxyproplumercapturic acid (HPMA).[92, 179] A specific metabolite of benzene is trans,trans-muconic acid (TTMA).[74] Both of these metabolites are found in the urine of humans and may potentially be utilized to assess exposure to their parent compounds.[74, 92, 179]

In recent years, the ability of trees and other vegetation to mitigate environmental air pollution has been investigated.[19] Vegetation has been shown to filter polluted air in the environment of specific air pollutants (O3, PM10, NO2, SO2, VOC's, CO).[30, 68] However, much remains to be understood about many aspects of this relationship, including which pollutants vegetation removes and to what extent, how this translates to changes in human exposure, and at what geographic scale this phenomena occurs.[145] Most of the limited research in this area has focused on the impact of vegetation on concentrations of particulate matter.[145] Little investigation has been performed on the

influence of vegetation on human exposure to anthroprogenically generated concentrations of harmful VOCs. The main objective of this aim is to determine the relationship between vegetation and air pollution. To do this, I will examine metabolites of benzene and acrolein as sensitive biomarkers to VOC exposure, in relation to residential area vegetation, and the geographic scale at which this relationship is most pronounced.

Materials and Methods

Study Population

Adult participants in the Louisville Healthy Heart Study (n=508) were recruited between October 2009 and May 2013 from the University of Louisville Hospital and clinic system. The Louisville Healthy Heart Study was designed to collect data on environmental factors that may influence prevalence, progression, and severity of CVD. The study is primarily supported through grants from the WellPoint Foundation and National Institutes of Health, with other support coming from the Center for Environmental Genomics and Integrative Biology and the National Institute of Environmental Health Sciences. Prior to participant enrollment, all study activities were approved by the University of Louisville Institutional Review Board (IRB 09.0174 and 10.0350). Participants were recruited through University of Louisville staff emails, posted flyers, and directly through relevant cardiology clinics. Many patients participating in The Healthy Heart Study were recruited from a secondary cardiology clinic, primarily treating patients with existing CVD, often with low socioeconomic status. Recruitment at clinics consisted of pre-screening patients (up to 1600) during

active enrollment and in-person recruitment of potential participants during waiting periods at the clinics. Due to IRB constraints, we were unable to collect data on patients prescreened who chose not to participate. All enrolled participants provided written informed consent after being provided thorough information on study and enrollment activities. Study exclusion criteria included inability to provide written informed consent; vulnerable populations (e.g. pregnant or lactating women and prisoners); lung, liver, kidney, or hematological disease; coagulopathies; substance abuse; chronic cachexia; and severe comorbidities. Enrollment in The Louisville Healthy Heart Study was originally planned to include 240 participants in order to detect a significant changes in circulating angiogenic cell levels. The study was subsequently expanded to include more participants in order to assess additional outcomes. A subset of study participants were enrolled to examine specific diabetic markers and were subject to additional exclusion criteria of type I diabetes, organ transplant, untreated thyroid disease, hormone replacement therapy, medications affecting bone marrow function or peripheral blood cell counts, those having received a blood transfusion in the past year, or those admitted to a hospital in the 5 months preceding enrollment. One individual chose to withdraw from the study after enrollment and was subsequently excluded from all data analysis.

Patients who met study enrollment criteria and consented to study participation were administered a questionnaire and provided blood and urine sample at the time of enrollment. Participant questionnaires collected demographic, health behavior, and medical information. Demographic and health behaviors included residential address and zip code, current and past tobacco smoking behavior, exposure to secondhand tobacco

smoke, alcohol consumption, and physical activity. Medical status information collected via questionnaire consisted of current medication usage and history of CVD including heart attacks, strokes, as well as various other cardiovascular events and conditions. Both blood and urine samples were collected from each study participant at the time of enrollment. Urine samples were analyzed for cotinine and creatinine. Blood samples were analyzed high-sensitivity C-reactive protein. The present analysis utilizes a subpopulation of the Healthy Heart Study cohort - participants who have complete covariate information, quantified HPMA or TTMA, and who were able to be successfully geocoded. After exclusions, 172 and 224 participants met criteria for HPMA and TTMA analysis, respectively.

Residential Proximity to Vegetation

Residential location of participants was identified by geocoding residential addresses provided during enrollment. Addresses were corrected for spelling errors, invalid characters in the data, and invalid or erroneous formats. ArcMap9.3+ GIS software was utilized to geocode these addresses using street and address data provided by the Louisville/Jefferson County Information Consortium. Addresses were automatically geocoded by GIS software and were manually validated against surrounding addresses. Addresses not assigned to actual residential locations (e.g. apartment complexes and mobile home communities) were manually located wherever possible. Addresses that could not to be automatically geocoded were located through zip codes, street names, and specific addresses of surrounding residences.

Overall vegetation was quantified by utilizing GIS software to calculate the average NDVI value of 30mx30m resolution satellite data within given radii from participant residential location. Satellite imagery was collected by NASA and USGS Landsat satellites and downloaded though the United States Geological Survey Earth Explorer Website. Summer imagery was collected for approximate peak greenness and subsequent NDVI calculation was performed utilizing imagery data from summer 2011, the approximate midpoint of study participant enrollment.

Buffer areas were calculated at distances of 50m, 100m, 200m, 300m, and 1km from residential addresses. NDVI data were then compiled within these buffer areas in order to obtain mean NDVI at the given buffer distance. In order to obtain NDVI at the participant residence, NDVI $30m^2$ (approximately 60% of Louisville mean residential lot area) pixel cell values were extracted at the geocoded point of participant residences. These data were then linked to participant records for analysis in statistical models.

Covariates

Covariates considered in the analysis included age, gender ethnicity, body mass index (BMI), tobacco exposure, median household income, proximity to roadways, and daily $PM_{2.5}$. Age, gender, ethnicity, BMI (calculated from height and weight variables), and tobacco exposure (verified by urinary cotinine concentration) were all collected through the participant questionnaire. Median household income, a proxy for neighborhood socioeconomic status and individual-level participant income and SES, was collected at

the U.S. Census Bureau block group level. Roadway exposure was calculated by quantifying the total length of roadways within a 50m buffer area from geocoded participant residential address.

Ambient levels of $PM_{2.5}$ from EPA validated monitors from the 24hrs prior to the day of blood and urine collection were used to estimate exposure. The average of daily $PM_{2.5}$ values for all monitors in the Louisville Metropolitan Area were calculated to estimate daily ambient $PM_{2.5}$. These daily ambient levels were then applied to estimate $PM_{2.5}$ exposure for participants enrolled on the following day. Little between-monitor variation of $PM_{2.5}$ was observed, indicating that there is little intra-urban variation.

Quantification of Benzene and Acrolein Metabolites

Urinary concentrations of TTMA were quantified by GC/MS using methods adapted from Waidyanatha et al.[180] Urinary levels of HPMA were quantified using a gas chromatography/ mass spectrometric (GC/MS) analysis method according to methods described previously by Carmella et al.[181] and subsequently modified by Conklin et al.[182] Levels of TTMA and HPMA were normalized to urinary creatinine, accounting for the effect of dilution in the urine.

Statistical Analysis

All statistical analyses were performed using SAS, version 9.4, software (SAS Institute, Inc., Cary, North Carolina). Participant characteristics are expressed as n (%) for

categorical variables in Table 1, and mean (SD) for continuous variables in Table 2. Participant demographics, CVD risk factors, and biomarkers were compared between high and low NDVI values using independent samples t-tests or Chi-squared tests as appropriate. High NDVI was classified as those above the median NDVI value of participants – 0.36.

Multiple linear regression was used to assess the relationship between outcome variables (HPMA, TTMA) and the primary exposure variable NDVI values at different radii surrounding participant residential locations. HPMA and TTMA were log-transformed for normality. The independent association of each demographic variable in Tables 1 and 2 were examined one at a time using simple linear regression with HPMA and TTMA as the dependent variables. All independent variables that were significant at the $p<0.05$ level were examined as potential covariates in the fully adjusted model. To determine covariates included in the final model, a stepwise procedure was used to exclude the least associated potential covariates. The final models include only those covariates that remained significant at the $p<0.05$ level. The TTMA models in table 3 were adjusted for ethnicity, smoking status, diuretic use, household income, total length of roads within 50 meters of residence, and residential distance to gas station. The HPMA models in table 4 were adjusted for ethnicity, cotinine, diuretic use, residential distance to gas station, and $PM_{2.5}$ levels. A subset analysis for smokers along and non-smokers alone was then performed in the models to test whether the association increased between NDVI values and the secondary dependent variables of HPMA and TTMA. The subset analysis used the same adjustment as the full models with the exception of adjusting for smoking.

Results

Geographic Distribution

Data from the Louisville Jefferson County Information Consortium (LOJIC) was utilized to geocode participant addresses into ArcMap 9.3+ Geographic Information Systems (GIS) software. Overall distribution of participant residential locations are represented in Figure 1. All displayed points are geographically masked and not of actual residential locations. The geographic distribution of participants largely fell within low greenness neighborhoods located in Northwestern Jefferson County, commonly referred to as West Louisville. A less dense, but relatively even, distribution of participants was observed throughout the rest of the county. Those falling outside of the county were excluded from all analyses due to incomplete single-image satellite coverage, substantial differences in urban environment between urban and rural areas, and incomplete covariate data. Attributes of landcover and vegetation varied widely within Jefferson County. Most participants resided with neighborhoods consisting of single family residential homes and lots containing both grass and trees.

Participant Characteristics

Participants examined in the present study were recruited and enrolled between October 2009 and May 2013. The study cohort included 251 persons, consisting of 51% males, 54% Caucasian, 44% African American, and 2% of another race(s) (Table 1). Much of the study population was hypertensive (83%), hyperlipidemic (67%), and at a high risk for CVD (77%). The cohort was 52±10 years old and mostly overweight or obese with a

mean BMI of 33±8 (Table 2). African American participants were significantly more likely to reside in areas with less vegetation than white participants. No significant differences were observed with CVD risk factors, cardiovascular history, medication use, age, sex, BMI, sum of CVD risk factors, or a variety of biomarkers. Participants residing in low vegetation areas were significantly more likely to live in a lower income neighborhood and have a lower distance to a major roadway. Study participants with quantified TTMA (n=224) and those with quantified HPMA (n=172) largely overlapped. There were no significant demographic differences between them.

Association between air pollution and vegetation

To examine the relationship between area vegetation and benzene exposure, TTMA, a metabolite of benzene, was compared with average vegetation at the residential level. The date of June 27, 2011 was utilized for NDVI calculation, which represents the approximate midpoint of participant enrollment and peak greenness for that year. Average NDVI within varying buffer area sizes around participant residences were compared to evaluate the effect of vegetation proximity on this relationship. After adjustment for race, smoking status, diuretic use, household income, nearby roadways, and distance to a gas station, TTMA was significantly inversely associated (p=0.0002-0.01) with average NDVI at 50m, 100m, 200m, 300m, and 1km buffer areas around residential addresses (Table 3). This relationship was not significant for the average 30x30m cell value overlaying participant address points. Observed change in TTMA per 0.1 change in average NDVI value ranged from 22.9% to 26.9%, depending on buffer areas used. Because smoking is a source of benzene, the analysis was also stratified into

non-smoking participants. These associations remained significant among non-smokers (p=0.001-0.022) with observed differences ranging from 22.1% to 31.5%. When the analysis was stratified to smokers, the association between NDVI and TTMA did not remain significant in any of the assessed buffer areas. (Table 5.)

To examine the relationship between area vegetation on acrolein exposure, HPMA, a metabolite of acrolein, was compared with average NDVI at the residential level. Average NDVI within varying buffer area sizes around participant residences were compared to evaluate the effect of vegetation proximity on this relationship. After adjustment for ethnicity, smoking, diuretics, residential distance to a gas station, and particulate matter, HPMA was significantly associated (p=0.001-0.038) with average NDVI at the participant address, 50m, 100m, 200m, 300m, and 1km buffer areas (Table 4). Observed change in HPMA per 0.1 increase in average value ranged from 12.2% to 15%. With the exception of NDVI at the 1km buffer area (p=0.172), these associations of vegetation and HPMA became more pronounced (p=0.0002-0.005) when stratified to non-smokers. The observed change in HPMA levels remained significant and increased with larger buffer areas, with observed changes ranging from a 20.1% to 28.9% per 0.1 unit NDVI increase. When the analysis was stratified to smokers, these associations did not remain significant. (Table 8.)

Discussion

The major finding of this study is that vegetation at residential location and in surrounding areas is associated with decreased concentrations of urinary metabolites of pollutant exposure in an at-risk study population. The analyses indicate that nearby vegetation is significantly and inversely associated with biomarkers of VOC exposure, even when controlling for roadway exposure. These results suggest that exposure to benzene, acrolein, and associated pollutants may be mitigated by nearby vegetation.

The major strength of this investigation is the use of VOC urinary metabolites to measure individual specific exposure to air pollutants, as opposed to other investigations utilizing various types of exposure estimates. Additionally, the use of cotinine as a variable in the statistical models, as opposed to self-reported smoking, to adjust for tobacco smoke exposure is an important strength given the high concentrations of VOCs present in tobacco smoke. NDVI is a common metric of greenness, enabling direct comparison of results with other findings. Few prior studies have evaluated vegetation at address of study participants, which may have differing influences on health than metrics of larger areas.

There are several limitations to this study. Importantly, airborne pollutant concentrations were not measured or estimated at participant residential locations. Also, while the measured urinary metabolite concentrations do reflect personal exposure to VOCs, total human exposure is not entirely through exposure to airborne VOC concentrations. This

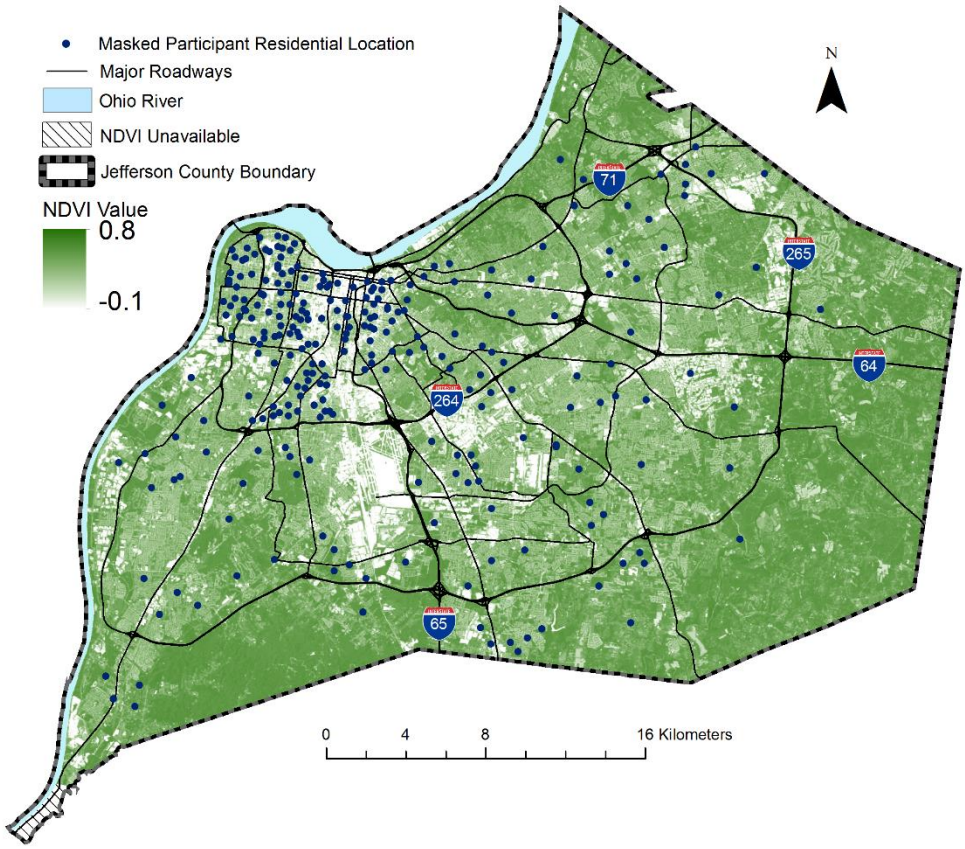
study assessed a satellite imagery-based measure of surrounding vegetation, but this cannot fully account for leaf area, biomass density, height, overlapping vegetation layers, speciation, and other important characteristics of vegetation and subsequent pollutant removal. Only residential vegetation was assessed, accounting for a large, but incomplete, portion of participants overall exposure while at home or elsewhere. A disproportionate number of participants suffered from elevated CVD risk, therefore, these results cannot be extrapolated to the overall population. Finally, due to the crossectional design of this study, the link between vegetation and exposure to airborne VOCs is limited to association. To further understand these associations, a longitudinal prospective study is needed to assess how vegetation affects pollutant concentration, exposure, and cardiovascular outcomes.

Significance

Results of this study suggest that proximate vegetation is associated with decreased levels of biomarkers of VOC exposure. These findings suggest that vegetation may mitigate exposure to air pollution, thereby decreasing health adverse health consequences of exposure. While most public health policy to address health effects of air pollution exposure revolve around reduction of emissions, vegetation potentially represents an important public health intervention to reduce airborne pollutant concentrations after emission.

Figures and Tables

Figure 4. Masked distribution of Healthy Heart Study Participants included in Aim 2 and vegetation.



Categorical Variable – n (%)	Total n = 251 (%)	Low Green n=126	High Green n=125	P-Value
Sex				0.486
Male	129 (51%)	62 (49%)	67 (54%)	
Race				0.010
White	135 (54%)	56 (44%)	79 (63%)	
Black	110 (44%)	67 (53%)	43 (34%)	
Other	6 (2%)	3 (2%)	3 (2%)	
CVD Risk Factors				
Hypertension	205 (83%)	106 (84%)	99 (81%)	0.536
Hyperlipidemia	166 (67%)	78 (63%)	88 (72%)	0.148
Diabetes	75 (30%)	42 (33%)	33 (27%)	0.263
Current smoker	100 (40%)	54 (43%)	46 (37%)	0.380
High CVD risk category	149 (77%)	70 (78%)	79 (76%)	0.765
Cardiovascular History				
Myocardial Infarction	98 (39%)	53 (42%)	45 (37%)	0.376
Stroke	33 (13%)	18 (14%)	15 (12%)	0.627
CABG/PCI/stents	84 (34%)	49 (39%)	35 (28%)	0.082
Heart Failure	48 (19%)	26 (21%)	22 (18%)	0.583
Medications				
ACE/ARB	153 (62%)	76 (61%)	77 (63%)	0.832
β-Blocker	163 (66%)	87 (70%)	76 (62%)	0.165
Calcium-channel blocker	56 (23%)	26 (21%)	30 (24%)	0.521
Diuretics	99 (40%)	50 (40%)	49 (40%)	0.938
Statins	140 (57%)	69 (56%)	71 (58%)	0.742
Aspirin	142 (57%)	74 (60%)	68 (55%)	0.485

Table 4. Demographics and cardiovascular disease history in the Louisville Healthy Heart Study stratified by high/low residential vegetation.

Abbreviations: CVD, cardiovascular disease; ACE, angiotensin-converting enzyme inhibitor; ARB, angiotensin-receptor blockers; CABG, coronary artery bypass graft; PCI, percutaneous coronary intervention.

Continuous Variable – mean (SD)	Total n=251	Low Green n=126	High Green n=125	p-value
Age (years)	51.8 (10.0)	52.2 (9.9)	51.4 (10.2)	0.529
BMI	32.7 (7.8)	33.1 (8.3)	32.4 (7.3)	0.499
Systolic Blood Pressure (mmHg)	131.9 (21.3)	134.3 (22.8)	129.9 (19.7)	0.162
Diastolic Blood Pressure (mmHg)	81.2 (12.2)	82.8 (13.0)	79.8 (11.4)	0.089
Cotinine (μg/g creatinine)	549.8 (1102.2)	660.0 (1252.2)	440.4 (922.2)	0.118
3-HPMA (μg/g creatinine)	371.8 (517.0)	406.5 (655.3)	342.5 (362.2)	0.418
trans,trans-Muconic acid (mg/g creatinine)	0.2 (0.3)	0.18 (0.20)	0.21 (0.34)	0.410
Creatinine (mg/dL)	142.6 (98.2)	149.9 (111.3)	135.3 (82.6)	0.238
Lipid Levels (md/dL)				
Cholesterol	193.7 (57.5)	191.8 (53.6)	195.4 (61.1)	0.697
HDL	44.5 (13.3)	45.6 (15.4)	43.4 (10.9)	0.306
LDL	107.3 (44.7)	105.4 (46.8)	109.1 (42.9)	0.609
Triglycerides	199.8 (234.1)	197.2 (156.9)	202.2 (288.2)	0.893
Thrombosis				
Fibrinogen (mg/dL)	345.6 (111.8)	341.3 (112.6)	349.3 (111.6)	0.624
Platelet-leukocyte aggregates	10.7 (5.9)	10.9 (6.5)	10.5 (5.3)	0.676
Inflammation				
hsCRP (mg/L)	4.9 (4.8)	4.6 (4.8)	5.2 (4.8)	0.404
CVD risk				
Sum of CVD risk factors	3.5 (1.2)	3.5 (1.3)	3.4 (1.2)	0.698
Median Household Income X 10^{3} ††	30.7 (17.0)	25.7 (12.6)	36.2 (19.4)	<.0001
Distance to Major Roadway	228.2 (236.7)	166.1 (154.7)	296.4 (287.9)	<.0001

Table 5. Demographics and cardiovascular disease history in the Louisville Healthy Heart Study Stratified by High/Low residential vegetation.

Abbreviations: 3-HPMA, 3-Hydroxypropylmercapturic acid; BMI, body mass index (weight(kg)/height(m)^2); hsCRP, high sensitivity C-reactive protein; $PM_{2.5}$, particulate matter with an aerodynamic diameter < 2.5 μm; SD, standard deviation.

	Trans,trans-muconic acid		
NDVI radius, n=224	Change (%)	95% C.I.	P-Value
0 meter NDVI	-9.9	(-26.6, 7.2)	0.257
50 meter NDVI	-25.0	(-37.9, -12.0)	0.0002
100 meter NDVI	-25.5	(-39.2, -11.9)	0.0002
200 meter NDVI	-23.4	(-38.0, -8.8)	0.002
300 meter NDVI	-22.9	(-40.3, -5.5)	0.010
1 kilometer NDVI	-26.9	(-43.8, -9.9)	0.002

Table 6. Association between trans,trans-muconic acid and vegetation at different radii in the Louisville Healthy Heart Study.

Percent change in TTMA per 0.1 increase in NDVI. Adjusted for ethnicity, smoking status (cotinine confirmed), diuretics, household income, total length of roads within 50 meters of residence, residential distance to gas station.

	Trans,trans-muconic acid, non-smokers		
NDVI radius, n=131	Change (%)	95% C.I.	P-Value
0 meter NDVI	-22.8	(-54.9, 9.3)	0.164
50 meter NDVI	-26.9	(-42.8, -11.0)	0.001
100 meter NDVI	-31.5	(-48.6, -14.3)	0.0003
200 meter NDVI	-22.1	(-39.9, -4.2)	0.015
300 meter NDVI	-25.3	(-46.9, -3.7)	0.022
1 kilometer NDVI	-28.7	(-49.5, -8.0)	0.007

Table 7. Association between trans,trans-muconic acid and vegetation at different radii among nonsmokers in the Louisville Healthy Heart Study.

Percent change in TTMA per 0.1 increase in NDVI. Adjusted for ethnicity, smoking status (cotinine confirmed), diuretics, household income, total length of roads within 50 meters of residence, residential distance to gas station.

	Trans,trans-muconic acid, smokers		
NDVI radius, n=93	Change (%)	95% C.I.	P-Value
0 meter NDVI	-2.3	(-17.2, 12.7)	0.766
50 meter NDVI	5.16	(-13,2, 23.6)	0.583
100 meter NDVI	3.51	(-14.0, 21.0)	0.695
200 meter NDVI	8.77	(-13.5, 31.0)	0.440
300 meter NDVI	12.0	(-11.4, 35.4)	0.315
1 kilometer NDVI	4.2	(-22.4, 30.7)	0.759

Table 8. Association between trans,trans-muconic acid and vegetation at different radii among smokers in the Louisville Healthy Heart Study.

Percent change in TTMA per 0.1 increase in NDVI. Adjusted for ethnicity, smoking status (cotinine confirmed), diuretics, household income, total length of roads within 50 meters of residence, residential distance to gas station.

	Hydroxypropylmercapturic acid		
NDVI radius, n=172	Change (%)	95% C.I.	P-Value
0 meter NDVI	-13.5	(-21.2, -5.8)	0.001
50 meter NDVI	-13.5	(-23.1, -3.9)	0.006
100 meter NDVI	-13.5	(-22.5, -4.40)	0.004
200 meter NDVI	-15.0	(-25.5, -4.5)	0.005
300 meter NDVI	-15.0	(-25.3, -4.7)	0.004
1 kilometer NDVI	-12.2	(-23.7, -0.6)	0.038

Table 9. Association between HPMA and vegetation at different radii in the Louisville Healthy Heart Study.

Percent change in hydroxypropylmercapturic acid per 0.1 increase in NDVI. Adjusted for ethnicity, cotinine, diuretics, residential distance to gas station, and particulate matter.

	Hydroxypropylmercapturic acid, non-smokers		
NDVI radius, n=105	Change (%)	95% C.I.	P-Value
0 meter NDVI	-20.1	(-32.2, -8.1)	0.001
50 meter NDVI	-28.1	(-42.8, -13.5)	0.0002
100 meter NDVI	-24.6	(-38.3, -10.8)	0.005
200 meter NDVI	-28.9	(-45.9, -11.9)	0.001
300 meter NDVI	-24.7	(-41.5, -8.0)	0.004
1 kilometer NDVI	-13.4	(-32.6, 5.8)	0.172

Table 10. Association between hydroxypropylmercapturic acid and vegetation among nonsmokers at different radii in the Louisville Healthy Heart Study.

Percent change in HPMA per 0.1 increase in NDVI. Adjusted for ethnicity, cotinine, diuretics, residential distance to gas station, and particulate matter.

	Hydroxypropylmercapturic acid, smokers		
NDVI radius, n=67	Change (%)	95% C.I.	P-Value
0 meter NDVI	-9.2	(-18.7, 0.3)	0.060
50 meter NDVI	-7.5	(-19.2, 4.2)	0.209
100 meter NDVI	-7.5	(-18.8, 3.7)	0.190
200 meter NDVI	-11.6	(-23.4, 0.22)	0.055
300 meter NDVI	-13.8	(-25.6, -2.1)	0.021
1 kilometer NDVI	-11.1	(-24.3, 2.15)	0.101

Table 11. Association between hydroxypropylmercapturic acid and vegetation among smokers at different radii in the Louisville Healthy Heart Study.

Percent change in HPMA per 0.1 increase in NDVI. Adjusted for ethnicity, cotinine, diuretics, residential distance to gas station, and particulate matter.

CHAPTER IV: SPECIFIC AIM 3

Introduction

Circulating angiogenic cells have recently been identified as a potentially important contributor to cardiovascular integrity and a sensitive biomarker of CVD risk.[121] Circulating angiogenic cells are recruited into the blood to aid in vascular healing and regeneration.[100-103] These cells not only repair damaged vasculature, but have been found to be predicative of CVD risk, severity, and mortality.[105-112] Various signaling pathways may trigger the release of these cells into circulation from the bone marrow, which then go on to serve a variety of functions including vascular repair and angiogenesis. [103, 183, 184] However, levels of these cells vary based both on vascular damage as well as chemical insults inhibiting release from the bone marrow.[183, 185] Previous work has shown that these cells may also be a sensitive biomarker of exposure to air pollutants, perhaps reflecting need for repair of ongoing vascular damage from exposure to pollutants.[119, 124]

Vegetation is thought to be one of the few common environmental features with the ability to passively reduce human exposure to previously emitted air pollutants. Given that roadway traffic is a nearly ubiquitous source of air pollution in urban environments, vegetation may represent an important mediator of urban residential pollution exposure. Vegetation has also shown to positively affect other risk factors for CVD including stress, social cohesion, and health behaviors.[19] This confluence of risk factors, all affected by greenness, substantially complicates investigation into mechanistic links between

greenness and CVD. This aim seeks to evaluate the relationship between exposure to greenness and levels of circulating angiogenic cells.

Materials and Methods

Study Population

Participant recruitment and the Louisville Healthy Heart Study are as described in chapter 3. The subpopulation included in this analysis consists of participants in the Louisville Healthy Heart Study who have complete covariate information, quantified circulating angiogenic cells, and who were able to be successfully geocoded. After exclusions, 250 participants met criteria for analysis.

Residential Proximity to Vegetation

Quantification of exposure to vegetation, measured by NDVI, is as described in chapter 3. Percent tree canopy was quantified by determining the average canopy coverage in a 100m buffer from participant residential locations. Canopy coverage data was available through Louisville Metro Government and quantified from satellite imagery by the Davey Resource Group as part of the 2015 Louisville Urban Tree Canopy Assessment.[174]

Covariates

Covariates included in the analysis are as described in chapter 3

Quantification of Circulating Angiogenic Cells

Characterization and measurement of specific circulating angiogenic cell populations was performed using a 7-color flow cytometry procedure. Phenotypic cell surface markers CD31+, CD34+, CD45+/dim, and AC133+ were used to identify and classify a total of 15 endothelial and stem/progenitor cell populations.[119, 186] Surface marker CD34+ identifies stem cells, AC133+ identifies early progenitor cells, CD31+ identifies endothelial cells, and CD45+ identifies hematopoietic cells. Combinations of these markers were used to identify cell sub-populations within these main categories.(Table 1) Circulating angiogenic cells were identified using a modification of a previously described approach.[187] Participant blood samples were collected in a CPT mononuclear separator tube and subsequently separated by centrifugation, within 24h of collection, for 30 min at 1700xg. Serum was collected and centrifuged for 10 min at 400xg to separate mononuclear cells. Cells were twice washed with 2%FBS in PBS and incubated for 10 minutes with FcR blocking reagent and 2% FBS/PBS while on ice and in the dark. The cells were further incubated with fluorescently-conjugated antibodies that include: anti-CD41a (Becton Dickinson) and anti-CD235a (Becton Dickinson), APC-AlexaFluor 750-labeled anti-CD45 (Invitrogen), APC-labeled anti-AC133 (Miltenyi Biotec), PE-Cy5.5-labeled anti-CD14 (Abcam), FITC-labeled anti-CD31 (Becton Dickinson), Pacific Blue (Pacific Blue monoclonal antibody labeling kit; Invitrogen), PE-Cy7-labeled anti-CD16 (Becton Dickinson), PE-labeled anti-CD34 (Becton Dickinson), and a marker for dead cells (LIVE/DEAD fixable dead cell stain; Invitrogen). Cells were then re-suspended in 1% FCS Formaldehyde following pelleting and washing with FBS/PBS.[119]

A LSR II Flow cytometer was then used to collect data on 500,000 events. Unstained controls were used to determine positive and negative gating boundaries. The markers CD235a and CD41a, as well as pacific blue staining to identify dead cells, were used to determine cell lymphocyte populations. From this population, a subpopulation of CD14 and CD16 negative cells were then selected. The final population was then selected by identifying stem cell with the CD34+ marker and endothelial cells with the CD31+ marker. This population was then sub-classified into monocytes and non-monocytes with the CD45+ marker as well as early or mature progenitors with the AC133+ marker. Circulating angiogenic cell counts for each sub-population were normalized to sample volume and analyzed with FloJo software.

Statistical Analysis

Generalized linear models were used to examine the association between the primary outcome variables (cell counts) and the primary exposure variable (NDVI values). Because circulating angiogenic cell (CAC) counts are positive, continuous values and are positively skewed, the Gamma distribution with the log link function was used in the generalized linear model. Unadjusted models were performed on all 15 cell types with NDVI values at multiple radii (0M, 50M, 100M, 200M, 300M, and 1KM) as shown in Table 3. Beta values were converted to % change per 0.1 unit increase in NDVI. Because of the strong unadjusted association with NDVI, CAC-5, CAC-10, and CAC-11 were further analyzed in the fully adjusted models. The independent association of each demographic variable in Tables 2 and 3 were examined individually using generalized linear models with cells 5 (early progenitor/endothelial), 10 (endothelial), and 11 (early

progenitor) as the dependent variables. All independent variables that were significant at the $p<0.05$ level were examined as potential covariates in the fully adjusted model. Potential covariates were excluded from the models in a stepwise fashion until all remaining covariates were associated at the $p<.05$ level. The final models for CAC-5 were adjusted for sex, age, BMI, creatinine levels, and beta-blocker use. The final models for CAC-10 were adjusted for sex and cotinine levels. The final models for CAC-11 were adjusted for BMI, diabetes, myocardial infarction, creatinine levels, and beta-blocker usage.

Results

Geographic Distribution

Participants in the present analysis largely overlapped with participants included in chapter 3; geographic distribution is similar. However, there were slightly fewer participants from the Northwestern area of the county and more in the suburban areas. (Figure 1)

Participant Characteristics and Demographics

Participant characteristics and demographics are similar to those described in chapter 3. Participants examined in the present aim were recruited and enrolled between October 2009 and May 2013. The study cohort included 250 persons, consisting of 51% males, 54% Caucasian, 41% African American, and 6% of another race(s) (Table 2). Much of the study population was hypertensive (69%), hyperlipidemic (61%), and at a high risk

for CVD (59%). The cohort was 50±10 years old and mostly overweight or obese with a mean BMI of 33±8 (Table 3). African American participants were likely to reside in areas with less vegetation than white participants. Participants residing in high green areas were significantly more likely to have hyperlipidemia, an observation different from the participant subgroup in chapter 3. However, no differences were observed with other CVD risk factors, cardiovascular history, medication use, age, sex, BMI, sum of CVD risk factors, or a variety of biomarkers. Participants residing in low vegetation areas were more likely to live in a lower income neighborhood and have a shorter distance to a major roadway.

Unadjusted Associations between Circulating Angiogenic Cells and Vegetation

Unadjusted, significant inverse associations were found between NDVI at address points and circulating angiogenic cells (CAC) 2 (Hematopoietic), 5 (early progenitor), 6 (endothelial), 8 (Hematopoietic), 9 (stem), 10 (endothelial), and 11 (early progenitor) (Table 4). Significant inverse associations were also found between percent tree canopy coverage within a 100m buffer area and cells 5, 9, and 10.

Adjusted Model of Associations between Circulating Angiogenic Cells and Vegetation

The 3 most highly significant cells at the residential level, cells 5, 10, and 11 were selected for assessment of cell levels in relation to vegetation at the residential level in fully adjusted models. Stepwise regression was utilized to identify relevant covariates utilized in final models that are specific to cell type. Cell 5 was adjusted for sex, age,

BMI, creatinine, and beta blocker. Cell 10 was adjusted for sex and cotinine. Cell 11 was adjusted for diabetes, MI, beta blocker, BMI, creatinine. Results of these adjusted models show that each cell type remained highly significant with vegetation at the residential level. (Table 5) A similar percent change was observed between unadjusted and adjusted models.

Discussion

The major finding of this study is that measures of vegetation surrounding homes are statistically associated with some populations of circulating angiogenic cells in an at-risk population. Importantly, vegetation was not significantly associated with hsCRP, a marker of inflammation, suggesting that changes in circulating angiogenic cells observed with nearby vegetation are not being caused by systemic inflammation, which is known to affect cell levels. Another relevant result is that both NDVI and surrounding canopy were significantly associated with levels of some cell types and not others. The effect size of results for nearby percent tree canopy is not as high as with NDVI, suggesting that factors driving these associations are not only associated with trees, but also with other types of vegetation surrounding participant residences.

Significant inverse associations were observed with cell types from three parent classification categories of circulating angiogenic cells: stem cells (CD34+), early progenitor cells(AC133+), and endothelial cells(CD31+). Previous studies have observed that chronic exposures to high levels of pollutants such as $PM_{2.5}$ and tobacco smoke are

associated with decreased levels of circulating angiogenic cells.[188] CVD risk has also been associated with lower levels of circulating angiogenic cells.[189] Previous findings among this study cohort have shown that circulating angiogenic cell levels, particularly cells early in the differentiation process, are positively associated with roadway exposure and may represent a sensitive biomarker of exposure.[124] The results of the present study are consistent with the hypothesis that vegetation may reduce overall pollutant exposure.

These results add to a limited body of evidence on biological mechanisms of decreased CVD risk associated with greenness. The present study utilized 15 antigenically defined subpopulations of cells, potentially yielding greater insights into mechanisms. There was also a relatively large participant population in comparison to previous studies. This investigation contributes to the overall literature about populations already at high risk for CVD. Another important strength of this analysis is the use of individual level health data to account for confounding factors. The use of cotinine to confirm smoking status and to account for smoke exposure intensity in the statistical model is an important strength of the analysis. The analysis included a wide geographic distribution of participants with variable residential exposure to vegetation using address-linked metrics of greenness. These results are directly comparable to many other greenness studies, since NDVI is a common greenness exposure metric.

There are a number of limitations to this analysis. It is a cross sectional analysis limited to tests of association. Another important limitation is the lack of individually linked

socioeconomic covariates, likely leading to a degree of residual confounding in the analysis. Exposure to vegetation outside of the residential location cannot be accounted for. While two metrics of vegetation were utilized in the analysis, these cannot account for the many varied facets of overall greenness that include important characteristics such as total leaf area, species makeup, accessible greenspace, aesthetic quality, or overlapping layers of vegetation.

Significance

Results of this analysis indicate that levels of circulating angiogenic cells, in an at-risk population, are inversely associated with residential vegetation, after adjustment for relevant covariates. These results may indicate that nearby vegetation is affecting cardiovascular risk, reflected by these cells, or that there is another driver of this relationship which has not be accounted for in this analysis. Results of this work may aid in understanding mechanisms of CVD, both in terms of vegetation's influence on risk factors and the role of circulating angiogenic cells therein. Further study is needed to better evaluate these relationships and seek to identify other associated factors.

Figures and Tables

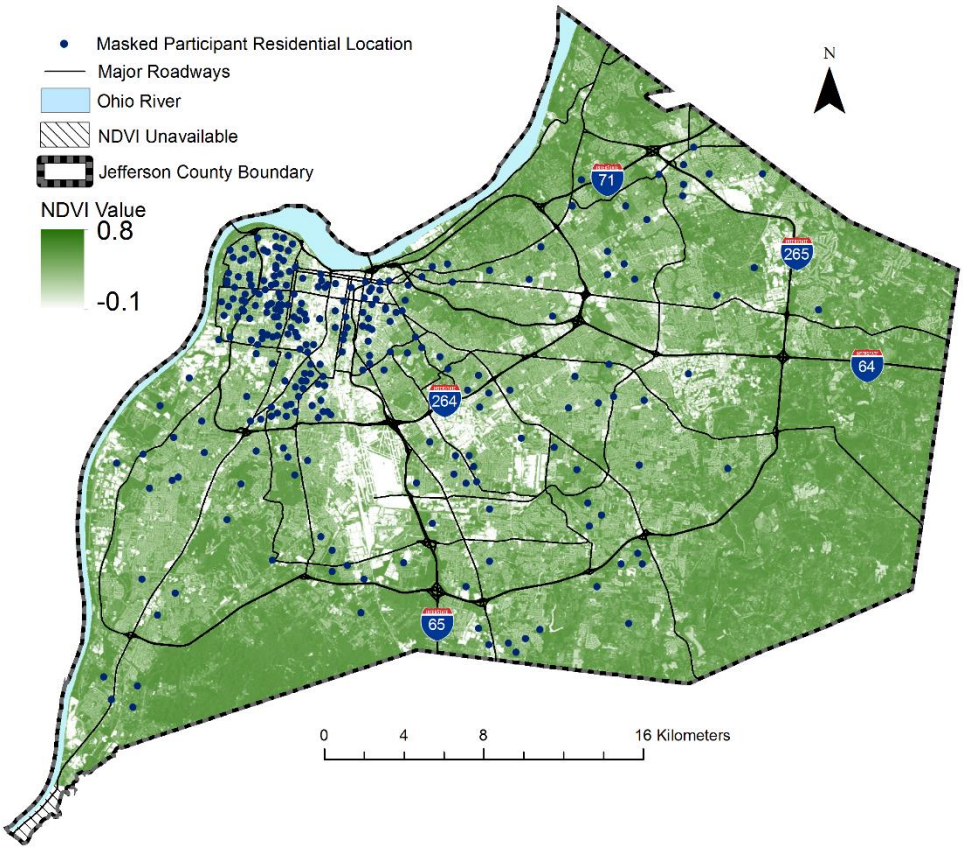


Figure 4. Masked Distribution of Healthy Heart Study Participants Included in Aim 3.

Cell type	Cell population	Antigenic Markers
Stem	CAC-9	CD34+
	CAC-13	CD34+/AC133+
	CAC-15	CD34+/AC133+/CD45dim
Early Progenitor	CAC-11	AC133+
	CAC-5	AC133+/CD31+
	CAC-4	AC133+/CD34+/CD31+/CD45+
	CAC-14	AC133+/CD34+/CD45+
Endothelial	CAC-10	CD31+
	CAC-6	CD31+/CD34+
	CAC-1	CD31+/CD34+/CD45dim
	CAC-7	CD31+/CD34+/AC133-/CD45dim
	CAC-3	CD31+/CD34+/AC133+/CD45dim
Hematopoietic	CAC-12	CD45+
	CAC-2	CD45+/CD34+/CD31+
	CAC-8	CD45+/CD34+/CD31+/AC133-

Table 12. EPC population classification and antigenic markers.

Categorical Variable – n (%)	Total n = 250 (%)	Low Green n=125	High Green n=125	P-Value
Sex				
Male	128 (51%)	63 (50%)	65 (52%)	0.800
Race				
White	134 (54%)	50 (40%)	84 (67%)	<0.0001
Black	102 (41%)	65 (52%)	37 (30%)	
Other	14 (6%)	10 (8%)	4 (3%)	
CVD Risk Factors				
Hypertension	170 (69%)	84 (68%)	86 (69%)	0.785
Hyperlipidemia	150 (61%)	63 (51%)	87 (70%)	0.002
Diabetes	79 (32%)	39 (31%)	40 (32%)	0.892
Current smoker	88 (35%)	43 (35%)	45 (36%)	0.791
High CVD risk category	140 (59%)	68 (57%)	72 (60%)	0.654
Cardiovascular History				
Myocardial Infarction	65 (26%)	32 (26%)	33 (27%)	0.885
Stroke	18 (7%)	8 (6%)	10 (8%)	0.625
CABG/PCI/stents	48 (19%)	24 (19%)	24 (19%)	1.000
Heart Failure	35 (14%)	17 (14%)	18 (15%)	0.876
Medications				
ACE/ARB	136 (55%)	65 (53%)	71 (57%)	0.486
β-Blocker	120 (49%)	59 (48%)	61 (49%)	0.847
Calcium-channel blocker	49 (20%)	28 (23%)	21 (17%)	0.251
Diuretics	93 (38%)	49 (40%)	44 (35%)	0.480
Statins	117 (47%)	51 (41%)	66 (53%)	0.064
Aspirin	121 (49%)	60 (49%)	61 (49%)	0.948

Table 13. Demographics and cardiovascular disease history in the Louisville Healthy Heart Study stratified by high/low residence level vegetation.

Abbreviations: CVD, cardiovascular disease; ACE, angiotensin-converting enzyme inhibitor; ARB, angiotensin-receptor blockers; CABG, coronary artery bypass graft; PCI, percutaneous coronary intervention.

Continuous Variable – mean (SD)	Total n=250	Low Green n=125	High Green n=125	p-value
Age (years)	49.74 (10.22)	49.90 (10.34)	49.58 (10.15)	0.805
BMI	33.29 (8.34)	33.89 (9.54)	32.71 (7.00)	0.270
Systolic Blood Pressure (mmHg)	131.13 (20.88)	133.1 (21.84)	129.2 (19.77)	0.138
Diastolic Blood Pressure (mmHg)	80.60 (11.90)	81.59 (12.51)	79.62 (11.23)	0.194
Cotinine (μg/g creatinine)	558.53 (1208.87)	603.6 (1253.1)	514.6 (1167.8)	0.574
3-HPMA (μg/g creatinine)[#]	379.62 (523.17)	407.1 (651.9)	354.2 (368.6)	0.538
trans,trans-Muconic acid (mg/g creatinine)[#]	0.20 (0.32)	0.19 (0.26)	0.22 (0.38)	0.537
Creatinine (mg/dL)	139.18 (96.01)	146.1 (104.7)	132.4 (86.50)	0.272
Lipid Levels (md/dL)				
Cholesterol	193.15 (53.92)	190.0 (48.26)	196.0 (58.67)	0.454
HDL	45.53 (12.97)	46.92 (14.41)	44.28 (11.45)	0.179
LDL	106.00 (39.50)	103.7 (40.15)	108.1 (39.01)	0.465
Triglycerides	176.52 (217.65)	161.9 (134.8)	189.7 (271.8)	0.380
Thrombosis				
Fibrinogen (mg/dL)[#]	350.40 (115.01)	346.2 (114.0)	354.4 (116.5)	0.643
Platelet-leukocyte aggregates[#]	10.41 (5.84)	10.56 (6.44)	10.28 (5.29)	0.798
Inflammation				
hsCRP (mg/L)	4.59 (4.67)	4.46 (4.75)	4.72 (4.59)	0.671
CVD risk				
Sum of CVD risk factors	3.31 (1.39)	3.22 (1.46)	3.40 (1.33)	0.334
Median Household Income X 10^3 ††	32.61 (18.37)	27.70 (16.39)	38.00 (18.98)	<0.0001
Distance to Major Roadway	272.39 (340.71)	189.8 (205.9)	362.2 (426.3)	<0.0001

Table 14. Demographics and cardiovascular disease history in the Louisville Healthy Heart Study stratified by high/low residence level vegetation.

Abbreviations: 3-HPMA, 3-Hydroxypropylmercapturic acid; BMI, body mass index (weight(kg)/height(m)^2); hsCRP, high sensitivity C-reactive protein; $PM_{2.5}$, particulate matter with an aerodynamic diameter < 2.5 μm; SD, standard deviation. #Anthem only.

	% Change	
CAC, n=250	NDVI	100m %Canopy
CAC-1 ($CD31^{+}/34^{+}/45^{dim}$)	-6.39	-0.77
CAC-2 ($CD31^{+}/34^{+}/45^{+}$)	-15.00*	-0.98
CAC-3 ($CD31^{+}/34^{+}/45^{dim}/AC133^{+}$)	-7.21	-0.91
CAC-4 ($CD31^{+}/34^{+}/45^{+}/AC133^{+}$)	-15.05	-0.38
CAC-5 ($CD31^{+}/AC133^{+}$)	-13.72**	0.24
CAC-6 ($CD31^{+}/34^{+}$)	-10.19*	-1.58**
CAC-7 ($CD31^{+}/34^{+}/45^{dim}/AC133^{-}$)	-5.12	-0.57
CAC-8 ($CD31^{+}/34^{+}/45^{+}/AC133^{-}$)	-15.00*	-1.07
CAC-9 ($CD34^{+}$)	-9.51*	-1.48**
CAC-10 ($CD31^{+}$)	-11.88**	-1.46**
CAC-11 ($AC133^{+}$)	-10.83*	0.08
CAC-12 ($CD45^{+}$)	-7.6	-1.06
CAC-13 ($CD34^{+}/AC133^{+}$)	-6.49	-0.74
CAC-14 ($CD34^{+}/45^{+}/AC133^{+}$)	-10.16	-1.4
CAC-15 ($CD34^{+}/45^{dim}/AC133^{+}$)	-8.04	-0.83

Table 15. Unadjusted association between circulating angiogenic cell levels and residential vegetation in the Louisville Healthy Heart Study.

*p-Value <0.05, **p-value <0.01. Represents % Change per .1 NDVI increase, or 1% increase in tree coverage.

CAC	Change (%)	95% C.I.	P-Value
CAC-5 (CD31+/AC133+)	-14.86	(-23.18, -6.54)	0.0005
CAC-10 (CD31+)	-10.07	(-16.70, -3.43)	0.003
CAC-11 (AC133+)	-14.73	(-23.54, -5.93)	0.001

Table 16. Association between circulating angiogenic cells and residential vegetation in fully-adjusted models.

Percent change in cell population per 0.1 increase in NDVI. Cell 5 Adjusted for sex, age, BMI, creatinine, and beta blocker. Cell 10 adjusted for sex and cotinine. Cell 11 adjusted for diabetes, MI, beta blocker, BMI, creatinine.

CHAPTER V: OVERALL DISCUSSION

Highlights

The preceding chapters describe means by which vegetation may be associated with cardiovascular risk. All analyses performed assess these relationships in Louisville, Kentucky, which is similar to many mid-sized U.S. cities. In the second chapter, CVD hospitalization rate at the zip code level was associated with zip code level vegetation. Associations by specific CVD admission types as well as length of hospital stay are described. Few previous investigations into these relationships have been performed. The third chapter reports associations between residential vegetation and metabolites of benzene and acrolein among participants in the Louisville Healthy Heart Study. These results may contribute relevant perspective regarding the potential link between vegetation and exposure to ambient air pollution, a key hypothesized mechanism of relationships between greenness and CVD. The fourth chapter describes associations between circulating angiogenic cell levels and residence-level vegetation. The three most highly associated cells were further assessed with fully adjusted regression models. Results of this analysis may indicate that nearby vegetation affects cardiovascular risk, reflected through associated levels of these cells. These investigations reveal some of the many potential associations between environmental vegetation and human health, which may also contribute to cardiovascular risk. Further study is needed to better understand these relationships.

Strengths

Results of these chapters contribute to a limited body of evidence concerning relationships between vegetation, CVD, and overall health. The use of NDVI in these analyses provides an objective estimate of overall residential and surrounding greenness. The use of NDVI to assess greenness also allows cross comparison of greenness to other studies in the field, many of which also utilize NDVI. An important strength of aim 1 is the ability to assess rate of all CVD admissions in the study area, as opposed to a subset of admissions that is not necessarily representative of overall demographic and geographic distribution. The availability of yearly vegetation and admissions data in aim 1 enables accounting for yearly changes in both of these metrics in a repeated measures statistical model, reducing the likelihood that these relationships may be driven or confounded by changes over time. An important strength of aims 2 and 3 is the availability of participant address, allowing use of NDVI for assessment of residential level vegetation at high spatial resolution. Another important strength of aims 2 and 3 is the availability of detailed health covariate data including CVD risk factors, CVD history, medication use, and biomarker data, including markers of smoking and inflammation. The at-risk patient populations assessed in these analyses includes a range of individuals at high risk of CVD morbidity and mortality. Analysis of these relationships in Louisville, Kentucky allows assessment of vegetation in a wide range of urban environments that include residential, industrial, and open spaces. Louisville is also similar in demographic and environmental makeup to many other cities across the U.S., potentially enabling limited extrapolation of these findings to similar urban areas.

Limitations

Each aim has substantial limitations, an important consideration for interpretation of results. Aim 1 examines associations between greenness and CVD hospital admissions at the zip code level. An important limitation of this aim is the inability to assess relationships between greenness and hospital admissions at a fine geographic resolution. The wide variation in demographics, greenness, built environment, and various risk factors at this scale cannot be accounted for, potentially leading to residual confounding in the analysis. Another important limitation of aim 1 is the inability to account for many individual level covariates, including residential address, socioeconomic factors, and date of admission. Limitations of the Louisville Healthy Heart Study affect both aims 2 and 3. This includes the lack of individual level covariates of socioeconomic status, which were instead assessed from the block group statistics of participant residences. Another important limitation is that this is a generally low socioeconomic status cohort already at high CVD risk, so results cannot be extrapolated to the overall population.

Aim 2 describes associations between greenness and metabolites of VOCs among participants in the Louisville Healthy Heart Study. Levels of urinary VOC metabolites may be indicative of exposure to parent compounds in the environment. However, other sources of exposure to acrolein and benzene, besides exposure to ambient pollution, limit the usefulness of urinary concentrations to assess exposure to air pollution. This analysis does not include monitoring data to validate these measurements as a metric of exposure from air pollution.

Aim 3 examines associations between residential level vegetation and circulating angiogenic cells among participants in the Louisville Healthy Heart Study. Other investigations have associated levels of these cells with cardiovascular health, risk, and exposure to harmful pollutants. However, links between vegetation and these factors cannot be extrapolated through the present analysis. This cohort was already largely affected by, or at high risk for CVD, potentially affecting the response of circulating angiogenic cells to various influences.

All aims cannot account for pollution exposure both inside the residential environment and during time spent away from the home, including occupational exposures. The analyses were unable to account for socioeconomic covariates, which potentially influences many risk factors affecting both residential vegetation and CVD risk. All aims are also subject to several limitations of NDVI as a metric of greenness. NDVI cannot account for multiple layers or vertical thickness of vegetation. Due to the 16 day satellite pass time of sensors utilized to collect NDVI data in the present analysis and cloud cover, there are limitations to the temporal availability of imagery. While summer greenness generally plateaus, identification and assessment of NDVI on the precise day of peak greenness or seasonal averaging is not possible from the varied temporal availability of NDVI. Importantly, analyses described in all aims are crossectional and limited to tests of association. Additional investigation is needed to further explore these associations and utilize study designs suitable for establishment of causation.

Future Studies

Future investigation may improve upon the current analyses in a number of ways. A key limitation of all aims is the inability to assess exposure of participants to air pollutants both inside and outside the residential environment. To overcome this limitation, indoor air monitoring of pollutant concentrations would provide detailed information about indoor air pollution exposure affected by a variety of factors, including building materials, household cleaners, cooking, window opening, filtration use, and infiltration of outdoor pollutants. Participant questionnaires may be used to strengthen this assessment or to account for these factors at a lower cost than air monitoring. Questionnaires are also a potential way to assess exposure outside the home and may include address of occupation, type of occupation, total time spent outside the home, time spent driving, and other frequently visited locations in order to account for other potentially important exposures outside of the home. Personal air monitoring, location logs, and GPS location tracking also represent potential ways to account for both exposures and location outside of participant residential location.

Individual level socioeconomic considerations are an important consideration in human studies that are not assessed in the present aims. Socioeconomic influences may affect and confound investigations into cardiovascular risk. While there is a considerable association between small area population level socioeconomic status and most individuals living in that area, assessment of individual level socioeconomic status would likely strengthen future analyses into the relationships between greenness and

cardiovascular health. These socioeconomic factors are often assessed with participant questionnaires at the time of enrollment.

While limitations exist, NDVI is a very useful, widely available, and objective measure of vegetation. An important facet of greenness that cannot be fully accounted for through NDVI is perception of environmental vegetation by nearby residents. To assess this perceived measure, participant questionnaires, interviews, or focus groups may be utilized. These qualitative assessments may provide useful measures of perceptions of environmental vegetation in terms of quality, stress, safety, and potential effects on health behavior – none of which can be assessed though NDVI or other measures of greenness. The inability of NDVI to assess multiple layers or thickness of vegetation could be mitigated in part using either mobile laser radar or human-conducted assessments. Satellite-derived and ground gathered vegetation data may also be combined to calculate a leaf area index score, which may be useful for calculating overall leaf surface area and potential for pollutant capture. Future analyses may incorporate other metrics of greenness into an overall greenness index to conduct a more comprehensive assessment.

To address limitations of Aim 1, data from existing longitudinal studies may be useful. Retrospective data from these studies could be employed in concert with historical NDVI data to assess participant residential and surrounding level greenness in relation to detailed CVD outcomes already being assessed though existing longitudinal study designs. Through this approach, natural changes in vegetation, or change in residential

location, may be tested for associations with differences in NDVI, CVD risk factors, and outcomes. Investigations within existing longitudinal cohort studies may have added benefits of substantial information regarding individual level covariates of socioeconomic status, health indicators, and biomarkers. Certain longitudinal studies, or studies with common outcome metrics, with participants in varied geographic areas may potentially be utilized to assess differences in response to vegetation over time between diverse environments.

A prospective longitudinal cohort study could be tailored provide a range of useful evidence in assessing relationships between greenness and CVD. At baseline, a wide variety of metrics of greenness and associated factors would be collected. Greenness may be assessed through multiple variables that include NDVI, leaf area index, distance to a park, and perceptions of greenness among participants. Participant exposure to air pollutants could be assessed both at baseline and follow-up. Other important hypothesized mechanisms including stress, health behaviors, and social cohesion may also be assessed at baseline and follow-up though a mixture of objective measurements and questionnaires. Many biomarkers of exposure and CVD may be collected to assess pollutant exposure, CVD risk, and outcomes. These baseline and follow-up measurements would then be compared with changes in greenness around participant residence and other locations and could be used to assess changes in residential, occupational, and other common locations.

Other investigations may be performed in a natural experiment scenario. There are examples in which weather, development, or invasive insect species have substantially altered greenness at varying geographic scales. In the case of development or invasive insect species, prospective studies could be designed and implemented before expected changes in greenness take place in order to assess effects of changes on the surrounding built environment and inhabitants. These natural experiments may also be assessed retrospectively using historical NDVI or other greenness data among existing study cohorts and at the population level.

The influence of greenness on many factors, especially air pollution, could be assessed with high geographic resolution in intervention studies. Vegetation could potentially be planted within neighborhoods, in participant residential locations, near schools, or along roadways. These interventions could be utilized to assess changes that result from interventions in terms of pollutant concentration, participant exposure, behavior, stress, and other hypothesized influences of vegetation on human health. Results and other lessons learned from these investigations may be utilized for design of large scale intervention studies.

To examine the effects of large scale vegetation-based public health interventions, a neighborhood scale vegetation experimental intervention could be staged. Similar in design to a prospective longitudinal study, this study design would incorporate aspects of a case control study to test the effects of an intervention area in comparison with a control

area. This design could potentially implement greening strategies in a single neighborhood area, or several smaller areas, and compare this intervention area and inhabitants with that of a control area. To do this, measurements of study outcome variables, possibly including air pollution, health behaviors, and biomarkers of health, would first need to be examined. Assessing baseline attributes of the built environment potentially affected be greenness interventions would also need to be performed, as resulting environmental changes may also influence health outcomes. Interventions may be comprehensive or focused on assessment of specific key hypothesized mechanisms in the influence of vegetation on health. Participant and environmental follow-up would be performed at regular intervals to assess changes in study outcome variables in relation to changing greenness. This design also has the potential to be leveraged with expected natural experiments. An intervention area could be shielded from an expected decrease in greenness, such as pesticide treatment in advance of expected tree loss from invasive insects, and then compared with an area with no intervention. The potential difference in greenness between intervention and control areas observed over time could be maximized by combining vegetation planting in the intervention area with protection from tree loss.

Controlled experiments could be performed to assess the role vegetation on air pollution by passing polluted air through vegetated areas and monitoring changes in pollutant concentrations. Attributes of plant health, species, and spatial arrangement on pollutant concentration could be assessed and compared in similar experiments. This approach could also be utilized to assess the role of vegetation on specific air pollutants, pollutant classifications, or concentrations. These experiments might then be expanded upon in

existing animal exposure models to assess the role of air pollution on cardiovascular health, risk, and specific biomarkers in a controlled setting.

In order to thoroughly estimate effects of the potential use of vegetation as a public health intervention, potential detriments of vegetation to the environment and health must also be assessed. There is little reported data on detrimental effects of vegetation in the built environment. Vegetation may lead to an increase in rates of allergies, accidents, VOCs, and secondary formation of air pollutants from VOC release. These are important ethical considerations for greenness interventions and exposure among humans, for which little data exists to consider. Future investigation could assess these outcomes and identify potential risk imparted by specific plant species, which may be compared with expected benefits.

Conclusion

These chapters describe associations between exposure to greenness and cardiovascular risk. The first chapter examines existing evidence linking cardiovascular health with greenness, and reviews potential mechanisms of these relationships. The second chapter identifies associations between neighborhood level greenness and CVD hospital admissions in Jefferson County, Kentucky. The third and fourth chapters utilize the existing Louisville Healthy Heart Study to evaluate links between human biomarkers and greenness. The third chapter describes the association between greenness and metabolites of benzene and acrolein. The fourth chapter describes associations between greenness and

populations of circulating angiogenic cells. The fifth and final chapter summarizes this work and discusses ways in which relationships between greenness and CVD risk could be further explored.

In the second chapter, existing hospital admissions records from 2005 through 2014 were assessed in relation to NDVI, calculated from historical satellite imagery for the same years. Neighborhood level covariates including socioeconomic status, age, ethnicity, and built environment were factored into this analysis. A repeated measures modeling approach was utilized to incorporate multiple observations of greenness and admissions over time as well as covariates to assess relationships between greenness and CVD admissions. This analysis found statistically significant inverse associations between greenness, all CVD admission rate, emergency CVD admission rate, stroke admission rate, CHD admission rate, and hospital length of stay from admissions. This analysis expands on previous work by examining specific types of admissions as well as length of stay. Results are consistent with limited previous ecological studies reporting associations between residential vegetation as well as green spaces and CVD.[18, 21, 35]

The third chapter describes associations between residential level greenness and levels of metabolites of both acrolein and benzene in the Louisville Healthy Heart Study. Individual level covariates of age, gender, ethnicity, cardiovascular risk, and medication use were factored into this analysis. Attributes of the localized built environment including roadway usage, residential land, and other potential sources of VOC exposure

were calculated and included. A multiple linear regression modeling approach was utilized to assess the relationship between greenness and VOC metabolites, while accounting for relevant covariates. Statistically significant inverse associations between VOC metabolites and greenness at participant residence as well as surrounding buffer areas was found. This analysis expands on previous studies showing associations between environmental vegetation and air pollution exposure by analyzing VOC metabolites in the urine of human study participants. These results are consistent with previous studies showing associations between vegetation and reductions in air pollutant concentration.[30-33, 137, 139, 140, 148-150]

In the Fourth Chapter, residential level greenness was associated with circulating angiogenic cell populations in the Louisville Healthy Heart Study. Individual level covariates including age, gender, ethnicity, and medication use were considered in this analysis. Participant location was also used to examine environmental covariates potentially affecting relationships between greenness and CVD risk. Generalized linear modeling was employed to examine relationships between residential greenness and circulating angiogenic cells while accounting for relevant covariates. This analysis found statistically significant inverse associates between residential level greenness and some populations of circulating angiogenic cells. This analysis provides insights into associations between the environment, particularly vegetation, and circulating angiogenic cells. Little information exists to directly compare these results with previous findings.

These chapters describe potentially important ways in which urban greenness may be linked to cardiovascular risk. There are numerous strengths of these analyses including objective and high spatial resolution metric of greenness, incorporation of many relevant covariates, and the assessment of multiple outcomes related to cardiovascular risk. Limitations include a lack of complete covariate information and longitudinal data. As crossectional data and methods were utilized, these analyses are limited to tests of association. Further investigation is needed to better understand these relationships.

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pressure in 10-year old children: results from the GINIplus and LISAplus studies. *BMC public health*. 2014;14:477.

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CURRICULUM VITAE

Ray Yeager
University of Louisville, School of Medicine

Email: Rayeag01@Louisville.edu
Phone: (502)681-6633
Address: 580 South Preston Street
Delia Baxter Building, Rm. 427
Louisville, KY 40202

Education

Doctorate of Philosophy in Public Health Sciences – Environmental Health, May 2016
University of Louisville – Louisville Kentucky
Dissertation: *Environmental Vegetation and Cardiovascular Disease Risk*

Master of Public Health, Environmental and Occupational Health Sciences, May 2010
University of Louisville - Louisville, Kentucky
Practicum: *A Geographic Analysis of Access to Healthy Nutrition and Recreation*

Bachelor of Science, Geography and Geosciences, May 2008
University of Louisville - Louisville, Kentucky
Thesis: *A Geographic Analysis of Bone Cancers in Fluoridated Areas*

Experience

Graduate Research Assistant, 2015 - 2016
University of Louisville School of Medicine, Division of Cardiology

- Served as a research assistant in the University of Louisville Diabetes and Obesity Center
- Developed manuscripts for scientific publication
- Assisted in grant writing and development
- Presented relevant research materials and preliminary analyses to research groups
- Analyzed geographic metrics of human subject biomarker data

Graduate Research Assistant, 2013 - 2015
University of Louisville School of Public Health and Information Sciences, Department of Environmental Health

- Served as a research assistant in the University of Louisville Diabetes and Obesity Center
- Assisted in teaching a variety of Public Health Courses
- Presented relevant research materials and preliminary analyses to research groups
- Analyzed geographic metrics of human subject biomarker data

Research Data Specialist, 2011 – 2013 (Part-Time)
University of Louisville School of Medicine, Division of Cardiology

- Analyzed human subject biomarker data for indicators of clustering and other geographic trends
- Identified, procured, and cleaned data relevant to exposure estimation
- Measured and quantified geographic variables for analyses in ongoing environmental cardiology research
- Reviewed literature to identify and improve current best practice techniques of exposure assessment
- Developed geographic masking techniques to enable display of HIPAA protected study subject distribution

Environmental Health Geographer, 2012 – 2013
Tetra Tech, Inc.

- Created maps and figures for reports, display, and analysis in technical and public outreach documents
- Performed a variety of environmental sampling in air, ground, and water medias
- Wrote site preparation documents, including health and safety plans
- Assisted with GIS, Public Health, and Geographic aspects of proposal writing
- Managed sub-contractor operations during various field sampling activities
- Served as a member of the EPA's Superfund Technical Assessment and Response Team (START), responding to environmental emergencies throughout EPA region four

Independent Health Geography Consultant, 2010 – 2012
University of Louisville Research Foundation

- Provided display tools for the evaluation of a $7.9m ARRA grant targeted at reducing childhood obesity
- Critically evaluated health data to determine suitability as a measure of intervention effectiveness
- Aided evaluation researchers in identifying, gathering, cleaning, and reporting necessary data
- Utilized GIS to determine areas and populations positively impacted by grant funded interventions

REACH of Louisville

- Performed epidemiological services using neighborhood level data to characterize health inequities
- Created a series of maps for clients to display risk indicators in Kentucky
- Assisted in performing rigorous data cleaning of geo-databases created from state health records
- Gathered and synthesized various data necessary for geographic display and analysis

University of Louisville School of Nursing
- Created maps displaying issues of access and transportation to specified at-risk populations
- Analyzed crime incidence data relevant to specified at-risk populations

Louisville Metro Department of Public Health and Wellness
- Worked with various departments within the agency to provide geographic products for specific needs
- Utilized vital statistics and BRFSS survey data to identify disparities in disease rates across Louisville
- Created maps displaying health and program indicators for communication to stakeholders and public
- Worked to train staff on geographic analysis and display practices through the use of GIS
- Assisted with policy planning by providing display tools and making recommendations based on geographic research

Center for Health Equity Intern, 2009 – 2010
Louisville Metro Department of Public Health and Wellness
- Analyzed community access to nutrition and recreation through cartographic modeling using GIS
- Assisted in policy development and geographically targeting interventions to address childhood obesity
- Collaborated with outside agencies in preparing and analyzing data
- Aided various city organizations and agencies with evidence based geographic research for grant writing

Mapping Technician, 2007 – 2008 (Part-Time)
The Jefferson County Property Valuation Administrator's Office
- Performed geographic research relating to wide variety of analytical projects
- Prepared presentations on geographic research methods and results for professional conferences
- Utilized numerous data sources through GIS to create customized maps for a variety intra-office uses
- Presented information and materials to peers and the public about organizational uses of geographic information

Achievements and Professional Development

Recognitions

- Winner, Research!Louisville, Public Health Doctoral Student Award, 2014
- Third Place - Student Achievement Poster Award, American Public Health Association Annual Meeting, 2014
- Winner, Research!Louisville Best Poster in Research & Practice in Public Health, 2013
- Featured Speaker – Louisville Sustainability Forum, 2011
- Second Place – Kentucky GIS conference, Map Gallery Competition, 2011
- Second Place – ESRI Social Media Challenge, 2010
- Contributor – *The State of Food*, 2010
  City of Louisville, Food in Neighborhoods Committee Report
- Winner, PVA Certificate of Service, 2008

Presentations at Professional and Academic Conferences

- Poster Presentation at Research!Louisville, 2015
  Residential Exposure to Vegetation Correlated with Hospital CVD Admission Rates
- Poster presentation at the Annual Meeting of the American Public Health Association, 2015
  Geographic Disparities of Pediatric Obesity Clinic
- Poster Presentation at the Kentucky GIS Conference, 2014
  Levels of Early Circulating Angiogenic Cells Associated with Geographic Metrics of Roadway Exposure
- Poster Presentation at Research!Louisville, 2014
  Geographic Disparities of Pediatric Obesity Clinic Attendance
- Poster presentation at the Annual Meeting of the American Public Health Association, 2014

Levels of Early Circulating Angiogenic Cells Associated with Geographic Metrics of Roadway Exposure

- Poster Presentation at the Annual Meeting of Ohio Valley Society of Toxicology, 2013
  Levels of Early Circulating Angiogenic Cells Associated with Geographic Metrics of Roadway Exposure

- Poster Presentation at Research!Louisville, 2013
  Levels of Early Circulating Angiogenic Cells Associated with Geographic Metrics of Roadway Exposure

- Oral Presentation at the Kentucky GIS conference, 2012
  Assessing Markers of Pollution from Exposure to Vehicle-Based Air Pollution

- Poster Presentation at the Annual Meeting of the Association of American Geographers, 2010
  A Geographic Analysis of Access to Healthy Foods

- Poster presentation at the Annual Conference of the Kentucky Public Health Association, 2010
  A Geographic Analysis of Access to Healthy Foods

- Poster presentation at the Annual Meeting of the American Public Health Association, 2009
  A Geographic Analysis of Bone Cancers in Fluoridated areas

- Poster presentation at the Annual Meeting of the Association of American Geographers, 2008
  Using Interpolation for Valuation of Vacant Residential Land

<u>Training</u>

Laboratory Research Courses

- *NIH Recombinant DNA*
- *Blood borne Pathogens*
- *HIPAA Privacy, Privacy and Research Fundamentals, Security Fundamentals*
- *CITI Human Subjects Biomedical Training*

OSHA 40-hour Hazwoper Training, 2012

FEMA Emergency Management Institute Courses, 2012

- *Introduction to Incident Command System (ICS-100)*
- *ICS for Single Resources and Initial Action Incident (ICS-200)*

- *National Incident Management System (NIMS) An Introduction (IS-00700.a)*
- *National Response Framework, An Introduction (IS-00800.b)*
- *Emergency Management Preparedness Fundamentals* (IS-00910)
- *Applications of GIS for Emergency Management* (IS-00922)

CASPER Emergency Response Survey Training. November 2010

Cooper/Clayton Smoking Cessation Facilitator Training, April 2009

Publications

Factors Associated with Attendance after Referral to a Pediatric Weight Management Program.

Laura A. Shaffer; Kyle B. Brothers; Thomas A. Burkhead; Ray Yeager; John A. Myers; Brooke Sweeney. The Journal of Pediatrics. 2 March 2016.

Residential Proximity to Major Roadways Is Associated With Increased Levels of AC133+ Circulating Angiogenic Cells.

Natasha DeJarnett, Ray Yeager, Daniel J. Conklin, Jongmin Lee, Timothy E. O'Toole, James McCracken, Wes Abplanalp, Sanjay Srivastava, Daniel W. Riggs, Ihab Hamzeh, Stephen Wagner, Atul Chugh, Andrew DeFilippis, Tiffany Ciszewski, Brad Wyatt, Carrie Becher, Deirdre Higdon, Kenneth S. Ramos, David J. Tollerud, John A. Myers, Shesh N. Rai, Jasmit Shah, Nagma Zafar, Sathya S. Krishnasamy, Sumanth D. Prabhu, Aruni Bhatnagar. Arteriosclerosis, Thrombosis, and Vascular Biology. 20 August 2015.

Acrolein Exposure Is Associated With Increased Cardiovascular Disease Risk.

Natasha DeJarnett; Daniel J. Conklin; Daniel W. Riggs; John A. Myers; Timothy E. O'Toole; Ihab Hamzeh; Stephen Wagner; Atul Chugh; Kenneth S. Ramos; Sanjay Srivastava; Deirdre Higdon; David J. Tollerud; Andrew DeFilippis; Carrie Becher; Brad Wyatt; James McCracken; Wes Abplanalp; Shesh N. Rai; Tiffany Ciszewski; Zhengzhi Xie; Ray Yeager; Sumanth D. Prabhu; Aruni Bhatnagar. Journal of the American Heart Association. 6 August 2014.

Manuscripts in Development

Residential Greenness Associated with Cardiovascular Injury and Exposure to Volatile Organic Compounds.

Ray Yeager, Daniel Riggs, Gilbert Liu, Jeffrey Wilson, Daniel Riggs, Ray Yeager, Natasha DeJarnett, Rachel Keith, Daniel Conklin, Sanjay Srivastava, Timothy

O'toole. Ihab Hamzeh, Stephen Wagner, Atul Chugh, Andrew Defilippis, Carrie Becher, Deidre Higdon, Shesh Rai, Sumanth Prabhu, Aruni Bhatnagar

Neighborhood Greenness Associated with Cardiovascular Disease Hospital Admissions.

Ray Yeager, Daniel Riggs, Gilbert Liu, Jeffrey Wilson, Aruni Bhatnagar

Tobacco Exposures, Glycemic Control, and insulin resistance in Hispanic Community Health Study/Study of Latinos (HCHS/SOL).

Ray Yeager, Daniel Riggs, Rachel Keith, Shesh Rai, Aruni Bhatnagar

Residential Greenspace is Associated with Arterial Stiffness and QT interval.

Daniel W. Riggs, Ray Yeager, Alex P. Carll, Affan Irfan, Natasha DeJarnett, Rachel J. Keith, Daniel J. Conklin, Sanjay Srivastava, Shesh N. Rai, Sumanth D. Prabhu, and Aruni Bhatnagar.

Associations between Ozone and Arterial Stiffness, SEVR, and Central Hemodynamics in the Louisville Healthy Heart Study.

Daniel Riggs, Ray Yeager, Natasha DeJarnett, Rachel Keith, Daniel Conklin, Sanjay Srivastava, Timothy O'toole. Ihab Hamzeh, Stephen Wagner, Atul Chugh, Andrew Defilippis, Carrie Becher, Deidre Higdon, Shesh Rai, Sumanth Prabhu, Aruni Bhatnagar

Endothelial Dysfunction and Inflammation Associated with Particulate Matter.

Daniel W. Riggs, Nagma Zafar, Ray Yeager, James McCracken, Wesley Abplanalp, Daniel J. Conklin, Timothy E. O'Toole, Sathya S. Krishnasamy, Shesh N. Rai, and Aruni Bhatnagar.

Association between Benzene Exposure, Circulating Angiogenic Cell Levels, and Cardiovascular Disease Risk in the Louisville Healthy Heart Study.

Natasha DeJarnett, Wesley Abplanalp, Daniel Conklin, Daniel Riggs, Timothy O'Toole, James McCracken, Petra Haberzettl, Ray Yeager, Sanjay Srivastava, Ihab Hamzeh, Stephen Wagner, Atul Chugh, Andrew DeFilippis, Tiffany Ciszewski, Brad Wyatt, Carrie Becher, Deidre Higdon, Zhengzhi Xie, Kenneth Ramos, David Tollerud, John Myers, Shesh Rai, Sumanth Prabhu, Aruni Bhatnagar