Smoking and CVD in the Multi-Ethnic Study of Atherosclerosis: Functional Form, Mediating Pathways, and Implications of Intensity Reduction

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Abstract

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Smoking as an epidemiological exposure can be quantified in terms of duration, intensity, pack-years, recency, and time of life. It is not clear which of these are important for cardiovascular disease, and how they should be modeled. Using the Multi-Ethnic Study of Atherosclerosis, generalized additive models for time to incident CVD were used to investigate the functional form of each aspect of smoking. Cox models were compared including various combinations of smoking intensity, duration, pack-years, and compound smoking indices. Duration was not associated with CVD, while current intensity was. Former smokers, regardless of duration, intensity or recency, were not at increased risk. We looked for mediating factors for current smoking and found modest mediation through coronary artery calcium, IL-6, tumor necrosis factor alpha, and fibrinogen. Unlike mortality outcomes, the association of smoking with incident CVD events was well captured by including merely a term for current smoking intensity.

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1 Background

1.1 Modeling Smoking as an Exposure

Smoking increases cardiovascular disease (CVD) risk, and it is of interest to know which aspects of smoking are associated with CVD outcomes. Even if a study is not investigating the smoking as an exposure directly, smoking is so strongly related to CVD that it is important to adjust for this exposure in the data analysis. However, there are many different ways to quantify smoking, including whether the person is currently smoking, how long the person has smoked, what type of tobacco product is used, how much the person smokes each day, and the period of life during which the tobacco exposure occurred. Due to this variety of ways to measure exposure to smoking, how smoking should be modeled is not clear. That is, what aspects of smoking are associated with CVD outcomes, and how should these aspects be used to best model smoking?

Never/Former/Current

One of the most basic ways to model smoking is by dividing subjects into never, former, and current smoker categories. In the prospective cohort Atherosclerosis Risk in Communities Study (ARIC), which included both white and African American subjects, 14,200 people were asked about their smoking and followed for up to 20 years for cardiovascular disease outcomes (myocardial infarction (MI), coronary heart disease (CHD) death, coronary revascularization, stroke) (1). Stratified by race and sex, former smokers were not at significantly increased risk of CVD compared to never smokers except in the white female group (1). The hazard ratios (HR) for current smokers were all larger than the former smoker HR, were significantly greater than 1, and ranged from 1.67 to 2.69 (1). In the Tehran Lipid Glucose Study (TLGS), which studied

3059 Iranian men for 9 years, compared to never smokers former smokers had a statistically significantly greater than 1 HR for CVD, but did not for CVD death or CHD (2). Current smoking is a clear risk factor for CVD, but whether former smoking status is a significant risk factor differs by population and outcome, and estimates for current smoker HRs vary, so efforts have been made to refine the definition of smoking as an epidemiological exposure.

Intensity

One way that heterogeneity can enter into smoking status categories is intensity, otherwise known as cigarettes/day or packs/day. Multiple studies have tackled the question of how CVD event risk changes depending on smoking intensity. In the ARIC study, comparing low intensity smokers, which were defined as those who smoked 1-14 cigarettes/day, to never smokers, Huxley *et al* found that the HR for CVD was 1.8 (95% CI 1.3, 2.6) (1). This is evidence that even low intensity smoking is a risk factor for CVD. When they looked at higher intensity smokers compared to never smokers the HR increased at 15-24 cigarettes/day (HR 2.7 (95% CI 1.9, 3.9), but then did not further increase at even higher intensity categories (HRs 2.7) (1).

In the TLGS study, for the CVD outcome and compared to never smokers, the HR for 1-9 cigarettes/day was 2.12, the HR for 10-19 cigarettes/day was 4.65, and the HR for greater than 20 cigarettes/day was 6.05 (2). The increasing HR with increasing intensity trend also held for the CHD and CVD death outcomes (2). This study supports the result that increasing intensity in current smokers is associated with an increase in CVD risk (2).

Another study that examined the impact of smoking intensity was the Nurses' Health Study, which collected smoking information at baseline and followed over 100,000 women for over 20 years (3). Using the time to CHD death endpoint and adjusting for age at starting smoking, the HR compared to never smokers for current smokers who smoke 1-14 cigarettes/day was 3.0 (95% CI 2.5, 3.7) and increased with increasing intensity category to a HR of 4.7 (95% CI 3.5, 6.3) for those who smoke 35 or more cigarettes/day (3). This trend was statistically significant with a p-value of <.001 (3). These results suggest that though there is increased hazard for even the lowest intensity category smokers, there is also increased risk with increased intensity.

Pope *et al* studied the functional form of how CVD death risk varies over a range of intensities using the large Cancer Prevention Study II (4). The authors examined both risks associated with categories of current cigarette smoking intensity and risks associated with environmental smoke exposure, which represented very low intensities (4). The authors conclude that a non-linear model of smoking intensity fits the data best (4). It seems that the linear model was rejected due to the very low intensity data points from the environmental smoke exposure risks (4). However, environmental smoke exposure may be very different from active exposure and so it is possible that these two data types should not have been modeled together. A good way to evaluate the functional form of the intensity-CVD relationship would be in a prospective cohort with continuous, not categorical, smoking intensity data and model the hazard of CVD versus intensity in a smoothed manner.

Duration

Duration of smoking is another important aspect of smoking as an exposure. In the Cancer Protection Study II, CHD death rates were calculated in intensity, duration, age, and sex categories (5). Though in many age, sex and intensity strata rates were higher in groups with higher duration, this was not the pattern in all strata (5). The limitations of the study include the lack of tests for trend and reported confidence intervals, and the large variation within strata, since the duration categories were 9 years and some intensity categories were quite heterogeneous and grouped 1-19 cigarettes/day smokers together (5). Another challenge with studying the effect of duration on heart disease is that age and duration are correlated and age is strongly related to heart disease risk (6). Additionally, in the Framingham Offspring Heart Study, Mannan *et al* modeled time to CVD with different combinations of aspects of smoking, adjusted for classic cardiovascular risk factors (7). Adjusted for time since quitting, within categories of smoking intensity, the HRs of increasing duration category showed no pattern of increasing hazard of CVD. There is not definitive evidence that duration of smoking is an important risk factor for CVD.

Pack-Years

Pack-years is a cumulative measurement of smoking and is generally calculated by multiplying average packs smoked per day by the number of years spent smoking (8). Using this measurement implies that 1 year of exposure to 10 cigarettes/day is equivalent to 10 years of exposure to 1 cigarette/day. However, with no evidence that duration is significantly associated with CVD risk, the question of the utility of using pack-years is a valid one, especially since this is a common way of adjusting for smoking in studies (8,16). Mannan *et al* showed that pack-years of smoking is associated with CVD risk (7). Again adjusted for time since quitting, less than 20 pack-years of smoking was not significantly associated with CVD, but the HR for 20-39 pack-years was 2.3 (95% CI 1.5, 3.5) and the HR for 40 or more pack-years was 2.2 (95% CI 1.6, 3.1) (7). In addition, this study reported the Akaike information criteria (AIC) for two models: one with time since quitting and pack-year categories, and the second with time since quitting and combinations of categories of intensity and duration (7). The AIC was 5824 for the pack-year model and 5833 for the intensity/duration model, indicating that the pack-years variable is slightly preferable (7). AIC is a function of the number of covariates and it is possible that this difference is a result of categorizing the variables since the intensity/duration model has six more covariates than the pack-years model. As part of this thesis, we model pack-years, intensity and duration continuously and compare models that differ by fewer covariates.

Non-Cigarette Forms of Tobacco Exposure

Secondhand Smoke

Forms of tobacco exposure other than active cigarette smoking, including secondhand smoke exposure, cigar use, and pipe use could also be associated with CVD. To investigate the effect of secondhand smoke, Barnoya and Glantz pooled 29 studies of the ischemic heart disease endpoint and performed a meta-analysis (9). They found that among never smokers, the relative risk of ischemic heart disease comparing groups with secondhand smoke exposure to those without was 1.3 (95% CI 1.2, 1.4) (9). This indicates that secondhand smoke exposure could impact CVD risk. Another study looked at risk of CHD across different levels of secondhand smoke exposure by measuring serum cotinine in non-smoking males in Britain (10). This study found that increasing cotinine category was associated with increasing hazard of CHD (10). This suggests that a secondhand smoke-CHD dose-response relationship may be present, though this study used a biomarker and did not find such a relationship with stroke (10).

Cigars/Pipes

Cigar smoking is another important non-cigarette form of tobacco exposure. In the Cancer Prevention Study II, the impact of cigar smoking was analyzed among the men who did not use cigarettes or pipes (11). This study found that, among the 30-74 year olds, compared to those who never smoked cigars the rate of CHD death was 30% higher for current cigar smokers (95% CI 5%-62%) (11). The rates were not significantly different for never and former cigar smokers (11). Pipe smoking among men who did not smoke cigarettes or cigars was also investigated in the Cancer Protection Study II (12). Compared to those who never smoked pipes, this study found that the hazard of CHD death was 30% higher for current pipe smokers (95% CI 18%-43%), but the hazards were not significantly different for never and former pipe smokers (12). These studies imply that both current cigar and pipe use impact the risk of CVD, though they did not look at cigar or pipe use on top of cigarette use as well.

Age at Smoking Initiation

The age at which a person begins smoking is an often ignored aspect of smoking, but it is possible that starting smoking at younger ages is more harmful than starting later in life due to developmental stage. Huxley *et al* looked at categories of age at starting smoking as a predictor of time to CVD adjusted for socioeconomic status (SES) variables, traditional cardiovascular risk factors, intensity and time-varying duration (1). Compared to starting smoking at 22 years or older, there was a significant trend of higher risk of CVD the younger the age at starting smoking was (1). For example, the HR for starting between 13 and 15 years of age was 1.34 (95% CI 1.01, 1.78) and for 12 or younger was 2.51 (95% CI 1.74, 3.61) (1). Another study that examined this aspect of smoking was the Nurses' Health Study (3). After adjusting for standard cardiovascular risk factors, age, and intensity, the hazard ratios for CHD death did not show a significant trend over categories of age at starting smoking ranging from 17 or younger to 26 or older in current smokers (p-value=0.67) (3). It is possible that the reason these studies differ is due to the different age categories, and perhaps the effect is mostly in the youngest starters, since the HR was so high for the 12 and younger category in Huxley *et al* (1,3). So there is conflicting evidence about whether age at starting smoking affects heart disease risk.

Time Since Quitting Smoking

Time since quitting is a widely acknowledged risk factor for CVD, as it even appears on websites dedicated to promoting quitting smoking (13). Huxley *et al* looked at hazard of time to CVD by categories of time since quitting, adjusted for classic cardiovascular risk factors, SES factors, and cigarette-years (1). None of the categories of time since quitting HRs were significantly different from a current smoker and there was no evidence of a trend (1). The much larger Nurses' Health Study reached a different conclusion (3). Compared to current smokers, and adjusted as before including the smoking covariates of age at starting smoking and intensity, there was a significant trend of decreasing risk of both vascular death and CHD death as time since quitting increased (3). The CVD outcome HR compared to current smokers for quitting less than 5 years ago was 0.53 (95% CI 0.41, 0.67) and for quitting 20 or more years ago was 0.23 (0.19, 0.28) (3). The quitting 20 or more years ago HR was very close to the never smoker HR of 0.26, indicating that risk of CHD could decay to the risk of a never smoker over enough time (3). These results also indicate that the time since quitting is an important factor and the impact of adjusting for former smoking status could vary greatly based on how long the subjects have to have not smoked before being included in this category (8). In addition, age, age at starting, duration, and time since quitting are often additive combinations of the other variables, and so care must be taken not to adjust for all of these in the same model (8).

Compound Smoking Indices

In part due to the problem of collinear aspects of smoking and in an attempt to reduce the number of covariates that need to be adjusted for in studies, a few single smoking indices have been proposed (14,15,16). One such index attempts to combine age at starting smoking (a), intensity(x_i), duration (n_i), a measure of how much smoke is inhaled (p_i), and the time since quitting (y) in a function that seems reasonable given current knowledge of different aspects of smoking (14). I indexes years when different intensities or forms of smoking were used (14). The proposed index is thus:

$S=(3-a/15)^{1/2}*\sqrt{(\sum(p_i n_i x_i)-0.5)}-y$, where S is set to 0 if the formula is not-evaluable or negative (14). The use of the p_is is particularly challenging as it is an attempt to weight how much smoke is inhaled for non-cigarette forms of smoking, like secondhand smoke, cigars, or pipes relative to a cigarette (14). This index was tested in a very small group of subjects and the index contributed a larger amount to the chi-square of the logistic model for a coronary artery disease outcome than intensity and duration together or pack-years in different models (14). Another set of two indices was proposed by Leffondré *et al* to model smoking in lung cancer incorporating time since quitting (T), duration (D), intensity (I), and two parameters that can be based on features of the dataset in use or on earlier scientific findings/hypotheses (15). These are delta (δ), which is "lag time" or time from exposure to disease occurrence, and tau (τ), which is "half-life" or a measure of how long it takes for a quitter's risk to return to that of a never smoker (15, 16). The first index is (15):

$$X1=\ln[(1-0.5^{(\max(D+T-\delta,0)-\max(T-\delta,0))/\tau})*0.5^{(\max(T-\delta,0)/\tau)}*I+1]$$

The second index is (15):

$$X2=(1-0.5^{(\max(D+T-\delta,0)-\max(T-\delta,0))/\tau})*(0.5^{\max(T-\delta,0)/\tau})*\ln(I+1)$$

Hudson *et al* evaluated the X2 index in a cross-sectional study of 606 systemic sclerosis (SSc) sufferers where they collected self-reported smoking data and measured vascular disease-related outcomes common in SSc patients (16). They then performed regression analyses for these outcomes using five models which were all adjusted for SSc specific variables as well as age, sex, and race, and compared model fit using the AIC (16). They found that for two out of three outcomes the X2 index model performed better than using ever versus never indicators or using a never indicator, time since quitting, pack-years, and the interaction of the two (16). So there is evidence that a smoking index is a good way to model smoking in some vascular-related outcomes, but this type of analysis has not been done in CVD or CHD outcomes, which are also vascular related. As suggested in (16), in this thesis we will use CVD/CHD outcomes and directly compare different ways of modeling smoking as an exposure, including combinations of the different aspects of smoking, pack-years, and the two proposed smoking indices.

1.2 Mediation – Smoking-CVD Pathways

Cigarette smoking increases the risk of CVD events. Some of the pathways by which smoking affects this increase in risk are known, but some are not. The general pathways of interest are ones that are well known to impact CVD risk, including atherosclerosis, hemodynamics, inflammation, and coagulation (6). However how much of the effect of cigarette smoking which goes through these pathways is unclear. Mediation analysis can be used to answer the question of what percentage of the current cigarette smoking effect is mediated through each pathway and the various pathway components.

Atherosclerosis

One pathway that is affected by cigarette smoking is atherosclerosis. Coronary artery calcium (CAC) is a marker of subclinical atherosclerosis. A study in Denmark showed that current smoking intensity was related to both CAC incidence and CAC progression (22). This study compared three groups: ex-smokers, current smokers with lower intensities and current smokers with higher intensities of at least 17 cigarettes/day (22). Among those with no CAC at the start of the study, there was a statistically significant trend of a higher percentage of incident CAC among those with higher current smoking intensity (22). There was also a statistically significant trend of a higher percentage of CAC progression among those with higher current smoking intensity (22). Therefore it will be relevant to examine both whether subjects have any or no CAC and the extent of the CAC as measured by the Agatston score.

Hemodynamics

Hemodynamics, including systolic blood pressure, heart rate, and cardiac output, may be a pathway through which cigarette smoking impacts CVD risk. High systolic blood pressure is a classic risk factor for CVD. In Barutcu *et al*, non-smokers were asked to smoke a single cigarette and hemodynamics measurements were taken both before and after (23). They found that common carotid artery flow volume did not significantly differ across time points (23). However, both systolic blood pressure and heart rate changed significantly, with systolic blood pressure increasing by 6 mmHg on average and heart rate increasing by 4 beats/min on average

(23). So the hemodynamic system is related to current cigarette smoking status and these components are valid targets of analysis.

Inflammation

Many proteins are involved in inflammation, including C-reactive protein (CRP), tumor necrosis factor alpha (TNFA), and interleukin-6 (IL-6). Those with a high risk of CVD also tend to have high amounts of CRP, TNFA and IL-6, indicating that the inflammation pathway is associated with CVD risk (24). Smoking status is also associated with inflammatory pathway proteins (25). In a study of chronic pancreatitis sufferers, despite the limited sample size of 28 patients, the researchers found that those who did not use tobacco on average had much lower levels of IL-6 (1.4 +/- 0.5) than those who smoked (10.7, +/- 5.4) (25). Inflammation proteins are related both to smoking status and CVD risk and so are interesting targets for a mediation analysis.

Coagulation

Coagulation is involved in clot formation, which directly contributes to CVD risk since MIs are caused by clots blocking blood flow to the heart. Many proteins are part of the coagulation system, including fibrinogen, D-dimer, Factor VIII, and Plasmin Anti-Plasmin complex (6). Tuut and Hense found that smokers had about 400mg/dl concentrations of fibrinogen, which was increased relative to non-smokers among persons of the same age, sex, and body size (26). In the Cardiovascular Health Study it was found that, among those at the same site and of the same age, race, and sex, compared to those who did not go on to have a CHD event, those who did had statistically significantly higher values of fibrinogen and Factor VIII (27). So proteins in the coagulation pathway are also associated with the CHD outcome.

This indicates that that coagulation pathway is possibly a strong mediator of the smoking-CVD relationship.

There is evidence that coagulation, inflammation, hemodynamic and atherosclerotic pathways all have known biomarkers that are associated with smoking status. These four pathways also include biomarkers that are known to be associated with CVD risk. Therefore these biomarkers are possible mediators for the smoking-CVD relationship, and there would be benefit in estimating the percentage of the effect that goes through these biomarkers and pathways.

1.3 Smoking Intensity Reduction

Coronary heart disease (CHD) causes many deaths each year, and there were 405,000 CHD deaths in the United States in 2008 (17). Smoking is a risk factor for CHD death (CHDD), and the fact that the CHDD outcome improves if people quit smoking is well-supported and widely accepted (6,13,18). However, it is unknown if CVD outcomes, including CHDD, will also be improved if smokers do not quit entirely, but instead decrease their smoking intensity. Answering this question is important because it will impact how smokers are advised to change their habits by health care professionals and public health messages.

Impact of Quitting

To support the idea that quitting smoking is beneficial and to get an estimate of the effect size, studies have been done to compare risk of CHDD in current smokers, former smokers, and never smokers (3). In the Nurses' Health Study using the CHDD outcome, the hazard ratio (HR) compared to never smokers was 1.2 (95% CI 1.1, 1.4) for former smokers and 3.9 (95% CI 3.4, 4.5) for current smokers (3). This indicates that the risk of CHDD is much lower for former smokers, or those who quit smoking at some point in the past, than for those who continue to smoke, and lends support to the practice of encouraging people to quit smoking (3).

Smoking Intensity-CVD Dose-Response

In order to lend credence to the idea of encouraging smoking reduction, there must first be evidence that higher smoking intensity is associated with greater risk of CVD outcomes. As stated previously, there is good evidence to support this point (1,2,3,4). The next step is to directly examine the impact of reducing smoking intensity.

Direct Studies of Smoking Reduction

Three studies have examined the effect of reduced smoking intensity on the CVD death outcome. First, a 20,000 subject combined study in Denmark used self-reported smoking data collected once and then again about five years later (19). Adjusted for factors including duration of smoking, the HR for continuing smokers who reduced intensity by one half or more from at least 15g of tobacco/day compared to continuing smokers who smoked at least 15g of tobacco/day at both time points was not significant at 1.01 (19). So this study does not support the hypothesis that reducing intensity will improve the CVD death outcome. Second, a 4,600 subject study in Israel recorded smoking information two years apart, and 1-10, 11-20, and 21 or more cigarettes/day groups were used for current smoking intensity (20). Adjusted for factors including initial intensity category and including competing risks, the HR for continuing smokers who moved from one smoking intensity category to a lower one compared to continuing smokers who did not change category was significant at 0.77 (95% CI 0.66, 0.94) (20). This result indicates that there is a decreased hazard of CVD death for those who reduced their smoking intensity. Finally, a third analysis of about 5,000 people was done in Scotland which combined two studies which collected the same smoking intensity categories as the Israel study at two time points (21). This study performed the same analysis as the Israel study but obtained a different result, namely a non-significant HR of 1.08 (95% CI 0.93, 1.25) for continuing smokers who reduced intensity category compared to continuing smokers who did not change intensity category (21). There was some evidence of differences in this HR by initial intensity category, indicating that perhaps the magnitude of the intensity reduction is important, though this has not been examined (21).

All together, these three studies provide conflicting and inconclusive results about the effect of smoking intensity reduction on CVD death risk. This area is ripe for further research, and it would be important to quantify the effect of the amount of cigarette/day change on CVD risk.

The question of how changes in smoking intensity are associated with the CVD outcome is an open one, but has great public health implications. While there is evidence that current smoking intensity is associated with CVD, the functional form is not clear. Cohort studies of smoking intensity changes using the CVD death outcome come to different conclusions, and so further work should be done to answer this important health question.

1.4 Statistical Background

In this section we provide a description of the statistical methods used in this thesis, namely the Cox proportional hazards model, generalized additive models, and mediation models. Details of precisely how these techniques were used appear in Section 2, Methods.

Cox Proportional Hazard Model

In 1972, Cox introduced the Cox proportional hazards model (28). In survival data, the failure time (T) is defined as the time from the start of follow-up until the event occurs or until censoring occurs (28). The hazard is a function of time (t) and is defined as (28):

$$\lambda(t) = \lim_{\Delta t \to 0} pr(t \le T \le t + \Delta t | t \le T)/\Delta t$$

The hazard is an instantaneous failure rate, or the probability of failure over an infinitely small time window. In the Cox proportional hazards model, the log hazard given a set of covariates **x** is modeled as (28):

$$\log\left(\lambda(t|\boldsymbol{x})\right) = \log\left(\lambda_0(t)\right) + \boldsymbol{\beta x}$$

$\lambda_0(t)$ is the baseline hazard, or the hazard when all covariates **x** are equal to zero, and the vector of coefficients associated with the covariates is **β** (28). The log hazard is a linear combination of the baseline hazard, which does not have to have a specified form, and the covariate parameters (28,40). For this reason, this is a semi-parametric model (40). In addition, this is a proportional hazards model because the hazards for different covariate values are assumed to be proportional due to the formulation of the model (28,40). To solve for **β**, the maximum of the conditional log partial likelihood is found, with i indexing the observed events, $R(t_i)$ defined as the set of all subjects at risk at event time i, and k indexing the unique times of failure (28):

$$\text{Log Likelihood}(\boldsymbol{\beta}) = \sum_{i=1}^{k} \boldsymbol{x}_{(i)}\boldsymbol{\beta} - \sum_{i=1}^{k} \log[\sum_{l \in R(t_i)} \exp(\boldsymbol{x}_{(l)}\boldsymbol{\beta})]$$

The resulting **β** parameters, when exponentiated, are interpreted as hazard ratios (40).

Generalized Additive Model

The generalized additive model (GAM) is an extension of the generalized linear model (GLM), which is in turn an extension of the standard linear model (29). In GLMs, the outcome (Y) can take on any exponential family density, such as the gamma distribution (29). GLMs also allow different links between "random components" and "systematic components", but still have the outcome depend on linear combinations of covariates and coefficients as in this model:

$$g(E(Y|\mathbf{x}))=\boldsymbol{\beta}\mathbf{x}$$

where g(u) is the link function, $\mathbf{x}$ is the vector of covariates, and $\boldsymbol{\beta}$ is the vector of corresponding coefficients (29). GAMs extend the GLM by additionally allowing the linear combination to include non-parametric functions of the predictors $\mathbf{x}$ (29). So one or more of the $\beta_i x_i$ may be replaced with $s_i(x_i)$ where s is any smooth function (29). Therefore these models may be non-parametric or semi-parametric (29). A scatterplot smoother is used to estimate the $s_i(x_i)$, which is constrained to have a mean of zero (29). This can be done via taking localized averages in a sliding window, using a running mean, or fitting a spline (29,30). The GAM package in STATA uses a cubic spline smoother (30).

Mediation

In a mediation model, the treatment affects the outcome, as well as inducing a change in another variable called the mediator (31). The mediator in turn also effects the outcome. In this model, the total effect of the treatment on the outcome can be broken into two parts: the direct effect which does not go through the mediator, and the mediated effect which goes through the pathway of the mediator (32). The following ideas and methods in this paragraph are presented in Imai, Tingley and Keele (31). The direct effect of one unit of treatment is estimated by the average difference in outcome if treatment is increased by one unit but the mediator is kept the same. The mediated effect of one unit of treatment is estimated by the average difference in outcome if treatment is held constant, but the mediator is changed from what it is to what it would be if the treatment was increased by one unit. However, each subject only experiences one combination of treatment and mediator, so to estimate the effects simulations of the counterfactual situations can be used. For this simulation method to work, "sequential ignorability" assumptions must be made. These assumptions are that the treatment is not affected by the mediators or outcomes conditional on designated covariates, and in turn that the mediator is not affected by outcomes conditional on the treatment and designated covariates. To estimate the effects, first a model with the mediator as the outcome predicted by the treatment and any covariates and a model with the outcome as the outcome predicted by the mediator, treatment, and covariates are fit from the data. For each subject, draw a simulated mediator value for different treatment values, then draw a simulated outcome for the different treatment values and simulated mediators. This is repeated a large number of times, and the estimated direct, mediated and total effects can be calculated as discussed above across all subjects. This method is implemented in STATA by Hicks and Tingley (32).

The organization of the remaining sections is as follows. In section 2 we describe the Multi-Ethnic Study of Atherosclerosis (MESA) population and then the methods used in this thesis, starting with modeling smoking as an exposure, then mediation analyses, then the impact of intensity reduction. In section 3 we describe the results of the methods described in section 2. Again the order is modeling smoking as an exposure, then mediation, and the impact of intensity reduction is last. Section 4 contains a discussion of the results with smoking modeling first, mediation second, and intensity reduction third. Section 5 lists the references and section 6 contains the tables and figures.

2 Methods

Study Population

The MESA study is a cohort of 6814 participants initially free of clinical heart disease at baseline in 2000-2002 (33,35). MESA participants were recruited at six sites across the United States, and recruiting minorities was a particular focus (33). Recruited participants had an age range of 45-84, with a mean of 62 and a standard deviation of 10 years (33,35). The participants were 47% male, and 38% Caucasian, 12% Chinese-american, 28% African-american, and 22% Hispanic (33). MESA collected questionnaire data on duration, intensity, and ages at starting and stopping smoking for cigarette, cigar, pipe, snuff and chewing tobacco use (33,35). This data was collected at baseline as well as at four other follow-up exams (33,35). Reported intensities of greater than 100 cigarettes/day were set to missing (4 intensities were set to missing). A pack of cigarettes was defined to be 20 cigarettes. Pack-years was calculated by multiplying intensity in packs/day by duration in years. MESA also collected a number of CVD endpoints by either calling or sending mail to the participant or a family member and inspecting hospitalization records every year or less (33,34,35). These endpoints included: CHD hard (myocardial infarction (MI), resuscitated cardiac arrest, CHD death), CHD all (MI, resuscitated cardiac arrest, definite angina, probable angina if followed by revascularization, CHD death), CVD hard (MI, resuscitated cardiac arrest, stroke, stroke death, CHD death), CVD all (MI, resuscitated cardiac arrest, definite angina, probable angina if followed by revascularization, stroke, stroke death, CHD death, other atherosclerotic death, other CVD death), stroke, CVD death, all death, and deep vein thrombosis or pulmonary embolism (DVTPEM) (http://www.mesa-nhlbi.org/MesaInternal/EventsData.aspx).

2.1 Modeling Smoking as an Exposure

Functional Form - GAMs

Generalized additive models for time to the four CVD endpoints of primary interest (CVD hard, CVD all, CHD hard, and CHD all) were used to investigate the functional form of each aspect of smoking. All models were linearly adjusted for age, and sex and race/ethnicity were adjusted for as categorical variables in order to remove the strongest confounders while keeping the number of covariates as small as possible. Current smokers were modeled separately from former smokers. To investigate intensity, the GAMs were additionally adjusted for duration, and the intensity smoothed log hazard ratio (HR) was plotted. To investigate duration, the GAMs were additionally adjusted for intensity, and the duration smoothed log hazard ratio (HR) was plotted. The pack-year GAMs were only adjusted for age, sex, and race/ethnicity. To explore any non-linearity in the age at starting smoking using GAMs, current and former smokers were modeled together and adjusted for age, sex, race/ethnicity, duration and intensity. In order to see the effect of years since quitting smoking, GAMs for former smokers only were run and were adjusted for age, sex, race/ethnicity, intensity and duration.

Intensity, Duration and Pack-years

Cox models were used to investigate the hazard ratio of each exposure definition of smoking on time to four different CVD endpoints (CVD hard, CVD all, CHD hard, and CHD all). Initial models were stratified by current versus former smoking status, with a second set of models that pooled all participants and controlled for status. All Cox models were adjusted for age, sex, race/ethnicity, education category, and income category. Education and income were adjusted for in order to account for confounding by socioeconomic status. Duration, intensity, and pack-years were all centered stratified by smoking status, and these centered variables were used for all further modeling. The effect of smoking status was examined first in the pooled sample by including only smoking status and adjustment variables. Status stratified models including only one of duration, intensity, and pack-years were examined next. The interaction between duration and intensity was also tested.

Both pooled and status stratified Cox models were compared which used intensity and duration alone, in combination, and expressed as pack-years. Model performance was summarized using the pseudo- R^2 statistic, the area under the receiver-operator characteristic curve (AUC), and the Akaike information criterion (AIC). AIC is calculated as (41):

-2*ln(maximum likelihood)+2*number of estimated parameters.

Two pooled Cox models including all MESA participants were run which adjusted for smoking status. One of the models additionally adjusted for intensity, duration, and the multiplicative interaction of intensity and smoking status (8). The other additionally adjusted for pack-years and the interaction of pack-years with smoking status.

Age at Starting/Time since Quitting

The age at starting smoking was examined by running Cox models adjusted for intensity separately for current and former smokers. Duration was not adjusted for due to co-linearity issues with duration and age. To see if years since quitting is associated with CVD outcomes, a

Cox model adjusted for intensity was run in only former smokers, since this is the only group that has a non-zero time since quitting. Years since quitting was modeled as a linear effect in this model. A second model additionally tested for an interaction of year since quitting and gender. A third model looked at years since quitting categorically as was done in (3). This model pooled all participants and adjusted for current smoking status and former smoking status with <5 years, 5 to <10, 10 to <15, 15 to <20 and 20 or more years since quitting smoking.

Non-Cigarette Forms of Tobacco Exposure

To investigate non-cigarette smoking types of tobacco exposure, Cox models were run which looked at pipe smoking status, cigar smoking status, snuff use status, chewing tobacco use status, secondhand smoke exposure status, and secondhand smoke exposure intensity one at a time. These models pooled all MESA participants, except for the secondhand smoke models which only included non-smokers. These models were additionally adjusted for cigarette smoking status, cigarette smoking intensity, cigarette smoking duration, and the interaction of cigarette smoking status and cigarette smoking intensity.

Risk Prediction

Classical CVD risk factors include age, sex, total cholesterol, HDL, diabetes, systolic blood pressure, and current smoking status (42). In order to see if current smoking intensity improves risk prediction over these classical risk factors, two Cox models with different predictor sets were fit for time to CVDH: one with the classical risk factors only, and one with the classical risk factors plus current smoking intensity. Only participants with complete data on all of these covariates were included in these models. The fitted values of these models were used to generate ROC curves and the corresponding AUCs were calculated to measure how well the models discriminate.

Additional Outcomes – Intensity and Smoking Status

After determining the functional form and significance of all six aspects of smoking for the four primary cardiovascular outcomes, it was of interest to see how the these forms translated to four additional CVD-related outcomes: stroke, CVD death, all death, and DVTPEM. Based on the previous results, pooled Cox models including all MESA participants were run on all eight outcomes which adjusted for smoking status, intensity, duration, and the multiplicative interaction of intensity and smoking status. A forest plot of the effect of current smoking intensity was used to visually summarize these results. This plot was made in R (R 2.9.2, rmeta package). Further analyses continued to examine all eight outcomes.

Compound Smoking Indices

Two smoking indices proposed by Leffondré *et al* were examined. Both indices incorporate time since quitting, duration, and intensity, and require the estimation of the τ and δ parameters (15). The indices are (15):

$$X1=\ln[(1-0.5^{(\max(D+T-\delta,0)-\max(T-\delta,0))/\tau})*0.5^{(\max(T-\delta,0)/\tau)}*I+1]$$

and

$$X2=(1-0.5^{(\max(D+T-\delta,0)-\max(T-\delta,0))/\tau})*(0.5^{\max(T-\delta,0)/\tau})*\ln(I+1)$$

These indices were calculated for all MESA participants using each integer δ and τ combination from δ=0 to 9, and τ=1 to 20, as suggested previously (15). Then Cox models adjusted for the index, age, sex, race, income, and education were fit for all the indices for all eight outcomes including all subjects with non-missing pack-years, smoking status, and years since quitting. The ability of the indices to capture smoking as an exposure for the eight CVD outcomes was investigated by calculating the AIC for these Cox models. The values of the δ and τ parameters that minimized the AIC for each index for each outcome were recorded, and AICs for different combinations of δ and τ for each outcome were plotted.

Comparing Models of Smoking

In order to directly compare how well different ways of modeling smoking as an exposure capture the effect of smoking on the eight outcomes, the AIC for different Cox models was calculated. These models all included the same participants who had non-missing pack-years, smoking status, and years since quitting information, and they were all adjusted for age, sex, race, income, and education. Seven models were compared, with the following measures of smoking. The first model was smoking status only. The second model was never smoking status, pack-years, years since quitting, and the interaction of time since quitting and pack-years. The third model was smoking status, intensity, and the interaction of smoking status and intensity. The fourth model was smoking status and current smoking intensity (which is set to zero for former and never smokers). The fifth model was current smoking intensity only. The sixth model was X1, using the δs and τs that were calculated to give minimum AICs for each outcome. The seventh and final model was X2, using the δs and τs that were calculated to give minimum AICs for each outcome. This type of comparison was done in (16), and the second model used was used in this paper as well.

Updating Smoking Intensity

Current smoking status and current smoking intensity information are collected up to five times in MESA. To test whether these measurements should be updated or not, a time-varying Cox model was compared to a baseline only Cox model. In both models, baseline age, sex, race, income and education were adjusted for, current smoking intensity was set to zero for never and former smokers, and participants with missing baseline smoking status were excluded. In the baseline only model, the baseline measurements for both current smoking status and current smoking intensity were used. In the time-varying model, both current smoking intensity and current smoking intensity were updated at each available exam. If a smoking covariate or exam was missing, the measurements were carried forward or backwards. For all eight endpoints, the fit of the two models were compared via the AIC.

2.2 Mediation – Smoking-CVD Pathways

In order to examine how current smoking effects the risk of the eight CVD endpoints, four major pathways and twelve possible mediators were examined. The first pathway is atherosclerosis, and the mediators from this pathway are binary and continuous CAC. The second pathway is coagulation, which is represented by four possible mediators: Factor VIII, D-Dimer, IL-6, and Plasmin-antiplasmin complex (PAP). The third pathway is inflammation, including the three potential mediators CRP, TNFA, and IL-6. The fourth and final examined pathway is hemodynamics, including systolic blood pressure (SBP), heart rate, and cardiac output.

Mediator Data Collection

Between 2000 and 2002, all MESA participants underwent a baseline examination (33). During this exam, baseline values of all of the putative mediators of the smoking-CVD relationship were collected (33,35). Participants fasted before the data was collected. Systolic blood pressure (n=6811) was obtained by taking the mean of the last two of three measurements taken while subjects were sitting (33). Participants were considered hypertensive if they had a history of hypertension along with a history of taking a medication for hypertension, or their diastolic bp was greater than or equal to 90, or their sbp was greater than or equal to 140 (36). Factor VIII (n=6765), D-Dimer (n=6769), IL-6 (n=6622), PAP (n=6627), TNFA (n=2871), fibrinogen (n=6767), and CRP (n=6762) were measured at a central laboratory (33,35). Mean CAC in terms of the Agatston score (n=6814) was calculated from the computed tomography scan (33,35). This value was transformed into two possible mediators: binary CAC (zero or more than zero), and log CAC, which was calculated as log (mean CAC +25). 12-lead electrocardiograms provided heart rate (n=6766) data, and cardiac magnetic resonance imaging provided cardiac output (n=4985) data (33,35,37).

Mediation Analyses

In order for significant mediation to occur through a given mediator, the predictor of interest should influence the mediator, and the mediator should influence the outcome of interest. To test the first piece, whether current smoking status is related to the level of the twelve mediators, twelve linear regression models were used. The predictor of interest was current smoking status (binary), and the outcome of interest was one of the twelve mediators. Each model was adjusted for age, sex, race, income, education and alcohol use. Alcohol use was adjusted for in order to make the sequential ignorability assumptions more reasonable. Alcohol use is associated with smoking, both are risky behaviors, and alcohol use is also associated with CVD. To test the second piece, whether the mediators are related to the outcomes of interest, Cox models were used. The predictor of interest was one of the twelve mediators, and the outcome of interest was the time to one of the eight CVD outcomes, with each combination tested separately. Each model was adjusted for age, sex, race, income, education, and alcohol use.

Estimated Mediation Percentage

To estimate the percentage of the current smoking effect on the binary CVD outcomes that is mediated through each mediator, the STATA *mediation* package was used. Each mediator/outcome combination was tested separately. Linear structural equation modeling was used, and each was simulated 800 times. The “treatment” variable was the current smoking status indicator.

2.3 Smoking Intensity Reduction

Data Collection

To investigate the effect of changing cigarette smoking intensity, data from MESA exam 2 was used. 6,233 participants returned for exam 2. At exam 2, participants answered the same questions about smoking behavior as they did at exam 1. The median time between exams 1 and 2 was 1.6 years. Subjects who reported being current smokers at both exam 1 and exam 2 were included in these analyses. The Framingham CVD risk score (42) was calculated for all participants and used as a way to adjust for many traditional CVD risk factors as one summary covariate. Times to events were considered starting from exam 2, and participants who dropped out or had events prior to exam 2 were excluded.

Intensity Change – Continuous

The effect of a smoking intensity change was examined by running Cox models which included the change in intensity as a continuous variable. This was calculated by subtracting the intensity at exam 1 from the intensity at exam 2. Two Cox models were compared for each endpoint: one model which included the intensity change and intensity at exam1 variables, and one model which included only intensity change. These Cox models were run for the CVDH, CVDA, CHDH, CHDA, stroke, CVD death and all death endpoints, and were adjusted for age, sex, race, Framingham CVD risk score, and log(CAC+25). CAC is a strong predictor of CVD risk so CAC was adjusted for to improve precision. The DVTPEM endpoint was not considered since there were only seven events among the subset of interest. It is important to adjust for initial intensity as well as age, sex, and race as in (20,21). Adjusting for initial intensity will prevent the interpretation of considering a 60 to 50 reducer to be at the same risk as a 40 to 30 reducer. Using the actual change in cigarettes/day is preferable to just using "reducer" or "maintainer" categories as in previous studies (19, 20, 21) for two reasons: a person who reduced their intensity from 40 to 30 would be considered a "maintainer" while a 25 to 15 reduction would be a "reducer", while they actually both have the same intensity change, and a person who reduced their intensity from 40 to 5 and a person who reduced from 21 to 20 would both be considered "reducers" while having vastly different intensity changes as well as different baseline risks.

Intensity Change – Categorical

To replicate the analyses done by (20,21), the change in intensity was also studied categorically. At exams 1 and 2, participants were categorized as low (0-10 cigarettes/day), medium (11-20 cigarettes/day), or high (21+ cigarettes/day) intensity smokers. These smokers were then classified as "reducers" if they decreased in category and as "increasers" if they increased in category. Cox models were then run, adjusted for age, sex, race, Framingham CVD risk score, and log(CAC+25), which included reducer and maintainer as binary variables.

STATA 11 was used for all analyses, including the GAM and *mediation* packages (38).

3 Results

Cohort Characteristics

There were 2487 former and 887 current smokers at baseline. Among former smokers, the average intensity was 17 cigarettes/day with a standard deviation of 14. Average duration in former smokers was 23 years (sd=14). Among current smokers, average intensity was 13 cigarettes/day with a standard deviation of 11. Current smokers had an average duration of 40 years (sd=10). The median follow-up time was 10 years. Among all 6814 participants, there were 450 CVD hard events, 639 CVD all events, 284 CHD hard events, 449 CHD all events, 180 stroke events, 161 CVD death events, 709 deaths, and 100 DVTPEM events.

3.1 Modeling Smoking as an Exposure

Never/Former/Current

In Cox models with all participants, the effect current smoking status was similar across the four primary cardiovascular endpoints (Table 1). Compared to never smokers, the hazard ratios for former smokers were non-significant, providing no evidence for an association between former smoking status and risk of CVD endpoints. In contrast, the hazard ratios for current smoking status were significant for all four endpoints, and ranged from 1.66 for CHDA to 1.98 for CVDH.

Functional Form - GAMs

There was no significant non-linearity detected via the GAM plots for age at start or years since quit with respect to risk of any of the CVDH, CVDA, CHDH or CHDA endpoints (Figure 1). No significant non-linearity was detected as well for intensity, duration, and pack-years among former smokers. Among current smokers, risk appears linear for duration, intensity and pack-years except for the very longest duration or highest intensity smokers. Among these smokers, it appears that the additional risk beyond 2 packs/day, 50 pack-years, or 40 years duration was small, however there are very few current smokers above this level of intensity. We display the smoothed association and 95% CIs for CVDH for each aspect of smoking in figure 1.

Intensity/Duration/Pack-Years

Stratified Models

In the status stratified Cox models, duration was not significantly associated with any of the four CVD endpoints examined in either the current or former smokers (Table 2). Higher intensity was associated with higher hazard of all four endpoints in the current smokers (HRs packs/day: CVD hard 1.92, CVD all 1.69, CHD hard 2.00, CHD all 1.83), but intensity was not associated with any of the four endpoints among the former smokers. Pack-years was also associated with higher hazard of all four endpoints in the current smokers (HR 1.01 for all four endpoints), but not in the former smokers. There was no evidence of an interaction between duration and intensity for any endpoint or any smoking status.

Comparing models with intensity alone, intensity and duration, and pack-years alone among the current smokers yielded some patterns across the four endpoints (Table 3). In the CVD hard endpoint, among current smokers, the model including intensity alone had an R^2 of 0.264, an AUC of 0.696 and an AIC of 1020. The model adding duration had very similar results with an R^2 of 0.264, an AUC of 0.696 and an AIC of 1022. The model with pack-years had worse performance than the model with intensity alone, with an R^2 of 0.256, an AUC of 0.693, and an AIC of 1021. This relationship among the three models in the information measures generally held across endpoints, with the pack-years model having the lowest R^2 and AUC.

Pooled Models

In the pooled models which adjusted for smoking status, duration, intensity and status/intensity interactions, former smoking and duration were non-significant (CVD hard HRs ,0.89 and 1.00 respectively) for all endpoints (Table 4). Current smoking status (CVD hard HR 1.98, $p<0.0005$) and intensity (CVD hard HR packs/day 1.85, $p<0.0005$) were significantly associated with all endpoints. Like current smoking and intensity, the interaction of former smoking status and intensity (CVD hard HR 0.61, $p=0.013$) was significant and less than 1 for CVD hard, CVD all, CHD hard, and CHD all. In the pack-years models, current smoking status and higher pack-years were associated with increased hazard of all four endpoints. There was no significant association of any endpoint with the interaction of pack-years and former smoking status. Thus, the pooled models yield similar conclusions to the status stratified models.

Age at Starting Smoking

Age at starting smoking was not significantly associated with any of the four outcomes in either former or current smokers (Table 5). For the CVDH outcome, the hazard ratio associated with a one year increase in age at starting smoking was 1.00 (0.96,1.04) among current smokers, and was 0.99 (0.96,1.02) among former smokers.

Time since Quitting Smoking

Modeled as a continuous linear variable, years since quitting was not significantly associated with CVD hard, CVD all, CHD hard or CHD all after adjusting for age, race, sex, income, education and intensity in former smokers (Table 6). There was also no evidence of an interaction between time since quitting and gender. When former smokers were broken into categories of time since quitting in a pooled model, none of the time since quitting categories was significantly different from never smokers (Table 7). In addition, the hazard ratios for the ordered categories did not follow the expected pattern of increasing risk as time since quitting decreased. For example, for the CVDH endpoint, the hazard ratios for former smokers with decreasing time since quitting category were 0.91, 0.88, 0.47, 0.90, and 1.11.

Other Forms of Smoking

None of the non-cigarette tobacco exposures were significantly associated with any of the four outcomes (Table 8). There were very few current cigar (131), pipe (42), and chewing tobacco (21) users, so this may have limited the power to detect any of these effects. There were so few current chewing tobacco users that there were no CHDH or CHDA events among these people. The effect of current chewing tobacco use could not be estimated for these endpoints.

Risk Prediction

The AUC for the classical risk factor model was 0.735, while the AUC for the classical risk factor plus current smoking intensity model was 0.738. Adding current smoking intensity improved the AUC by 0.003, and the difference between the two was borderline significant at p=0.067.

Additional Endpoints – Intensity and Smoking Status

In the pooled models which adjusted for smoking status, duration, intensity and status/intensity interactions, like for the primary cardiovascular endpoints, former smoking and duration were not significant for the additional four endpoints of stroke, CVD death, all death, and DVTPEM (Table 9). Current smoking status was significantly associated with stroke, CVD death, and all death but not DVTPEM, though the HR was in the same direction (DVTPEM HR 1.37, p=0.359). Intensity was significantly associated with all death but not stroke, CVD death, and DVTPEM. The intensity effects on the CVDH, CVDA, CHDH, CHDA, stroke, CVD death and all death endpoints are pictured in figure 2. The intensity HRs for stroke (HR 1.45, p=0.184), CVD death (HR 1.46, p=0.202) and DVTPEM (HR 1.72, p=0.225) were again in the direction of increasing hazard with increasing intensity but did not reach significance.

Compound Smoking Indices

After calculating the compound indices X1 and X2 for δs from 0 to 9 and τs from 1 to 20 and using these indices to model the smoking as an exposure and find the AIC for these models, the patterns in the AICs looked similar for many of the eight endpoints (Figure 3). Generally the AIC improved as both τ and δ decreased for both X1 and X2 for the CVDH, CVDA, CHDH, stroke, CVD death, and death outcomes. For CHDA, the AIC improved as both δ and τ decreased for X1, but for X2 as δ decreased the AIC decreased, but the AIC had a U-shape across the range of τs. The DVTPEM outcome had a markedly different pattern of AICs for both X1 and X2. For this outcome, AIC generally increased as δ decreased and again there was a U-shape across the range of τs. As for the δs and τs associated with the minimum AIC, for both X1 and X2, the best δ was the minimum tested δ (0) and the best τ was the minimum tested τ (1) for most of the outcomes (Table 10). CVDA was best modeled with the minimum δ (0) and a slightly larger τ (2 for X1 and 3 for X2). CHDA had a minimum AIC with an X1 with a δ of 0 and a τ of 2, and a minimum AIC with an X2 with a δ of 0 and a mid-range τ of 10. The death outcome reached a minimum AIC with minimum τs (1) but slightly larger δs (1 for X1 and 2 for X2). DVTPEM was the only outcome which was best modeled with the maximum δ (9) for both indices and slightly larger τs (2 for X1 and 5 for X2).

Comparing Models of Smoking as an Exposure

Seven different ways to model smoking were tested by comparing model fit as measured by the AIC for all eight outcomes. For all of the outcomes, both compound indices captured the exposure to cigarette smoking very well, and if one of the compound indices did not have the minimum AIC, it was within 2 units of the minimum. Overall X2 performed slightly better than X1, but X1 and X2 were never more than 2 units apart. The worst model for CVDH, CVDA, CHDH, stroke, and CVD death was the never/pack-years/time since quitting/pack-years-time since quitting interaction model. For all of the outcomes except death and DVTPEM, the models which use intensity fit quite well. Of the models including intensity, the simpler models fit better, with the current intensity only model having the lowest AIC. For example, for CHDH the ordered AICs were 4405, 4404, and 4402. The current intensity only models, if they did not have the minimum AIC, were within 2 units of the minimum. For the death outcome, the best fitting models by more than 10 units were the compound index models. For the DVTPEM outcome, while the compound index models were within 3 units of the minimum AIC, the best fitting model was the never/pack-years/time since quitting/pack-years-time since quitting interaction model.

Updating Smoking Intensity

Comparing time updated versus baseline measurements of current smoking status and intensity, the HRs were quite similar for CVDH, CVDA, CHDH, CHDA, and DVTPEM (Table 12). The estimates for the HRs differed between the two models for the other endpoints (stroke, CVD death, all death). Current smoking status was not significant for CVDH, CVDA, CHDH, or CHDA, but current smoking intensity was. In terms of model fit, the AIC did not differ by more than 5 for any endpoint, and the model with the lower AIC varied by endpoint. For example, CVDH baseline had AIC 7123 and time updated had AIC 7126, while for CHDH baseline had AIC 4499 and time updated had AIC 4494.

Summary

To summarize, only current smokers had increased hazard of CVD outcomes. Former smokers, regardless of the time since quitting or amount smoked, were no longer at higher risk. Among the current smokers, of the six aspects of smoking only intensity and pack-years were significantly associated with the outcomes, and there was no evidence of non-linearity. Intensity models fit better than pack-year models, and adding current smoking intensity to classical CVD risk factors improved CVDH risk prediction. Compared to current smoking intensity models,

models with compound smoking index X2 as well as models with time updated current smoking intensity fit about equally well.

3.2 Mediation – Smoking-CVD Pathways

Mediation Analyses

In table 13, the associations between twelve potential mediators and current smoking status were tested. CRP, SBP, and heart rate were not significantly associated with current smoking status, but the other nine potential mediators were. Current smokers had significantly lower levels of hypertension. At least one mediator from all four pathways was strongly associated with current smoking status. Table 14 displays the results of Cox regression models which tested the association of the mediators with the eight CVD outcomes. In the hemodynamics pathway, cardiac output was not significantly associated with any outcome, while SBP and heart rate were significantly associated with all outcomes except for DVTPEM. Heart rate was also associated with death. The inflammation pathway mediators were all associated with all outcomes, except for CRP with DVTPEM. In the coagulation pathway, D-dimer, fibrinogen, and factor VIII were all associated with most outcomes, except for factor VII and stroke, D-dimer and stroke, and fibrinogen and DVTPEM. The last coagulation mediator, PAP, followed a different pattern and was only associated with death and DVTPEM. Both atherosclerosis mediators, CAC yes/no and log(CAC+25) were strongly associated with all outcomes, with the exception of DVTPEM.

Estimated Mediation Percentage

For most of the mediators the percentage of the current smoking effect on the eight CVD outcomes that is mediated through them was small (Table 15). Factor VIII, D-Dimer, PAP, SBP, CRP, cardiac output and heart rate all showed very small percent mediation for all outcomes except DVTPEM. There was a moderate amount of mediation through fibrinogen, IL-6, and TNFA for all outcomes. The highest percentage of mediation was through log(CAC+25), with CAC yes/no being slightly lower. The two significant inflammation mediators, IL-6 and TNFA, showed especially strong mediation in the stroke, CVD death, death, and DVTPEM outcomes. Current smoking was weakly mediated through both CAC mediators for the DVTPEM outcome. The percent mediation for the CHDA, stroke, and all death outcomes are pictured in figure 4.

Summary

Current smoking was at least moderately mediated though at least one mediator from three of the four pathways (atherosclerosis: CAC, inflammation: IL-6 and TNFA, coagulation: fibrinogen).

3.3 Smoking Intensity Reduction

Intensity Change – Continuous

There were 621 continuing smokers at exam 2, and the mean change intensity was a decrease of 1 cigarette/day with a standard deviation of 6. The change in intensity was moderately correlated with the intensity at exam 1 (r=0.41). The numbers of events were very low for the stroke and CVD death outcomes at 21 and 16 events respectively. For the CHDA endpoint, the HR associated with a decrease of 1 cigarette/day was 0.98 and not significant when the model was not adjusted for starting intensity, but the HR decreased to 0.95 and was significant when adjusted for the starting intensity (Table 16). This pattern of the 1 cigarette/day HR being lower for the models adjusted for starting intensity held for the other endpoints, but the HRs did not reach statistical significance.

Intensity Change – Categorical

Among the continuing smokers, there were 314 low intensity smokers, 235 medium intensity smokers, and 72 high intensity smokers at exam 1. After exam 2, 89 were classified as reducers and 41 as increasers. Compared to medium initial intensity smokers, the low intensity smokers had HRs less than 1 for all endpoints except stroke, and these HRs were significant for the CVDH, CVDA, CHDH, and CHDA endpoints (Table 17). In the same comparison, the high intensity smokers had HRs greater than 1 for all endpoints, but only for the death outcome was this HR significant. Compared to maintainers, or those who did not change category, the HRs for reducers were less than 1 except for stroke and death. The increasers had the opposite pattern, with HRs greater than 1 except for the stroke endpoint. However, none of the increaser or reducer HRs were statistically significant.

Summary

A reduction in current cigarette smoking intensity, modeled either categorically or continuously, was not associated with a reduction in CVD risk when not adjusted for starting intensity. After adjustment for starting intensity, a reduction in intensity of 1 cigarette/day was associated with a HR of 0.99 to 0.95 for the four main CVD endpoints, though the association was only statistically significant for CHDA.

4 Discussion

4.1 Modeling Smoking as an Exposure

Never/Former/Current

Smoking status is an important factor for CVD risk in this study as well as in previous studies. Current smokers are at a significantly higher risk for CVDA, CHDA, CVDH, and CHDH events compared to never smokers, but former smokers are not (Table 1). This agrees with the findings of Huxley *et al* and the TLGS cohort in terms of the CVD endpoint (1,2). These results imply that the main group that experiences increased CVD risk from smoking are the current smokers. So in the setting of limited degrees of freedom, the former smoker indicator variable could be omitted.

Intensity/Duration/Pack-Years

In smoking status stratified models, intensity of smoking is significantly associated with CVD outcomes in current smokers but not in former smokers in stratified models (Table 2). This conclusion is also supported by the pooled models for CVDH, CVDA, CHDH, and CHDA (Table 9). In these models, intensity in current smokers is significantly greater than one, and the interaction of former status and intensity is significantly less than one. These results indicate that the intensity effect is much weaker among former smokers and is effectively null. The effect of current smoking intensity is similar across endpoints (Figure 2). Previous studies agree that higher intensity increases risk of CVD in current smokers, but the impact in former smokers was not investigated (1,2,3,4). The impact of intensity increases linearly as no non-linearity was detected in this study (Figure 1). That is, at any level of smoking an additional 20 cigarettes/day is associated with an estimated 70-100% higher cardiovascular risk. This differs from some previous studies which observe a leveling off of the risk at high intensities, but this may be due to the use of categorical instead of continuous intensity or a different range of investigated intensities (1,4). Current smoking intensity is not significant for stroke, CVD death, or DVTPEM, which may be due to decreased power since there are fewer events in these endpoints, or differences in biological pathways. Higher smoking intensity increases the risk of death for both former and current smokers. The significance of former smoking is probably due to the large variety of reasons for death, including cancer. The impact of intensity on the competing risks of death from cancer, CVD, and other outcomes is a direction for future work.

Higher duration of smoking is not associated with CVD outcomes in either current or former smokers (Table 2,9). This is consistent with previous studies, which have also failed to detect this association (5,7). Taken together with the intensity and status findings, this suggests that former smokers, regardless of how long or how much they smoked, are no longer at increased CVD risk.

Pack-years of smoking follows the same pattern as intensity, in that for CVDH, CVDA, CHDH, and CHDA higher pack-years is associated with higher risk in current but not former smokers (Table 2). Also like intensity, no non-linearity is detected in the pack-years effect (Figure 1). Since duration is not associated with outcomes, the pack-years effect is likely due to the intensity component. In fact, in current smokers when models with pack-years are compared to models with intensity, pack-years performs worse in both the R^2 and AUC measurements for CVDH and CVDA, and has approximately the same performance for CHDH and CHDA (Table 3). In addition, adding duration to intensity in these models does not improve the AIC. These results suggest that duration does not add information and that the use of the pack-years variable can result in a loss of information since it does not estimate the intensity effect as accurately. Mannan *et al*, in agreement with our study, found that among current smokers increased categories of pack-years were associated with increased CVD risk (7). However, Mannan *et al* reports that a model including pack-years fit better than a model including intensity/duration in terms of AIC (7). The difference between our study and Mannan *et al* may be due to the fact that Mannan *et al* use categories instead of continuous measurements which inflate the number of variables used in the intensity/duration model, as well as the fact that the pack-years model is additionally adjusted for time since quitting (7). These results suggest that only current smoking intensity is significant for CVD risk, not cumulative exposure.

Age at Starting Smoking

In this study, there was no effect of age at starting smoking in any smoking status group on risk of any CVD outcome (Table 5). This result agrees with the results from the Nurses' Health Study, and implies that smoking at an earlier age does not pose an additional risk for CVD later in life compared to those who start later (3).

Time Since Quitting Smoking

Whether it was modeled as a continuous or categorical variable, time since quitting smoking was not associated with CVDH, CVDA, CHDH or CHDA (Table 6, 7). This contrasts with the Nurses' Health Study, which reaches the intuitive conclusion that risk of CVD is higher for people who quit more recently (3). This could represent a difference in the effect of time since quitting between women and men since the NHS only included women and MESA is half men (3). However, there was no evidence of an interaction between gender and time since quitting in MESA. In addition, the impact of smoking may vary significantly within the first year of quitting. Since MESA only measures time since quitting in one year increments, this possible source of variation cannot be explored.

Other Forms of Tobacco Exposure

Secondhand smoke, pipe, cigar, and chewing tobacco exposure were not significantly associated with CVD risk, suggesting that exposure to these alternative forms of tobacco are not harmful in terms of CVD outcomes (Table 8). This does not agree with multiple other studies, which have found increased CVD risks associated with secondhand smoke exposure and current cigar and pipe use (10,11,12). The MESA results may differ since they were additionally adjusted for cigarette use. There were also very few current cigar (131), pipe (42), and chewing tobacco (21) users, so there was limited power to detect any of these effects. Secondhand smoking was also self-reported (as was all of the smoking information) and this may be particularly hard to estimate accurately. So there may be a large amount of reporting error in the secondhand smoking estimates as compared to studies which measure a biomarker of tobacco exposure, such as Pope *et al* (4).

Risk Prediction

In CVD risk scores, current smoking status is often the way that smoking is incorporated into the estimation of future risk (18). We found that adding current smoking intensity to classic cardiovascular risk factors including current smoking status increased the AUC by 0.003. This difference was close to statistical significance and suggests that current smoking intensity may improve CVD risk prediction, but only quite modestly.

Compound Smoking Indices

Fitting the compound smoking indices X1 and X2 from Leffondré *et al* (15) revealed some interesting patterns among the eight examined endpoints (Table 10). First, the best fitting X1 and X2 in the same endpoint have very similar AICs and similar values of the parameters δ and τ. There are not major differences between the two forms of the index so X2 will be focused on. One pattern that emerged is that the δ parameter for all endpoints except for death and DVTPEM is zero. This means there is no lag time between the exposure to smoking and the outcome, implying that smoking is immediately increasing the risk for these outcomes. For death, the lag time is a bit longer at two years, which might be due to the effect on cancer. Cancer takes longer to develop after smoking and since death involves both CVD deaths and cancer deaths this may have increased the best fitting δ. The best fitting δ for DVTPEM was the maximum tested of nine years. This indicates that it takes longer after the exposure to cigarette smoking for DVTPEM to occur.

For many endpoints (CVDH, CHDH, Stroke, CVD death, and death), the best fitting index involved a τ of one, the minimum tested value. As τ is the "half-life" parameter, this implies that the risk of these endpoints drops very quickly after the exposure to smoking (15,16). This agrees with the previously stated conclusion that only current smoking intensity is important, not pack-years or duration of exposure. The best fitting τ is larger than one for CVDA, CHDA, and DVTPEM. For these endpoints, the risk associated with smoking decreases more slowly, or lingers longer, after the last exposure to smoking. Angina is included in the CVDA and CHDA endpoints but not the CVDH or CHDH endpoint, and this may have impacted the estimated τ. It may be valuable in future studies to fit the X1 and X2 indices for angina endpoints separately. DVTPEM has a long lag time and half-life compared to the other endpoints, and this may be due to the difference in etiology between the CVD endpoints and

DVTPEM. DVTPEM is a major venous clotting event, while CVD is a disease of the arteries. One mechanism that could link smoking to DVTPEM is the increase in atherosclerosis in smokers, leading to slower blood return and venous stasis. This pathway through venous atherosclerosis and decreased blood flow almost certainly plays a greater role in venous clotting events relative to MI or stroke, and this may be why the DVTPEM outcome has such different δs and τs than other CVD events.

Comparing Models of Smoking as an Exposure

It is important to know the best way to model smoking, both to understand what aspects of smoking impact CVD risk, as well as to properly adjust for smoking as a confounder in any study that uses CVD as an outcome. After comparing multiple models (Table 11), for CVDH, CVDA, CHDH, CHDA, and CVD death the best model fit comes from adjusting for only one measurement of smoking. For all five of these outcomes, using only current smoking intensity or the compound index X2 produces the lowest AIC and the best fitting model. X2 and current smoking intensity models have AICs within 2 units of the other, indicating that there is no substantial difference in goodness of fit (16,39; page 70). This again supports the idea that current smoking intensity alone is significant for CVD risk. In addition, the model involving only smoking status (never/former/current) was often 10 or more units higher than the best fitting model, indicating that using smoking status alone is a poor way to adjust for smoking. Since current smoking intensity requires no extra calculation, for these outcomes it is best to use current smoking intensity alone as the measurement of smoking.

The stroke outcome has a very similar pattern to the five previously discussed outcomes, but the best fitting model for stroke is the X2 only model and the current smoking intensity only model has an AIC 3 units larger. This difference is marginally important and though current smoking intensity provides a very good model fit, fitting the X2 index is slightly better.

For death, X1 and X2 have the lowest AIC by 13 points, suggesting that these measures better capture the effect of smoking on the many causes of death, including CVD and cancer. The compound indices may better model the importance of former and current smoking intensity, and the lag time and half-life parameters for the death outcome. For DVTPEM, the X1 and X2 models are within 3 points of the best fitting model, which is the rather complex model including never smoking status, pack-years, years since quitting, and the interaction of pack-years and years since quitting. Again, this suggests that DVTPEM involves different biological pathways than the other CVD outcomes since factors such as time since quitting that are not significant for other CVD outcomes improve model fit. For death and DVTPEM, it is probably best to fit the X2 index and use this to adjust for smoking.

Updating Smoking Intensity

Using time-updated current smoking status and intensity did not produce very different estimates of the effect of these variables compared to using baseline measurements only (Table 12). There was no pattern of improvement in the AIC from using the time-updated covariates, though the time-updated models did fit slightly better for the CHDH and CHDA outcomes. There is no compelling evidence that the smoking as an exposure is captured more effectively if the smoking status and intensity variables are updated over time. In addition, in the baseline model, there was current smoking status was not significantly associated with CVD risk after adjusting for current smoking intensity. These results also support the conclusion that using current smoking intensity alone is the best way to adjust for smoking in models where the outcome is CVD.

Conclusions – Modeling Smoking as an Exposure

In conclusion, in the MESA study only current smoking intensity is significant for CVD risk. For coronary outcomes, including CVDH, CVDA, CHDH, CHDA, and CVD death, in order to adjust for smoking it is recommended to adjust for only current smoking intensity as a linear covariate. For vascular-related outcomes such as stroke, death, and DVTPEM, estimating the δ and τ parameters and using the X2 index is the best way to adjust for smoking.

4.2 Mediation – Smoking-CVD Pathways

Atherosclerosis

The effect of current smoking on CVDH, CVDA, CHDH, CHDA, stroke, and CVD death is strongly mediated through both the presence of any CAC and the amount of CAC (Table 14). The percentage mediated is larger through the amount of CAC than just the presence or absence of CAC. Smoking increases risk of these outcomes through the atherosclerosis pathway both through promoting the initiation of CAC formation and the progression of CAC over time. CAC is not as strong a mediator for death or DVTPEM since it is not as strongly associated with these outcomes. This is most likely due to death being a composite outcome of cardiac and non-cardiac events, and DVTPEM being a major clotting event in the veins instead of in the arteries and having a different etiology than the four primary cardiovascular endpoints.

Hemodynamics

None of the examined hemodynamics measurements are significant mediators of the current smoking effect on any outcome. Higher heart rate and systolic blood pressure are associated with higher risks of the CVD outcomes, but current smokers do not have significantly higher levels of these factors (Table 13, 14). It is unexpected that current smokers do not have higher systolic blood pressure on average than non-smokers, but this may be due to the way the mediators were measured. In MESA, participants were instructed to fast overnight before their labs were taken, and this fasting included smoking. Therefore if a smoked cigarette influences the mediator only temporarily and the levels return to normal within eight hours or so, this impact on the mediator might be missed. It is also unexpected that current smokers have lower average systolic blood pressure as well as lower rates of hypertension than the non-current smokers, and this disagrees with the findings of Barutcu *et al* (23). This may be the result of a "healthy smoker bias", which is discussed in Hudson *et al,* where people who continue smoking are able to survive the harmful effects of smoking by being slightly healthier in other cardiovascular risk factors such as systolic blood pressure (16). Current cigarette smoking increases cardiac output, but cardiac output is not associated with any of the outcomes. Therefore there is no evidence that the harmful effects of smoking on CVD are acting through the hemodynamics pathway as measured by systolic blood pressure, cardiac output, and heart rate.

Inflammation

The effect of current smoking status is moderately mediated through TNFA and IL-6, but not through CRP. CRP is not a strong mediator since it is not statistically significantly associated with current smoking status. The mediation percentage through IL-6 and TNFA is higher for stroke, CVD death, death, and DVTPEM, so raised levels of these two proteins may be more important in promoting these outcomes. IL-6, TNFA, and the whole inflammation pathway could be promising targets for future studies of the impact of smoking on CVD.

Coagulation

Out of the four possible mediators in the coagulation pathway, only fibrinogen is a moderate mediator of the smoking-CVD relationship for all eight outcomes. Though D-dimer and Factor VIII are associated with CVD outcomes, Factor VIII has very low mediation percentages for all outcomes, and D-dimer only shows moderate mediation for CVD death and death. The number of events for DVTPEM is low and the confidence interval around the mediation percentage is large. Perhaps D-dimer is a mediator for the outcomes where death results and not the less lethal events, while fibrinogen affects all CVD events.

Conclusions – Mediation – Smoking-CVD Pathways

Current smoking status increases CVD risk by affecting the atherosclerosis, inflammation, and coagulation pathways. CAC, IL-6, TNFA, fibrinogen, and D-dimer are the main mediators and would be possible targets for future studies or interventions. These may be the largest mediators due to their functions in the pathways of interest or because they were measured more accurately. A limitation of this study is that mediators were measured hours after the last smoked cigarette and so short-lived effects may have been underestimated. Future studies may want to measure the potential mediators sooner after cigarettes are smoked.

4.3 Smoking Intensity Reduction

Quitting smoking is very beneficial for CVD risk, as former smokers do not have significantly higher hazards than never smokers, while current smokers do (Table 1). In addition, evidence is present in MESA for increasing CVD risk with increasing current smoking intensity (Table 2). Among participants with the same initial smoking intensity, those who decrease their intensity tend to have a lower hazard for all events. This effect did not reach statistical significance, but we hypothesize that this is due to the small sample size. The only exceptions are the stroke outcome, which has very few events and so strong conclusions cannot be drawn, and CHDA, which reaches statistical significance. For CHDA, the effect of a decrease of one cigarette/day is a 5% decrease in hazard (Table 16). Controlling for initial intensity was important since when initial intensity was added to the models, the HRs associated with intensity reduction decreased for every examined endpoint. The expected trends of reducers having lower risk and higher initial intensity smokers having higher risk are observed when categories are used but these results also failed to reach statistical significance (Table 17). These results indicate that decreasing intensity may decrease risk of CVD, but this study was not powered to detect the difference in most outcomes. Two previous studies detected no effect of intensity reduction on CVD death, while a third study did find a beneficial effect of reduction (19,20,21). The limited number of events and the small range of observed smoking intensity changes in this study make it difficult to detect the impact of intensity reduction. Though the relationship between current smoking intensity and CVD outcomes is demonstrated in this and previous studies, more work is still needed to quantify the possible benefits to public health that may result from intensity reduction strategies.

Overall Conclusions

In the MESA study, the best way to model smoking for CVD outcomes is either current smoking intensity or compound smoking index X2 from (16). Smoking indices X1 or X2 seem to be more appropriate for all-cause mortality. Using smoking status alone (never/former/current) alone is insufficient. Duration was not associated with CVD, while current intensity was, and so pack-years is not an optimal way to model smoking as an exposure for CVD endpoints. Changes in CAC, IL-6, TNFA, fibrinogen, and D-dimer due to smoking partially explain the impact of current smoking status on CVD; though more work needs to be done to confirm these results.

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6 Figures and Tables

Figure 1. GAM plots for Intensity, Duration, Pack-Years, Age at start, and Years since quitting for the CVDH endpoint.

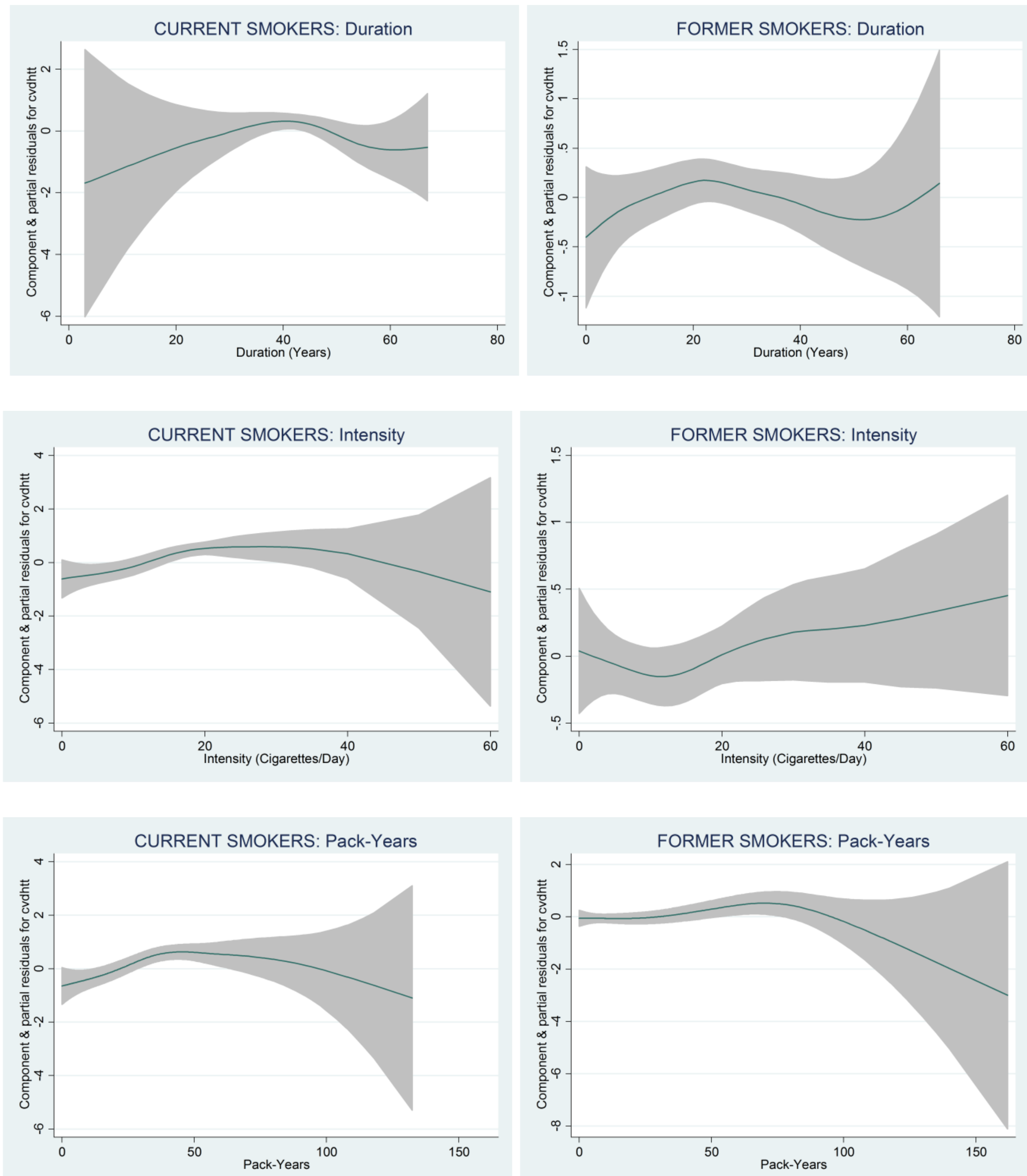


Table 1. Cox models adjusted for age, gender, race, education, income and cigarette smoking status (never smokers are the comparison group).

Hazard Ratio 95% CI P-value	CVDH	CVDA	CHDH	CHDA
Current Smoker	1.98 1.51,2.60 <0.0005	1.80 1.42,2.29 <0.0005	1.94 1.38,2.74 <0.0005	1.66 1.23,2.22 0.001
Former Smoker	0.89 0.72,1.11 0.308	1.06 0.89,1.27 0.496	0.91 0.69,1.20 0.507	1.13 0.92,1.40 0.251

Table 2. Cox models adjusted for age, gender, race, education, income and one of duration, intensity, and pack years.

Hazard Ratio 95% CI P-value	CVDH		CVDA		CHDH		CHDA	
	Current	Former	Current	Former	Current	Former	Current	Former
Intensity (Packs/day)	1.92 1.33,2.76 <.0005	1.11 0.90,1.38 0.328	1.69 1.20,2.38 0.003	1.06 0.90,1.27 0.477	2.00 1.29,3.09 0.002	1.17 0.90,1.52 0.235	1.83 1.23,2.73 0.003	1.12 0.92,1.36 0.260
Duration	1.00 0.96,1.04 0.849	1.00 0.99,1.01 0.827	1.00 0.97,1.04 0.816	1.00 0.99,1.01 0.463	1.03 0.97,1.09 0.289	1.01 1.00,1.03 0.135	1.04 0.99,1.10 0.140	1.01 1.00,1.02 0.034
Pack-Years	1.01 1.01,1.02 0.001	1.00 1.00,1.01 0.597	1.01 1.00,1.02 0.007	1.00 1.00,1.01 0.299	1.01 1.01,1.02 0.001	1.00 1.00,1.01 0.26	1.01 1.00,1.02 0.003	1.00 1.00,1.01 0.089

Table 3. Information Comparing Duration & Intensity (Packs/day) versus Pack Years Within Current Smokers.

Outcome - # outcomes	Age, Gender, Race, Education, Income Adjusted		
n=823	AIC	R^2	AUC
CVDH - 79			
Intensity	1020	0.264	0.696
Intensity & Duration	1022	0.264	0.696
Pack Years	1021	0.256	0.693
CVDA - 98			
Intensity	1257	0.270	0.698
Intensity & Duration	1259	0.271	0.700
Pack Years	1259	0.262	0.696
CHDH - 51			
Intensity	670	0.262	0.677
Intensity & Duration	671	0.267	0.679
Pack Years	670	0.264	0.676
CHDA - 66			
Intensity	858	0.277	0.695
Intensity & Duration	858	0.287	0.700
Pack Years	858	0.273	0.697

Table 4. Cox models pooled with former, never, and current smokers, in both the intensity/duration and pack-years models.

Model Used: All MESA members, the listed variables plus age, sex, race, education, and income				
Hazard Ratio 95% CI P-value	CVDH	CVDA	CHDH	CHDA
Former Smoker	0.89 0.72,1.12 0.321	1.06 0.88,1.27 0.559	0.88 0.66,1.16 0.362	1.10 0.89,1.37 0.368
Current Smoker	1.98 1.48,2.64 <0.0005	1.77 1.37,2.28 <0.0005	1.83 1.27,2.65 0.001	1.54 1.13,2.10 0.007
Intensity (Packs/day)	1.85 1.33,2.57 <0.0005	1.63 1.20,2.22 0.002	1.99 1.34,2.96 0.001	1.76 1.23,2.51 0.002
Duration	1.00 0.99,1.01 0.527	1.00 0.99,1.01 0.830	1.00 0.99,1.02 0.481	1.01 1.00,1.02 0.090
Former*Intensity	0.61 0.41,0.90 0.013	0.64 0.45,0.91 0.012	0.57 0.36,0.91 0.019	0.59 0.40,0.89 0.011
Current*Intensity	Omitted	Omitted	Omitted	Omitted
Model Used: All MESA members, the listed variables plus age, sex, race, education, and income				
	CVDH	CVDA	CHDH	CHDA
Former Smoker	0.88 0.71,1.10 0.268	1.05 0.88,1.26 0.593	0.88 0.66,1.16 0.360	1.11 0.90,1.38 0.324
Current Smoker	1.89 1.43,2.52 <0.0005	1.76 1.37,2.25 <0.0005	1.85 1.30,2.66 0.001	1.59 1.17,2.16 0.003
Pack-years	1.01 1.00,1.02 0.007	1.01 1.00,1.01 0.019	1.01 1.00,1.02 0.007	1.01 1.00,1.02 0.007
Former*Pack-years	0.99 0.98,1.00 0.099	0.99 0.99,1.00 0.171	0.99 0.98,1.00 0.199	0.99 0.98,1.00 0.163
Current*Pack years	Omitted	Omitted	Omitted	Omitted

Table 5. Age at starting cigarette smoking. Current and former smokers modeled separately, adjusted for age, gender, race, education, income, and intensity.

Hazard Ratio 95% CI P-value	CVDH	CVDA	CHDH	CHDA
Age at start - current smokers	1.00 0.96,1.04 0.884	0.99 0.95,1.03 0.580	0.98 0.93,1.04 0.509	0.97 0.92,1.02 0.268
Age at start - former smokers	0.99 0.96,1.02 0.632	0.99 0.96,1.01 0.287	0.98 0.94,1.02 0.402	0.97 0.94,1.01 0.110

Table 6. Years since quitting cigarette smoking. Only former smokers were included, adjusted for age, gender, race, education, income, and intensity.

Hazard Ratio 95% CI P-value	CVDH	CVDA	CHDH	CHDA
Years since quitting - former smokers	1.00 0.99,1.02 0.705	1.00 0.99,1.01 0.920	0.99 0.98,1.01 0.426	0.99 0.98,1.00 0.224

Table 7. Time since quitting cigarette smoking categories. All subjects were included, adjusted for age, gender, race, education, income, and intensity.

Hazard Ratio 95% CI P-value	CVDH	CVDA	CHDH	CHDA
Categories -				
Never (n=3418)	1.00(Ref)	1.00(Ref)	1.00(Ref)	1.00(Ref)
Former (n=1399) 20 or more years since quitting	0.91 0.71,1.18 0.491	1.05 0.85,1.29 0.661	0.88 0.63,1.21 0.423	1.06 0.83,1.36 0.649
Former (n=292) 15 to <20 years since quitting	0.88 0.53,1.45 0.621	1.10 0.74,1.63 0.645	1.04 0.58,1.85 0.907	1.34 0.87,2.06 0.190
Former (n=280) 10 to <15 years since quitting	0.47 0.23,0.96 0.039	0.74 0.45,1.20 0.222	0.65 0.30,1.39 0.263	0.86 0.50,1.49 0.593
Former (n=228) 5 to <10 years since quitting	0.90 0.51,1.58 0.710	1.22 0.80,1.88 0.353	0.76 0.35,1.63 0.477	1.39 0.85,2.27 0.186
Former (n=241) <5 years since quitting	1.11 0.64,1.92 0.719	1.30 0.83,2.02 0.247	1.10 0.55,2.19 0.786	1.31 0.78,2.20 0.309
Current (n=887)	2.00 1.52,2.63 <0.0005	1.82 1.43,2.32 <0.0005	2.00 1.41,2.82 <0.0005	1.70 1.27,2.28 <0.0005

Table 8. Other forms of smoking: Pipe, cigar, chewing tobacco, secondhand smoke. Adjusted for age, gender, race, cigarette smoking status, cigarette intensity, cigarette duration, interaction of cigarette status and intensity, education and income.

Hazard Ratio 95% CI P-value	CVDH	CVDA	CHDH	CHDA
Cigar use former n=505	0.77 0.53,1.12 0.174	0.82 0.61,1.11 0.195	0.71 0.45,1.13 0.146	0.82 0.58,1.14 0.233
Cigar use current n=131	1.42 0.79,2.56 0.241	1.05 0.61,1.80 0.858	1.09 0.51,2.34 0.831	0.71 0.35,1.45 0.350
Pipe use former n=526	0.93 0.65,1.32 0.670	0.90 0.68,1.20 0.466	0.86 0.56,1.32 0.485	0.84 0.60,1.16 0.283
Pipe use current n=42	1.42 0.52,3.84 0.495	1.25 0.55,2.82 0.595	1.57 0.49,4.98 0.445	0.81 0.26,2.55 0.722
Chewing tobacco use former n=82	1.54 0.79,3.03 0.208	1.51 0.86,2.64 0.149	1.40 0.61,3.20 0.427	1.15 0.56,2.33 0.709
Chewing tobacco use current n=21	0.63 0.09,4.51 0.645	0.37 0.05,2.66 0.325	Cannot be estimated	Cannot be estimated
Secondhand smoke exposed n=1208	0.78 0.57,1.06 0.115	0.80 0.61,1.05 0.104	0.67 0.44,1.00 0.052	0.77 0.56,1.07 0.120
Secondhand smoke Intensity (hours per week)	0.99 0.97,1.00 0.111	1.00 0.98,1.01 0.425	0.98 0.96,1.01 0.137	0.99 0.98,1.01 0.342

Table 9. Cox models pooled with former, never, and current smokers, comparing all eight outcomes.

Model Used: All MESA members, the listed variables plus age, sex, race, education, and income				
Hazard Ratio 95% CI P-value	CVDH	CVDA	CHDH	CHDA
Former Smoker	0.89 0.72,1.12 0.321	1.06 0.88,1.27 0.559	0.88 0.66,1.16 0.362	1.10 0.89,1.37 0.368
Current Smoker	1.98 1.48,2.64 <0.0005	1.77 1.37,2.28 <0.0005	1.83 1.27,2.65 0.001	1.54 1.13,2.10 0.007
Intensity (Packs/day)	1.85 1.33,2.57 <0.0005	1.63 1.20,2.22 0.002	1.99 1.34,2.96 0.001	1.76 1.23,2.51 0.002
Duration	1.00 0.99,1.01 0.527	1.00 0.99,1.01 0.83	1.00 0.99,1.02 0.481	1.01 1.00,1.02 0.090
Former*Intensity	0.61 0.41,0.90 0.013	0.64 0.45,0.91 0.012	0.57 0.36,0.91 0.019	0.59 0.40,0.89 0.011
	Stroke	CVD Death	Death	DVTPEM
Former Smoker	0.89 0.63,1.27 0.528	0.86 0.59,1.25 0.424	1.11 0.92,1.32 0.274	1.05 0.66,1.69 0.831
Current Smoker	1.88 1.18,3.00 0.008	1.77 1.03,3.02 0.038	2.07 1.62,2.65 <0.0005	1.37 0.70,2.70 0.359
Intensity (Packs/day)	1.45 0.84,2.49 0.184	1.46 0.82,2.59 0.202	1.54 1.21,1.96 <0.0005	1.72 0.72,4.14 0.225
Duration	0.99 0.97,1.00 0.103	1.00 0.98,1.01 0.694	1.01 1.00,1.02 0.058	1.00 0.98,1.02 0.905
Former*Intensity	0.67 0.35,1.29 0.233	0.82 0.42,1.61 0.560	0.77 0.58,1.03 0.079	0.99 0.39,2.50 0.978

Figure 2. Forest plot of the intensity HRs across endpoints from the pooled models including smoking status, intensity, duration, and the interaction of intensity and smoking status.

Event	No. Events current smokers	No. Events not current	HR current smoker	SE
CVDH	84	366	1.98	0.29
CVDA	104	535	1.77	0.23
CHDH	53	231	1.83	0.34
CHDA	69	380	1.54	0.24
Stroke	31	149	1.88	0.45
CVD Death	25	136	1.77	0.48
All Death	125	584	2.07	0.26

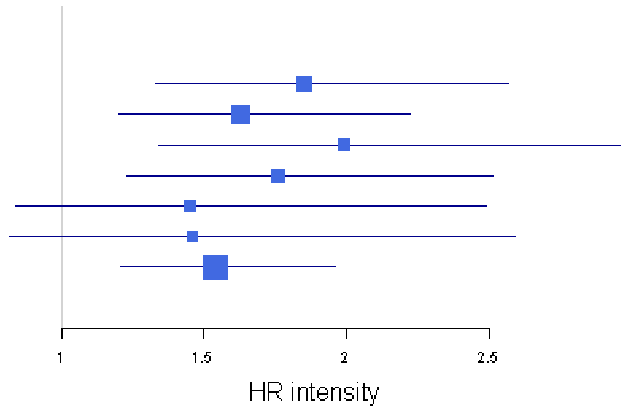
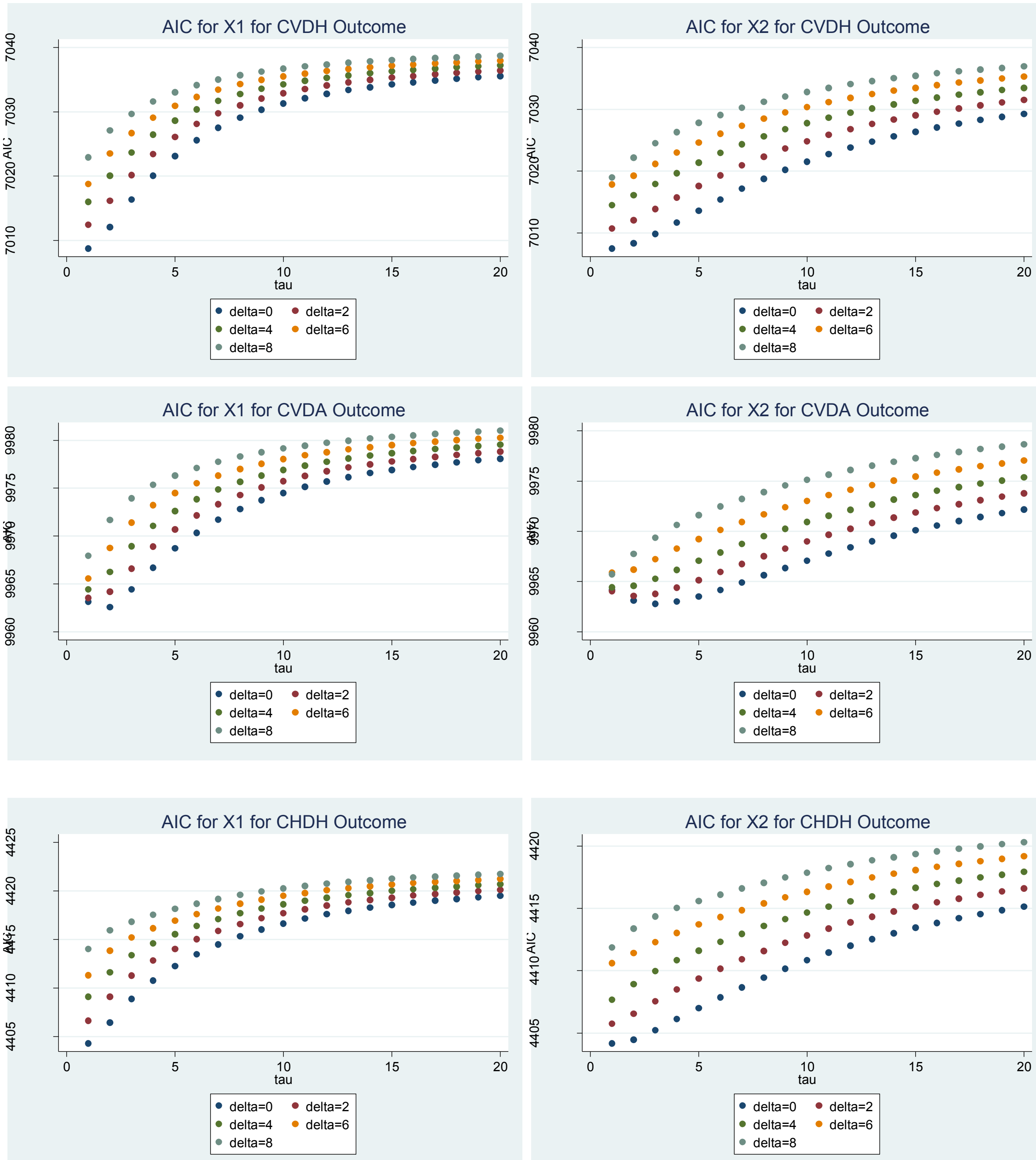
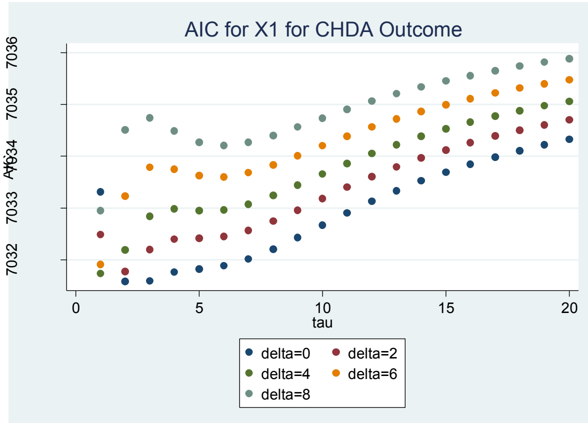
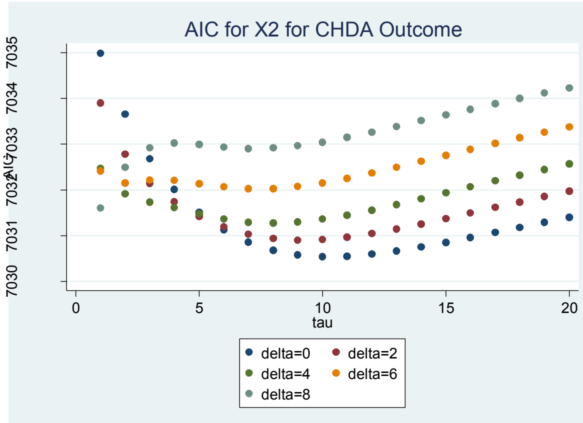
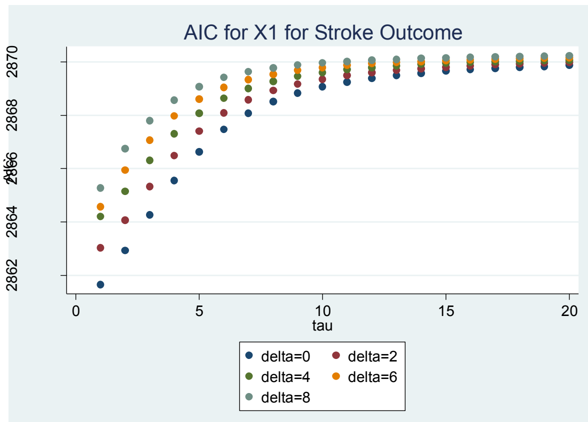


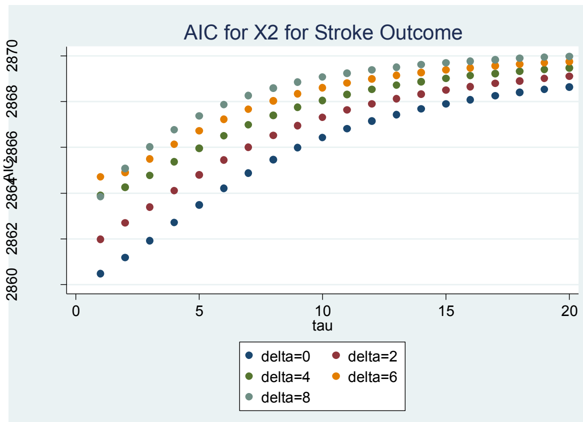
Figure 3. Scatterplots of the AIC from Cox models using X1 or X2 with a variety of δs and τs, and adjusted for age, sex, race, income, and education.




AIC for X1 for CHDA Outcome
7032
7033
7034
7035
7036
AIC
0
5
10
15
20
tau
delta=0
delta=2
delta=4
delta=6
delta=8


AIC for X2 for CHDA Outcome
7030
7031
7032
7033
7034
7035
AIC
0
5
10
15
20
tau
delta=0
delta=2
delta=4
delta=6
delta=8


AIC for X1 for Stroke Outcome
2862
2864
2866
2868
2870
AIC
0
5
10
15
20
tau
delta=0
delta=2
delta=4
delta=6
delta=8


AIC for X2 for Stroke Outcome
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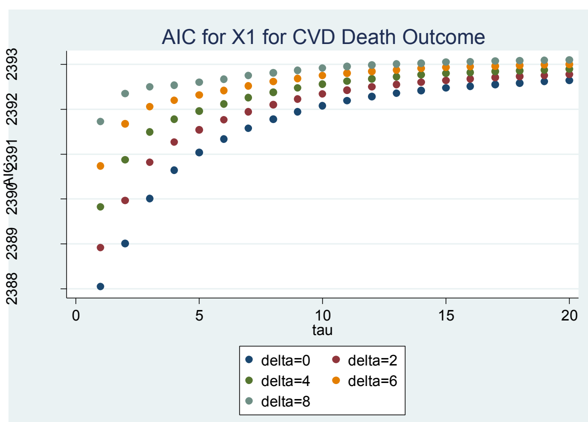
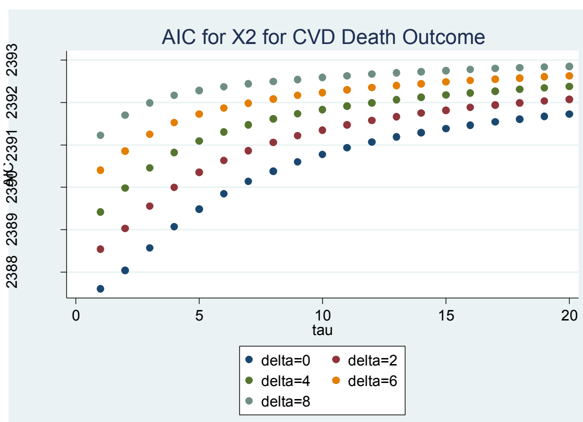
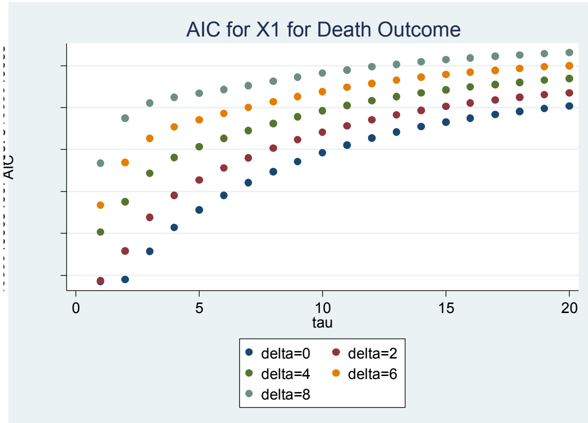
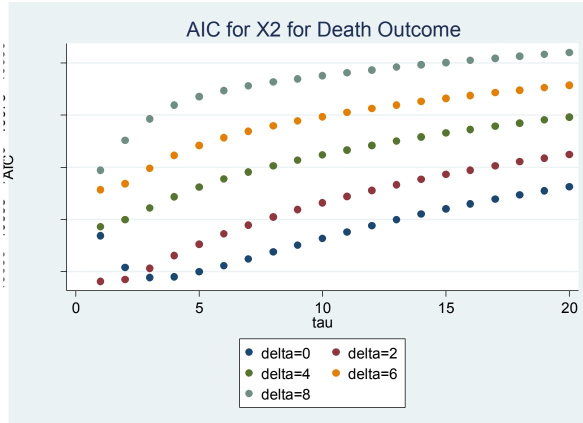
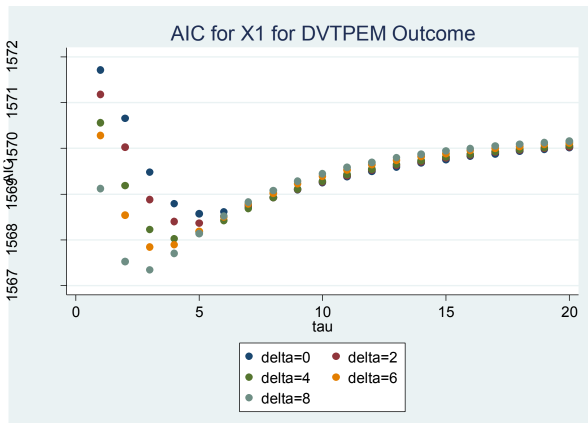
2862
2864
2866
2868
2870
AIC
0
5
10
15
20
tau
delta=0
delta=2
delta=4
delta=6
delta=8


AIC for X1 for CVD Death Outcome
2388
2389
2390
2391
2392
2393
AIC
0
5
10
15
20
tau
delta=0
delta=2
delta=4
delta=6
delta=8


AIC for X2 for CVD Death Outcome
2388
2389
2390
2391
2392
2393
AIC
0
5
10
15
20
tau
delta=0
delta=2
delta=4
delta=6
delta=8







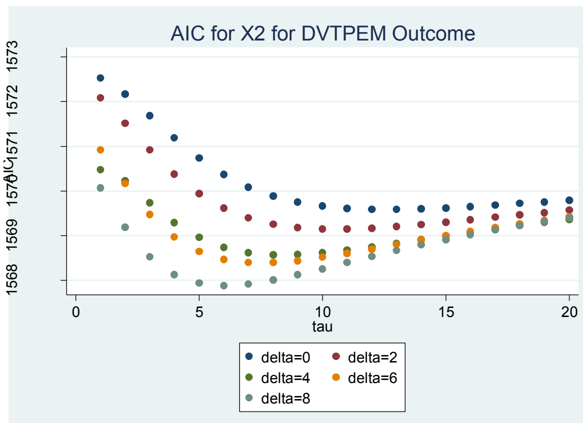


Table 10. Minimum AIC Associated δ and Tau.

	X1			X2		
Endpoint	δ	τ	AIC	δ	τ	AIC
CVDH	0	1	7009	0	1	7007
CVDA	0	2	9963	0	3	9963
CHDH	0	1	4404	0	1	4404
CHDA	0	2	7032	0	10	7031
Stroke	0	1	2862	0	1	2860
CVD Death	0	1	2388	0	1	2388
Death	1	1	10659	2	1	10659
DVT/PEM	9	2	1567	9	5	1568

Table 11. Comparing models of smoking in terms of AIC. All models were also adjusted for age, sex, race, income, and education, and only subjects with complete smoking status, intensity, pack-years, and time since quitting information were included.

AIC	CVDH	CVDA	CHDH	CHDA	Stroke	CVD Death	Death	DVT PEM
Never/Former/Current	7018	9973	4412	7040	2865	2390	10684	1574
Never, Pack-years, Years since quitting, Packyears*Years since quitting	7035	9980	4418	7037	2874	2397	10683	1565
Never/Former/Current, Intensity, Intensity*Former	7010	9968	4405	7035	2867	2392	10672	1569
Never/Former/Current, Current intensity	7009	9966	4404	7034	2865	2390	10676	1575
Current intensity	7009	9964	4402	7031	2863	2388	10683	1571
X1	7009	9963	4404	7032	2862	2388	10659	1567
X2	7007	9963	4404	7031	2860	2388	10659	1568

Table 12. Comparing baseline versus time updated models of current smoking status and intensity. Hazard ratios, 95% confidence intervals and p-values are displayed. Current smoking intensity is in units of packs/day, or 20 cigarettes/day.

	Baseline Only			Time Updated		
Outcome	Current Smoking Status	Current Smoking Intensity	AIC	Current Smoking Status	Current Smoking Intensity	AIC
CVDH	1.34 0.92,1.95 0.125	1.84 1.32,2.55 <0.0005	7123	1.51 1.01,2.25 0.042	1.79 1.20,2.67 0.005	7126
CVDA	1.23 0.88,1.72 0.232	1.63 1.20,2.21 0.002	10133	1.37 0.96,1.96 0.082	1.60 1.10,2.31 0.013	10133
CHDH	1.19 0.74,1.92 0.464	2.03 1.37,3.00 <0.0005	4499	1.34 0.83,2.19 0.235	2.23 1.43,3.47 <0.0005	4494
CHDA	1.00 0.66,1.51 0.997	1.79 1.25,2.55 0.001	7149	1.10 0.72,1.68 0.673	1.94 1.31,2.89 0.001	7145
Stroke	1.52 0.85,2.74 0.157	1.39 0.80,2.39 0.241	2883	1.83 0.93,3.60 0.080	0.97 0.41,2.31 0.951	2886
CVD Death	1.35 0.71,2.56 0.359	1.50 0.85,2.63 0.161	2423	2.04 0.99,4.20 0.052	1.07 0.41,2.79 0.882	2421
Death	1.48 1.11,1.98 0.007	1.56 1.23,1.98 <0.0005	10811	1.11 0.78,1.57 0.562	2.30 1.64,3.21 <0.0005	10816
DVTPEM	0.88 0.34,2.28 0.792	1.70 0.71,4.04 0.232	1591	0.83 0.29,2.33 0.721	1.88 0.70,5.06 0.210	1591

Table 13. Linear regression of mediators versus current smoking status adjusted for age, sex, race, income, education, alcohol use.

Mediator	N	Current Smoking Regression Coefficient	P-value
CAC (yes/no)	6517	0.081	<0.0005
log(CAC+25)	6517	0.168	<0.0005
Factor VIII	6472	-3.876	0.005
D-Dimer	6476	0.083	0.009
Fibrinogen	6474	10.871	<0.0005
PAP	6339	0.221	0.005
IL-6	6341	0.249	<0.0005
CRP	6470	0.418	0.055
TNFA	2769	72.487	0.001
SBP	6514	-1.122	0.133
Cardiac Output	4804	-0.233	<0.0005
Heart Rate	6470	0.345	0.343

Table 14. Cox regression models of how mediators are associated with outcomes, adjusted for age, sex, race, income, education, and alcohol use. HR/P-values are reported.

Mediator	CVDH	CVDA	CHDH	CHDA
CAC (yes/no)	2.57/<0.0005	3.23/<0.0005	3.57/<0.0005	4.79/<0.0005
log(CAC+25)	1.45/<0.0005	1.56/<0.0005	1.58/<0.0005	1.72/<0.0005
Factor VIII	1.00/0.033	1.00/0.001	1.00/0.014	1.00/0.001
D-Dimer	1.08/0.013	1.06/0.067	1.11/0.002	1.07/0.046
Fibrinogen	1.00/<0.0005	1.00/<0.0005	1.00/<0.0005	1.00/<0.0005
PAP	1.03/0.083	1.01/0.569	1.03/0.076	1.00/0.862
SBP	1.02/<0.0005	1.01/<0.0005	1.01/<0.0005	1.01/<0.0005
IL-6	1.16/<0.0005	1.14/<0.0005	1.12/0.005	1.10/0.004
CRP	1.01/0.022	1.01/0.006	1.01/0.096	1.01/0.015
TNFA	1.00/<0.0005	1.00/<0.0005	1.00/<0.0005	1.00/<0.0005
Cardiac Output	1.01/0.777	0.99/0.699	0.99/0.788	0.97/0.390
Heart Rate	1.02/<0.0005	1.02/<0.0005	1.02/<0.0005	1.02/<0.0005
Mediator	Stroke	CVD Death	All Death	DVT/PEM
CAC (yes/no)	1.66/0.006	1.78/0.007	1.35/0.002	1.15/0.550
log(CAC+25)	1.26/<0.0005	1.34/<0.0005	1.20/<.00005	1.05/0.580
Factor VIII	1.00/0.476	1.00/0.017	1.00/<0.0005	1.01/<0.0005
D-Dimer	0.99/0.926	1.13/0.001	1.13/<0.0005	1.20/<0.0005
Fibrinogen	1.00/0.001	1.00/0.002	1.00/<0.0005	1.00/0.058
PAP	1.01/0.803	1.03/0.293	1.05/<0.0005	1.07/0.002
SBP	1.02/<0.0005	1.01/0.003	1.00/0.202	1.00/0.961
IL-6	1.22/<0.0005	1.24/<0.0005	1.23/<0.0005	1.30/<0.0005
CRP	1.02/0.031	1.02/0.042	1.02/<0.0005	1.01/0.280
TNFA	1.00/<0.0005	1.00/0.112	1.00/<0.0005	1.00/0.001
Cardiac Output	1.07/0.273	1.03/0.633	0.99/0.722	1.04/0.688
Heart Rate	1.02/0.005	1.03/0.001	1.02/<0.0005	1.01/0.419

Table 15. Percentage of current smoking effect mediated through twelve mediators for eight outcomes.

Mediator	CVDH	CVDA	CHDH	CHDA
CAC (yes/no)	8.5%(6.1%, 13.8%)	15.1%(10.3%,28.1%)	10.9%(7.3%,21.7%)	24.1%(14.0%,81.3%)
log(CAC+25)	11.9%(8.6%,19.2%)	21.9%(14.9%,39.8%)	15.2%(10.1%,29.8%)	33.8%(19.7%,109.6%)
Factor VIII	-1.5%(-2.5%,-1.1%)	-3.1%(-5.8%,-2.1%)	-2.3%(-4.5%,-1.5%)	-4.7%(-15.7%,-2.7%)
D-Dimer	1.5%(1.1%,2.4%)	1.2%(0.8%,2.2%)	2.9%(2.0%,5.7%)	2.3%(1.3%,7.6%)
Fibrinogen	4.9%(3.5%,7.9%)	7.1%(4.8%,13.2%)	5.3%(3.5%,10.4%)	10.0%(5.8%,34.2%)
PAP	1.0%(0.7%,1.5%)	0.3%(0.2%,0.5%)	1.3%(0.9%,2.6%)	-0.1%(-0.4%,-0.1%)
IL-6	5.7%(4.1%,9.0%)	7.7%(5.3%,13.9%)	4.4%(3.0%,8.5%)	7.2%(4.2%,23.4%)
CRP	0.9%(0.6%,1.4%)	1.5%(1.0%,2.9%)	0.8%(0.5%,1.6%)	2.3%(1.3%,7.8%)
TNFA	5.3%(3.8%,8.6%)	4.6%(3.3%,7.7%)	5.2%(3.4%,10.5%)	4.5%(3.0%,8.7%)
SBP	-3.3%(-5.3%,-2.3%)	-4.0%(-7.5%,-2.7%)	-2.2%(-4.4%,-1.5%)	-3.9%(-13.1%,-2.2%)
Cardiac Output	-0.8%(-1.2%,-0.6%)	0.0%(0.0%,0.1%)	0.2%(0.1%,0.4%)	1.4%(0.9%,3.8%)
Heart Rate	0.7%(0.5%,1.2%)	0.9%(0.6%,1.7%)	0.7%(0.5%,1.5%)	1.0%(0.6%,3.7%)
Mediator	Stroke	CVD Death	All Death	DVT/PEM
CAC (yes/no)	6.0%(3.4%,22.3%)	6.4%(3.1%,39.3%)	2.7%(2.1%,3.9%)	2.5%(-34.9%,31.5%)
log(CAC+25)	9.1%(5.1%,35.4%)	14.0%(6.8%,79.2%)	6.8%(5.2%,9.9%)	1.8%(-27.1%,23.4%)
Factor VIII	-0.8%(-2.9%,-0.5%)	-3.9%(-22.0%,-1.9%)	-2.4%(-3.5%,-1.8%)	-14.6%(-247.1%,170.1%)
D-Dimer	-0.9%(-3.5%,-0.5%)	6.4%(3.1%,34.3%)	4.9%(3.7%,7.1%)	12.3%(-202.1%,139.3%)
Fibrinogen	6.5%(3.7%,23.9%)	6.6%(3.2%,36.8%)	3.8%(2.9%,5.6%)	7.0%(-95.7%,86.8%)
PAP	0.2%(0.1%,0.5%)	2.1%(1.1%,8.8%)	3.1%(2.4%,4.7%)	8.0%(-220.2,106.3%)
IL-6	9.9%(5.8%,32.2%)	15.7%(6.8%,132.8%)	11.2%(8.3%,17.3%)	23.8%(-404.4%,329.9%)
CRP	1.9%(1.1%,6.9%)	1.8%(0.9%,9.3%)	2.1%(1.6%,3.2%)	1.2%(-17.9%,16.0%)
TNFA	7.5%(4.4%,22.8%)	5.1%(-52.4%,64.7%)	12.6%(8.7%,22.3%)	14.1%(-290.8%,176.6%)
SBP	-5.5%(-20.0%,-3.1%)	-3.1%(-17.7%,-1.5%)	-0.4%(-0.5%,-0.3%)	-0.2%(-4.8%,3.4%)
Cardiac Output	-3.0%(-9.6%,-1.7%)	-1.4%(-3.8%,-0.8%)	-0.2%(-0.2%,-0.1%)	-1.2%(-9.2%,5.3%)
Heart Rate	0.9%(0.5%,3.2%)	1.1%(0.5%,6.5%)	0.6%(0.5%,0.9%)	0.5%(-6.9%,7.4%)

Figure 4. The percentage of the effect of current smoking mediated through atherosclerosis, inflammation, coagulation, and hemodynamics pathways.

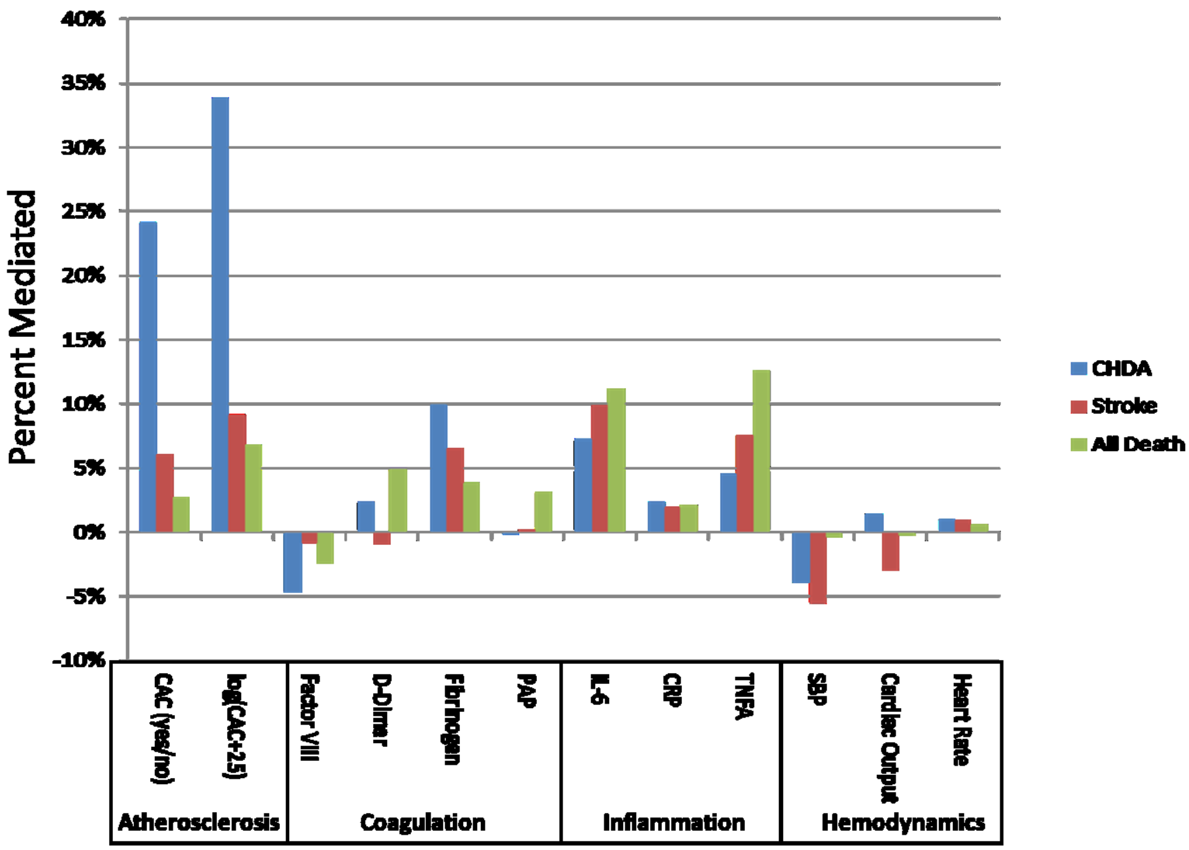


Table 16. Impact of 1 cigarette/day intensity reduction, modeled continuously.

HRs adjusted for age, race, gender,log(CAC+25), and Framingham risk score.			
Outcome	No. Events	Cig/day change adjusted for starting intensity	Cig/day change not adjusted for starting intensity
CVDH	54	0.99(0.95,1.04) p=0.775	1.02(0.98,1.06) p=0.296
CVDA	69	0.98(0.94,1.02) p=0.288	1.00(0.97,1.04) p=0.890
CHDH	33	0.96(0.91,1.01) p=0.138	0.99(0.94,1.05) p=0.695
CHDA	44	0.95(0.91,1.00) p=0.037	0.98(0.93,1.03) p=0.436
Stroke	21	1.05(0.98,1.12) p=0.158	1.06(1.01,1.11) p=0.023
CVD Death	16	0.98(0.92,1.06) p=0.674	1.01(0.94,1.07) p=0.883
All Death	74	0.99(0.96,1.03) p=0.592	1.02(0.99,1.06) p=0.153

Table 17. Impact of intensity change, modeled categorically.

HRs adjusted for age, race, gender,log(CAC+25), and Framingham risk score.				
Outcome	Reduction in Category	Increase in Category	Initial Intensity Category Low (<=10 Cigs/day)	Initial Intensity Category High (21+ cigs/day)
CVDH	0.90(0.45,1.80) p=0.762	1.22(0.37,4.02) p=0.744	0.37 (0.18,0.76) p=0.006	1.10(0.52,2.35) p=0.797
CVDA	0.75(0.38,1.47) p=0.123	1.97(0.83,4.69) p=0.123	0.56(0.31,1.00) p=0.051	1.38(0.70,2.71) p=0.354
CHDH	0.57(0.22,1.47) p=0.244	1.41(0.32,6.13) p=0.650	0.25(0.09,0.66) p=0.005	1.23(0.49,3.10) p=0.655
CHDA	0.54(0.23,1.28) p=0.162	2.09(0.72,6.07) p=0.176	0.38(0.17,0.82) p=0.014	1.62(0.73,3.59) p=0.239
Stroke	1.69(0.57,5.00) p=0.341	0.96(0.12,7.41) p=0.967	0.68(0.23,1.99) p=0.478	1.03(0.27,3.94) p=0.966
CVD Death	0.69(0.14,3.51) p=0.654	2.94(0.61,14.06) p=0.177	1.27(0.39,4.20) p=0.691	2.62(0.57,12.00) p=0.213
All Death	1.05(0.54,2.01) p=0.894	1.93(0.82,4.58) p=0.134	0.71(0.41,1.21) p=0.209	2.55(1.25,5.19) p=0.010