IDENTIFYING AND OVERCOMING BARRIERS IN THE TRANSLATION OF ETIOLOGIC CANCER BIOMARKERS

by
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A dissertation submitted to Johns Hopkins University in conformity with the requirements for the degree of Doctor of Philosophy

Baltimore, Maryland

July 2017

Abstract

Background: With the goal to improve the translation of cancer biomarkers, this dissertation examined strategies to overcome two barriers in the practice of translational epidemiology: Aim 1) practice of multidisciplinary team science to overcome threats to validity, and Aim 2) use of quantitative metrics to determine the influence of continued investigation to improve utility of information from future biomarker investigations. Aim 1 and 2 strategies were used in Aim 3 to inform the analysis of multiple biomarkers of glycemia to better characterize the relationship between the natural history of diabetes and prostate cancer mortality. **Methods:** Aim 1 examined the impact of the practice of multidisciplinary team science on identifying and overcoming threats to validity. A case-study of a multidisciplinary team's investigation of three tissue biomarkers where threats to validity were identified along with appropriate solutions through the practice of team science was carried out. In Aim 2 the impact of continued investigation of a biomarker-cancer relationship was quantified by adapting established research synthesis and clinical trial metrics – fail-safe number and conditional power analysis. To document how these metrics can be adapted to overcome the lack of utility of information from continued investigation, they were applied to a previously curated set of 98 meta-analyses of prospective studies investigating biomarkers and cancer risk. The strategies evaluated in Aims 1 and 2 were applied to Aim 3, by first identifying evidence gaps about the relationship between diabetes and prostate cancer followed by assembling a team with expertise in glycemia biomarkers and diabetes, and prostate cancer etiology. The team developed a refined strategy of incorporating multiple biomarkers of glycemia to better define normo- and hyperglycemia to investigate the relationship between the natural history of diabetes and prostate cancer in the Atherosclerosis Risk in Communities study. **Results:** Aim 1: Through the practice of team science, the

multidisciplinary team consisting of a pathologist, cancer biologists, a biostatistician, and epidemiologist identified measurement error in the pre-analytic and analytic phase of the biomarker measurement, and was able to overcome the threats to validity by implementing appropriate corrections in the data analyses. Aim 2: Applying the fail-safe number and conditional power calculation to the 98 meta-analyses, we observed patterns in the characteristics of the existing evidence and the values of each of these metrics including the size of the observed summary estimate, the number of studies included in the observed meta-analysis, and the extent of between-study heterogeneity. Aim 3: After incorporating strategies from Aims 1 and 2, creating joint categories of the three glycemia biomarkers, and using men without diagnosed diabetes who had normal values for all three biomarkers as the reference group, men without diagnosed diabetes high on all three markers had close to a 5-fold increase in risk of prostate cancer death (HR: 4.80; 95% CI: 1.11 to 20.95). Men with diagnosed diabetes had a non-statistically significant 3-fold increase in risk of prostate cancer death. **Conclusions:** The inferential benefit achieved through the practice of multidisciplinary team science coupled with the adaptation of the fail-safe number and conditional power analysis to quantify the impact of continued biomarker investigation provide two strategies for the more efficient practice of translational epidemiology. Using these strategies to inform the analysis of biomarkers of glycemia and prostate cancer mortality, revealed an elevated risk of prostate cancer death in men without diagnosed diabetes with elevated glycemia and in men with diagnosed diabetes. These findings speak to the overall importance of diabetes prevention and good glycemic control in men with diagnosed diabetes.

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Preface

This dissertation is divided into five chapters. The first chapter provides an introduction to the translation of cancer biomarkers and the practice of translational epidemiology with a focus on two barriers in the translation of cancer biomarkers – threats to validity and lack of utility of information. The following two chapters describe meta-research investigations of strategies aimed at overcoming each of these barriers in the early phase of the translational continuum, including the practice of multidisciplinary team science to overcome threats to validity and the use of existing quantitative metrics (e.g., the fail-safe number and conditional power analysis) to determine the impact of continued investigation on the current evidence based summarized in a meta-analysis. Each of these practice-based strategies were incorporated into the conceptualization and design of the fourth chapter which describes the analysis incorporating multiple biomarkers of glycemia to better characterize the relationship between the natural history of diabetes and prostate cancer mortality. The final chapter summarizes the results of the preceding three chapters and as well as the overall strengths and limitations. I also describe a series of next-steps to incorporate the strategies aimed at overcoming barriers in the practice of translational epidemiology, as well as future directions for research that focus on exploring additional strategies to improve the translation of cancer biomarkers and for the analysis of biomarkers of glycemia and prostate cancer. The overall public health implications emanating from the results of each chapter are also described in the final chapter.

Acknowledgements

I am most grateful to my mentoring team, Drs Elizabeth Platz and Corinne Joshu, for their patience and confidence throughout my time as a doctoral student. Without their support, I could not have realized my potential to accomplished as much as I have during my time as a doctoral student. I owe them special thanks in providing the flexibility for me to pursue my interests in my dissertation research and throughout my doctoral training. I am thankful to have had to opportunity to work with Dr. Stephan Ehrhardt on this dissertation and am thankful for his thoughtful feedback and constant support and encouragement. It has been a pleasure to work with one of Dr. Platz's former doctoral students, Dr. Konstantinos Tsilidis who has been an inspiration as I begin to chart my career path.

To all of the faculty in the cancer epidemiology track directed by Dr. Kala Visvanathan, who helped me to refine and focus my thinking to identifying the most important research questions in cancer prevention and control. Their dedication and commitment to their work on all aspects of cancer prevention and control has been inspiring. I would also like to thank Dr. Liz Selvin for allowing me to work with her data and for constantly challenging my thinking and application of epidemiologic methods in my analyses. I have learned a great deal from her in both the class room and through collaborating on part of this dissertation. I owe special thanks to Drs. Allan Meeker, Shawn Lupold and Christopher Heaphy for responding to my questions regarding their work in the tissue biomarker analyses. I have learnt a great deal from attending group meetings with them and am thankful for their comments and suggestions on my work.

Prior to beginning the doctoral program, I am grateful for the opportunity to work with thought-leaders in various fields that helped me to formulate my interests leading to this dissertation including Dr. Muin Khoury, Director of the Office of Public Health Genomics at the Centers for Disease Control and Prevention and Dr. Kay Dickersin, Director of the US Cochrane Center.

I would like to acknowledge the funding I have received from the Department of Epidemiology throughout my time as a doctoral student, and especially the Distinguished Epidemiology Scholars funding I received in my last year. I am also thankful for the Harvey M. Meyerhoff Fellowship in Cancer Prevention I was awarded in my first year in the doctoral program.

Finally, I would like to thank my family, friends, and fellow doctoral students for their unconditional support through this process. The one person who I owe the most thanks to is my wife Sarah. For so many reasons I could not have accomplished this dissertation without her.

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Chapter 1.

Introduction

There is no other phase in the cancer research enterprise that generates as much excitement and enthusiasm than the discovery of a novel biomarker. In order to capitalize on such discoveries in a timely and efficient manner, the National Institutes of Health (NIH) introduced a series of initiatives to catalyze the translation of basic science discoveries into practice and improved health.[2] Despite such initiatives, there remains some disagreements in what translational research actually means. Some hold the view of the "bench-to-bedside" continuum at the confluence of basic science and clinical practice with a drug or device as the final product of translational research. While others view translational research more broadly through a population-based lens at the intersection of research with practice and the successful implementation in the patients (clinical) and populations (public health) in which the research targeted as the end result.[3]

Translational Epidemiology Framework

For the purpose of this dissertation, we will draw on the translational framework (Figure 1.1) introduced by Khoury et al.,[1] which recognizes epidemiologic methods and study designs as central to all phases in the translational continuum, referred to as translational epidemiology. In Figure 1.1, the discovery phase – T0, links the current understanding of a biological process in the form of a biomarker with a specific health outcome. This is followed by T1, which aims to determine the clinical validity by replicating and characterizing the T0 biomarker in clinical or population setting. T2 seeks to determine the clinical utility or efficacy of applications emerging from T0 and T1 investigations, and is envisioned to support evidence-based recommendations and guidelines. T3 aims to evaluate the factors related to the implementation and uptake of evidence-based guidelines built on T2 evidence. Finally, T4 sets out to determine the real-world effectiveness of the intervention at improving population health. At the center

of the T0 to T4 continuum is knowledge integration, which incorporates knowledge synthesis, management, and translation. The aim of knowledge integration is to maximize the existing evidence base to facilitate progression through the translational continuum.[4] It is important to note that knowledge integration is not specific to any one phase, but can be equally applied to evidence generated throughout the T0-T4 continuum.

Evolution of Biomarker Definitions

In early molecular epidemiologic investigations, biomarkers were categorized as biomarkers of internal dose; markers of biologically effective dose; markers of early response/effect; markers of susceptibility; or markers of altered structure and function.[5] While this model of biomarker classification is particularly relevant in the continuum of molecular alterations leading to cancer, the introduction of modern high-throughput –omic technologies have led to new opportunities for biomarker applications for cancer prevention and control.[6] The definition from the National Cancer Institute offers a modern view of cancer biomarkers including biomarkers of treatment response and multi-marker panels: "A biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease. A biomarker may be used to see how well the body responds to a treatment for a disease or condition. Also called molecular marker and signature molecule".[7] For the purposes of this dissertation, we will use the definition endorsed by the Institutes of Medicine[8]: "A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, or pharmacologic responses to a[n]…intervention".

Biomarker Failures and Successes

Despite the remarkable advances in high-throughput technologies, exponential increase in knowledge of basic cancer biology, and investment in biomedical research the current landscape of biomarker discoveries is riddled with failed attempts at producing viable cancer-related applications.[9-11] Contributing to the ineffective translation of promising discoveries is the absence of a clear clinical scenario or clinical decision process in which the application is intended for (i.e., etiologic risk prediction, early detection/screening, diagnostic, prognostic, or treatment response prediction). Identifying how the biomarker will be deployed and used in clinical and public health practice will determine the study design, population, and biospecimens required to answer the research questions.[12]

Four general types of biomarker failures have been described,[10] pointing to various aspects in the practice of translational epidemiology contributing to biomarker failures. Ioannidis[10] describes a Type A failure when a biomarker implemented into routine practice is later shown to be ineffective or harmful (e.g., PSA for early detection of prostate cancer). A Type B failure is when a biomarker shows promise in the early phases of discovery, but eventually fails the later phases of validation. When a biomarker has demonstrated robust validation (e.g., T1), but has not gone through subsequent investigation to determine its utility (e.g., T2) represents a Type C failure; common among gene-expression panels for cancer prognosis. Finally, a Type D failure occurs when a biomarker is promoted for clinical or population use despite no evidence of its usefulness (e.g., direct-to-consumer genetic testing services). Alternatively, the successful implementation of applications targeting several biomarkers aimed at cancer prevention and control are supported by evidence-based recommendations and guidelines. These include HPV testing and vaccination to prevent cervical cancer;

screening for microsatellite instability in relatives of individuals diagnosed with Lynch Syndrome (screening of high-risk individuals); and HER2 testing for targeting breast cancer treatment.

Barriers in the Practice of Translational Epidemiology

Within the translational continuum (e.g., T0 to T4), the validation phase (e.g., T1) can ultimately determine the fate of T0 biomarker discovery. Knowledge emanating from T1 investigations serves as the foundation for subsequent phases in the translational continuum. Questions addressed in validation studies are: *Is the hypothesized association between the biomarker and outcome reproducible? Can the biomarker be effectively and efficiently applied in a population setting?* Bypassing the critical validation steps may lead to the premature introduction of a biomarker (e.g., early detection of prostate cancer with PSA).[10] Equally daunting are the number of potentially viable applications of etiologic biomarkers that are lost in translation due to insufficient validation efforts. Defining the subsequent research questions and designing the studies to establish the newly-minted assay's performance characteristics as well as obtaining the appropriate clinical samples and funding are just a few of the methodological challenges encountered in the practice of T1 translational epidemiology.[13-16] A sobering reminder that up to 90% of biomedical research findings cannot be replicated points to fundamental flaws in the early stages of the translational research continuum.[17,18] The strongest line of defense against the potential biases within the discovery and validation of biomarkers is the use of well-defined study designs, populations and associated samples to address specific research questions.[13,14] Adopting principles rooted in population science will ensure reliability of reported associations and allow for more efficient translation.[19,20]

Deconstructing the practice of T1 translational epidemiology into the basic elements of the scientific method (Figure 1.2) will facilitate the identification of potential barriers as well as solutions for overcoming such barriers. Threats to validity occurring during study design and conduct and in the analysis and reporting of results, as well as insufficient efforts evaluating the utility of information from continued T1 biomarker validation have been cited as factors leading to promising application of biomarkers of cancer etiology, risk, prognosis, and treatment prediction (throughout called "cancer biomarkers") getting lost in translation.[1,14,16,21,22] A more efficient process, overcoming these barriers in translating T1 cancer biomarkers, would save time and allow researchers and funders to concentrate resources on promising biomarkers with the potential of improving population health outcomes. Current efforts to overcome threats to validity include data sharing, developing large-scale disease-specific consortiums, and multidisciplinary team-based research.[9,14,23] Disease-specific consortiums have also been cited as another way to target specific gaps in knowledge directly improving the utility of information.[23] Other systematic efforts include the Early Detection Network (EDRN) sponsored by the National Cancer Institute which aims to improve the discovery and translation of promising cancer biomarkers, and to eliminate biomarkers without added clinical value from further investigation.[24] Further consideration of the existing evidence-base outlined in the principles of knowledge integration mentioned above[4] is another approach for improving the utility of information.[25] I have conducted a series of interrelated aims applying meta-research methods[26] to empirically examine specific barriers in the practice of T1 translational epidemiology for cancer biomarkers. The primary objective of meta-research is to evaluate and improve research practices, and is broken down into 5 themes: methods; reporting; reproducibility; evaluation; and incentives.[26] Thus, a meta-research approach is ideal for investigating barriers in the practice of T1 translational epidemiology.

Driving Translational Epidemiology with Team Science

Aim 1. Adding the team into translational epidemiology: a case-study of team science in biomarker development. Given the challenges of translational research, multidisciplinary team science is promoted by funders and some researchers as a strategy to increase the likelihood of successfully moving from discovery to individual and population health impact. Many of the methodological challenges encountered in the practice of T1 translational epidemiology require a multidisciplinary team of researchers to successfully move a discovery from bench-to-bedside.[23,27] Some have viewed the diversity of the training and experience of those participating in translational research as contributing to the unsuccessful translation.[9,14,16,28] This might suggest the absence of well-defined epidemiology methods in all phases of translational research may contribute to the pervasive failure of discoveries from making a population impact.

In Aim 1, we present a case study documenting the utility of multidisciplinary team science from the epidemiologic perspective. I used primary research data from a team consisting of a pathologist, cancer biologists, a biostatistician, and epidemiologists specializing in prostate cancer biomarkers. I examined their contributions during each of phase of biomarker evaluation to identify where they, through the practice of team science, recognized and solved threats to internal validity. Then, I quantified extent of bias in the estimates avoided because the team recognized and solved the threats in evaluating the association of cancer biomarkers – Ki67 (IHC), stromal cell telomere length (FISH), and miRNA (miR-21, miR-141, miR-221, quantitative RT-PCR) – with prostate cancer risk or recurrence in nested case-control studies. In this case study, we were able to document an inferential benefit of multidisciplinary team science in biomarker evaluation.

Overcoming "Me Too Science" by Quantifying Utility of Information

Aim 2. When is enough, enough? Adapting the fail-safe number and conditional power for deciding whether more research is needed on biomarker-cancer associations. Repetitive epidemiologic investigations of biomarker-cancer associations that do not fill knowledge gaps are not uncommon in the literature and can be a resource drain. The studies are often referred to as "Me Too Science".[29] Such redundant investigations that lack clinical or public health significance or the potential to improve biological understanding represent a practice-based barrier in translation, including for etiologic cancer biomarkers for determining cancer risk.[11,16] The overall magnitude of the cost of research waste has been estimated to be as much as 85% of the $200 billion invested in biomedical research in 2010.[30,31] A significant factor contributing to research waste is overlooking what is already known, or ignoring what is currently under investigation.[31,32] Unlike the necessary and legitimate practice of research reproducibility, redundant research neglects the existing evidence base and the context in which the current result will be considered. Thus, redundant uninformative research can be minimized by determining whether or not further investigation will provide a meaningful contribution to the existing evidence. Recently, Ioannidis and Khoury[25] introduced a PQRST index composed of a series of metrics to appraise the quality and influence of existing biomedical research. The PQRST index includes general aspects that capture the productivity, quality, reproducibility, sharing of data, and translational influence of biomedical research. However, the PQRST index leaves room for a systematic process to quantify the impact of future biomedical research on the existing evidence base for a particular biomarker-cancer outcome relationship. A systematic method for stakeholders in translational epidemiology, including journal editors, grant reviewers, researchers, and research funders, can be used to determine whether more research on the same biomarker-outcome association is needed. We aim to introduce a systematic process for

determining whether further T1 investigation will influence the current evidence on that specific biomarker-outcome association.

In Aim 2, I adapted clinical trial research synthesis methods to quantify the impact of continued investigations. Two versions of the fail-safe number (FSN) were applied to 98 fixed and random effects meta-analyses on biomarkers and risk of 17 cancers. Rosenberg's FSN conditions on the statistical significance of each meta-analysis to determine number of future studies of null effect ($p \geq 0.05$) and average study weight needed to drive the summary result to null. Orwin's FSN conditions on the summary estimate of each meta-analysis to determine number of future studies of a specified effect size needed to be added to drive the combined summary estimate to a certain effect size. From this work, we concluded that together with traditional assessments of study quality and remaining knowledge gaps (e.g., subgroup associations), use of such metrics by researchers, funders, grant reviewers, and journal editors might help determine whether more research is needed *or not* on specific biomarker and cancer associations, potentially saving time, money, and allow researchers to focus efforts on biomarkers with the greatest promise of improving individual and population-level cancer burden.

Maximizing Efficiency & Impact: Biomarkers of Glycemia and Prostate Cancer

Aim 3. Glycemia is positively associated with prostate cancer mortality in white and black men without diabetes when better classifying hyper- and normo-glycemia using 3 biomarkers. The study conducted for Aim 3 is an example of the real-time execution of the strategies to overcome barriers in the practice of T1 translational epidemiology evaluated in Aims 1 and 2. The practice of translational epidemiology incorporating a team-science approach and considering alternative methods of

measuring biomarkers of important biologic processes will enhance the existing evidence base and maximize the utility of information generated. We first identified knowledge gaps and then assembled a multidisciplinary team to investigate the association of glycemia with prostate cancer mortality.

Gaps: Epidemiologic investigations have shown that diabetes is associated with an increased risk for many cancers, but not prostate cancer.[33-37] While diabetes is associated with a decreased prostate cancer risk, the association appears stronger the longer the duration of diabetes.[38] With respect to prostate cancer mortality, in men without the diagnosis at baseline, some studies reported that diabetes is inversely associated,[33,35,39] while more recent studies reported a positive association.[40,41] However, the exact mechanism(s) driving these diabetes associations are unclear. A joint consensus statement between the American Diabetes Association and the American Cancer Association highlighted the need to better understand the biologic mechanisms underlying the association between diabetes and cancer.[42] Biomarkers of hyperglycemia characterizing states early in the natural history of diabetes (e.g., prediabetes and undiagnosed diabetes) provide an opportunity to investigate this relationship with the most clinically relevant outcome, prostate cancer mortality. Prostate cancer mortality has become the most clinically relevant outcome following the recognition of overdiagnosis of prostate cancer stemming from the introduction of routine screening with PSA. And whether associations are similar across race is also unknown. Thus, we aimed to rigorously characterize the association of diabetes and hyperglycemia with prostate cancer mortality in the ARIC cohort, which consists of ~25% African-American men. We classified hyperglycemia with a combination of 3 blood biomarkers – fasting glucose, hemoglobin A1c (HbA1c), and glycated albumin (%GA), which capture complementary aspects of glycemia.

Team: We assembled a diverse team with expertise in the landscape of prostate cancer etiology and in hyperglycemia and factors influencing biomarkers of hyperglycemia.

Conclusions: We concluded that using 3 biomarkers that capture complementary aspects of glycemia and have different sensitivities to non-glycemic factors, men without diagnosed diabetes who have hyperglycemia have an increased risk of death from prostate cancer compared to men who have normal glycemia levels, independent of BMI and other factors. Our findings did not appear to be influenced by racial differences in hyperglycemia. The knowledge emanating from this efficient practice of translational epidemiology can be leveraged to identify more robust associations between modifiable risk factors in specific populations.

Following this chapter, this dissertation is organized into three research papers addressing each aim described above followed by a final chapter summarizing the findings, synthesizing and contextualizing them, and providing next steps for their implementation to improve T1 translation.

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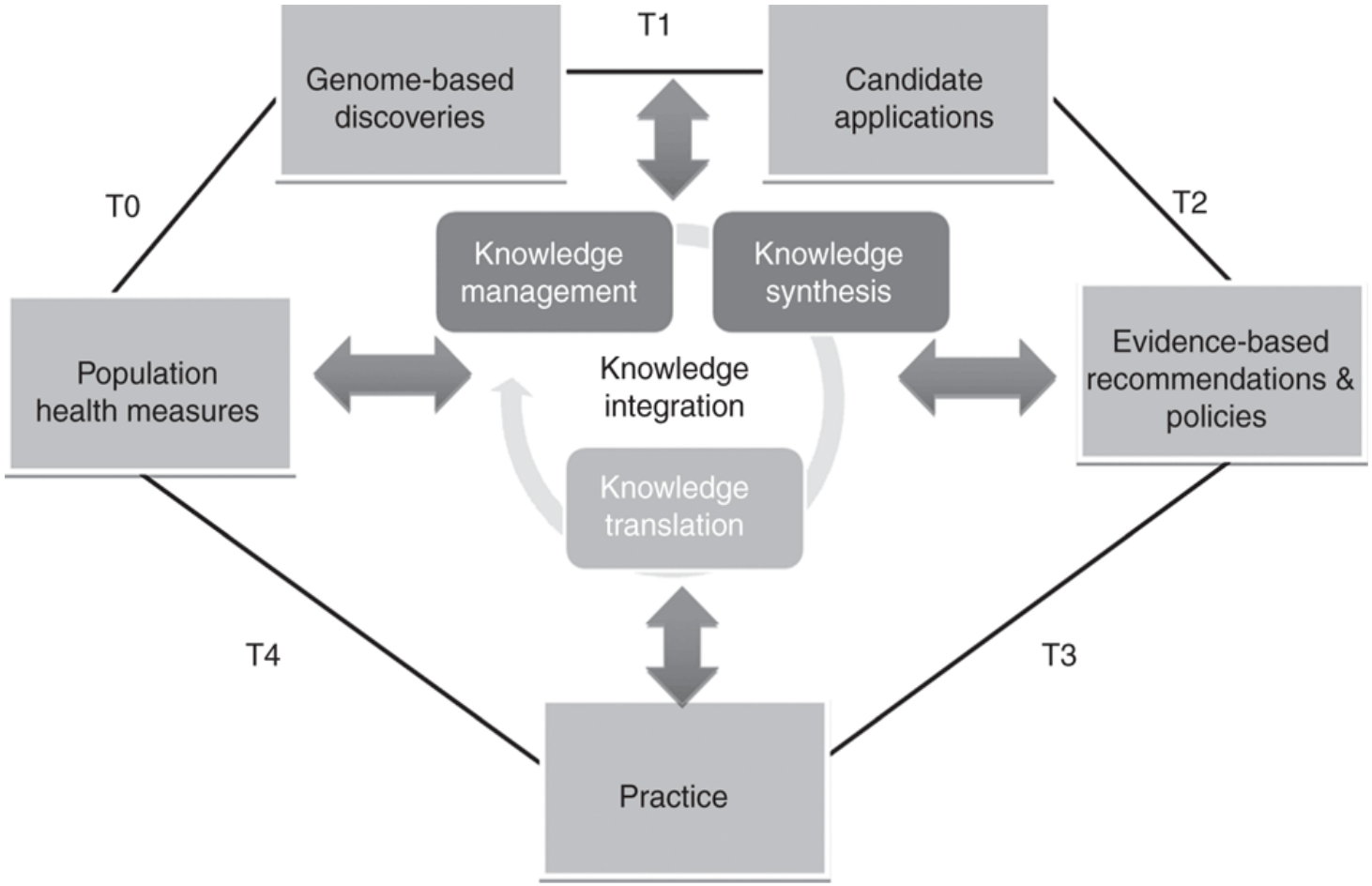

Figure 1.1 Translational epidemiology framework[1]

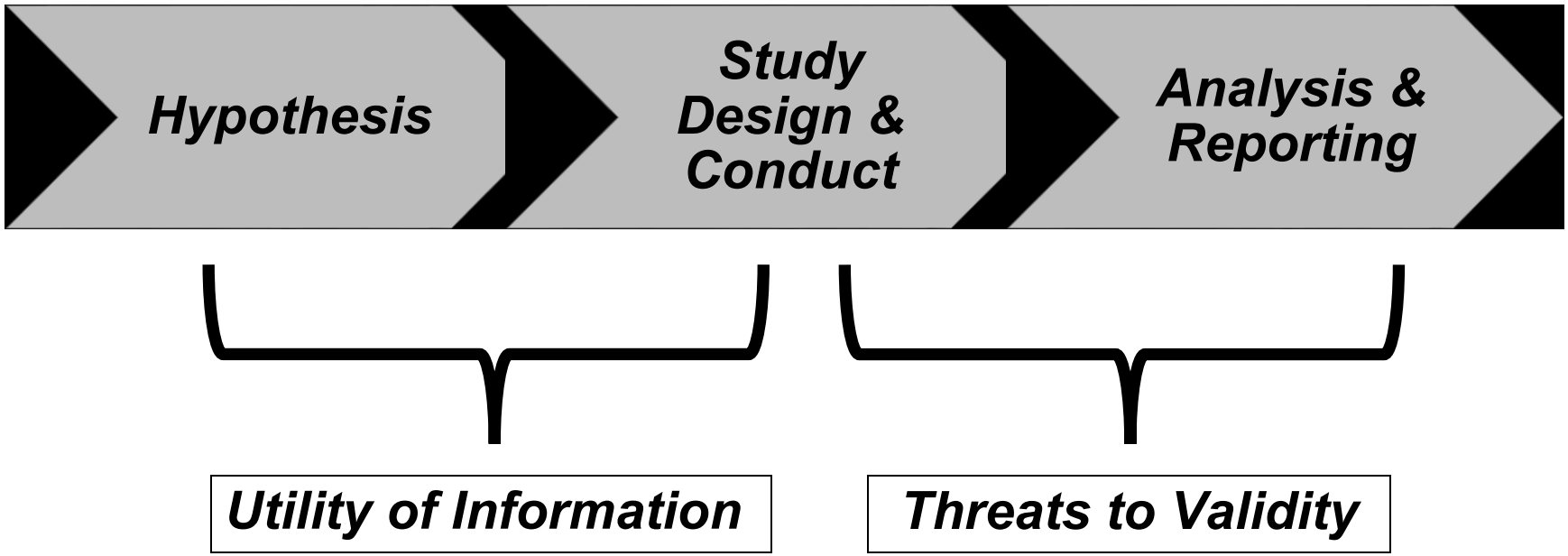

Figure 1.2 Barriers in the practice of T1 translational epidemiology

Chapter 2:

Adding the *team* into T1 translational research: a case study of team science that includes epidemiology in the evaluation of biomarkers of prostate cancer risk and prognosis

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2.1 Abstract

Background: Given the challenges of translational research, multidisciplinary team science is promoted by funders and some researchers as a strategy to increase the likelihood of successfully moving from discovery to individual and population impact. We present a case study documenting the utility of multidisciplinary team science from the epidemiologic perspective. **Methods:** We used primary research data from a team consisting of a pathologist, cancer biologists, a biostatistician, and epidemiologists specializing in prostate cancer biomarkers. We examined their contributions during each phase of biomarker evaluation to identify where each team member, through the practice of team science, recognized and solved threats to internal validity. Next, we quantified the extent of bias in the estimates (from logistic or conditional logistic regression) avoided because the team recognized and solved the threats in evaluating the association of cancer biomarkers – Ki67 (IHC), stromal cell telomere length (FISH), and miRNA (miR-21, miR-141, miR-221; quantitative RT-PCR) – with prostate cancer risk or recurrence in nested case-control studies. **Results:** Threats to validity resulting in measurement error were tissue storage time (Ki67, miRNA; pre-analytic) and laboratory equipment maintenance (telomeres; analytic). Solutions were all in the data analysis phase and involved using tissue storage-time specific cut points and/or batch-specific cut points. Bias in the beta coefficients for each biomarker association ranged from 24 to 423%, and for each test for trend ranged from 15 to 910%. Interpretation changed as follows: Ki67 – null to positive association; telomere length – null to positive association; and miR-21, miR-141 – null association remained, miR-221 – weak inverse to moderate inverse association. **Conclusions:** In this case study, we document an inferential benefit of multidisciplinary team science that includes epidemiology in T1 biomarker evaluation.

2.2 Introduction

Remarkable advances in biomedical technology have contributed improved understanding of the molecular mechanisms driving cancer development and progression. The rapidly expanding knowledge of the molecular landscape of cancer has been incorporated in biomarker applications to estimate risk of cancer recurrence (e.g., gene-expression profiling) and in new therapies targeting specific molecular alterations (e.g., trastuzumab for HER2 positive breast cancer). However, despite these remarkable advances, improved population-level health outcomes remain to be seen from biomarker applications targeted at cancer prevention.[1-3]

Within the translational continuum spanning discovery (e.g., T0) to population health impact (e.g., T4), the validation phase (i.e., T1) may ultimately determine the fate of T0 biomarker discovery. Knowledge emanating from T1 investigations serves as the foundation for subsequent phases in the translational continuum. Questions addressed in validation studies are: *Is the hypothesized association between the biomarker and outcome reproducible? Can the biomarker be effectively and efficiently applied in a population setting?* Bypassing the critical validation steps may lead to the premature introduction of a biomarker. The story of PSA exemplifies a scenario in which a validated biomarker that detects prostate cancers was introduced as marker for early detection without being formally evaluated in the setting of early detection to reduce prostate cancer mortality rates. The imbalance in benefits to harms from the routine use of PSA for early detection of prostate cancer to reduce prostate cancer mortality contributed to revised recommendations for shared decision making on the use of PSA.[4] Equally daunting are the number of potentially viable applications of etiologic biomarkers that are lost in translation due to insufficient validation efforts.

Barriers in the Practice of Translational Research Despite the disparity in research funding and attitudes toward the T1 to T4 stages in the translational continuum,[5,6] many of the methodological challenges encountered in the practice of translational epidemiology require a multidisciplinary team of researchers to successfully move a discovery from bench-to-bedside.[7,8] In the translation of promising biomarker discoveries it is critical to clearly define clinical or public health scenario or decision process in which the application is intended for (i.e., etiologic risk prediction, early detection/screening, diagnostic, prognostic, or treatment response prediction).[9,10] Identifying how the biomarker will be deployed and used in clinical and public health practice will determine the study design, population, and biospecimens required to answer the research questions. Designing the studies to establish the biomarker's performance characteristics as well as obtaining the appropriate clinical samples and funding are just a few of the methodological challenges in the validation phase of the translational continuum.[9-12]

Within the discovery and validation process (i.e., T0 and T1), attention should be paid to the pre-analytic (i.e., sample characteristics, collection, processing, and storage), analytic (i.e., specimen analysis, assay performance, and analytic method), and post-analytic (i.e., data analysis and interpretation) factors, which may influence the reliability of the hypothesized association. A systematic difference in the way samples and data are handled, for example between cases and controls, may inadvertently introduce artificial structure into the data and result in faulty interpretation of the results.[13] The artificial structure with no inherent biological plausibility adds variability in the groups being compared, which can lead to spurious associations that cannot be explained, and ultimately will not be reproduced. Ensuring that external quality control measures are in place and following standard laboratory protocols may not be sufficient to preclude such

hidden factors. Because it is difficult to detect and guard against the many known and unknown biases, incorporating an understanding of how potential biases relate to the specific technology used as well as the provenance of samples and underlying biological processes may help to mitigate the overall adverse impact on study results. That up to 90% of biomedical research findings cannot be replicated points to fundamental flaws in the translational research continuum.[14,15]

Some have viewed the diversity in experience of those participating in translational research with limited training in epidemiologic study design, fundaments of unbiased participant/sample selection, and high-throughput data analysis as contributing to the unsuccessful translation.[11,12,16-18] We see great value in this diversity of research perspective and approaches and instead postulated that an absence of well-defined epidemiology methods in all phases of translational research may contribute to the pervasive failure of discoveries from making a population impact. Like others,[19,20] we propose that the strongest line of defense against the potential biases within the discovery and validation of biomarkers is the use of well-defined study designs, populations and associated samples to address specific research questions.[9,11] Adopting principles rooted in population science will ensure reliability of reported associations and allow for more efficient translation.

Driving Translational Research with Team Science including Epidemiology

Multidisciplinary team science is the process through which researchers representing different disciplines work together to expand and integrate discipline-specific knowledge and methods to address a common research problem.[21] Leveraging the diverse and complementary research perspectives in a collaborative effort is an ideal model for efficient translation.[8,19,22-24] A robust multidisciplinary team, with a foundation in

fundamental epidemiology principles, will allow the cancer research community to capitalize on emerging technologies and investments in biomedical research. From our experience working in a multidisciplinary team, this approach to translational research has provided new insights and tools, including biomarkers, to tackle the current questions in cancer etiology, risk, prognosis, and treatment. To this end, large funding initiatives from the National Cancer Institute including the Cancer Center Support Grants and the Translational Research Program's SPORE initiative emphasize the practice of multidisciplinary team science. The dynamics of team science in cancer research have been cited as a driver of translational cancer epidemiology.[8] Team science is a contemporary topic of study currently supported though the mission of the Science of Research and Technology Branch in the Behavioral Research Program at the National Cancer Institute.[21,25] However, there has been no effort to empirically evaluate how the multidisciplinary contributions cultivated in the practice of team science contribute directly to the effective translation of T1 cancer biomarkers.

Thus, we followed the principles outlined in the methodology domain of meta-research,[26] which seeks to understand biases stemming from research conduct necessary to improve research practices, to perform a case study to illustrate the utility of multidisciplinary team science that includes epidemiology in the T1 translation of biomarkers and prostate cancer. We examined the team's individual and joint contributions during each phase of biomarker evaluation to identify where through the practice of team science they recognized and solved threats to internal validity. Next, we quantified the extent of bias in the estimates (from logistic or conditional logistic regression) avoided because the team recognized and solved the threats in evaluating the association of cancer biomarkers with prostate cancer risk or recurrence in nested case-control studies.

2.3 Methods

Our multidisciplinary team specializing in cancer biomarkers includes investigators with formal training and expertise in population science (e.g., epidemiology and biostatistics), clinical science (e.g., pathology and urology), and basic science (e.g., cancer biology, biochemistry, and molecular biology). The composition of the team is 33% female and includes senior members who have worked together for the past 20 years and mid-rank and junior members who joined the team initially as trainees who later went on to be appointed as faculty. The established working relationship between members of the team; success in receipt of extramural funding from the National Institutes of Health and Department of Defense; numerous peer-reviewed publications; prior reflection on practice of team science;[22] and willingness to share data provides the ideal opportunity to examine the utility of team science in overcoming barriers encountered in T1 translational epidemiology.

Members of the team participated in the investigation of three different etiologic tissue biomarkers and prostate cancer risk or recurrence including Ki67 expression (marker of cell proliferation);[27] microRNA expression (non-coding regulatory RNA);[28] and telomere length (marker of chromosome stability).[29] In each of the investigations threats to internal validity (e.g., systematic measurement error) in the measurement of each tissue biomarker were revealed through the practice of team science along with the appropriate solutions to correct for the measurement error (Table 1).

Key Indicators of Threats to Internal Validity A structured interview was conducted by an investigator not participating in the original investigations of the three biomarkers. Leading questions asked during team meetings and through individual correspondence

via email were designed to provoke team members to recall the events and circumstance raising suspicion of potential bias in the observed results for each biomarker association. Team members were asked to describe how the team jointly or individually was then able to identify the source of bias pointing to measurement error in the analytic pipeline (pre-analytic, analytic, and post-analytic). The corresponding solutions properly accounting for measurement error in the statistical analysis were also described during the team and individual interviews.

Measuring Bias and Changes in Inference Using the primary research data collected and analyzed in each of the three scenarios (Table 1), the amount of bias avoided by the team identifying and correcting for the measurement error was quantified by calculating the percent difference in the team-science corrected and biased beta coefficients ([corrected – uncorrected] / [corrected]) from adjusted unconditional (telomere length) and conditional (Ki67, microRNAs) logistic regression models. For each biomarker-outcome comparison, changes in the magnitude and direction of the measures of associations are described to show the impact of team science on the overall inference and study conclusions.

2.4 Results

Ki67 Expression and Prostate Cancer Recurrence The team investigated the association between Ki67 immunohistochemical (IHC) staining in tumor in formalin-fixed paraffin embedded tissue blocks from prostatectomy tissue sampled and arrayed on tissue microarrays (TMAs) and prostate cancer recurrence. The team used an established case-control study[30-33] nested in a clinical cohort of men with clinically localized prostate cancer undergoing prostatectomy between 1993 and 2001 at the Johns Hopkins Hospital followed through 2004.[34] Controls were sampled using incidence

density sampling[35] and were matched to cases of biochemical recurrence on age, race, stage and grade. A greater extent of biopsy prostate tissue Ki67 staining had previously been observed to be associated with a higher risk of prostate cancer progression in the setting of radiation therapy.[36] The team hypothesized that higher percentage of cell staining positive for Ki67 are associated with increased risk of prostate cancer recurrence. The team categorized men into quartiles of Ki67 staining, based on the distribution in the controls; and in initial analyses, they observed no association between higher quartiles of Ki67 staining compared with the first quartile and biochemical recurrence. Because this finding was inconsistent with published studies and with team member's clinical experience, the team suspected potential bias in their findings. Prior discussions among the team's epidemiologists and pathologists regarding the temporal decay in IHC staining in stored tissue blocks in a different context prompted the team to perform stratified analyses by date of tissue collection. The stratified analyses revealed a difference in the distribution of Ki67 staining confirming that the older tissue samples had weaker staining. While cases and controls were matched on follow-up time, they were not matched on year of surgery. The nested case-control study was originally designed to study germline DNA variants, which is unaffected by block storage time. Additionally, when the team realized the value of this nested case-control study for discovery and validation of tissue-based prognostic biomarkers, the complexities introduced by variation in block storage were not yet appreciated.

Based on these observations, emanating from team discussions and exploratory data analyses, the team detected measurement error in Ki67 staining attributed to tissue block storage time (Table 2), which was differential between cases and controls. To overcome this measurement error, the team used calendar-time specific cut points for Ki67 quartiles based on the distribution in controls. The bias in the beta coefficients from

the adjusted conditional logistic regression model for quartiles of the Ki67 staining distribution (vs Q1) the initial results relative to those from the team-science corrected results was 85% for Q2, 70% for Q3, 45% for Q4. The bias in the beta coefficient for an ordinal variable with median Ki67 for each quartile to test for trend was 910% (Figure 1). The inference from the initial results was a null association between Ki67 staining and prostate cancer recurrence. After accounting for the differential measurement error, the team-science corrected inference suggested a positive association with a strong dose response. The initial conversation between members discussing the temporal decay of tissue biomarkers, prior to the current analysis, is representative of the conducive environment emerging from the practice of team science, which allowed the team to recognize threats to validity and incorporate steps to overcome the corresponding measurement error.

Telomere Length and Prostate Cancer Risk The team investigated the association between prostate cell telomere length measured using telomere-specific fluorescence in situ hybridization (FISH) and the odds of prostate cancer overall and aggressive disease in a subset of a case-control study nested in the placebo arm of the Prostate Cancer Prevention Trial.[29] Telomere length was measured in stromal, luminal epithelial, and basal epithelial cells from prostate biopsy tissue. Cases were detected on biopsies performed for clinical indication or on biopsies performed during end-of-study biopsies per trial protocol in men free of disease during trial follow-up. Controls were men who had an end-of-study biopsy per trial protocol who did not have prostate cancer detected. The majority of the controls did not have a clinical indication for biopsy at the time of the end-of-study biopsy. This investigation was designed to test hypothesis that men with shorter telomeres in normal cells collected from diagnostic biopsies have a higher risk of having prostate cancer. During the analytic phase of measuring intensity of FISH

signals, the light guide on the fluorescent microscopy system was replaced due to normal deterioration over time. One member of the team performing telomere-specific FISH assay noticed inconsistent telomere FISH and DAPI signals after rerunning a single batch following replacement of the illumination system. After discussion with the rest of the team, significant batch-effects were observed in the distribution of telomere FISH and DAPI signals prior to and after replacement of the light guide. The team science environment facilitating an equal exchange of ideas, comments, and questions, whereby a member of the team noted an inconsistency in the data after routine maintenance of laboratory equipment allowed the entire team to concentrate their collective experience to undercover hidden structure in the data that could have otherwise gone unnoticed.

Batch-specific cut points based on the median telomere length in controls were used to compare telomere length across each of the three cell types and the odds of prostate cancer (Table 2). The bias in the beta coefficients from the adjusted unconditional logistic regression model for shorter telomere length (<median vs longer) from the initial results relative to those from the team-science corrected results was 128% for stromal cells, 45% for luminal epithelial cells, and 107% for basal epithelial cells (Figure 2). After correcting for the measurement error by using batch specific cut points, the association between telomere length and the odds of prostate cancer shifted from a non-statistically significant inverse association to a statistically significant positive association in the stromal cells, but remained null for luminal and basal epithelial cells.

microRNA Expression and Prostate Cancer Recurrence The team investigated the association between three index microRNAs (miR-21, miR-141, and miR-221) and prostate cancer recurrence[28] in the same nested-case control study used for the Ki67

investigation. This investigation was designed to test the hypothesis that differential expression of microRNAs is associated with an increased risk of prostate cancer recurrence following prostatectomy. The index microRNAs were measured by quantitative real-time RT-PCR after extraction of total RNA from cancer tissue. The ratio of the index miRNA to U6 non-coding small nuclear RNA (snRNA) was calculated to normalize quantities for any variation resulting from the RNA extraction and quantitative RT-PCR steps. U6 snRNA, which has been shown to have uniform expression, is often used in miRNA studies for normalization.[37] The team confirmed the abundance and quality of the extracted RNA. While protein degradation in tissue blocks is now recognized to be a problem in biomarker research, RNA is thought to be more stable in formalin-fixed paraffin embedded tissue blocks.[38] Assuming the stability of U6 snRNA in tissue blocks over time, the team performed stratified analyses for the association between the ratio of the index miRNA to the reference miRNA and recurrence by date of tissue collection after reviewing the initial results. The results still appeared contrary to the hypothesis. So, the team next evaluated the distribution of the index miRNAs and the reference U6 snRNAs separately by date of tissue collection. They observed different rates of decay among the index miRNAs and in U6 snRNA over time, with the greatest decay observed for U6 snRNA and miR-21.[39] Reflecting on the team's prior experience working with stored tissue samples in the same nested study as the Ki67 investigation, the member of the team conducting the statistical analyses together with guidance from other team members with expertise in the biology and measurement of mircoRNAs, led to the detection of time-dependent measurement error in the biomarker used to standardized expression of the index biomarkers.[39] Differential measurement error was introduced because of the difference in block storage time between the cases and controls as described in the Ki67 example above. In the analyses, the team corrected for the measurement error by using cut points for U6-normalized miRNA expression

(miRNA / U6) based on the distribution in controls to generate tertiles in across two calendar-time periods (Table 2). The bias in the beta coefficients from the adjusted conditional logistic regression model for tertiles of the index microRNAs (vs T3 [highest expression]) the initial results relative to those from the team-science corrected results was 190% for T1 (lowest) and 423% T2 (middle) for miR-21; 115% for T1 and 215% for T2 for miR-141; and 24% for T1 and 93% for T2 for miR-221 (Figure 2.3). The bias in the beta coefficient for an ordinal variable with values of 1, 2, and 3 corresponding to the lowest to highest tertile to test for trend was 283%; 87%; and 23% for miR-21; miR-141; and miR-221 respectively (Figure 2.3). After implementing the team-science correction in the data analysis properly accounting for the measurement error, the magnitude of the positive association for miR-221 was stronger in the team-science corrected association, while null associations remained for miR-21 and miR-141 (Figure 2.3).

2.5 Discussion

We presented a case study of a prostate cancer research team's research to illustrate the utility of multidisciplinary team science that includes epidemiology in the T1 investigation of etiologic tissue biomarkers for prostate cancer. A structured interview led by an investigator not a part of the original investigations provides a unique perspective to characterize the contributions from each team member in the initial suspicion of potential bias, how the team then was able to hone in on the exact source of bias in the analytic pipeline, followed by the implementation of appropriate solutions in the data analysis. The scenarios discussed herein highlight the shared responsibility in detecting problems and developing solutions; requiring active participation from team members with expertise in basic science, population science, and clinical science.

In each scenario, we compared the naïve inference based on the biased associations with the inferences from the team-science corrected associations to illustrate how a traditional, single discipline approach may have led to incorrect results and distorted inferences as compared to a multidisciplinary team-science approach. In all three scenarios, measurement error was detected after thoughtful and reflective consideration of the initial results followed by discussion among members of the team. The exact source of measurement error – degradation with tissue storage time coupled with differing distribution of block storage time (Ki67 and microRNA) and batch-effect (telomere length) points to the necessity of a multidisciplinary approach to detect errors that potentially would have gone unrecognized without the unique perspectives from a multidisciplinary team. The specific threats to validity encountered – all of which were types of measurement error – a core epidemiologic concept, points to the value of epidemiologic principles in a translationally-focused multidisciplinary team. The experience gained through these investigations has contributed methodological awareness in working with archived tissue samples in the T1 phase of biomarker validation.[39] Given this awareness, steps can be taken in early in the study-design phase to deal with these issues to ensure valid contributions to the evidence base with the goal of identifying valid biomarker targets for further clinical evaluation (e.g., T2).

Our case study further highlights the iterative process of evaluating initial results and formulating alternative, non-biological explanations before constructing plausible biological explanations for the observed results. In each scenario, contributions from team members with expertise in epidemiologic methods were coupled with team members with expertise in cancer biology and pathology establishing a synergy anchored by epidemiologic design and principles. The ability of the team to identify and correct for unforeseen errors in the biomarker pre-analytic and analytic phases via

corrections in the statistical analysis phase highlights the importance of population science and the impact that proper statistical analyses can have through uncovering hidden biases leading to new discoveries and strengthening findings.

In 2 of the 3 scenarios, the team-science corrected inference shifted from null to a significant association, one in the positive direction (Ki67 and prostate cancer recurrence) and one in the inverse direction (telomeres and prostate cancer risk). In both of these cases, a valid biomarker-cancer association would not have been detected and would not have the potential to be considered for further investigation in the translational continuum in the absence of team-science. Given the results of the telomere investigation, the team has received subsequent funding to study the telomeres-prostate cancer association in the full set of nested cases and controls. In the case of Ki67, the team is now looking in other settings because of its promise to inform prostate cancer outcomes, but also to provide information on how modifiable factors, such as obesity and smoking, influence recurrence as we observed in the source population for this nested case-control set.[40,41] In the other scenario (microRNA and prostate cancer recurrence), the team-science corrected inference became stronger for one index microRNA (miR-221), supporting its further investigation as a potentially valid biomarker of recurrence. The team-science corrected inference remained a null association for two index microRNAs (miR-21 and 141), and so the net effect on biomarker investigation is unchanged. While the implementation of the team science solutions produced associations corrected for measurement error, use of these solutions resulted in reduced precision of the estimates of these associations (Figures 1.1 to 1.3). Furthermore, the team science approach to correct for measurement error – use of batch or year specific cut points – did not provide an opportunity to determine optimal cut points in the

distribution of each biomarker for clinical or public health use. Calibration of the using known biomarker levels was not possible.

This case study focused on documenting the utility of the team science model in identifying and overcoming, what turned out to be, different types of measurement error in the investigation of tissue biomarkers for prostate cancer. Using a quantitative approach designed to evaluate the impact of systematic measurement error, we determined the extent of the bias and the change of the inferences resulting from team science corrected estimates to document the potential benefits of the multidisciplinary approach. While the extent of the bias and the nature of the shift in the inferences are specific to these particular studies, the utility of team science is not limited to these specific examples. The disciplines represented by team members (pathology, cancer biology, biostatistics, and epidemiology) reflect the scope and focus of this team's research, but we do not mean to suggest these specific disciplines are prerequisites for a multidisciplinary team to be successful. Rather, we provide some evidence that the multidisciplinary team science approach can be a useful model for translational research. Because this team is focused on T0 to T1 phases of the translational continuum, in this case study, we could not assess the impact of team science on the subsequent phases of the translational continuum, specifically clinical evaluation of efficacy, implementation, and impact assessment. The subsequent stages in the translational continuum (T2 and T3) evaluating the real-time use of biomarkers applications to inform clinical and public health decisions may not be vulnerable to the same threats to validity encountered during the T1 phase (i.e., increasing biomarker waning with longer block storage time). While different in scope and focus, epidemiology principles applied in a multidisciplinary team science approach will continue to provide a vital contribution to overcome barriers in the practice of translational research. Additional

meta-research drawing on the existing T0 to T4 evidence base of cancer biomarkers could be designed to comprehensively evaluate the influence of the practice of team science on moving through each phase of the translational continuum. This approach, would also help identify other practice-based barriers in the efficient translation of cancer biomarkers – from discovery to implementation.

The team's success, illustrated through each scenario discussed, can be attributed to the ability to coordinate discipline-specific activities including epidemiology, into an effective team process along with similar attitudes and beliefs among team members; factors that have been cited to facilitate successful team science initiatives.[25] While this case study focused on the utility of a successful multidisciplinary team, there are also limitations to implementing team science. Previous qualitative evaluation of multidisciplinary team science identified integration of conceptual and scientific efforts, differences in methods, terminology, and work-styles as some of the challenges in multidisciplinary team science.[25]

2.6 References

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Table 2.1 Characteristics of three tissue-based biomarker and prostate cancer risk and recurrence: T1 translation

	Ki67	Telomeres	miRNA
Study design	• Nested case-control of retrospective clinical cohort with cases and controls matched on pathologic stage, Gleason sum, race, and age	• Prospective analysis of sample from placebo arm of randomized controlled trial	• Nested case-control of retrospective clinical cohort
Index biomarker	• IHC-detected Ki67 protein	• Telomere length measured by telomere-specific FISH	• quantitative RT-PCR detected miR-21, 141, and 221 expression
Tissue sample	• Tissue microarrays constructed from tumor foci in prostatectomy tissue samples	• Benign areas of prostate tissue biopsies performed irrespective of indication[42]	• Tumor cores from FFPE prostatectomy tissue samples
Outcome	• Prostate cancer recurrence defined as PSA >0.2 ng/mL, local recurrence of disease, development of distant metastases, or death from prostate cancer	• Incident prostate cancer relative to entering parent study	• Prostate cancer recurrence defined as PSA >0.2 ng/mL, local recurrence of disease, development of distant metastases, or death from prostate cancer
Threat to validity	• Temporal decay of Ki67 protein	• Decreased intensity and replacement of illumination system used to microscopically measure telomere length	• Different temporal decay of reference RNA (U6 snRNA) used to normalize expression of index miRNAs relative to that of the index miRNAs
Analytic measurement error	• Differential tissue storage times between cases and controls	• Batch-specific telomere length introduced by variable intensity of illumination system	• Differential tissue storage times between cases and controls

	• Systematic difference in Ki67 expression between cases and controls (differential error)	• Systematic difference in telomere length by batch (non-differential error)	• Systematic difference in relative normalized expression levels of miRNA between cases and controls (differential error)
Team-science solution	• Calendar time-specific Ki67 cut points in cases and controls based on calendar time-specific Ki67 distribution in controls	• Applied batch-specific telomere cut points (e.g., short vs. long) to both cases and controls based on the distribution in controls	• Calendar time-specific miRNA cut points in cases and controls based on calendar time-specific miRNA distribution in controls

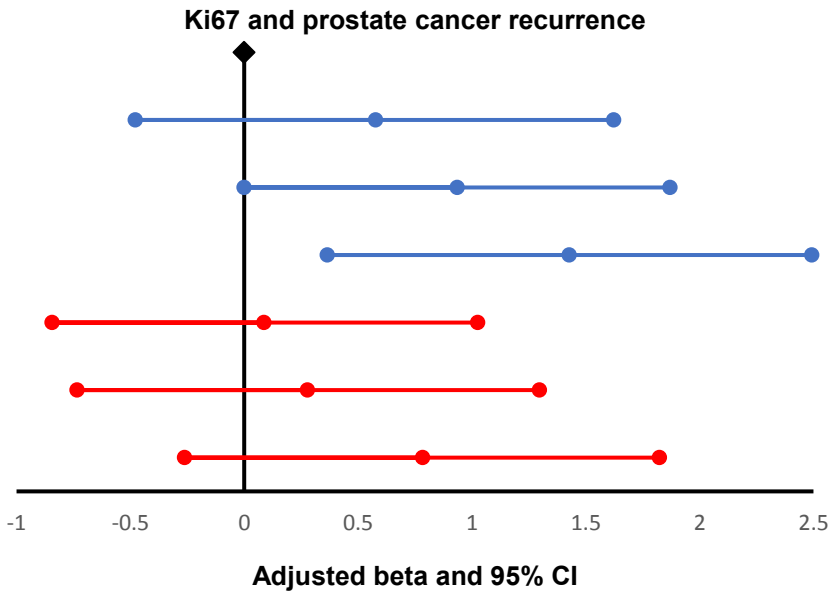
IHC – immunohistochemistry; FISH - fluorescence in situ hybridization; FFPE – formalin-fixed paraffin embedded

Table 2.2 Key indicators of threats to validity and source of measurement error identified by the practice of team science in three investigations of tissue-based biomarker and prostate cancer risk and recurrence: T1 translation

	Ki67	Telomeres	miRNA
Indicator of threat to validity			
Data-analysis & Interpretation	• Observed null association between increased Ki67 expression and prostate cancer recurrence; lack of consistency with hypothesis based on prior studies in other settings	• Observed null association between short telomere length and prostate cancer risk; lack of consistency with hypothesis	• Observed weak or null associations between the index miRNAs and prostate cancer recurrence; lack of consistency with hypothesis based on prior studies in other settings
		• Observed structure in the data indicative of batch-effects due to variable intensity of the illumination system	• Observed inconsistent patterns in associations for the index miRNA relative to U6 snRNA based on calendar time of tissue collection
			• Temporal differences in index miRNAs and especially in U6 snRNA with higher values observed in tissue samples collected in earlier calendar years compared to later years in cases and controls separately
Source of measurement error			
Tissue collection & storage	• Proteins, including Ki67, as detected by IHC decay over time in FFPE tissue blocks		• Observed temporal decay of U6 snRNA used to normalize expression of index miRNAs
	• Observed temporal decay of Ki67 in the study TMA spots		

Biomarker assay technologies		• Waning intensity of the microscopy-based illumination system used to digitally measure fluorescent telomere FISH signals	
		• Replacement of illumination source	
		• Rerun single batch after replacing illumination system with changed values for telomere signals	

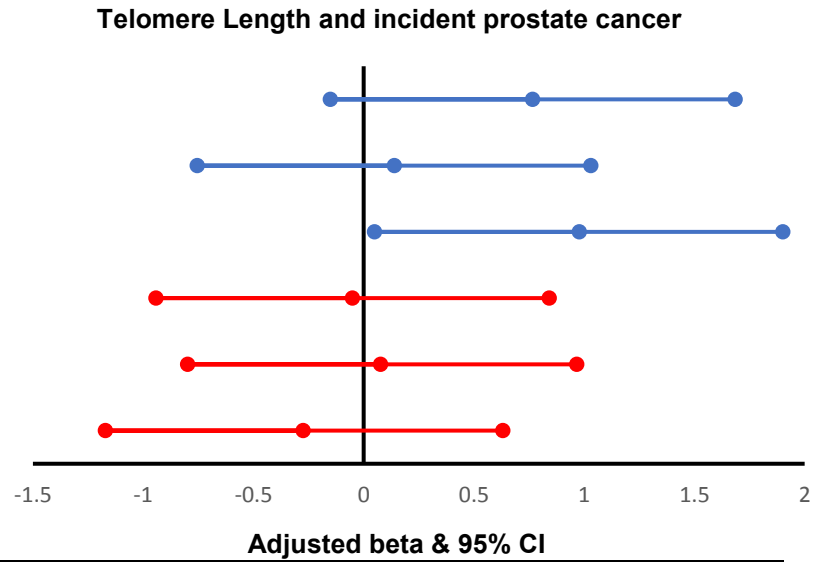
	Result	Ca/Co	Adjusted beta*	95% CI	% Bias**
Q1	NA	29/45	0.00	Ref	
Q2	Corrected	30/37	0.58	-0.48 to 1.62	85%
Q3	Corrected	44/43	0.94	0.0 to 1.87	70%
Q4	Corrected	64/42	1.43	0.36 to 2.49	45%
Q2	Biased	30/37	0.09	-0.84 to 1.03	
Q3	Biased	44/43	0.28	-0.73 to 1.30	
Q4	Biased	64/42	0.78	-0.26 to 1.82	
p-trend	Corrected	Beta:	0.46314	p = 0.007	910%
	Biased	Beta:	4.67842	p = 0.003	



Ca/Co – number of cases and controls
*ln(odds ratio)
** Percent difference in the team-science corrected and biased beta coefficients ([corrected – uncorrected] / [corrected])

Figure 2.1 Comparison of biased and team science-corrected associations between Ki67 quartiles and prostate cancer recurrence

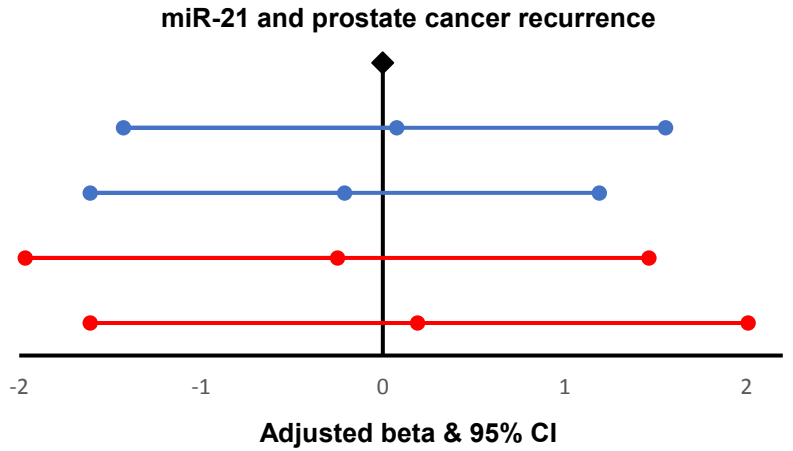
	Result	Ca/Co	Adjusted beta**	(95% CI)	% Bias***
Basal	Corrected	32/50	0.77	-0.15 to 1.68	107%
Luminal	Corrected	32/50	0.14	-0.76 to 1.03	45%
Stromal	Corrected	32/50	0.98	0.05 to 1.90	128%
Basal	Biased	32/50	-0.05	-0.94 to 0.84	
Luminal	Biased	32/50	0.08	-0.80 to 0.97	
Stromal	Biased	32/50	-0.27	-1.17 to 0.63	



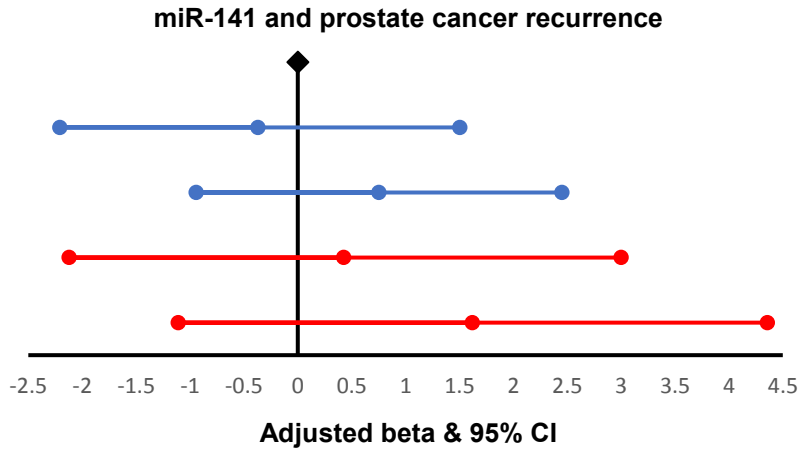
Ca/Co – number of cases and controls
*Below the median versus at or above
**ln(odds ratio)
*** Percent difference in the team-science corrected and biased beta coefficients ([corrected – uncorrected] / [corrected])

Figure 2.2 Comparison of biased and team science-corrected association between short* telomere length and incident prostate cancer

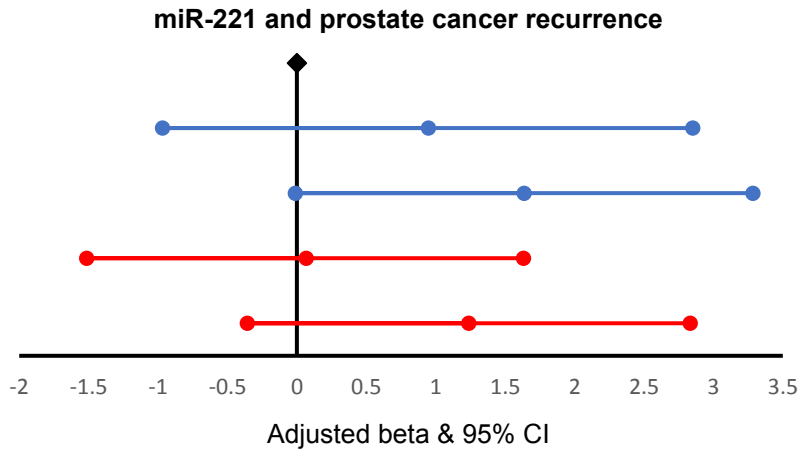
	Result	Ca/Co	Adjusted beta*	(95% CI)	% Bias**
T3	NA	24/22	0.0	Ref	
T2	Corrected	19/18	0.08	-1.43 to 1.56	423%
T1	Corrected	16/19	-0.21	-1.61 to 1.19	190%
T2	Biased	19/18	-0.25	-1.97 to 1.46	
T1	Biased	16/19	0.19	-1.61 to 2.00	
p-trend	Corrected	Beta:	0.09856	p = 0.7813	233%
	Biased	Beta:	-0.13134	p = 0.7573	



	Result	Ca/Co	Adjusted beta*	(95% CI)	% Bias**
T3	NA	14/21	0.0	Ref	
T2	Corrected	19/20	-0.37	-2.21 to 1.50	215%
T1	Corrected	26/18	0.75	-0.94 to 2.45	115%
T2	Biased	19/20	0.43	-2.12 to 3.00	
T1	Biased	26/18	1.62	-1.11 to 4.35	
p-trend	Corrected	Beta:	-0.48533	p = 0.2371	87%
	Biased	Beta:	-0.90576	p = 0.1772	



T3	NA	10/22	0.0	Ref	
T2	Corrected	21/19	0.95	-0.97 to 2.85	93%
T1	Corrected	28/18	1.64	-0.01 to 3.28	24%
T2	Biased	21/19	0.07	-1.51 to 1.63	
T1	Biased	28/18	1.24	-0.36 to 2.83	
p-trend	Corrected	Beta:	-0.80259	p = 0.0502	15%
	Biased	Beta:	-0.68547	p = 0.0870	



Ca/Co – number of cases and controls
**ln(odds ratio)
*** Percent difference in the team-science corrected and biased beta coefficients ([corrected – uncorrected] / [corrected])

Figure 2.3 Comparisons of biased and team science-corrected associations between micro-RNA tertiles and prostate cancer recurrence

Chapter 3.

When is enough, enough? Adapting the fail-safe number and conditional power for deciding whether more research is needed on biomarker-cancer associations.

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3.1 Abstract

Repetitive epidemiologic investigations of biomarker-cancer associations that do not fill knowledge gaps are not uncommon and can be a resource drain. We adapted clinical trial synthesis methods to quantify the impact of continued investigations. Two versions of the fail-safe number (FSN) and conditional power calculations were applied to 98 fixed and random effects meta-analyses on biomarkers and risk of 17 cancers. Rosenberg's FSN conditions on the statistical significance of each meta-analysis to determine the number of future studies of null effect and average study weight needed to drive the summary P value to > 0.05. Orwin's FSN conditions on the effect size in each meta-analysis to determine the number of future studies of a specified effect size needed to be added to drive the summary result to a certain, trivial effect size. Example 1: For the random effects meta-analysis of 15 studies on *H. pylori* infection and gastric cancer (OR=2.29; 95% CI 1.71-3.05), Rosenberg's FSN was 805; and Orwin's FSN was 240 future studies with an average OR=1.00 to drive the combined association from positive to null (OR to 1.05). The large number of additional studies needed to change the inference illustrates the futility of further study of this established association. Example 2: For the random effects meta-analysis of 7 studies on dehydroepiandrosterone sulfate and prostate cancer risk (OR=1.29; 95%CI: 0.99 to 1.69; I^2 = 17%), only 5 studies would need to be added to drive the combined association from null to statistically significantly positive (OR=1.29). This result suggests additional investigation could change the inference. Together with traditional evidence synthesis and appraisal methods, including assessments of between-study heterogeneity, small-study effects bias, study quality and remaining knowledge gaps (e.g., subgroup associations), use of such metrics by researchers, funders, grant reviewers, and journal editors might help determine whether more research is needed *or not* on specific biomarker and cancer associations, potentially saving time, money, and

allowing researchers to focus efforts on biomarkers with the greatest promise of improving individual and population-level cancer burden.

3.2 Introduction

Biomarkers of cancer etiology, risk, prognosis, and prediction are of contemporary interest in current cancer epidemiology, prevention and control research efforts. Yet, it is quite common to observe in the published literature, conference abstract proceedings, submitted grant applications, and submitted manuscripts repetitive investigations of established biomarker-cancer associations, referred to as *Me-too Science*.[1] Such redundant investigations that lack clinical or public health significance or the potential to improve biological understanding represent a practice-based barrier in translation, including for etiologic cancer biomarkers for determining cancer risk.[2,3] The overall magnitude of the cost of research waste has been estimated to be as much as 85% of the $200 billion invested in biomedical research in 2010.[4,5] A significant factor contributing to research waste is overlooking what is already known, or ignoring what is currently under investigation.[5,6] Unlike the necessary and legitimate practice of research reproducibility, redundant research neglects the existing evidence base and the context in which the current result will be considered. Thus, redundant uninformative research can be minimized by determining whether or not further investigation will provide a meaningful contribution to the existing evidence.

This brings into focus the importance of up-to-date systematic reviews and meta-analyses, which contribute to the domain of knowledge integration centrally positioned within the translational epidemiology framework.[7,8] The objective of knowledge integration is to synthesize the existing scientific evidence to accelerate the translation of discoveries into clinical and public health applications, and contribute to evidence-based recommendations by supporting or not supporting the implementation of the application into routine practice.[8] In cases where additional biomarker investigations are unlikely to provide a meaningful contribution to the current evidence, investigator's attention should

be focused on addressing existing evidence gaps in the biologic understanding of the biomarker-cancer relationship by evaluating new or improved methods to measure the biomarker or using other markers correlated and more specific to the studied biomarker, evaluating clinically meaningful outcomes, and reducing heterogeneity and imprecision in the observed associations by investigating the biomarker-cancer relationship in important subpopulations.

Existing quantitative metrics developed to determine how future investigations will contribute to the current evidence base can be adapted into a systematic process to overcome uninformative and redundant cancer biomarker investigations. A common practice contributing to non-reproducibility of biomedical research is the file-drawer problem[9], where investigators file away their uninteresting or nonsignificant results; ultimately leading to a selected sample of statistically significant results in the published literature (e.g., publication bias). To quantify the impact of selectively unpublished research on the existing meta-analysis, Rosenthal[9] introduced the fail-safe-number indicating the number of unpublished studies with an average null effect needed to be included in an updated meta-analysis to drive the combined P value to ≥ 0.05. In the context of redundant uninformative biomarker investigations, the fail-safe number can be adapted to determine whether the inference based on the existing meta-analysis for a statistically significant biomarker-outcome relationship will likely change with the addition of further research. Additional quantitative strategies have been developed to determine the sample size of a future study, based on the existing meta-analysis, needed to provide sufficient power in the combined meta-analysis.[10,11] In the context of an existing statistically non-significant meta-analysis, conditional power analysis can be used to determine the size and number of future studies needed to provide sufficient power to observe a statistically significant finding if one exists.[12] Conditional power analysis can

be adapted to determine the feasibility of conducting a sufficient number of future investigations to provide sufficient power to detect a significant association in the combined meta-analysis.

The objective of the current study is to adapt the fail-safe number and conditional power analysis to quantify the impact of further investigation of individual biomarker-cancer relationships on the existing evidence base, and to encourage the translational research community to consider existing evidence gaps to overcome uninformative redundant investigations. Utilizing a collection of 98 meta-analyses[13] describing the relationship between an array of cancer biomarkers and risk for multiple types of cancer provides an opportunity to demonstrate the empirical application of these metrics. The implementation and characterization of these metrics as applied to the 98 meta-analyses is followed by a summary of these metrics in the context of a well-established biomarker-cancer relationship (e.g., *H. pylori* and gastric cancer) and in a more uncertain biomarker-cancer relationship (e.g., androgens and prostate cancer).

3.3 Methods

We used an existing sample of 98 meta-analyses of prospective observational studies describing the association between a diverse range of non-genomic biomarkers and cancer risk including: Insulin-like growth factor(IGF)/insulin markers (21 meta-analyses); sex hormones (13 meta-analyses); dietary markers (31 meta-analyses); inflammatory markers (3 meta-analyses); infectious agents (22 meta-analyses); and environmental markers (8 meta-analyses).[13] The 98 meta-analyses described biomarker associations and risk of 17 specific cancer types. The most common cancer sites include breast (28 meta-analyses); prostate (24 meta-analyses); lung (10 meta-analyses); and colorectal (8 meta-analyses). As described by Tsilidis et al.[13] a comprehensive search

strategy was used to search PubMed between 1996 and 2010 to identify the 98 meta-analyses described in 37 publications which included a total of 847 individual studies with a median of seven included studies in each meta-analysis (range: 2 to 42). For each of the 98 meta-analyses both fixed- and random effects summary estimates are available as well as the corresponding 95% confidence intervals, number of included studies, total number of cases and non-cases, effect estimate from the study with the largest sample size included in each meta-analysis, and the I^2 statistic describing the between study heterogeneity. Based on random-effects models, 44 (45%) of the meta-analyses reported statistically significant summary odds ratios (OR), whereas fixed-effect models revealed 54 (55%) statistically significant meta-analyses. The summary ORs described in the 98 meta-analyses ranged from 0.41 to 65, with meta-analyses comparing infectious diseases with cancer risk at the extreme end of this distribution.

Fail-Safe Number (FSN) We applied two variations of the FSN to both fixed and random effects summary estimates in the 98 meta-analyses. We used Rosenberg's FSN[14] to quantify the number of future studies with an average null effect and average weight (e.g., inverse variance), needed to be included in the observed statistically significant meta-analysis, to drive the combined meta-analysis to null ($p \geq 0.05$) when the original meta-analyses observed statistically significant results. To overcome the restriction of statistical significance at the meta-analysis level, we used Orwin's FSN[15] to calculate the number of future studies with a specified average effect needed to be included in all meta-analyses to reduce to combined effect to a specified trivial value. The added utility of Orwin's FSN is the ability to test specific values of the effect in the future studies against a range of values for the effect in the combined analysis. We took an approach consistent with the underlying assumptions in Rosenberg's FSN by assuming the average effect in the future studies was null (OR = 1.00). We applied our approach to

Orwin's FSN to a range of effect estimates (OR = 1.05; 1.10; 1.25; 1.5; and 2.00) for the combined summary estimate.

Conditional power analysis For the meta-analyses not reaching statistical significance ($p \geq 0.05$), we conducted conditional power analysis to determine the number of future studies needed to achieve sufficient power to detect a statistically significant summary effect when added to the observed meta-analysis. We set the targeted power to 0.8 and took a pragmatic approach declaring an alternative hypothesis equivalent to the observed summary fixed and random effects OR and assumed the future studies were of average weight as those included in the observed meta-analysis. Our conditional power analyses were based on two approaches described by Roloff et al.[12] In approach 1, we assumed there was no heterogeneity between studies included in the observed meta-analysis ($I^2 = 0\%$) and that the future studies will not introduce heterogeneity. In the second approach, we focused on the random-effects meta-analyses to determine the conditional power analysis while still taking a pragmatic approach with an alternative effect size equivalent to the OR observed in the meta-analysis and average weight of the studies included in the meta-analysis. When using the random effects meta-analysis, we assumed between-study heterogeneity was equivalent in the new studies so that the new studies will not introduce additional between-study heterogeneity when combined with the observed meta-analysis. We calculated the two variations of the FSN and both conditional power analysis approaches in STATA version 13 (STATA Corp, College Station, TX).

3.4 Results

Among the 54 and 44 statistically significant fixed and random effects meta-analyses with a median $I^2 = 42\%$ and 36% (range: 0 to 94%) respectively, the median

number of included studies was 9 (range: 2 to 42) and the median total number of participants was 3,727 (range: 357 to 1,381,129) for the fixed-effect meta-analyses and 3,781 for the random effects meta-analysis (Table 3.1). For the statistically significant fixed-effect meta-analyses with a median summary OR of 1.28 (range 0.41 to 65), the median value of Rosenberg's FSN, the number of additional studies of average weight and average null effect needed to be included in the observed meta-analysis to drive the combined summary estimate to null ($p \geq 0.05$), was 31.5 (range 3.2 to 24,939). For the random effects meta-analyses with a median OR = 1.52 (range 0.41 to 61.2), the median value of Rosenberg's FSN was 31.1 (range 3.2 to 3,464). The median FSN for meta-analyses with no heterogeneity ($I^2 = 0\%$; 15 fixed and random effects meta-analyses; median OR = 1.7; median number of included studies - 4) was 17.0 for both fixed and random effects meta-analyses; 53 and 45 for fixed and random effects meta-analyses with low heterogeneity (I^2: 1 to 29%; 6 and 5 meta-analyses; median ORs = 1.35 and 1.54; median number of included studies - 12); 31 and 26 for fixed and random effects meta-analyses with moderate heterogeneity (I^2: 30 to 59%; 17 and 11 meta-analyses; median ORs = 1.19 and 1.28; median number of included studies – 8 and 9); 30 and 17 for fixed and random effects meta-analyses with high heterogeneity (I^2: 60 to 80%; 9 and 5 meta-analyses; median ORs = 1.24 and 1.99; median number of included studies - 11); and 1497 and 148 for fixed and random effects meta-analyses with extreme heterogeneity (I^2: > 80%; 7 and 8 meta-analyses; median ORs = 3.88 and 3.13; median number of included studies – 15 and 21) (Figure 3.1).

The influence of between-study heterogeneity on Rosenberg's FSN is illustrated by comparing the FSN for fixed and random effects meta-analyses from the same study. The FSN for the fixed-effect model tends to be larger compared to the corresponding FSN for random effects meta-analysis given the assumption of no between-study heterogeneity resulting in tighter confidence intervals around the fixed-effect summary

estimates (Figure 3.1). Smaller values for the FSN in the random effect meta-analyses are indicative of the between-study heterogeneity incorporated into the summary estimates which introduces additional uncertainty in the precision of the random effects estimates. With the additional uncertainty in the random effects meta-analysis, fewer studies would be needed to drive the combined meta-analysis to null, compared to the corresponding fixed-effect meta-analysis. The pattern of larger values of Rosenberg's FSN with increased precision of the summary estimate is illustrated further when conditioning on the number of studies included in the observed meta-analysis within strata of between-study heterogeneity (Figure 3.2). Among meta-analyses with similar between-study heterogeneity, meta-analyses with more included studies tend to have high values for the FSN.

Conditioning on summary estimates observed in the existing meta-analyses revealed larger values for the FSN for meta-analyses with larger summary estimates (Figure 3.3). Among statistically significant fixed and random effects meta-analyses with summary ORs between 1.00 and 1.10, the median FSNs were 24 and 17 respectively; 49 and 16 for meta-analyses with summary ORs between 1.11 and 1.25; 68 and 27 for meta-analyses with summary ORs between 1.26 and 1.50; 14 and 17 for fixed and random effects meta-analyses with summary ORs between 1.51 and 2.00; and 795 and 155 for meta-analyses with summary ORs greater than 2.00.

Conditioning on the summary estimates in the observed meta-analyses, the size of the effect in the future studies, and size of the summary effect in the combined result, Orwin's FSN does not take into account within- or between-study variance in the observed meta-analysis. Therefore, we only considered the values of Orwin's FSN for the fixed-effect meta-analyses and assumed the average effect in the future studies was null (OR = 1.00). We observed a pattern where larger values for Orwin's FSN were

observed for the smaller combined summary estimates (Figure 3.4). To reduce the combined effect size to 1.05 among 38 meta-analyses with a summary OR greater than 1.05, Orwin's FSN was 271. The median FSN was 140; 58; 29; and 33 to reach a combined OR of 1.10; 1.25; 1.50; and 2.00 respectively among meta-analyses with summary ORs greater than the combined effect size (e.g., 1.10, 1.25, 1.50, and 2.00).

Our first approach to conducting conditional power analysis was to determine the number of future studies of average weight as those included in the observed meta-analysis assumed no between-study heterogeneity in the observed and combined meta-analysis. The assumption of no between-study heterogeneity in both the observed and combined meta-analyses leads to equivalent results in the fixed and random effects meta-analyses. Therefore, we only considered the 18 fixed-effect meta-analyses not reaching statistical significance with summary ORs greater than 1.01 in our first approach. With a median power of 15% (range: 0.5% to 50%) for the existing meta-analysis with a median of 6 (range: 2 to 14) included studies, we observed a median of 78 (range: 4 to 994) future studies of average weight with no between-study heterogeneity needed to be included in the combined meta-analyses to achieve 80% power to detect the observed summary OR reported in the existing meta-analysis (Table 3.1). In our second approach, between-study heterogeneity in the future studies was assumed to be equivalent to the between-study heterogeneity in the observed meta-analysis. Based on 21 statistically non-significant random effects meta-analyses with summary ORs greater than 1.01, a median power of 21% (range: 6% to 47%), with a median I^2 of 50% (range: 0% to 92%) and a median of 7 included studies (range: 2 to 14), 103 future studies (range 5 to 6,656) of average weight and equivalent between-study heterogeneity as in the observed meta-analysis would be required to achieve 80% power in the combined meta-analysis (Table 3.1). The greater number of future studies

required to achieve 80% for the random effects meta-analyses compared with the fixed-effect meta-analyses is consistent with the different assumptions regarding between-study heterogeneity incorporated into our two approaches. By taking into account the between-study heterogeneity, our second approach incorporated additional uncertainty into the summary estimates, thereby increasing the number of future studies. With respect to the size of the observed summary estimates conditioned on in our power analysis, we observed a decrease in the number of future studies with increasing size of the summary estimate in the both fixed and random effect meta-analyses (Figure 3.5).

3.5 Discussion

In this paper, we adapted two established metrics – the fail-safe number (FSN)[14] and conditional power analysis[12] – to quantify the impact of future investigations on the inferences drawn from existing meta-analyses. Each of these metrics provides a heuristic approach to determine if continued investigation is warranted versus sufficient evidence is available to establish or refute a biomarker-cancer association. We applied these metrics to 98 meta-analyses of observational epidemiologic studies evaluating the associations between non-genomic biomarkers and cancer risk to demonstrate the ability of these metrics to identify situations where future research would not provide a meaningful contribution in an update meta-analysis. The results of the FSN and conditional power analysis are consistent with the underlying computation of each metric, which summarize characteristics of the cumulative evidence, including the number of studies included in the meta-analysis, the summary estimate and the precision of the summary estimate, and the heterogeneity in the findings of the studies included in the meta-analysis. We saw the values of the FSN to increase with decreasing levels of heterogeneity, increasing number of included studies, and larger summary estimates, while the size of the summary estimate appeared to be the

strongest factor influencing the results of the conditional power analysis. Our motivation to adapt these metrics as a means of quantifying the impact of further investigation stems from the abundance of wasteful biomedical research.[5] We envision the application of this process along with traditional assessments of study quality and remaining knowledge gaps (e.g., subgroup associations) by stakeholders engaged in translational epidemiologic research including principle investigators, funding agencies, grant reviewers, journal editors, and peer-reviewers, will lead to more informative research.

Application of FSN and conditional power analysis to cancer biomarker investigations

We describe here the application of these adapted methods to two example biomarker-cancer meta-analyses.

FSN – H. pylori and gastric cancer In 1994 the International Agency for Research on Cancer (IARC) classified *Helicobacter pylori* as a Group 1 carcinogen.[16] At the time, the evidence supporting IARC's classification included four cohort studies and nine case-control studies evaluating the relationship between *H. pylori* infection and gastric cancer. In the time since the initial classification, the accumulation of evidence suggests the relationship between *H. pylori* and gastric cancer has been well established. This is reflected in the greater than 2-fold increase in risk of gastric cancer described in the meta-analysis of 15 studies with more than 5,000 cases and controls reported by Huang et al.[17] Rosenberg's FSN indicates 805 future studies would be required to reduce the reported fixed-effect summary OR of 2.05 (95% CI: 1.79 to 2.35; I^2= 76%) to null ($p \geq 0.05$) and 224 future studies based on the random effects meta-analysis (summary OR: 2.29; 95% CI 1.71 to 3.05; I^2= 76%). Based on Orwin's FSN, a total of 615 future studies averaging null effect (OR = 1.00) would be required to drive the observed fixed-effect

summary OR of 2.05 to a trivial 1.05. The implementation of each FSN to the example of *H. pylori* and gastric cancer illustrates the futility of further investigation of the association between *H. pylori* and gastric cancer, while the extreme between study heterogeneity (I^2= 76%) suggests the need for further subgroup analysis. To this end, further investigations have shown associations between dietary factors and the risk of gastric cancer, which help explain the geographic and ethnic differences in the distribution of gastric cancer. This line of research has shown a substantial increase in risk associated with diets high in salt after accounting for *H. pylori* infection, which provides evidence that dietary salt intake acts to modify the effect of *H. pylori* infection on gastric cancer.[18,19] The role of dietary salt intake as a modifier of the effect of *H. pylori* is supported by additional research that identified cagA gene expression in *H. pylori*, a marker of higher risk of gastric cancer, is modified by dietary salt intake.[20] These findings further illustrate the importance of examining subgroups or different populations once the main effect of the etiologic cancer biomarker has been established, which can provide additional understanding of the underlying biology driving the biomarker cancer association. Finally, greater public health impact could be gained from developing and examining approaches targeting *H. pylori* prevention and treatment. To this end, future investigations could be designed to examine factors influencing the successful implementation of such approaches and the impact of these strategies on reducing the burden of gastric cancer.

Conditional power analysis – androgens and prostate cancer In 1993 the Prostate Cancer Prevention Trial was launched to investigate the effects of finasteride, a drug that blocks the transformation of testosterone into dihydrotestosterone (DHT), in preventing prostate cancer,[21] which eventually was stopped early in 2003 given a 25% reduction in the period prevalence of prostate cancer in the treatment group receiving

finasteride.[22] The reduction in prostate cancer incidence in the finasteride group provided additional evidence supporting the underlying hypothesis that DHT is an etiologic factor in prostate cancer development. However, several methodological challenges encountered in population-based epidemiologic investigations including adequacy of measuring circulating hormones, difficulty integrating multiple components of the androgen pathway, difficulty incorporating clinical and population health import outcomes, and detection bias (e.g., differential opportunity to be screened with PSA by exposure; and differential detection of prostate cancer due to relationship between exposure and screening with PSA), have contributed to the inconsistent associations between circulating androgens and prostate cancer incidence.[23] The relationship between individual components in the androgen pathway and prostate cancer have been summarized in the meta-analyses by Roddam et al.[24] (Table 3.2). In 6 of the fixed-effect meta-analyses not reaching statistical significance with no between-study heterogeneity (I^2 = 0%), conditional power analysis revealed 18 to 1173 future studies of average weight as those included in the observed meta-analysis required to achieve 80% power to detect the observed summary OR in the combined meta-analysis (Table 3.2). For these comparisons, the large number of future studies needed to achieve sufficient power may not be within reach of existing resources, and points to a situation where further research should be aimed at overcoming the methodologic challenges mentioned above[23] to fill important evidence gaps with respect to androgens and prostate cancer.

In the case of the random effects meta-analysis with 7 included studies comparing dehydroepiandrosterone sulfate and prostate cancer (summary OR: 1.29; 95% CI: 0.99 to 1.68; I^2: 17%), the 5 future studies required to achieve 80% power to detect the observed summary OR in the combined meta-analysis may be within reach of existing resources, and points to a scenario where additional research could provide a

meaningful contribution to the existing meta-analysis. However, we caution against the inappropriate interpretation of the results of the conditional power analysis applied to the example of androgens and prostate cancer incidence. Our approach assumed the number of future studies required to achieve sufficient power are of average weight as those already included in the observed meta-analysis and will not introduce additional between-study variance into the combined meta-analysis. Overlooking the composition of the existing evidence base and failure to consider the methodological issues previously cited as factors leading to inconsistent associations when considering future investigations would reflect practice of *Me too* science we are trying to overcome. With respect to molecular epidemiologic investigations, measurement error in the index biomarker assay may introduce between-study heterogeneity in the summary estimates. In settings where the FSN or conditional power analysis indicate potential benefit from continued research, future research should be designed to account for the known measurement error in the existing evidence in order to maximize the contributions from the future investigations.

Furthermore, sufficient biological plausibility of the biomarker-cancer relationship, and applicability of strategies incorporating the biomarker into cancer prevention and control efforts are necessary for future research to be informative. The meta-analyses with sufficiently low number of future studies, determined by the FSN or conditional power analysis, suggesting future research may influence the observed association (Table 3.2) may not be relevant given the current state of cancer prevention efforts and are not in step with the progress that has been made by the cancer research community. To this end, Emmons and Colditz[25] made the point of focusing on the implementation of current strategies aimed at cancer prevention with sound evidence supporting their implementation. This scenario is analogous to the example of *H. pylori* and gastric

cancer previously described, where future research should be targeted to enhance efforts aimed at the subsequent stages within the translational continuum from discovery to population health impact.

Adapting research synthesis metrics for translational research

For this work, we selected the FSN, a metric previously developed for use in settings different from ours, specifically to quantify potential publication bias in a systematic review and meta-analysis. However, the Cochrane Collaboration does not recommend its use for this purpose.[26] This critique stems from the reliance on statistical significance rather than on clinical significance of an observed effect. Other metrics endorsed by the Cochrane Collaboration for evaluating publication bias,[26] include inspection of the funnel plots for asymmetry and corresponding metrics developed by Egger et al.[27] to statistically test for the presence of such asymmetry. Our use of the FSN for a different purpose (to determine if future research can provide meaningful information to the current evidence base) is not subject to this critique.

For this work we also adapted conditional power analysis in the context of a null meta-analysis, which was developed to determine the sample size and number of future studies based on an existing meta-analysis necessary to provide sufficient power to detect a specific effect in the combined meta-analysis.[12] While our use is consistent with the intent of the method, other methods, which we did not consider here, are available for planning and designing future research. In the context of randomized controlled trials of intervention, for example, Sutton et al. evaluated Barrowman's n and a simulation approach to determine the number of additional participants needed to be included in an updated meta-analysis to achieve sufficient power, and demonstrated the application of such metrics to prioritize updating systematic reviews of interventions.[11] In that review,

Sutton et al. noted the computational challenges associated with the simulation based approach, which may impede the use of this metric.[10,11] In contrast, conditional power analysis, which we used, has straightforward implementation and interpretation.

To our knowledge no method has been introduced to directly quantify the impact of continued observational epidemiologic research on the current evidence base. While our motivation was to show how both the FSN and conditional power analysis could be used to quantify the impact of future research, additional work is needed to incorporate the FSN and conditional power analysis into a formal framework prioritizing observational epidemiologic research. Constructing evidence maps by plotting the level of evidence determined by the FSN and/or conditional power analysis for each biomarker and cancer against the relevant outcomes and subgroups would provide a formal approach incorporating these metrics with other factors to consider when planning future research. However, the application of the FSN and conditional power analysis for such purposes would need to be supported by further evaluation to determine explicit stopping points based on the FSN and conditional power analysis. Exploring the distribution of each metric in the context of symmetrical (e.g., unbiased sample of studies included in the meta-analysis) and asymmetrical (e.g., potentially biased sample of included studies) effects of the included studies quantified by Egger's test[27] may serve as starting point for developing such an approach.

We do not intend for the use of these methods by stakeholders to stop new research on biomarkers considered to be established (number of future studies needed to change a significant result to non-significant is very large) or not establishable (number of future studies needed to change a non-significant result to significant is very large). A goal is to prevent research that is *Me-too science* that does not advance

knowledge. Instead, we intended for these metrics to encourage translational researchers to not simply perform the "same" biomarker study in their cohort, but to consider designing a study that addresses the association in different populations and subpopulations than previously studied, use different or novel approaches to measuring the biomarker in accessible tissue (e.g., blood) or the biological pathway or process directly in the target organ, and refined endpoints, including those defined based location within the organ, histology, molecular phenotype, and step in the natural history of the cancer. Such practices would help inform more productive research and will provide valuable insights by addressing existing evidence gaps with greater potential for navigating the translational continuum from discovery to population-health impact.

While our work was focused on determining whether additional individual studies are needed to inform a biomarker-cancer association, these adapted methods could also be easily applied to determine when an updated meta-analysis on the same biomarker-cancer association is needed. A comparison of the results for the FSN and conditional power analysis would provide an opportunity to explore the impact of updating an existing meta-analysis with new studies. This would have allowed us to extend our approach to informing and prioritizing the conduct of meta-analyses. The conduct and dissemination of uninformative meta-analyses is an analogous problem as redundant uninformative primary research. Recently, Ioannidis[28] described two issues observed in the exponential increase in the number of uninformative systematic reviews and meta-analyses: 1) methodologic flaws in the conduct of the systematic review and meta-analysis, and 2) the absence of a significant number of primary studies in the relevant systematic review. We see the application of the FSN and conditional power analysis to serve in a similar capacity for prioritizing observation epidemiologic analyses as we

demonstrated in the current analyses, as well as prioritizing the conduct of meta-analyses of observational epidemiologic research.

We recognize that application of these adapted methods to existing meta-analyses is not the only strategy to minimizing the problem of *Me-too science*. The *Me-too Science* problem arises when individual investigators conduct and publish on the same topic without coordination of the sufficiency of what has already been published. We highlight a quantitative approach to determine whether another study is needed. An alternative approach to avoid the *Me-too science* problem is a coordinated effort among individual investigators to collectively determine which biomarkers require additional investigations, to share and pool their data and biospecimens, and to standardize the biomarker's measurement and harmonize the outcome and covariate data. Using this approach, research on particular biomarkers is prioritized through consensus, biomarker-cancer associations can be investigated in subpopulations of the pooled studies, and power is maximized. This practice-based approach has been used over the past 15 years by large consortia including the Cancer Cohort Consortium (>50 cohorts with 7 million participants) and the Early Detection Research Network both supported by the National Cancer Institute (NCI),[29] and the Endogenous Hormones, Nutritional Biomarkers and Prostate Cancer Collaborative Group (35 studies with biomarker data on 23,000 men with prostate cancer and 35,000 controls).[24]

Several aspects of the design of this work warrant further consideration in terms of the application of the FSN and conditional power analysis in determining contribution of future investigations on the current evidence base. With respect to the conditional power analysis, we declared the alternative hypothesis equivalent to the summary estimate reported in the observed meta-analysis, and assumed no additional between-

study heterogeneity was introduced by the new studies. The veracity of these assumption could have been tested through an iterative approach evaluating different alternative hypotheses (e.g., using the OR of the largest study included in the observed meta-analysis) that would be more sensitive to small study effect on the summary OR. Given the focus on observational epidemiologic studies, different degrees of between-study heterogeneity in the future studies would also be informative. However, our intention was not to investigate the impact of an unlimited number of conditions on the composition of the future studies. While the focus of our work was to apply the fail-safe number and conditional power analysis to an existing list of meta-analyses of observational epidemiology studies of etiologic cancer biomarkers and cancer risk, these methods are equally applicable to observational epidemiology studies on any exposure-outcome association, including non-biomarker exposures and other important outcomes such as mortality, and prognosis.

In summary, we show how the fail-safe number and conditional power analysis can be adapted to quantify the impact of future investigations of individual biomarkers and cancer risk on the current evidence base summarized in the corresponding meta-analysis. In the context of a well-established biomarker-cancer relationship, and a less certain biomarker-cancer relationship, we show how these metrics can precipitate investigator's consideration of characteristics of the existing evidence related to the populations previously studied, different approaches to measuring the underlying biologic construct, and meaningful endpoints, thereby contributing to more informative research. We envision the systematic application of these metrics by stakeholders engaged in validation and replication of cancer biomarkers including journal editors, grant reviewers, funding agencies, and principal and junior investigators will ultimately

lead to more productive research with greater potential for navigating the translational continuum form discovery to population-health impact.

3.6 References

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Table 3.1 **Results for Rosenberg's and Orwin's FSN and conditional power analysis for the 98 meta-analysis**

Area	Author & Year	Cancer	Biomarker	No.of studies	No.cases/ controls	I^2	Fixed-effect				Random effects		
							OR (95% CI)	FSN[1]	FSN[2]	M[3]	OR (95% CI)	FSN[1]	M[4]
Diet	Chen 2010[30]	BrCA	1a,25(OH)2 vitamin D	3	3627	47	1.02 (0.81-1.29)	NA	NA	858	0.99 (0.68-1.44)	NA	27210
Diet	Saadatian-Elahi 2004[31]	BrCA	Arachidonic acid	5	2226	0	0.89 (0.65-1.22)	NA	NA	79	0.89 (0.65-1.22)	NA	181
Diet	Saadatian-Elahi 2004[31]	BrCA	Linoleic acid	8	3081	60	0.88 (0.69-1.12)	NA	NA	67	0.85 (0.57-1.26)	NA	457
Diet	Saadatian-Elahi 2004[31]	BrCA	MUFA	5	2291	67	1.33 (0.98-1.81)	NA	NA	4	1.44 (0.82-2.53)	NA	31
Diet	Saadatian-Elahi 2004[31]	BrCA	Palmitic acid	7	2802	59	1.04 (0.81-1.35)	NA	NA	621	1.05 (0.69-1.58)	NA	6656
Diet	Saadatian-Elahi 2004[31]	BrCA	Palmitoleic acid	2	798	81	1.09 (0.68-1.74)	NA	NA	123	1.26 (0.41-3.89)	NA	301
Diet	Saadatian-Elahi 2004[31]	BrCA	SFA	6	2570	0	1.05 (0.79-1.39)	NA	NA	410	1.05 (0.79-1.39)	NA	1430
Diet	Saadatian-Elahi 2004[31]	BrCA	Stearic acid	7	2802	14	0.93 (0.71-1.23)	NA	NA	200	0.93 (0.69-1.26)	NA	937
Diet	Saadatian-Elahi 2004[31]	BrCA	a Linolenic acid	8	3444	39	0.82 (0.65-1.03)	NA	NA	12	0.80 (0.59-1.08)	NA	39
Diet	Saadatian-Elahi 2004[31]	BrCA	n-3 PUFA	8	2946	37	0.79 (0.60-1.03)	NA	NA	11	0.79 (0.56-1.11)	NA	51
Diet	Saadatian-Elahi 2004[31]	BrCA	n-6 PUFA	7	2667	16	0.75 (0.55-1.03)	NA	NA	36	0.75 (0.53-1.06)	NA	369
Diet	Chen 2010[30]	BrCA	25(OH) vitamin D	7	11330	86	0.58 (0.51-0.66)	230	75	NA	0.55 (0.38-0.80)	29	NA
Diet	Saadatian-Elahi 2004[31]	BrCA	Docosahexanoic acid	7	3262	36	0.76 (0.59-0.99)	5	106	NA	0.73 (0.53-1.02)	NA	9
Diet	Saadatian-Elahi 2004[31]	BrCA	Eicosapentanoic acid	5	2291	0	0.91 (0.87-0.95)	48	88	NA	0.91 (0.87-0.95)	48	NA
Diet	Buck 2010[32]	BrCA	Enterolactone	12	7710	71	0.84 (0.74-0.96)	24	200	NA	0.79 (0.61-1.02)	NA	14
Diet	Larsson 2007[33]	BrCA	Folate	6	3584	41	0.69 (0.53-0.90)	18	79	NA	0.67 (0.46-1.00)	6	NA
Diet	Saadatian-Elahi 2004[31]	BrCA	Oleic acid	9	3723	70	0.83 (0.71-0.98)	14	144	NA	0.99 (0.70-1.38)	NA	184370
Diet	Larsson 2010[34]	CRC	Vitamin B6	4	2307	0	0.52 (0.38-0.71)	31	39	NA	0.52 (0.38-0.71)	31	NA
Diet	Yin 2009[35]	Colon CA	25(OH) vitamin D	7	2944	46	0.77 (0.59-1.00)	7	103	NA	0.78 (0.53-1.13)	NA	46
Diet	Gallicchio 2008[36]	Lung CA	A-carotene	5	5618	53	0.91 (0.69-1.19)	NA	NA	65	0.88 (0.59-1.33)	NA	438
Diet	Gallicchio 2008[36]	Lung CA	B-cryptoxanthin	5	5618	75	0.87 (0.62-1.21)	NA	NA	44	0.82 (0.40-1.69)	NA	529
Diet	Gallicchio 2008[36]	Lung CA	Lutein/zeaxanthin	4	5066	11	0.95 (0.68-1.33)	NA	NA	342	0.95 (0.67-1.36)	NA	1192
Diet	Zhuo 2004[37]	Lung CA	Selenium	6	2687	42	0.80 (0.63-1.02)	NA	NA	6	0.77 (0.56-1.08)	NA	17
Diet	Gallicchio 2008[36]	Lung CA	B-carotene	10	37629	41	0.83 (0.73-0.94)	31	160	NA	0.84 (0.66-1.07)	NA	36
Diet	Gallicchio 2008[36]	Lung CA	Carotenoids	4	7803	45	0.70 (0.50-0.97)	5	53	NA	0.70 (0.44-1.11)	NA	12
Diet	Gallicchio 2008[36]	Lung CA	Lycopene	4	5294	0	0.71 (0.51-0.99)	5	54	NA	0.71 (0.51-0.99)	5	NA
Diet	Yin b 2009[38]	PrCA	25(OH) vitamin D	11	7806	26	1.03 (0.97-1.10)	NA	NA	82	1.03 (0.95-1.11)	NA	536
Diet	Collin 2010[39]	PrCA	Folate	7	9920	39	1.04 (0.98-1.11)	NA	NA	25	1.11 (0.96-1.28)	NA	17
Diet	Collin 2010[39]	PrCA	Total Homocysteine	4	7015	14	0.93 (0.74-1.17)	NA	NA	77	0.91 (0.70-1.19)	NA	123
Diet	Collin 2010[39]	PrCA	Vitamin B12	6	9401	45	1.09 (1.03-1.14)	24	127	NA	1.10 (1.01-1.19)	10	NA
Diet	Simon 2009[40]	PrCA	a Linolenic acid	6	2361	16	1.51 (1.17-1.94)	26	181	NA	1.54 (1.16-2.06)	21	NA
Environment	Khanjani 2007[41]	BrCA	Cis-nonachlor	3	1387	0	1.09 (0.72-1.64)	NA	NA	137	1.09 (0.72-1.64)	NA	290
Environment	Lopez-Cervantes 2004[42]	BrCA	DDT	24	11369	17	0.97 (0.87-1.09)	NA	NA	668	0.97 (0.85-1.11)	NA	8663
Environment	Khanjani 2007[41]	BrCA	Dieldrin	5	3223	43	1.18 (0.89-1.58)	NA	NA	26	1.15 (0.77-1.69)	NA	288
Environment	Khanjani 2007[41]	BrCA	Trans-nonachlor	6	3248	0	0.86 (0.68-1.07)	NA	NA	23	0.86 (0.68-1.07)	NA	35
Environment	Khanjani 2007[41]	BrCA	Oxychlordane	5	2718	51	0.75 (0.57-0.98)	4	73	NA	0.77 (0.51-1.14)	NA	38
Environment	Veglia 2008[43]	CA (cur smokers)	DNA adducts	8	916	94	3.88 (3.31-4.54)	1146	628	NA	3.76 (1.75-8.05)	39	NA
Environment	Veglia 2008[43]	CA (for smokers)	DNA adducts	7	632	0	0.94 (0.71-1.25)	NA	NA	291	0.94 (0.71-1.25)	NA	1041
Environment	Veglia 2008[43]	CA (nev smokers)	DNA adducts	9	564	79	1.20 (0.88-1.64)	NA	NA	41	1.64 (0.72-3.77)	NA	103
IGF/insulin	Pisani 2008[44]	BrCA	C-peptide	11	3517	64	1.26 (1.07-1.48)	27	269	NA	1.35 (1.01-1.81)	11	NA
IGF/insulin	Morris 2006[45]	CRC	IGFBP-3	7	3501	60	1.00 (0.77-1.30)	NA	NA	NA	0.98 (0.64-1.51)	NA	47178
IGF/insulin	Pisani 2008[44]	CRC	C-peptide	12	5542	54	1.36 (1.15-1.62)	64	322	NA	1.51 (1.14-1.99)	39	NA
IGF/insulin	Pisani 2008[44]	CRC	Glucose	11	1381129	47	1.19 (1.07-1.32)	49	257	NA	1.28 (1.06-1.54)	26	NA

IGF/insulin	Rinaldi 2010[46]	CRC	IGF-1	11	7828	0	1.07 (1.01-1.14)	17	230	NA	1.07 (1.01-1.14)	17	NA
IGF/insulin	Morris 2006[45]	CRC	IGF-2	3	1685	0	1.95 (1.26-3.00)	11	117	NA	1.95 (1.26-3.00)	11	NA
IGF/insulin	Pisani 2008[44]	Endometrial CA	C-peptide	4	862	69	1.09 (0.74-1.62)	NA	NA	141	1.18 (0.57-2.43)	NA	642
IGF/insulin	Chen 2009[47]	Lung CA	IGF-1	6	12515	41	1.05 (0.80-1.37)	NA	NA	361	0.98 (0.68-1.41)	NA	21602
IGF/insulin	Chen 2009[47]	Lung CA	IGFBP-3	6	12515	67	0.89 (0.68-1.15)	NA	NA	54	0.96 (0.59-1.56)	NA	10376
IGF/insulin	Pisani 2008[44]	Pancreas CA	C-peptide	2	692	0	1.70 (1.11-2.61)	4	68	NA	1.70 (1.11-2.61)	4	NA
IGF/insulin	Pisani 2008[44]	Pancreas CA	Glucose	5	1334539	0	1.98 (1.67-2.35)	152	198	NA	1.98 (1.67-2.35)	152	NA
IGF/insulin	Rowlands 2009[48]	PrCA	IGFBP-1	3	1553	92	0.93 (0.80-1.09)	NA	NA	72	1.20 (0.65-2.22)	NA	251
IGF/insulin	Rowlands 2009[48]	PrCA	IGFBP-2	5	2670	78	1.07 (0.95-1.21)	NA	NA	36	1.18 (0.90-1.54)	NA	56
IGF/insulin	Rowlands 2009[48]	PrCA	IGFBP-3	29	17160	81	0.97 (0.93-1.01)	NA	NA	80	0.88 (0.79-0.98)	57	NA
IGF/insulin	Rowlands 2009[48]	PrCA	IGF-1	42	19347	88	1.18 (1.14-1.23)	1497	974	NA	1.21 (1.07-1.36)	159	NA
IGF/insulin	Rowlands 2009[48]	PrCA	IGF-1/BP-3	11	9677	80	1.07 (1.02-1.13)	30	230	NA	1.10 (0.97-1.24)	NA	46
IGF/insulin	Rowlands 2009[48]	PrCA	IGF-2	10	2797	77	1.24 (1.12-1.36)	81	242	NA	1.17 (0.93-1.47)	NA	75
IGF/insulin	Key 2010[49]	postmenopausal BrCA	IGF-1	15	8185	0	1.30 (1.13-1.49)	92	385	NA	1.30 (1.13-1.49)	92	NA
IGF/insulin	Key 2010[49]	postmenopausal BrCA	IGFBP-3	15	8012	31	1.21 (1.04-1.41)	32	357	NA	1.22 (1.01-1.49)	16	NA
IGF/insulin	Key 2010[49]	premenopausal BrCA	IGFBP-3	11	5927	0	0.99 (0.83-1.19)	NA	NA	7367	0.99 (0.83-1.19)	NA	50352
IGF/insulin	Key 2010[49]	premenopausal BrCA	IGF-1	11	6033	29	1.18 (1.00-1.40)	10	255	NA	1.21 (0.98-1.49)	NA	12
Infection	Gutierrez 2006[50]	Bladder CA	HPV (DNA)	13	657	6	2.29 (1.37-3.84)	53	597	NA	2.30 (1.33-4.00)	45	NA
Infection	Gutierrez 2006[50]	Bladder CA	HPV (no DNA)	3	379	0	2.98 (1.65-5.40)	18	180	NA	2.98 (1.65-5.40)	18	NA
Infection	Zhao 2008[51]	CRC	H. pylori	14	3581	58	1.41 (1.22-1.65)	127	391	NA	1.49 (1.16-1.90)	57	NA
Infection	Mandelblatt 1999[52]	Cervical CA	HPV	12	3657	27	8.07 (6.49-10.0)	2338	1978	NA	8.08 (6.04-10.8)	1249	NA
Infection	Zhang 1994[53]	Cervical CA	T. vaginalis	2	65764	0	1.88 (1.29-2.74)	9	75	NA	1.88 (1.29-2.74)	9	NA
Infection	Islami 2008[54]	ESCC	H. pylori	9	3664	73	1.08 (0.92-1.27)	NA	NA	73	1.10 (0.78-1.55)	NA	1356
Infection	Islami 2008[54]	ESCC	cagA	4	2327	0	1.01 (0.79-1.27)	NA	NA	NA	1.01 (0.79-1.27)	NA	NA
Infection	Islami 2008[54]	Esophageal adeno CA	H. pylori	13	3730	15	0.56 (0.48-0.67)	275	136	NA	0.57 (0.47-0.69)	207	NA
Infection	Islami 2008[54]	Esophageal adeno CA	cagA	5	1472	17	0.41 (0.29-0.59)	54	37	NA	0.41 (0.28-0.62)	42	NA
Infection	Huang 2003[17]	Gastric CA	H. pylori	15	5054	76	2.05 (1.79-2.35)	805	615	NA	2.29 (1.71-3.05)	224	NA
Infection	Huang 2003[17]	Gastric CA	cagA	10	3831	85	2.65 (2.29-3.05)	888	531	NA	2.87 (1.95-4.22)	137	NA
Infection	Zhuo 2008[55]	Laryngeal CA	H. pylori	3	357	0	2.02 (1.27-3.23)	10	121	NA	2.02 (1.27-3.23)	10	NA
Infection	Hobbs 2006[56]	Larynx CA	HPV	8	1133	50	1.71 (1.11-2.64)	17	281	NA	2.01 (0.96-4.22)	NA	6
Infection	Donato 1998[57]	Liver CA	HBV (HCV-)	28	9199	86	17.9 (15.7-20.5)	24939	10279	NA	21.9 (14.9-32.3)	3464	NA
Infection	Donato 1998[57]	Liver CA	HBV + HCV	9	2437	37	65.0 (35.0-121)	784	12315	NA	61.2 (27.0-139)	440	NA
Infection	Donato 1998[57]	Liver CA	HCV (HBV-)	26	7694	86	16.8 (14.1-20.0)	13151	9822	NA	20.3 (12.2-33.7)	1924	NA
Infection	Zhuo 2009[58]	Lung CA	H. pylori	4	430	79	2.31 (1.46-3.65)	22	185	NA	3.24 (1.11-9.41)	6	NA
Infection	Hobbs 2006[56]	Oral CA	HPV	8	3976	62	1.68 (1.36-2.08)	76	274	NA	1.99 (1.17-3.38)	17	NA
Infection	Hobbs 2006[56]	Oropharynx CA	HPV	5	2199	56	3.01 (2.11-4.30)	93	300	NA	4.31 (2.07-8.95)	35	NA
Infection	Taylor 2005[59]	PrCA	HPV	9	4864	35	1.37 (1.11-1.69)	31	246	NA	1.52 (1.12-2.06)	23	NA
Infection	Hobbs 2006[56]	Tonsil CA	HPV	8	380	0	15.1 (6.78-33.4)	173	2471	NA	15.1 (6.78-33.4)	173	NA
Infection	Wang 2007[60]	early Gastric CA	H. pylori	15	16698	83	4.83 (4.27-5.48)	4639	1467	NA	3.38 (2.15-5.32)	197	NA
Inflammation	Heikkila 2009[61]	CA	Interleukin-6	4	6785	21	1.01 (0.92-1.11)	NA	NA	718	1.01 (0.90-1.12)	NA	3321
Inflammation	Heikkila 2009[61]	CA	C-reactive protein	14	74545	73	1.09 (1.05-1.13)	150	299	NA	1.10 (1.02-1.18)	35	NA
Inflammation	Tsilidis 2008[62]	CRC	C-reactive protein	8	39145	51	1.10 (1.02-1.18)	20	172	NA	1.12 (1.01-1.25)	12	NA
Sex hormones	Barba 2009[63]	PrCA	2OHE1	2	536	0	0.76 (0.45-1.28)	NA	NA	13	0.76 (0.45-1.28)	NA	15
Sex hormones	Roddam 2008[24]	PrCA	A-diol G	8	5488	24	1.12 (0.96-1.31)	NA	NA	17	1.15 (0.95-1.38)	NA	28
Sex hormones	Roddam 2008[24]	PrCA	D4	6	4211	0	1.02 (0.85-1.21)	NA	NA	994	1.02 (0.85-1.21)	NA	3995
Sex hormones	Roddam 2008[24]	PrCA	DHES-S	7	3024	17	1.22 (0.98-1.53)	NA	NA	8	1.29 (0.99-1.68)	NA	5

Sex hormones	Roddam 2008[24]	PrCA	DHT	7	2455	0	0.88 (0.69-1.11)	NA	NA	41	0.88 (0.69-1.11)	NA	80
Sex hormones	Roddam 2008[24]	PrCA	E2	9	5225	0	0.92 (0.78-1.09)	NA	NA	62	0.92 (0.78-1.09)	NA	162
Sex hormones	Roddam 2008[24]	PrCA	Free E2	8	4778	0	0.97 (0.82-1.16)	NA	NA	1173	0.97 (0.82-1.16)	NA	5279
Sex hormones	Roddam 2008[24]	PrCA	Free T	14	9365	0	1.12 (0.98-1.27)	NA	NA	18	1.12 (0.98-1.27)	NA	20
Sex hormones	Roddam 2008[24]	PrCA	T	17	10324	0	0.98 (0.87-1.10)	NA	NA	502	0.98 (0.87-1.10)	NA	3602
Sex hormones	Barba 2009[63]	PrCA	16a-OHE1	2	536	0	1.82 (1.08-3.05)	3	73	NA	1.82 (1.08-3.05)	3	NA
Sex hormones	Barba 2009[63]	PrCA	2OHE1/16a-OHE1	2	536	0	0.52 (0.31-0.89)	4	19	NA	0.52 (0.31-0.89)	4	NA
Sex hormones	Roddam 2008[24]	PrCA	SHBG	15	9702	0	0.86 (0.76-0.97)	32	249	NA	0.86 (0.76-0.97)	32	NA
Sex hormones	Key 2002[64]	postmenopausal BrCA	E2	9	2365	42	1.29 (1.14-1.45)	72	227	NA	1.26 (1.07-1.49)	27	NA

IGF, insulin-like growth factor; CRC, colorectal cancer; IGFBP, insulin-like growth factor binding protein; CA, cancer; BrCA, breast cancer; PrCA, prostate cancer; ESCC, esophageal squamous cell carcinoma; T, testosterone; E2, estradiol; DHT, dihydrotestosterone; A-diol g, androstanediol glucuronide; DHES-S, dehydroepiandrosterone sulfate; D4, androstenedione; SHBG, sex hormone binding globulin; E1, estrone; SFA, total saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; H. pylori, helicobacter pylori; HPV, human papillomavirus; HBV, hepatitis B virus; HCV, hepatitis C virus; T. vaginalis, trichomonas vaginalis; DDT, dichlorodiphenyltrichloroethane; Cur, current; For, former; Nev, never; NA, non-statically significant meta-analyses not applicable to the FSN, and statically significant meta-analyses not applicable to the conditional power analysis.

1. Rosenberg's FSN – the number of future studies averaging null effect and average weight to reduce the summary OR to null
2. Owin's FSN – the number of future studies averaging null effect to reduce the summary OR to 1.05
3. Number of future studies of average weight and no between-study heterogeneity needed to be included in the combined meta-analysis to achieve 80% power to detect the observed fixed-effect summary OR
4. Number of future studies of average weight and average between-study heterogeneity need to be included in the combined meta-analysis to achieve 80% power to detect the observed random effects summary OR

Table 3.2 **Results of conditional power analysis for 9 meta-analyses comparing androgens and prostate cancer reported by Roddam 2008[24]**

Comparison	No. included studies	No. cases/ controls	I^2	Fixed-effect			Random effects		
				Odds ratio	95% CI	Future studies[1]	Odds ratio	95% CI	Future studies[2]
SHGB	15	9702	0	0.86	0.76 – 0.97	1	0.86	0.76 – 0.97	1
Free T	14	9365	0	1.12	0.98 – 1.27	18	1.12	0.98 – 1.27	20
DHT	7	2455	0	0.88	0.69 – 1.11	41	0.88	0.69 – 1.11	80
E2	9	5225	0	0.92	0.78 – 1.09	62	0.92	0.78 – 1.09	162
T	17	10324	0	0.98	0.87 – 1.10	502	0.98	0.87 – 1.10	3602
D4	6	4211	0	1.02	0.85 – 1.21	994	1.02	0.85 – 1.21	3995
Free E2	8	4778	0	0.97	0.82 – 1.16	1173	0.97	0.82 – 1.16	5279
DHES-S	7	3024	17	1.22	0.98 – 1.53	8	1.29	0.99 – 1.68	5
A-diol G	8	5488	24	1.12	0.96 – 1.31	17	1.15	0.95 – 1.38	28

T, testosterone; E2, estradiol; DHT, dihydrotestosterone; A-diol g, androstanediol glucuronide; DHES-S, dehydroepiandrosterone sulfate; D4, androstenedione; SHBG, sex hormone binding globulin.

1. Number of future studies of average weight as studies included in observed meta-analysis needed to achieve 80% in combined meta-analysis determined by conditional power analysis assuming no between-study heterogeneity
2. Number of future studies of average weight and equivalent between-study heterogeneity as studies included in observed meta-analysis needed to achieve 80% power in combined meta-analysis determined by conditional power analysis assuming equivalent between-study heterogeneity in combined meta-analysis

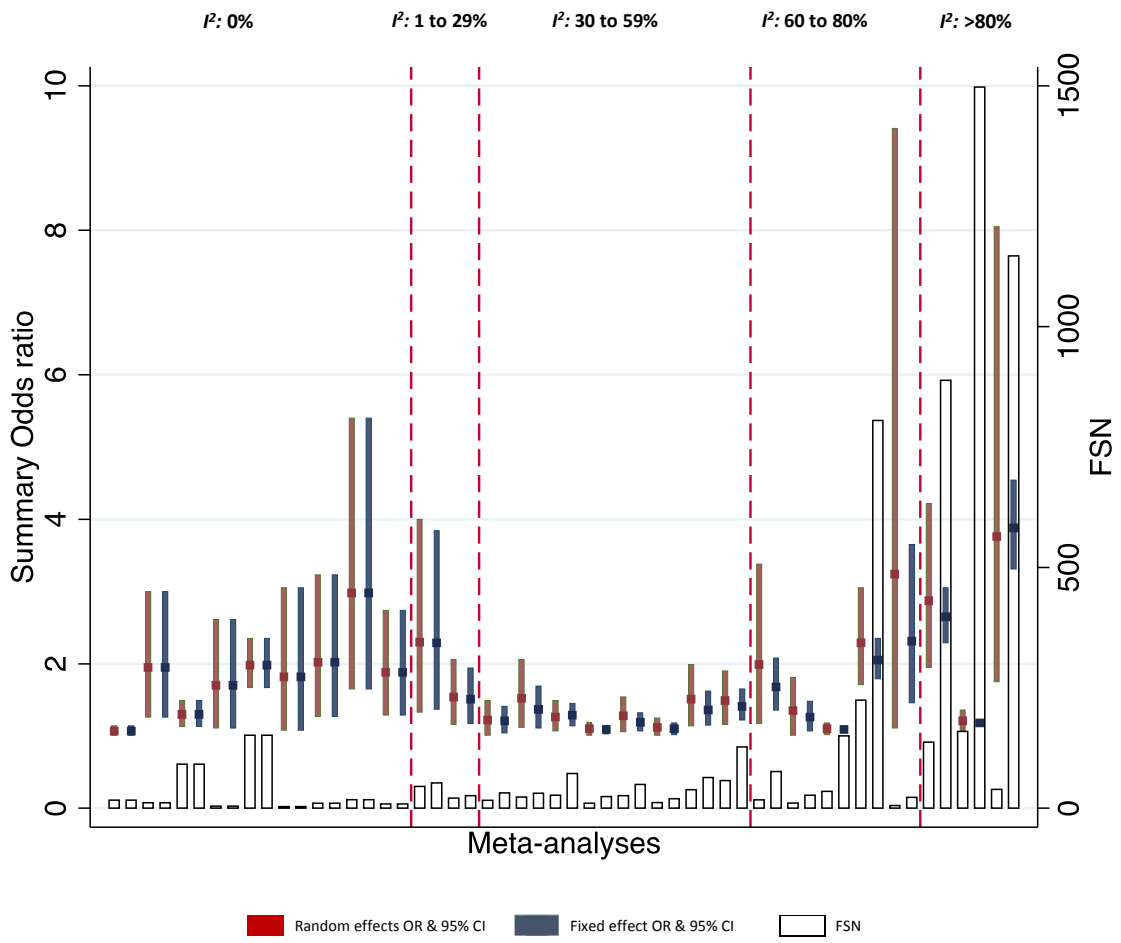

Figure 3.1 Rosenberg's Fail-Safe Number (FSN) for statistically significant fixed and random effects meta-analyses with summary odds ratios between 1.00 and 4.00 by increasing level of between-study heterogeneity (I^2)

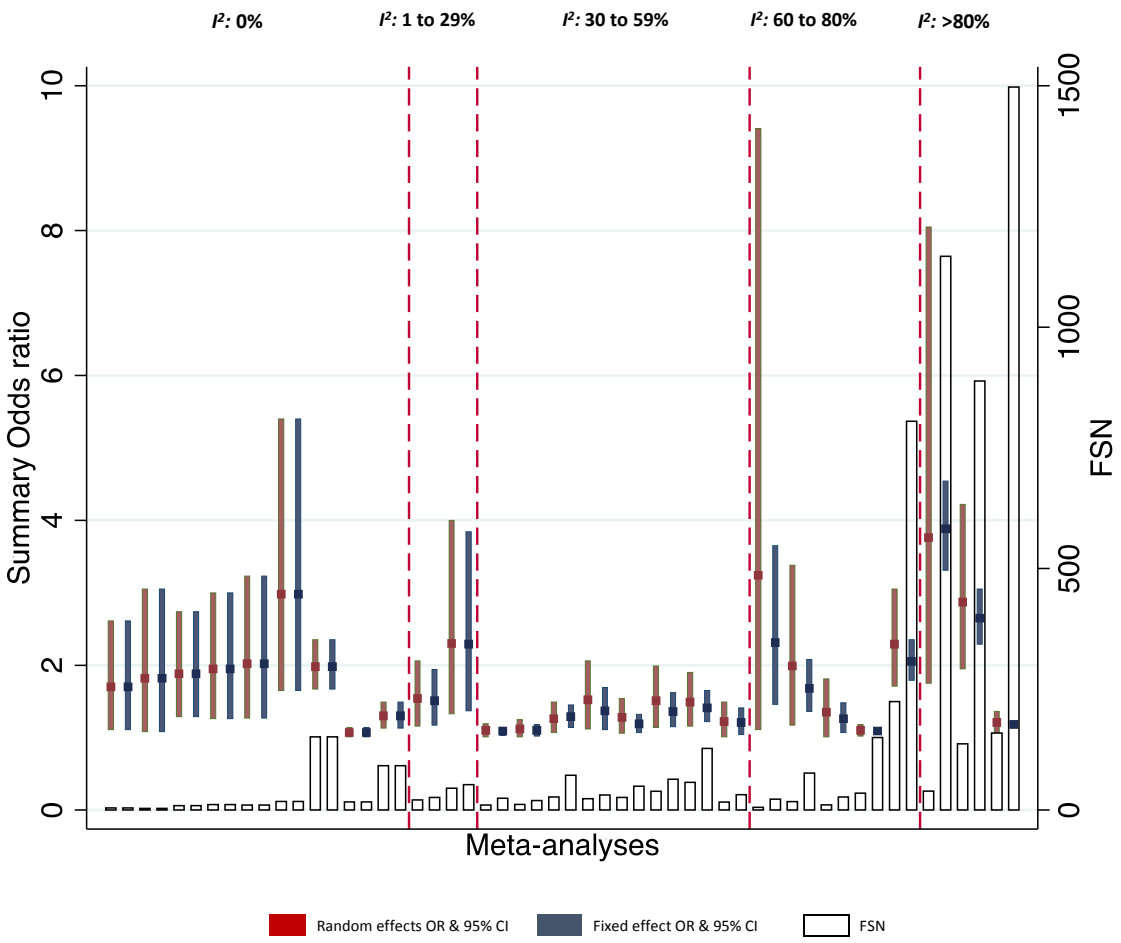

Figure 3.2 Rosenberg's Fail-Safe Number (FSN) for statistically significant fixed and random effects meta-analyses with summary odds ratios between 1.00 and 4.00 by number of studies included in each meta-analysis within strata of between-study heterogeneity (I^2)

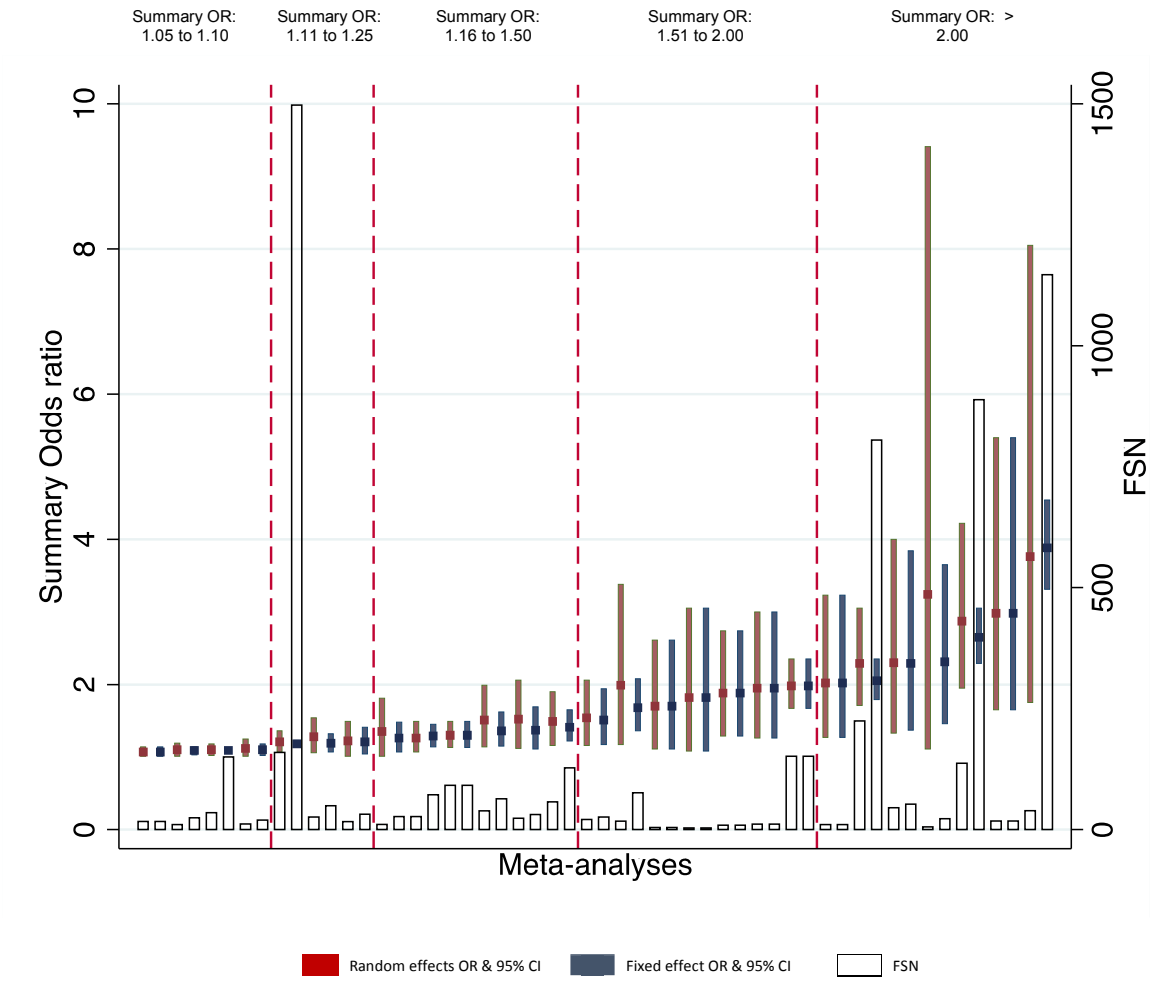

Figure 3.3 Rosenberg's Fail-Safe Number (FSN) for statistically significant fixed and random effects meta-analyses with summary odds ratios between 1.00 and 4.00 by total number of cases and controls within levels of summary effect estimates.

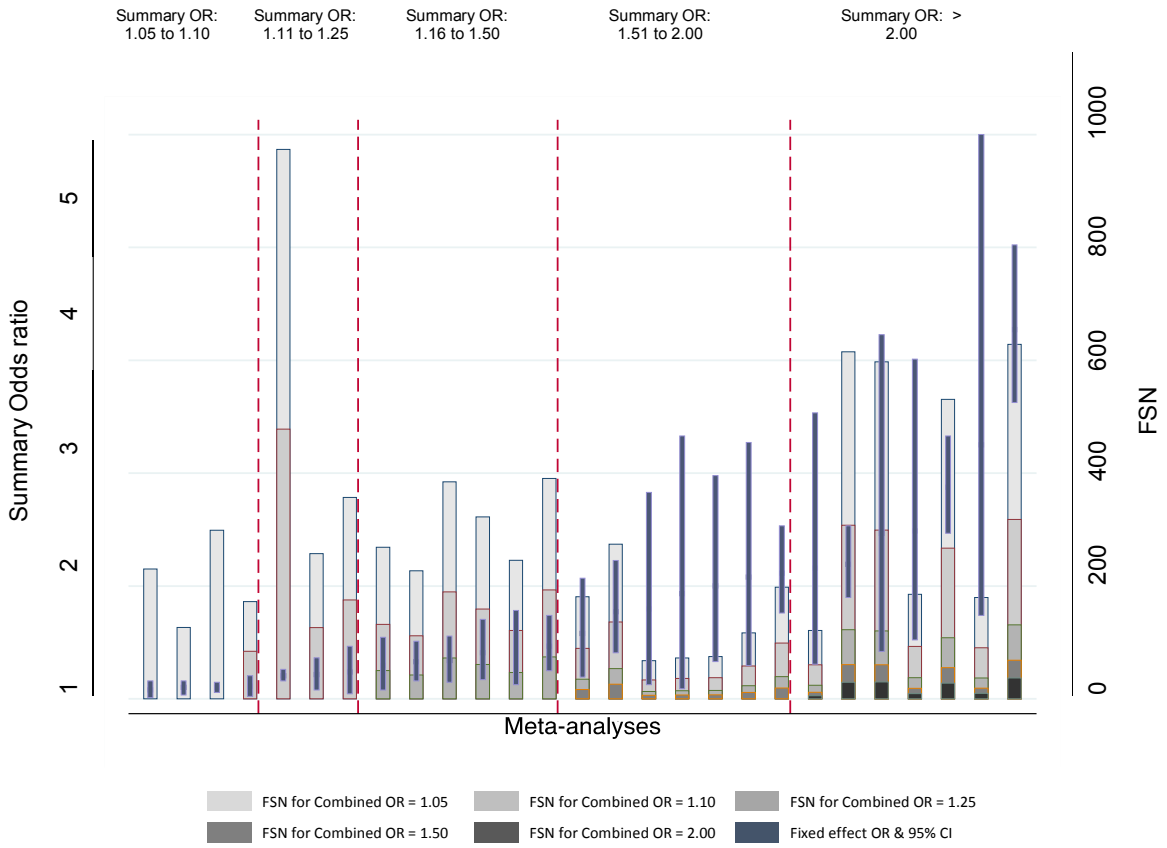

Figure 3.4 Orwin's Fail-Safe Number (FSN) for an average null effect (OR = 1) in future studies reducing the combined fixed effect summary odds ratio of 1.10; 1.25; 1.50; and 2.00 in 28 fixed effect meta-analyses with an observed summary OR ≥ 1.05

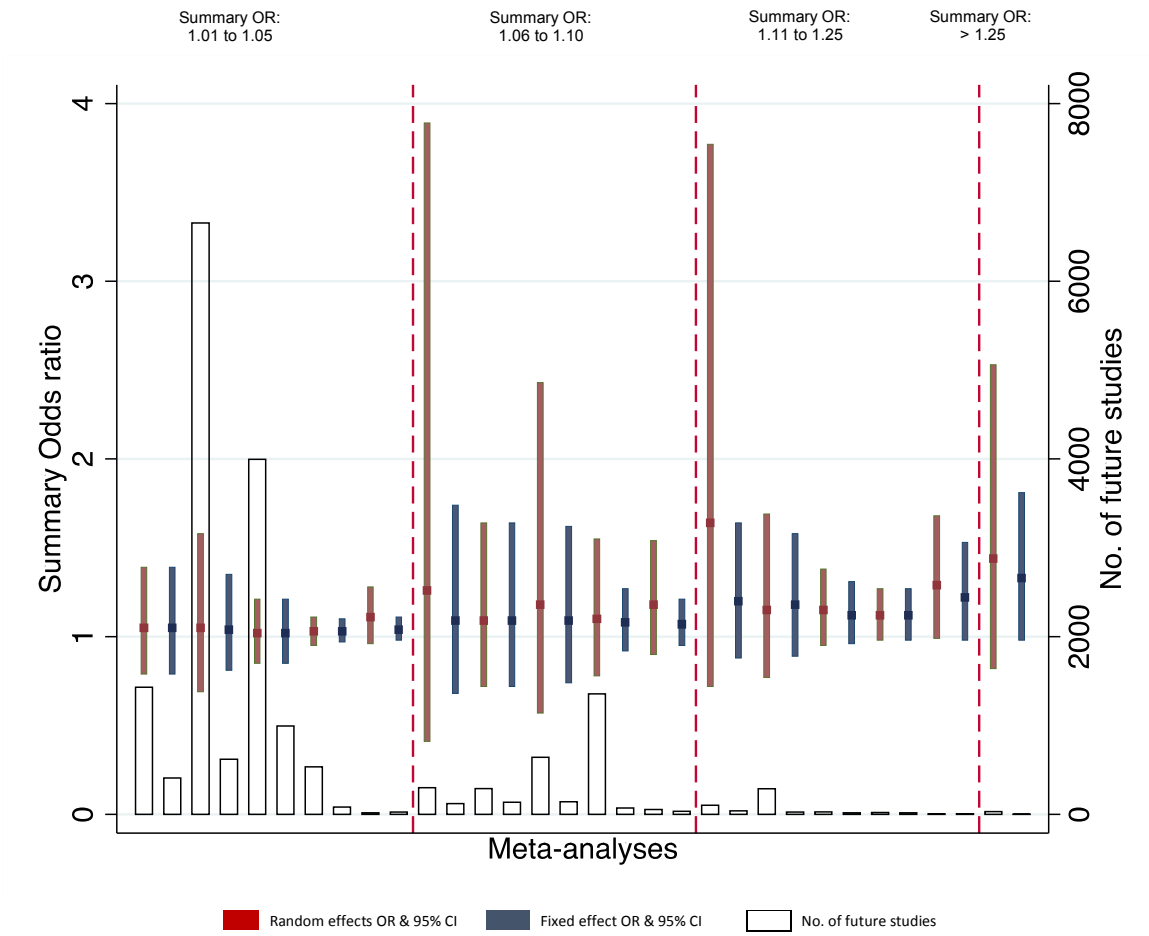

Figure 3.5 Number of future studies required to achieve 80% power to detect the observed summary odds ratio based on 2 approaches to conditional power analysis for statistically non-significant fixed and random effects meta-analyses with summary odds ratios between 1.01 and 4.00

Chapter 4.

Glycemia is positively associated with prostate cancer mortality in white and black men without diabetes when better classifying hyper- and normo-glycemia using 3 biomarkers

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Funding and Acknowledgments:

The Atherosclerosis Risk in Communities Study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, and HHSN268201100012C). The authors thank the staff and participants of the ARIC study for their important contributions. Studies on cancer in ARIC are also supported by the National Cancer Institute (U01 CA164975). The content of this work is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Cancer incidence data have been provided by the Maryland Cancer Registry, Center for Cancer Surveillance and Control, Department of Mental Health and Hygiene, 201 W. Preston Street, Room 400, Baltimore, MD 21201. We acknowledge the State of Maryland, the Maryland Cigarette Restitution Fund, and the National Program of Cancer Registries (NPCR) of the Centers for Disease Control and Prevention (CDC) for the funds that helped support the availability of the cancer registry data

4.1 Abstract

Background: Diabetes is associated with a decreased prostate cancer risk, but may increase prostate cancer mortality among men with this cancer. The association of diabetes or earlier hyperglycemic states (i.e. prediabetes) on the most clinically relevant outcome, prostate cancer mortality in men without this cancer at baseline, has not been well studied and whether associations are similar across race is unknown. Thus, we evaluated the association between hyperglycemia, classified by three different biomarkers (fasting glucose, HbA1c, and glycated albumin) individually and then jointly, and prostate cancer mortality. We hypothesized that the association between hyperglycemia and prostate cancer mortality would be the strongest when participants classified as hyperglycemic on all three biomarkers were compared to participants classified as normal on all three biomarkers (e.g., where there was maximum agreement across biomarkers). **Methods:** We conducted a prospective study of 5,276 cancer-free white and black men attending visit 2 (1990-1992) of the Atherosclerosis Risk in Communities (ARIC) Study and who were followed through 2012. Death from prostate cancer as the underlying cause was ascertained from death certificates. We identified 69 prostate cancer deaths in 96,617 person-years. Men were categorized as having diagnosed diabetes or jointly categorized using clinical or research-based cut points of the 3 biomarkers: low (fasting glucose <3.1 mmol/L, HbA1c <5.0% or glycated albumin <11.0%); normal on all 3 biomarkers (reference: <5.6 mmol/L, 5.0 to <5.7%; and 11.0 to <16.0% respectively); and high on any 1; on any 2; or on all 3 biomarkers. No men in the analytic study population had low fasting glucose. We used Cox proportional hazards regression to estimate the relative hazards (HR) of prostate cancer death and 95% confidence intervals jointly for the markers of glycemia and for diagnosed diabetes adjusting for age, education, body mass index (BMI), waist circumference, smoking, and race and field center, overall and by race. **Results:** At visit 2, mean age was 57 years

and mean BMI was 27.7 kg/m^2; 19% of men were African American. Compared to men without diabetes who were normal on all 3 biomarkers of glycemia, greater than 2-fold increased risk of prostate cancer mortality was observed among men high on any 1 biomarker (HR: 3.66; 95% CI: 1.42 to 9.48), any 2 biomarkers (HR: 2.58; 95% CI: 0.92 to 7.22), all 3 biomarkers (HR: 4.8; 95% CI: 1.10 to 20.95), and among men with diagnosed diabetes (HR: 3.18; 95% CI 0.94 to 10.73). Men without diagnosed diabetes with low glycemia also had an elevated risk of prostate cancer mortality (HR: 2.98; 95% CI: 0.98 to 8.90). Associations were similar in white and black men. **Conclusions:** When using 3 biomarkers to classify glycemia and reducing the potential for non-differential misclassification of a single biomarker, men without diagnosed diabetes who have hyperglycemia and men with low glycemia have an increased risk of death from prostate cancer compared to men who have normal glycemia levels, independent of BMI and other factors. Also, compared to men without diagnosed diabetes with normal glycemia, men with diagnosed diabetes have an increased risk of prostate cancer death. Our findings did not appear to be influenced by racial differences in hyperglycemia.

4.2 Introduction

In contrast to several other types of cancer,[1] diabetes is inversely associated with prostate cancer incidence,[1,2] and this association is stronger with a longer duration of diabetes.[3] Genetic association studies have also observed an inverse association between diabetes susceptibility genes and prostate cancer risk.[4,5] A number of hypothesized mechanisms driving the inverse relationship include decreased circulating androgens associated with long term diabetes;[6,7] pharmacological effects of diabetes medications; and microvascular effects of diabetes on the prostate.[8] With respect to prostate cancer mortality, some studies reported that diabetes is inversely associated,[9-11] while more recent studies reported a positive association.[12,13] However, the exact mechanism(s) driving these diabetes associations are unclear. A joint consensus statement between the American Diabetes Association and the American Cancer Society highlighted the need to better understand the biologic mechanisms underlying the association between diabetes and cancer.[1] The rise in the prevalence of diabetes in the US over the past two decades – with 1.5% US adults with undiagnosed diabetes (calibrated HbA1c $\geq$ 6.5%) between 2005 and 2010 up from 1.1% in 1988 to 1994; 12.4% with prediabetes (calibrated HbA1c 5.7-6.4%) between 2005 and 2010 up from 5.8% from 1988 to 1994; and 9.9% with diagnosed diabetes or calibrated HbA1c $\geq$ 6.5% between 2005 and 2010 up from 6.2% between 1998 and 1994[14] points to the importance of understanding the relationship between the natural history of diabetes and the etiology of prostate cancer. In addition to the diagnosis of diabetes, biomarkers of hyperglycemia, which characterize states both early (e.g., prediabetes) and later (e.g., undiagnosed diabetes) in the natural history of diabetes, provide an opportunity to investigate this relationship.

Several prospective epidemiologic studies have evaluated the association between glycemic biomarkers, most commonly fasting glucose, 2-hour glucose, and glycated hemoglobin (HbA1c), and prostate cancer risk. In a pooled analysis of six prospective cohorts, increased blood glucose (non-fasting glucose in four of the cohorts) was not significantly associated with prostate cancer incidence or mortality.[15] In contrast, a meta-analysis of three prospective cohort studies indicated a positive association between prediabetes, defined by fasting glucose 5.6 to 6.9 mmol/L or 6.1–6.9 mmol/l, depending on the study, or 2-hour glucose tolerance test value of 7.8 to 11.1 mmol/L, and risk of prostate cancer.[16] More recently, a population-based study of over 40,000 men without diabetes found an inverse association between higher levels of glucose (p-trend 0.04) following serum glucose 1 h after a 75-g oral glucose challenge test.[17] However, a recent study with repeated measures of glycemia also reported prostate cancer incidence was not associated with the standardized log mean of glucose, but was inversely associated with the standardized log mean of fructosamine, after mutual adjustment and adjustment for fasting status.[18] Fewer population-based epidemiologic studies have investigated the association between HbA1c and prostate cancer incidence and mortality. In the Atherosclerosis Risk in Communities (ARIC) Study with follow-up through 2006, we previously reported that compared with normal HbA1c neither elevated HbA1c or low HbA1c was significantly associated with prostate cancer incidence, but both had a suggestive positive association with prostate cancer mortality in men without a diabetes diagnosis.[9] In that study, diagnosed diabetes had a suggestive inverse association with prostate cancer incidence, but there were too few cases for a stable estimate for prostate cancer mortality.[9] A meta-analysis of four studies, which included the ARIC study, reported an inverse, dose-response association between elevated HbA1c and prostate cancer incidence.[19] Collectively, the evidence base in men without a diabetes diagnosis for the association between elevated glycemia, measured with

several different biomarkers, and prostate cancer incidence is inconsistent, and there is a dearth of information on the association between hyperglycemia and prostate cancer mortality.

Many factors may contribute to the inconsistent findings reported by these studies, including differences in biomarkers and analytic decisions. Studies use different biomarkers to capture glycemia. Although each of these biomarkers may be used to diagnose diabetes (fasting glucose: ≥ 7.0 mmol/L; or HbA1c: ≥ 6.7%) and/or monitor glycemic control in people diagnosed with diabetes, there is imperfect agreement in the classification of hyperglycemia across biomarkers. This may be, in part, because these biomarkers measure different aspects of glycemia and are susceptible to the influence of different, yet complementary, non-glycemic factors. Fasting glucose captures the lowest glycemia value, and can fluctuate throughout the day owing to moderate within-person variability.[20] HbA1c, which is formed through the bonding of glucose to hemoglobin in circulating red blood cells, captures glucose exposure over the previous two to three months. It is a non-fasting test that is susceptible to relatively rare conditions that affect the lifespan of red cells in circulation including hemoglobin variants, recent blood transfusions, hemolytic anemia and renal failure.[20,21] Glycated albumin, which has not been evaluated for its association with prostate cancer risk or mortality, is not used clinically in the US, but is used to monitor glycemic control in persons with diabetes in China, Japan, and South Korea.[22] Glycated albumin, which forms through the non-enzymatic bonding of glucose to serum albumin, captures average glycemia over the previous two to three weeks. It is a non-fasted test that may increase under conditions of decreased albumin metabolism, such as liver cirrhosis and hypothyroidism, and decrease under conditions of increased albumin metabolism, such as thyroid disease, glucocorticoid administration, and nephrotic syndrome.[23] Glycated albumin has also

been found to be lower in smokers and decreases with increasing adiposity.[23] In addition to differences in biomarkers, studies are also inconsistent in the classification of glycemia values. Some studies use clinical cut points, while others use study-specific quantiles. The use of clinical cut points, where possible, improves comparisons across study populations. Collectively, these differences in biomarkers and classification of the biomarkers could contribute to inconsistent findings across studies.

To further explore the association between hyperglycemia and prostate carcinogenesis, we capitalized on the unique features of the ARIC, a diverse, prospective cohort study that has measured multiple biomarkers of glycemia at the same time point and now has cancer follow-up through 2012. We evaluated the association between hyperglycemia, classified by three different biomarkers (fasting glucose, HbA1c, and glycated albumin) individually and then jointly, and prostate cancer mortality. We used clinical cut points for the biomarkers, where possible, to maximize comparability to prior and future studies. We hypothesized that the association between hyperglycemia and prostate cancer mortality would be the strongest when participants classified as hyperglycemic on all three biomarkers were compared to participants classified as normal on all three biomarkers (e.g., where there was maximum agreement across biomarkers). We also evaluated the association between diagnosed diabetes and prostate cancer mortality. We focused on prostate cancer mortality because in the PSA era, it is the most clinically relevant outcome and it is understudied with respect to glycemia.

4.3 Methods

Study Population ARIC is a prospective epidemiologic cohort that began in 1987 with the recruitment of 15,792 participants aged 45-64 years across four field centers:

Forsyth County., NC; Jackson, MS; Minneapolis, MN; and Washington County, MD that included high representation of African-Americans (27%).[24] A wealth of measured anthropometric, lifestyle, and medical data, biospecimens and biomarkers pertinent to cancer have been collected during five in-person clinical visits spanning 30 years of follow-up with greater than 90% response rate from annual phone calls. Fasting glucose, HbA1c, and glycated albumin are all available at the second study visit (1990-1992). The analytic study population for the current analysis is composed of 5,276 African American and Caucasian men without a cancer diagnosis by visit 2 (1990-1992) who were fasting for at least 8 hours, and who had complete information on fasting glucose, HbA1c, and glycated albumin. Men were followed from their visit 2 date until the date of death from any cause, loss-to-follow-up, or December 31, 2012, whichever came first.

Assessment and Categorization of Glycemia Participants were asked to fast for 12 hours, defined as nothing by mouth except for water, prior to venous blood draw at visit 2. Participants fasting for less than 8 hours were excluded from the current analysis. Fasting serum glucose was measured by a modified hexokinase/glucose-6-phosphate dehydrogenase procedure. HbA1c was measured in frozen whole blood collected during the visit 2 using high-performance liquid chromatography.[25] Glycated albumin measured in serum using a Roche Modular P800 system was reported as percent of total albumin. The inter-assay coefficients of variation for glycated albumin were previously reported, and were 2.3% at a concentration of 1.579 g/dL and 2.8% at a concentration of 0.426 g/dL.[26]

In the main analysis, men were classified as having a diagnosis of diabetes if they self-reported a doctor's diagnosis of diabetes and/or were taking diabetes medication at visit 2. Among men without a diagnosis of diabetes, clinical and research-

based cut points were used for each glycemia biomarker to classify the men. Clinical cut points were used 1) because we hypothesize clinically elevated glycemia is associated with prostate cancer mortality and 2) to yield findings more comparable to past and future studies using common cut points. Clinical cut points were used to define low glucose as <3.1 mmol/L; normal glucose as 3.1 to 5.6 mmol/L; and high glucose as >5.6 mmol/L, which includes men with pre-diabetes and diabetes.[27] No men in our analytic study population had low fasting glucose based on this clinical cut point, therefore we did not include a category for low glucose in the analyses. Because low HbA1c has been associated with an increased risk of all cause and cancer mortality,[9,28-30] in ARIC, we used these same research-based cut points to distinguished between low, defined as <5.0%, and normal, defined as 5.0 to 5.6% HbA1c. We used a clinical cut point to define high HbA1c as >5.6%, which includes men with pre-diabetes and undiagnosed diabetes.[27] We used assay manufacturer cut points to define low glycated albumin as < 11%; normal glycated albumin as 11 to 16%; and high glycated albumin as >16% (Asahi Kasei Lucica GA-L, Tokyo, Japan). The median values and 25th-75th percentile ranges of fasting glucose, HbA1c, and glycated albumin within each biomarker category are shown in Table S4.1. The cross classification of men within each category of each biomarker is shown in Table S4.2. Men without a diagnosis of diabetes were also jointly categorized using all three biomarkers. Men were first classified as low if they were low on any one of the three biomarkers (low HbA1c or low glycated albumin; no low fasting glucose in our study population); and then classified as normal if they were in the normal range on all three biomarkers. The remaining men were classified as high on any 1 biomarker; high on any 2 biomarkers; or as high on all three biomarkers.

Outcome Ascertainment Cancer incidence was ascertained from 1987 through 2012 via linkage with the four state cancer registries in which the majority of ARIC participants

live, abstraction of medical records collected following a cancer-specific telephone call, abstraction of archived hospital discharge summaries and medical records, and death certificates. Cancer mortality was ascertained from death certificates. Prostate cancer mortality was defined as death from prostate cancer as the underlying causes among men without a diagnosis of cancer at baseline.

Covariate assessment Participants self-reported their highest level of education (less than high school, high school or equivalent, college or above) and smoking status (never, current, former). Body weight (kg) and height (cm), from which body mass index (BMI, kg/m^2) was calculated, and waist circumference (cm) were measured by trained study personnel.

Statistical Analysis We calculated unadjusted and age and race adjusted means and proportions of demographic characteristics at baseline by fasting glucose level, HbA1c level, glycated albumin level, and the joint categories using regression models. We used Cox proportional hazards regression, with visit 2 as baseline, to estimate the relative hazard (HR) and 95% confidence interval (CI) of prostate cancer mortality for 1) high fasting glucose (>5.6 mmol/L) in men without diagnosed diabetes, and diagnosed diabetes both compared with normal fasting glucose (3.1 to 5.6 mmol/L) in men without diagnosed diabetes, 2) low (<5.0%) and high (>5.6%) HbA1c in men without diabetes, and diagnosed diabetes all compared with normal HbA1c (5.0 to 5.6%), 3) low (<11%) and high (>16%) glycated albumin in men without diagnosed diabetes, and diagnosed diabetes all compared with normal glycated albumin (11 to 16%) in men without diagnosed diabetes, and 4) joint classification of glycemia in men without diagnosed diabetes (low on any one marker; high on any 1 marker; high on any 2; and high on all 3 markers), and diagnosed diabetes all compared with men normal on all 3 markers

without diagnosed diabetes. To test for trend in the HR across categories of fasting glucose, HbA1c, and glycated albumin, we assigned men without diagnosed diabetes (1) the continuous value of each biomarker, expressed as a per one standard deviation change, (2) the median value for their category of glycemia, and (3) an ordinal value for each category of glycemia. These values were modeled as continuous variable, and a term for diagnosed diabetes was included. To test for trend for the joint glycemia categories, we assigned men without diagnosed diabetes an ordinal variable for normal (0), high on 1 marker (1), high on 2 (2), and high on all 3 (3) markers, modeled the variable as continuous and included a term for diagnosed diabetes. We adjusted all models for visit 2 age (continuous), joint categories for race and field center (Caucasian participants from Minnesota; Caucasian participants from Maryland and North Carolina; African American participants from Mississippi; African American participants from Maryland and North Carolina), visit 2 BMI (kg/m^2, continuous), waist circumference (cm, continuous), level of education (less than high school, high school or equivalent, more than high school), and cigarette smoking status (current, former, never). We conducted all analyses in the overall population and stratified by race, and tested for interaction between race and each biomarker category or between race and diagnosed diabetes using the likelihood ratio test. We confirmed the proportional hazards assumption was met in all models using a global test for the adjusted model. Statistical analyses were conducted using Stata 13.1 (StataCorp, College Station, TX). All tests were two-sided with $p<0.05$ indicating statistical significance.

To determine whether classification of diabetes influenced our findings, we conducted sensitivity analyses for each of the biomarkers in which (1) men with undiagnosed diabetes based on their biomarker value (fasting glucose ≥ 7.0 mmol/L and HbA1c ≥ 6.5%) where included in the category with men diagnosed with diabetes, and

(2) all men were categorized based on their biomarker value, irrespective of a prior diagnosis of diabetes, including a term for diagnosed diabetes in the model. To determine whether the presence of prostate cancer was influencing glycemia values, we conducted sensitivity analyses for all models in which we censored prostate cancer deaths occurring within the first three years of the visit 2 blood collection as their date of date of death and considered these as non-events.

4.4 Results

From 1990 to 2012, 69 deaths from prostate cancer were ascertained among 5,276 men contributing 96,617 person-years of follow-up. At visit 2, mean age was 57 years, mean BMI was 27.8 kg/m^2, and 19% of me were African American. At baseline, 419 (8%) men had diagnosed diabetes. We observed similar means and percentages of participants according to baseline demographic characteristics across categories of fasting glucose, HbA1c, glycated albumin after adjusting for age and race (Table 4.1) as well as across joint categories of all three biomarkers (Table 4.2). We observed consistent patterns in participant characteristics across unadjusted categories of fasting glucose, HbA1c, glycated albumin, and the joint categories with a higher percentage of African American men with elevated glycemia and diagnosed diabetes, higher BMI and larger waist circumference in categories of elevated glycemia and diagnosed diabetes (Tables S4.3 and S4.4). Among men without a diagnosis of diabetes, mean values of each biomarker appeared to increase with the number of biomarkers classified as high (Tables S4.4).

Fasting glucose and prostate cancer mortality Among men without diagnosed diabetes, men with high fasting glucose had twice the risk of dying from prostate cancer (HR: 1.99; 95% CI 1.09 to 3.63) compared to men with normal fasting glucose (Table

4.3). Men with diagnosed diabetes appeared to have 1.9 times the risk of prostate cancer mortality compared to men without diagnosed diabetes and normal fasting glucose. Race-specific HRs are shown in Table 4.3; interaction terms between race and high fasting glucose or diabetes were not statistically significant. When modeled as a continuous variable expressed as a 1-standard deviation increase in fasting glucose, the association was not statistically significant overall or in African American men, but was statistically significant in Caucasian men (Table 4.3). The p-value comparing categories of fasting glucose in men without diagnosed diabetes was statistically significant overall (p=0.03) and in Caucasian men (p=0.02), but not in African American men (Table 4.3).

HbA1c and prostate cancer mortality Among men without diagnosed diabetes, those with high HbA1c had a non-statistically significant 32% increase risk of prostate cancer mortality (HR: 1.32; 95% CI: 0.75 to 2.31) compared to those with normal HbA1c. Among men without diagnosed diabetes, those with low HbA1c had a non-statistically significant 68% increase in risk of dying from prostate cancer (HR: 1.68; 95% CI: 0.73 to 3.86) compared to those with normal HbA1c. Men with diagnosed diabetes had a non-statistically significant 36% increase in prostate cancer mortality (HR: 1.36; 95% CI: 0.55 to 3.38) compared to men with men without diagnosed diabetes and normal HbA1c. Race-specific hazard ratios are shown in Table 4.3; interaction terms between race and levels of HbA1c or diagnosed diabetes were not statistically significant. An increase in 1-standard deviation in HbA1c was not statistically significant overall or in Caucasian men, but was statistically significant in African American men (Table 4.3). The test for trend across categories of HbA1c in men without diagnosed diabetes was not statistically significant overall or by race (Table 4.3).

Glycated albumin and prostate cancer mortality Among men without diagnosed diabetes, those with high glycated albumin had a non-statistically significant 53% increase in risk of dying from prostate cancer (HR: 1.53; 95% CI: 0.47 to 5.00). Among men without diagnosed diabetes, those with low glycated albumin had a non-statistically significant 51% lower risk of dying from prostate cancer (HR: 0.49; 95% CI: 0.12 to 2.05) compared to those with normal glycated albumin. Men with diagnosed diabetes had a non-statistically significant 13% increase in risk of dying from prostate cancer (HR: 1.13; 95% CI: 0.48 to 2.65) compared to men without diagnosed diabetes and normal glycated albumin. Race-specific HRs are shown in Table 4.3; interaction terms between race and glycated albumin or diagnosed diabetes were not statistically significant. An increase in 1-standard deviation of glycated albumin was not statically significant overall or by race (Table 4.3). The test for trend across categories of glycated albumin in men without diagnosed diabetes was statistically significant in Caucasian men only (p=0.05) (Table 4.3).

Joint classification based on 3 biomarkers of glycemia and prostate cancer mortality When men without diagnosed diabetes were jointly classified using all 3 biomarkers, those high on any one biomarker had greater than 3-fold increase in risk of dying from prostate cancer compared to those normal on all three biomarkers (HR: 3.66; 95% CI: 1.42 to 9.48). Men high on any two biomarkers appeared to have greater than a 2-fold increase in risk of dying from prostate cancer, which was not statistically significant. Men high on all three biomarkers had close to a 5-fold increase in risk (HR: 4.80; 95% CI: 1.11 to 20.95). This association appeared to be similar in African American men, though not statistically significant, and was possibly stronger in Caucasian men (HR: 6.78; 95% CI: 1.21 to 38.02). Men low on any 1 of the 3 biomarkers had close to a 3-fold increase in risk of dying from prostate cancer (HR: 2.95;

95% CI: 0.98 to 8.90) compared to men in the normal range on all 3 biomarkers, although not statistically significant. When compared to men without diagnosed diabetes with normal glycemia on all three biomarkers, men with diagnosed diabetes had greater than a 3-fold increase in risk (HR: 3.18; 95% CI: 0.94 to 10.73). Race-specific HRs are shown in Table 4.3; interaction terms between race and the joint categories of glycemia or diagnosed diabetes were not statistically significant. The test for trend across the joint categories of glycemia was not statistically significant overall or by race (Table 4.3).

Sensitivity analysis In sensitivity analyses in which men with undiagnosed diabetes based on their biomarker value were included in the diabetes category, and in which all men were categorized based on their biomarker value, irrespective of a prior diagnosis of diabetes, inferences did not change overall or by race for fasting glucose, glycated hemoglobin, or glycated albumin (data not shown). To address the concern of possible reverse causation, when prostate cancer deaths occurring within the first three years of visit 2 blood collection were censored at date of death and considered non-events, inferences did not change in overall or by race for the individual biomarkers or the joint categories (data not shown).

4.5 Discussion

In this prospective investigation of the association between biomarkers of glycemia and prostate cancer mortality in over 5,000 men free from a cancer diagnosis at baseline at the time of blood collection, we found that men without diagnosed diabetes who had high fasting glucose had a significantly increased risk of prostate cancer mortality. High HbA1c and glycated albumin were not statistically significantly associated with prostate cancer mortality compared with normal levels of each biomarker. When better classifying hyperglycemia and normoglycemia by using all 3 glycemia biomarkers

simultaneously, men without diagnosed diabetes with hyperglycemia had a statistically significant 4.8 times higher risk of dying from prostate cancer as compared with men without diagnosed diabetes who had normal glycemia. This association was similar in African American and Caucasian men. Men with diagnosed diabetes had a non-statistically significant increased risk of prostate cancer mortality as compared with men without diagnosed diabetes with normal levels of each biomarker. However, when better classifying normoglycemia using all three biomarkers, this association was stronger. The attenuated associations for fasting glucose, HbA1c, and glycated albumin compared to the associations for the joint categories is suggestive of potential non-differential misclassification. To our knowledge, this is the first investigation to use this combination of glycemia biomarkers to jointly classify glycemia in relation to prostate cancer mortality. While the three biomarkers we used in our joint classification strategy are complementary measures of glycemia, each marker is susceptible to different sources of measurement error, which is evident when comparing the distributions of each biomarker (Table S4.3). HbA1c and glycated albumin together classified 62% men with normal fasting glucose as normal (Table S4.4), and together classified 3% of men with high fasting glucose as high (Table S4.4). Thus, in our joint classification strategy using all three markers we were able to better classify individuals as having normal or high glycemia with less error than using any one of these biomarkers.

Though no prior studies have investigated the relationship between glycated albumin and prostate cancer mortality, both glucose and HbA1c have been evaluated in association with prostate cancer mortality. A pooled analysis of six cohorts from Norway, Austria, and Sweden investigating the relationship between glucose (combining fasting and non-fasting participants) and cancer mortality, reported a non-statistically significant increased risk of a dying from prostate cancer in the 2nd (mean: 4.7 mmol/L) and 3rd

(mean: 5.1 mmol/L) quintiles of glucose compared with the 1st quintile (mean: 4.1 mmol/L), but an inverse association was reported for the 4th (mean: 5.5 mmol/L) and 5th (mean: 6.9 mmol/L) quintiles of glucose.[15] Our findings differ from these in that we found men without diagnosed diabetes had an increased risk of prostate cancer mortality for elevated fasting glucose (≥ 5.6 mmol/L) compared to men without diabetes with glucose in the normal range (3.1 to 5.6 mmol/L). The difference in findings could be due to the use of different reference groups and differences in the extent of fasting (pooled analysis: fasting times ranged from < 1 hour to at least 8 hours, ARIC: fasting time at least 8 hours). Thus, it is difficult to compare values between studies, though the reference group in the current study includes individuals in the 1st through 4th quintiles of the pooled analysis. With respect to HbA1c, the results of the current analysis revealed a non-statically significant increase in risk of dying from prostate cancer in men without a diagnosis of diabetes with low HbA1c (< 5.0%) and in men with elevated HbA1c (≥ 5.7%) compared to men without a diagnosis of diabetes with HbA1c in the normal range (5.0 to 5.6%). These results are consistent, although attenuated, with the non-statically significant increase in risk of dying from prostate cancer we observed in prior analyses (with deaths through 2006) comparing men without a diagnosis of diabetes with low HbA1c and elevated HbA1c.[9]

We observed a suggestive increased risk of dying from prostate cancer among men with diagnosed diabetes compared to men without diagnosed diabetes who had normal levels of each of the three biomarkers individually, and to men without diagnosed diabetes who had normal levels of all three biomarkers simultaneously in our joint classification. The rationale for including men with diagnosed diabetes in a separate category compared to men without diagnosed diabetes is based on the overall objective to use glycemia biomarkers to define states early in the natural history of diabetes.

Comparing men with diagnosed diabetes to men with normoglycemia without diagnosed diabetes allowed us to determine the relationship between advanced states in the natural history of diabetes with normal states. Our findings are consistent with a pooled analysis of Asian cohorts that reported slightly positive association between self-reported diagnosed diabetes and prostate cancer mortality when compared with men without diabetes.[12] In our prior ARIC analysis, we observed no statistically significant association between diagnosed diabetes and prostate cancer mortality when compared with men who had HbA1c values in the normal range.[9] However, this finding was based on follow-up through 2006; the current analysis includes follow-up through 2012. In the Cancer Prevention Study-II, there was a statistically significant inverse association between self-reported diabetes and dying from prostate cancer compared to those without a diagnosis.[11] In a pooled analysis that included ARIC participants, men who had diabetes had a non-statistically significant lower risk of prostate cancer mortality.[10] In two of the prior analyses showing an inverse association between diabetes and prostate cancer men with and without diabetes were not classified in the same way, which may help explain the differences in the observed associations. In the large pooled analysis including ARIC, men with diabetes, ascertained by self-report, medication use, or fasting glucose ≥126 mg/dL (≥7.0 mmol/L), were compared to men without diabetes.[10] Diabetes was ascertained based on self-report, and compared to men not self-reporting they had diabetes in the Cancer Prevention Study-II.[11] In contrast, our approach included a reference group based on the normal range of each biomarker in men without diabetes while also classifying men without diabetes as either low, or high leading presumably to less non-differential misclassification of the reference group.

Compared with men with normal levels, men with low HbA1c and men who were low on any one biomarker had non-statistically significant increased risk of prostate

cancer mortality, whereas low glycated albumin was associated with a non-statistically significant decreased risk of prostate cancer mortality. Our findings suggest that men who have elevated glycemia and potentially men who have a diagnosis of diabetes are at an increased risk for prostate cancer mortality. Previous research has suggested HbA1c < 5.0% in individuals without diabetes may be indicative of ill health in general,[31] with several studies showing increased risks of all-cause mortality, liver hospitalization, and cancer death in this group.[28-32] Our results revealed an increased risk of prostate cancer mortality in individuals without diabetes with glycemia levels below the normal the range based on our joint categories and with low HbA1c suggesting heterogeneity may be present in the reference groups of other studies that do not account for low glycemia levels.

Elevated glycemia could influence prostate carcinogenesis through the Warburg effect, which has established that non-differentiated proliferating cancer cells utilize the less efficient process of aerobic glycolysis for energy metabolism.[33] This creates a high dependency on glucose which is advantageous when abundant extracellular glucose is available. Although the exact reason for this preference of aerobic glycolysis by cancer cells still remains unclear, oncogenic activation in metabolic pathways may play a role, including the PI3K/Akt/mTOR pathway.[33,34] There is growing evidence that PI3K/Akt activation is associated with increased glycolysis.[33,34] Furthermore, metformin, a drug used commonly to treat type 2 diabetes activates AMPK, which regulates glucose metabolism and blocks the PI3K/Akt/ mTOR pathway,[33,35] has been inversely associated with prostate cancer-specific mortality and biochemical recurrence in men with prostate cancer.[36] However, metformin also stimulates the uptake of glucose by skeletal muscle leading to decreased circulating glucose. Nonetheless, the inverse relationship between metformin and prostate cancer-specific mortality and biochemical recurrence[36] provides

some insight into the potential biological mechanism underlying the relationship between elevated glycemia and prostate cancer mortality.

The significance of our findings of a positive relationship between elevated levels of glycemia and prostate cancer mortality are particularly relevant in the context of the relationship between obesity and prostate cancer mortality. Previous research has demonstrated obesity to be associated with an increased risk of prostate cancer mortality.[37] Measuring biomarkers of glycemia provides an opportunity to examine a direct link between the metabolic perturbations downstream of obesity. Serving as a potential mediator of this relationship, the strong positive relationship between elevated levels of glycemia captured in our joint classification strategy provides evidence to help explain the obesity-prostate cancer relationship. Given the multiple metabolic and hormonal pathways connecting obesity with increased risk of prostate cancer, which have been thoroughly reviewed previously,[38-40] additional phenotypes of obese metabolically normal individuals may be relevant to further characterize the obesity prostate cancer relationship. A strength of the current analyses is our ability to adjust for both BMI and waist circumference. This further supports the role of hyperglycemia in the complex relationship between obesity and prostate cancer mortality.

The ability to define a low category composed of men with glycemia levels below the normal range is an important strength of this analysis, which provides an opportunity to comprehensively characterize the relationship between glycemia and prostate cancer mortality. Further investigation is needed to determine whether non-glycemic factors are driving the J-shaped association between HbA1c and prostate cancer mortality in our results. We were unable to evaluate the association between low fasting glucose and prostate cancer mortality because no men in our study population had low values.

Another strength of our analysis includes the prospective design with measurement of glycemia biomarkers prior to prostate cancer diagnosis and mortality. This allows us to demonstrate the temporal relationship between states early in the natural history of diabetes (e.g., hyperglycemia in men without diabetes) and prostate cancer mortality. All ARIC participants included in the present analysis reporting having fasted for at least 8 hours prior to blood collection during visit 2, allowing us to evaluate fasting glucose. The advantage of having multiple biomarkers of glycemia in fasted individuals precludes the influence of non-glycemic factors on these values, which have been shown to contribute the observed racial differences in glycemia.[41] Diabetes was classified based on self-report and medication use, reducing the potential of misclassification.[42] Due to the small number of prostate cancer deaths (n=69), we observed wide confidence intervals around our estimates showing elevated risk of dying from prostate cancer. Our analyses also relied on biomarkers of glycemia measured at a single study visit preventing us from conducting analyses with updated values of each biomarker throughout follow-up.

Our findings using multiple markers of glycemia to reduce misclassification of both hyperglycemia and normoglycemia support that elevated glycemia and diabetes increase the risk for prostate cancer mortality. In the current investigation, we observed greater than a 4-fold increase in risk of dying from prostate cancer in men without diabetes who had elevated levels of glycemia compared to men with glycemia in the normal range based on a joint classification strategy incorporating three independent biomarkers of glycemia. Men diagnosed with diabetes appeared to have a 3-fold increase in risk of dying from prostate cancer compared to men without diabetes with glycemia in the normal range. Men with glycemia below the normal range also experienced what appears to be close to a 3-fold increase in risk of dying from prostate cancer that was not statistically significant. These findings are consistent in both African

American men and Caucasian men. These findings support abnormal glycemia in the etiology of the development of prostate cancer with a lethal phenotype, and point to yet another reason to prevent and intervene on the development of pre-diabetes and diabetes.

4.6 References

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Table 4.1 Baseline (visit 2) characteristics of participants without diabetes by categories of age and race adjusted biomarkers of glycemia and participants with diagnosed diabetes in the Atherosclerosis Risk in Communities Study, 1990-1992.

	Fasting Glucose mmol/L			**HbA1c %**				**Glycated Albumin %**			
	Normal < 5.6mmol/L (n=1,768)	**High ≥5.6mmol/L (n=3,090)**	**Diagnosed diabetes (n=418)**	**Low < 5.0% (n=443)**	**Normal 5.0 - 5.6% (n=2,896)**	**High ≥ 5.7% (n=1,519)**	**Diagnosed diabetes (n=418)**	**Low < 11% (n=396)**	**Normal 11 -16% (n=4,345)**	**High ≥ 17% (n=117)**	**Diagnosed diabetes (n=418)**
Mean Age, yr (SE)^	57.2 (0.08)	57.1 (0.08)	57 (0.08)	57.12 (0.08)	57.23 (0.08)	56.9 (0.08)	57 (0.08)	57.3 (0.08)	57.1 (0.08)	56.9 0.09	57 (0.08)
Mean BMI, kg/m2 (SE)	27.8 (0.060)	27.8 (0.060)	27.7 (0.060)	27.8 (0.060)	27.8 (0.06)	27.8 (0.06)	27.7 (0.06)	27.8 (0.06)	27.8 (0.06)	27.8 (0.07)	27.7 (0.06)
African American, % (SE)^	20 (0.005)	20 (0.005)	19 0.007	20 (0.006)	20 (0.005)	19 (0.006)	19 (0.006)	20 (0.006)	20 (0.006)	19 (0.006)	19 (0.006)
Mean Waist circumference, cm (SE)	100.4 (0.15)	100.3 (0.15)	100.1 (0.16)	100.4 (0.15)	100.5 (0.15)	100 (0.16)	100 (0.16)	100.7 (0.16)	100.4 (0.15)	99.9 0.17	100.1 (0.16)
Smoking status Never, % (SE)	27 (0.006)	27 (0.006)	26 (0.006)	27 (0.006)	27 (0.006)	26 (0.006)	26 (0.006)	27 (0.007)	27 (0.006)	27 (0.007)	26 (0.006)
Current, % (SE)	23 (0.006)	23 (0.006)	24 (0.006)	23 (0.006)	23 (0.006)	24 (0.006)	24 (0.006)	22 (0.006)	23 (0.006)	25 (0.007)	24 (0.006)
Former, % (SE)	50 (0.007)	50 (0.007)	50 (0.007)	49 (0.007)	50 (0.007)	49 (0.007)	50 (0.007)	51 (0.007)	50 (0.007)	48 0.008	50 (0.007)
Education Less than high school, % (SE)	20 (0.005)	20 (0.005)	25 (0.006)	19 (0.005)	18 (0.006)	25 (0.006)	25 (0.006)	17 (0.006)	21 (0.005)	27 (0.006)	25 (0.006)
High school/equivalent, % (SE)	37	37	35	38	38	35	35	39	37	34	35

	(0.007)	(0.007)	(0.007)	(0.007)	(0.007)	(0.007)	(0.007)	(0.007)	(0.007)	(0.007)	(0.007)
College or above, % (SE)	43 (0.007)	42 (0.007)	40 (0.007)	43 (0.007)	43 (0.007)	40 (0.007)	40 (0.007)	44 (0.007)	42 (0.007)	40 0.008	40 (0.007)

Fasting glucose: Normal <5.6mmol/L; High >5.6 mmol/L
HbA1c: Low <5.0%; Normal 5.0 to 5.6%; High ≥ 5.6%
Glycated albumin: Low < 11%; Normal 11% to 16%; High > 16%
*Means and proportions adjusted for age and race. ^Mutually adjusted for age and race.

Table 4.2 Baseline (visit 2) characteristics of participants without diagnosed diabetes by age and race adjusted joint category of glycemia, and for participants with diagnosed diabetes in the Atherosclerosis Risk in Communities Study, 1990-1992.

	Joint categories of glycemia among men without diagnosed diabetes					**Diagnosed**
	Low (n=780)	**Normal (n=1,089)**	**High 1 (n=1,828)**	**High 2 (n=1,059)**	**High 3 (n=102)**	**diabetes (n=418)**
Mean Age, yr (SE)^	57.2 (0.08)	57.2 (0.08)	57.2 (0.08)	57 (0.08)	57 (0.08)	57 (0.08)
African American, % (SE)^	20 (0.006)	20 (0.005)	20 (0.005)	19 (0.006)	19 (0.006)	19 (0.006)
Mean BMI, kg/m2 (SE)	27.8 (0.06)	27.8 (0.06)	27.8 (0.06)	27.8 (0.06)	27.8 (0.06)	27.8 (0.06)
Mean Waist circumference, cm (SE)	100.5 (0.16)	100.5 (0.15)	100.5 (0.15)	100 (0.16)	99.9 (0.17)	100.1 (0.16)
Smoking status						
Never, % (SE)	27 (0.006)	27 (0.006)	27 (0.006)	26 (0.007)	27 (0.007)	26 (0.006)
Current, % (SE)	23 (0.006)	23 (0.006)	23 (0.006)	24 (0.006)	25 (0.006)	24 (0.006)
Former, % (SE)	50 (0.007)	50 (0.007)	50 (0.007)	49 (0.007)	48 (0.008)	50 (0.007)
Education						
Less than high school, % (SE)	18 (0.006)	19 0.005	20 0.005	25 (0.006)	26 (0.006)	26 (0.006)
High school/equivalent, % (SE)	38 (0.007)	38 (0.007)	38 (0.007)	35 (0.007)	34 (0.007)	35 (0.007)
College or above, % (SE)	44 (0.007)	43 (0.007)	43 (0.007)	40 (0.007)	40 (0.008)	40 (0.007)

Joint category classification for men without diabetes: low – low on any one biomarker (HbA1c, fasting glucose, glycated albumin); normal – normal on all 3 biomarkers; High 1 – high on any 1 biomarker; High 2 – high on any 2 biomarkers; High 3 – high on all 3 biomarkers. *Means and proportions adjusted for age and race. ^Mutually adjusted for age and race.

Table 4.3 Association between biomarkers of glycemia and prostate cancer mortality in the Atherosclerosis Risk in Communities Study (ARIC) 1990-2012.

	Overall				African American men				Caucasian men			
	No. cases/ Person yrs	**HR[1]**	**95% CI**		**No. cases / Person yrs**	**HR[2]**	**95% CI**		**No. cases/ Person yrs**	**HR[2]**	**95% CI**	
Fasting Glucose												
Normal	14/33,247	1	Ref		7/5,401	1	Ref		7/27,846	1	Ref	
High	49/57,058	1.99	1.09 to 3.63		16/11,216	1.10	0.43 to 2.77		33/45,842	2.69	1.18 to 6.13	
Diabetes	6/6,563	1.91	0.72 to 5.10		2/1,945	0.77	0.15 to 3.89		4/4,618	2.98	0.85 to 10.46	
Per 1-SD increase		1.24	0.87 to 1.78			0.64	0.14 to 2.93			1.40	1.02 to 1.93	
Wald p-value[3]				0.03				0.85				0.02
Wald p-value[4]				0.03				0.43				0.02
HbA1c												
Low	7/8,474	1.68	0.73 to 3.86		2/1,341	1.26	0.25 to 6.36		5/7,134	1.83	0.69 to 4.84	
Normal	29/55,201	1	Ref		6/6,324	1	Ref		23/48,876	1	Ref	
High	27/26,797	1.32	0.75 to 2.31		15/8,981	1.42	0.54 to 3.78		12/17,815	1.19	0.58 to 2.42	
Diabetes	6/6,563	1.36	0.55 to 3.38		2/1,945	0.93	0.18 to 4.81		4/4,618	1.55	0.52 to 4.59	
Per 1-SD increase		1.25	0.90 to 1.73			1.65	1.07 to 2.55			0.88	0.48 to 1.64	
p-trend[5]				0.86				0.59				0.80
p-trend[6]				0.93				0.61				0.73
Glycated Albumin												
Low	2/7,138	0.49	0.12 to 2.05		1/393	3.23	0.40 to 26.30		1/6,745	0.27	0.04 to 2.02	
Normal	58/80,888	1	Ref		21/15,264	1	Ref		37/65,624	1	Ref	
High	3/2,048	1.53	0.47 to 5.00		1/841	0.98	0.13 to 7.55		2/1,207	2.56	0.61 to 10.82	
Diabetes	6/6563	1.13	0.48 to 2.65		2/1,925	0.74	0.17 to 3.22		4/4,618	1.34	0.47 to 3.38	
Per 1-SD increase		1.04	0.64 to1.68			0.55	0.14 to 2.18			1.21	0.77 to 1.90	
p-trend[5]				0.26				0.77				0.05
p-trend[6]				0.21				0.53				0.05
Joint Classification												
Low on any 1 marker	9/14,471	2.95	0.98 to 8.9		3/1,673	4.80	0.48 to 47.52		6/12,798	2.30	0.64 to 8.23	
Normal on all 3 markers	4/20,654	1	Ref		1/1,638	1	Ref		4/20,434	1	Ref	

High on 1 marker	30/34,410	3.66	1.42 to 9.48	8/5,196	4.50	0.56 to 36.45	22/31,744	3.25	1.12 to 9.49
High on 2 markers	16/18,748	2.58	0.92 to 7.22	10/6,302	3.90	0.48 to 31.53	5/12,091	1.78	0.5 to 6.42
High on 3 markers	3/1,791	4.80	1.11 to 20.95	1/690	4.21	0.25 to 71.76	4/2,269	6.78	1.21 to 38.02
Diabetes	6/6,453	3.18	0.94 to 10.73	2/1,925	2.74	0.24 to 31.80	4/4,618	3.32	0.81 to 13.65
p-trend[6]			0.09			0.34			0.18

HbA1c: Low <5.0%; Normal 5.0 to 5.6%; High > 5.6%; Fasting glucose: Normal ≤5.6mmol/L; High >5.6 mmol/L; Glycated albumin: Low < 11%; Normal 11% to 16%; High > 16%

[1]Model adjusted for age (visit 2, continuous), race by ARIC field center, education (less than high school, high school and college; graduate school), BMI (Visit 2, continuous), and smoking status (never; former; current).

[2]Model adjusted for age (visit 2, continuous), education (less than high school, high school and college; graduate school), BMI (Visit 2, continuous), and smoking status (never; former; current).

[3] Wald p-value comparing median biomarker value between categories among men without diagnosed diabetes

[4] Wald p-value comparing change in biomarker category among men without diagnosed diabetes

[5] trend for change in median value of biomarker category among men without diagnosed diabetes

[6] trend for change biomarker category among men without diagnosed diabetes

Table S4.1 Biomarker distribution (median and 25th – 75th percentile) across categories of individual biomarkers in men without diagnosed diabetes in the Atherosclerosis Risk in Communities Study, 1990-1992.

	Fasting glucose					
	Low		**Normal**		**High**	
	< 3.1 mmol/L		**3.1 to 5.6 mmol/L**		**≥ 5.7 mmol/L**	
Fasting glucose (mmol/L)	NA		5.3	(5.1 – 5.4)	6.1	(5.8 – 6.5)
HbA1c (%)	NA		5.3	(5.0 – 5.5)	5.5	(5.3 – 5.8)
Glycated albumin (%)	NA		12.3	(11.6 – 13.1)	12.6	(11.8 – 13.6)
	HbA1c					
	< 5.0%		**5.0 to 5.6%**		**≥ 5.7%**	
Fasting glucose (mmol/L)	5.6	(5.2 – 4.9)	5.7	(5.4 – 6.1)	6.1	(5.7 – 6.8)
HbA1c (%)	4.8	(4.7 – 4.9)	5.3	(5.2 – 5.5)	5.9	(5.8 – 6.1)
Glycated albumin (%)	12.1	(11.5 – 12.8)	12.3	(11.6 – 13.1)	13.1	(12.1 – 14.2)
	Glycated albumin					
	< 11%		**11 to 16%**		**≥ 17%**	
Fasting glucose (mmol/L)	5.7	(5.3 – 6.1)	5.8	(5.4 – 6.1)	8.3	(7.1 – 11.3)
HbA1c (%)	5.3	(5.1 – 5.6)	5.4	(5.2 – 5.7)	7.0	(6.2 – 8.2)
Glycated albumin (%)	10.6	(10.3 – 10.8)	12.6	(11.9 – 13.4)	17.6	(16.5 -21.7)

NA – not applicable; no men in the analytic population had low fasting glucose (< 3.1 mmol/L)

Table S4.2 Cross tabulation of number of men (%) by category of HbA1c and glycated albumin within strata of fasting glucose (normal and high) in men without diagnosed diabetes in the Atherosclerosis Risk in Communities Study, 1990-1992.

		Normal fasting glucose (3.1 – 5.6 mmol/L)					
		HbA1c					
		Low (< 5.0%)		**Normal (5.0 to 5.6%)**		**High (≥ 5.7%)**	
Glycated albumin	**Low (< 11%)**	31	(1.8)	131	(7.4)	18	(1.0)
	Normal (11 – 16%)	207	(11.7)	1,089*	(61.6)	286	(16.2)
	High (≥ 16%)	0	(0.0)	4	(0.2)	2	(0.1)
		High fasting glucose (≥ 5.6 mmol/L)					
		HbA1c					
		Low (< 5.0%)		**Normal (5.0 to 5.6%)**		**High (≥ 5.7%)**	
	Low (< 11%)	28	(0.9)	125	(4.1)	63	(2.0)
	Normal (11 – 16%)	177	(5.7)	177	(49.8)	1,048	(33.9)
	High (≥ 16%)	0	(0.0)	9	(0.3)	102**	(3.3)

*Number of men without a diagnosis of diabetes classified as within the normal glycemia range on all three biomarkers. ** Number of men without a diagnosis of diabetes classified as within the high glycemia range on all three biomarkers.

Table S4.3 Baseline (visit 2) characteristics of participants without diabetes by category of biomarkers of glycemia and participants with diagnosed diabetes in the Atherosclerosis Risk in Communities Study, 1990-1992.

	Fasting Glucose			**HbA1c**				**Glycated Albumin**			
	Normal < 5.6mmol/L (n=1,768)	**High ≥5.7mmol/L (n=3,090)**	**Diagnosed diabetes (n=418)**	**Low < 5.0% (n=443)**	**Normal 5.0-5.6% (n=2,896)**	**High ≥ 5.7% (n=1,519)**	**Diagnosed diabetes (n=418)**	**Low < 11% (n=396)**	**Normal 11-16% (n=4,345)**	**High ≥ 17% (n=117)**	**Diagnosed diabetes (n=418)**
Mean biomarker (SD)	5.26 (0.25)	6.32 (1.10)	10.44 (3.92)	4.75 (0.21)	5.32 (0.18)	6.10 (0.76)	7.97 (2.13)	10.46 (0.57)	12.72 (1.06)	20.23 (5.64)	20.57 (7.58)
Mean Age (SD)	56.78 (5.79)	57.15 (5.65)	58.44 (5.96)	56.11 (5.63)	56.70 (5.67)	57.80 (5.69)	58.44 (5.96)	56.34 (5.61)	57.06 (5.70)	57.77 (5.90)	58.44 (5.96)
African American - No. (%)	297 (16.80)	622 (20.13)	128 (30.62)	77 (17.4)	338 (11.7)	504 (33.20)	128 (30.62)	25 (6.31)	847 (19.49)	47 (40.17)	128 (30.62)
Mean BMI (SD)	26.60 (3.76)	28.19 (4.23)	29.76 (4.67)	27.00 (3.92)	27.13 (3.74)	28.72 (4.66)	29.76 (4.67)	29.04 (4.49)	27.42 (4.03)	29.83 (5.11)	29.76 (4.67)
Mean Waist circumference, cm (SD)	97.25 (10.10)	101.45 (10.92)	105.34 (12.04)	98.43 (10.27)	98.75 (9.93)	102.58 (12.06)	105.34 (12.04)	104.48 (10.80)	99.38 (10.62)	104.46 (13.23)	105.34 (12.04)
Smoking status Never – No. (%)	513 (29.02)	788 (25.50)	112 (26.79)	148 (33.41)	832 (28.73)	321 (21.13)	112 (26.79)	77 (19.44)	1,191 (27.41)	33 (28.21)	112 (26.79)
Current – No. (%)	438 (24.77)	700 (22.65)	88 (21.05)	59 (13.32)	612 (21.13)	467 (30.74)	88 (21.05)	127 (32.07)	981 (22.58)	30 (25.64)	88 (21.05)
Former – No. (%)	817 (46.21)	1,602 (51.84)	218 (52.15)	236 (53.30)	1,452 (50.10)	731 (48.12)	218 (52.15)	192 (48.48)	2,173 (50.01)	54 (46.15)	218 (52.15)
Education Less than high school – No. (%)	319 (18.07)	659 (21.38)	121 (29.02)	63 (14.25)	490 (16.95)	425 (28.05)	121 (29.02)	69 (17.56)	884 (20.38)	25 (21.37)	121 (29.02)

High school/equivalent– No. (%)	653 (37.00)	1,132 (36.72)	156 (37.41)	156 (35.29)	1,105 (38.22)	524 (34.59)	156 (37.41)	161 (40.97)	1,586 (36.56)	38 (46.15)	156 (37.41)
College or above – No. (%)	793 (44.93)	1,292 (41.91)	140 (33.57)	223 (50.45)	1,296 (44.83)	566 (37.36)	140 (33.57)	163 (41.48)	1,868 (43.06)	54 (46.15)	140 (33.57)

Table S4.4 Baseline (visit 2) characteristics of participants without diagnosed diabetes by joint category of glycemia, and for participants with diagnosed diabetes in the Atherosclerosis Risk in Communities Study,
1990-1992.

	Joint categories of glycemia among men without diagnosed diabetes					**Diagnosed Diabetes**
	Low (n=780)	**Normal (n=1,089)**	**High 1 (n=1,828)**	**High 2 (n=1,059)**	**High 3 (n=102)**	**(n=418)**
Mean fasting glucose (mmol/L) (SD)	5.65 (0.59)	5.26 (0.25)	5.94 (0.49)	6.44 (0.72)	10.08 (3.49)	10.44 (3.92)
Mean HbA1c (%) (SD)	5.04 (0.41)	5.29 (0.18)	5.43 (0.32)	6.00 (0.35)	7.86 (1.86)	7.97 (2.13)
Mean glycated albumin (%) (SD)	11.45 (1.25)	12.51 (0.93)	12.62 (0.99)	13.25 (1.26)	20.72 (5.87)	20.57 (7.58)
Mean Age (SD)	56.30 (5.61)	56.68 (5.71)	56.93 (5.69)	57.95 (5.64)	57.73 (5.91)	58.44 (5.96)
African American - No. (%)	101 (12.95)	143 (13.13)	283 (15.48)	353 (33.33)	39 (38.24)	128 (30.62)
Mean BMI (SD)	27.92 (4.46)	26.37 (4.10)	27.32 (4.37)	28.89 (4.67)	30.40 (5.06)	29.76 (4.67)
Mean Waist circumference, cm (SD)	101.12 (10.96)	96.56 (9.71)	99.30 (9.97)	102.95 (11.75)	106.12 (12.93)	105.34 (12.04)
Smoking status						
Never – No. (%)	208 (26.67)	340 (31.22)	503 (27.52)	223 (21.06)	27 (26.47)	112 (21.05)
Current – No. (%)	175 (22.44)	233 (21.4)	417 (22.81)	286 (27.01)	27 (26.47)	88 (21.05)
Former – No. (%)	397 (50.90)	516 (47.38)	908 (49.67)	550 (51.94)	48 (47.06)	218 (52.15)
Education						
Less than high school – No. (%)	128 (6.49)	168 (15.44)	360 (19.70)	300 (28.44)	22 (21.57)	121 (29.02)

High school/equivalent– No. (%)	289 (37.24)	415 (38.14)	676 (37.00)	372 (35.26)	33 (32.35)	156 (37.41)
College or above – No. (%)	359 (46.26)	505 (46.42)	791 (43.30)	383 (36.30)	47 (46.07)	140 (33.57)

Joint category classification for men without diabetes: low – low on any one biomarker (HbA1c, fasting glucose, glycated albumin); normal – normal on all 3 biomarkers; High 1 – high on any 1 biomarker; High 2 – high on any 2 biomarkers; High 3 – high on all 3 biomarkers.

Chapter 5.

Discussion

5.1 Summary and explanation of findings

Within the cancer research enterprise, the discovery of a novel biomarker generates much excitement and enthusiasm for new applications to improve clinical and population health. The discovery phase of translational research, referred to by some as "T0", links the current understanding of a biological process, in the form of a biomarker with a specific health outcome.[1] Despite the remarkable advances in high-throughput technologies, exponential increase in knowledge of basic cancer biology and investment in biomedical research the current landscape of etiologic cancer biomarker discoveries is riddled with failed attempts at producing viable applications targeting cancer prevention and control.[2-4] Threats to validity (e.g., systematic measurement error and bias) and redundant uninformative research (e.g., *Me too science*) represent two barriers in the practice of T1 translational epidemiology. Such barriers have been cited as factors leading to promising application of biomarkers of cancer risk, prognosis, and treatment prediction (throughout called "cancer biomarkers") getting lost in translation.[1,5-8] A more efficient process of translating T1 cancer biomarkers, overcoming each of these barriers would save time and allow researchers and funders to concentrate resources on promising biomarkers with the potential of improving population health outcomes.

For this dissertation, we conducted a series of interrelated aims applying meta-research methods[9] to empirically examine approaches to overcome two barriers mentioned above in the practice of T1 translational epidemiology for etiologic cancer biomarkers. We incorporated these strategies to investigate the natural history of diabetes in the etiology of the development of fatal prostate cancer. We adopted a team science approach to incorporate multiple biomarkers of glycemia to better classify the relationship between glycemia and prostate cancer mortality, the outcome of most importance in public health.

In the first aim of this dissertation, we conducted a case study to document improvements in the validity of results and the inferential benefit of multidisciplinary team science, including epidemiology, in tissue biomarker evaluation. Through the implementation of multidisciplinary team science in the practice of T1 translational research, the team was able to identify and overcome threats to internal validity. Sources of measurement error in the pre-analytic phase of each scenario of the case study included temporal decay of the biomarker associated with tissue block storage time, and batch effects introduced with maintenance of laboratory equipment. Once the threat to validity was identified, the team was able to overcome the threat to validity in the data analysis phase by implementing calendar-time specific cut points based on distribution in controls and batch-specific cut points based on controls.

The team participating in this case study is able to thrive in an atmosphere where multiple research perspectives are cultivated through an interactive and iterative process resulting in a team highly expert on multiple aspects of the team's research topic. The highly collaborative spirit allows researchers to accomplish tasks that could not be done individually to create a synergy whereby the total is greater than the sum of the parts. The diffusion of core information from the array of disciplines and unique perspectives has been instrumental in overcoming unavoidable inherent biases ingrained in their formal training and later experiences. This has allowed the team to be successful at coming up with creative solutions to solving problems and to develop novel questions that are more translational in nature, which would not have been considered prior to the team's interaction. The successful integration of diverse research perspectives into the experimental design, execution, interpretation, and presentation of results is necessary to meet the current challenges in cancer prevention and control. The team has been

able to tackle larger scale questions that are more translational in nature requiring more sophisticated methods compared to traditional single-investigator driven research questions that are more limited in scope.

The second aim of this dissertation was designed to overcome the practice of redundant uninformative observational epidemiologic research, we introduced a systematic process to quantify the impact of continued investigation on the current evidence base summarized in a meta-analysis. We adapted the fail-safe number to quantify the number of future studies that would need to be included in the current meta-analysis to change a statistically significant summary estimate to null ($p \geq 0.05$). We also adapted conditional power analysis to determine the number of future studies that would need to be included in the current meta-analysis to reach 80% power to detect the observed summary estimate in the combined meta-analysis. Applying each of these metrics to 98 meta-analyses of prospective studies addressing non-genomic biomarkers and cancer risk, we observed patterns between the characteristics of the existing evidence and the values of each of these metrics including the size of the summary estimate, the number of studies included in the observed meta-analysis, and the between-study heterogeneity.

We identified two biomarker-cancer outcome associations to illustrate how each of these metrics could be used to inform future research and to describe additional conditions when planning future research. In the context of an established biomarker-cancer association (e.g., *H. pylori* and gastric cancer) the fail-safe number suggested the number of future studies needed to change the current inference is out of reach of existing resource. However, further investigation of this association in populations with different prevalence of gastric cancer contributed additional evidence of effect modification through dietary salt intake which led to improved biologic understanding of

this association. We then described the results from the conditional power analyses for the meta-analyses comparing different circulating androgens and prostate cancer. The uncertainty in the meta-analysis of dehydroepiandrosterone sulfate suggested additional research may provide an inferential benefit (i.e., reasonable number of future studies needed to change the current inference is within reach of existing resources). While this result points to a potential area where future research might be needed, methodologic limitations in measuring circulating androgens points to additional considerations in the method to measure the biomarker when planning future investigations.[10] In both of these examples, the planning of future studies should be done in the context of the existing evidence with additional consideration of the method used to measure the index biomarker; populations or subpopulations not previously studied or in population-based studies with design characteristics that improve ability to make temporal comparisons and/or protect against certain types of bias (e.g., detection bias); outcomes of most public health significance with respect to cancer prevention and control.

For the third aim of this dissertation, we employed a team-science approach by bringing together experts in the etiology and prostate cancer, and experts in the clinical evaluation of biomarkers of glycemia. Building on the strengths and experience of this team, we developed a strategy to incorporate multiple biomarkers of glycemia (e.g., glycated hemoglobin, fasting glucose, and glycated albumin), each measuring complementary aspects of glycemia, but with different sensitivities to non-glycemic facts, to investigate the association between states early in the natural history of diabetes (e.g., pre-diabetes) and diagnosed diabetes with prostate cancer mortality. Through this approach using normal glycemia as the reference category defined as being normal on all three biomarkers, men classified as high on all three markers, had close to a 5-fold increase in risk of dying from prostate cancer (HR: 4.80; 95% CI: 1.11 to 20.95). Men

with diabetes appeared to have greater than a 3-fold increase in risk of dying from prostate cancer, although not statistically significant (HR: 3.18; 95% CI: 0.94 to 10.73). For the low category, defined as low on any one of the three biomarkers, we observed what appeared to be close to a 3-fold increase in risk of dying from prostate cancer, although not statistically significant (HR: 2.98; 95% CI: 0.98 to 9.80). The pattern in these associations was consistent with both African American men and Caucasian men with the interaction between race not statistically significant. Using this approach to define our reference group as normal on all three biomarkers, we observed an elevated risk of prostate cancer death among men with diagnosed diabetes, which is inconsistent with prior research.[11,12] This finding is biologically plausible in that cancer cells utilize glycolysis for energy metabolism that has a high dependency on available extracellular glucose, which is associated with the activation of the oncogenic PI3K/Akt/mTOR pathway.[13,14] Our findings also provide evidence of the direct association between glycemia and prostate cancer mortality, and points to glycemia as a potential mediator of the relationship between obesity and prostate cancer mortality which could be investigated in the future. Overall, in this aim, we employed strategies to overcome two barriers in the practice of translational epidemiology, and in doing so, we gained new insights into the relationship between a modifiable risk factor and prostate cancer mortality.

5.2 Strengths and limitations

A strength of the team science case study is the ability to examine empirically the real (not simulated) impact of multidisciplinary team science in the translation of T1 biomarkers. The sources of analytic measurement error identified (tissue storage time and batch-effects) pose important threats to analytic validity relevant to modern pathoepidemiology[15] using tissue samples to characterize exposure and outcomes. This

increases the generalizability of the application of team science across topic-areas engaged in T1 tissue biomarker research (i.e., neuroscience and environmental and occupational health). We limited this case study to one multidisciplinary team, which may not be generalizable to other teams, and covered only three scenarios of the implementation of team science. Our small sample and choice of scenarios limits the ability to capture variation in the sources of measurement error across the analytic pipeline (e.g., pre-analytic, analytic, and post-analytic). Lack of variability in the source of the analytic measurement error limited our ability to quantify the full impact of team science on identifying and correcting the error if the sources of measurement error in our sample have minimal effect on the biomarker measurements. Our sample is also limited to examples of tissue biomarkers with different analytic pipelines and methodological concerns compared to non-tissue biomarkers.

Utilizing the fully characterized 98 meta-analyses[16] provides several strengths to the proposed aim. Tsilidis et al. applied a robust search strategy to identify potentially eligible biomarker-cancer meta-analyses of prospective studies reflecting a comprehensive range of biomarkers and cancer types. The investigators also employed a process of double-data extraction enhancing the reliability of the corresponding study-level data. The full complement of meta-analytic metrics describing the 98 meta-analyses enhanced our ability to implement the proposed fail-safe number and conditional power analysis. For this work we adapted approaches, previously developed in for use in clinical trials and research synthesis,[17-19] as novel solutions to overcome practice-based barriers in the translation of cancer biomarkers. The prior development and use in other areas of biomedical research for similar purposes to quantity the influence of additional null studies and improve the usefulness of future research speaks to the utility of using these metrics for related purposes in other areas of research.

One limitation of applying our approach to the 98 meta-analyses is absence of duplicate biomarker-cancer meta-analyses. During the selection process, 16 meta-analyses were excluded that evaluated the same biomarker-cancer association as another meta-analysis. The investigators retained the larger meta-analysis. A comparison of the redundant meta-analysis including the magnitude and direction of the odds ratios and the included studies would provide an opportunity to explore the impact of updating an existing meta-analysis with recent studies that may contribute little to the current meta-analysis. Our approach utilizing the fail-safe number to quantify the impact of continued investigation of specific exposure-outcome association is not without limitations. As discussed in the previous chapter of this dissertation, the fail-safe number initially was developed as a metric to assess publication bias in the field of clinical trials. The Cochrane Collaboration does not recommend the use of the fail-safe number as a means to quantify the effect of publication bias on the findings of a systematic review and meta-analysis.[20] This critique stems from the reliance on statistical significance rather than on clinical significance of an observed effect. However, the fail-safe number does provide utility in putting the general issue of public bias into perspective by quantifying the number of unpublished studies necessary to change the overall significance level of a meta-analysis. With respect to the conditional power analysis, one limitation stems from setting the alternative hypothesis conditioned on as well as the degree of heterogeneity introduced by additional of the new studies. A number of iterative approaches can be used to test different alternative hypotheses and different degrees of heterogeneity in the observed meta-analysis as well as in the new studies. Our intention is not to characterize the full array of conditions that can influence the conditional power of a future study.

The major strength of the third aim is in the approach where we integrated the strategies for overcoming barriers in the translation of etiologic cancer biomarkers evaluated in aims 1 and 2. We cultivated a multidisciplinary team of experts in both prostate cancer etiology and glycemia biomarkers to develop a strategy to incorporate multiple glycemia biomarkers to better classify glycemia in the analysis of prostate cancer mortality, the outcome of most public health importance in a racially diverse cohort. Using this approach, we were able to fill existing evidence gaps using alternative methods for measuring glycemia biomarkers to better understand the relationship between states early in the natural history of diabetes and prostate cancer mortality. Additional strengths of this work are in the longitudinal design of the Atherosclerosis Risk in Communities (ARIC) study with prospectively collected biomarkers of glycemia. This is the ideal design for investigating the natural history of diabetes in relation to prostate cancer mortality. It is not possible to identify the same biomarker-outcome relationship in cross-sectional or case-control designs, which are often vulnerable to certain types of biases and confounding, and certainly do not provide the temporality to compare exposure prior to onset of the outcome. The availability of multiple glycemia biomarkers not available in other cohort studies was the foundation of our joint classification strategy, which is another strength of ARIC. Furthermore, ARIC is racially diverse with 25% African American participants, which allowed use to investigate the associations between glycemia and prostate cancer mortality in African American men and Caucasian men. One limitation in using ARIC to conduct molecular epidemiologic investigations of biomarkers of glycemia is the majority of the glycemia markers were measured only once, thus limiting our ability to examine the time-vary effects of each biomarker. Another limitation is the relatively small sample size in ARIC with only 69 prostate cancer deaths, which is apparent in the wide confidence intervals around our estimates.

5.3 Public health implications

Both aims one and two have immediate relevance in that they each provide empirical evidence of strategies to improve research practices in an effort to overcome barriers in translational research. The adoption of such practices by the translational research community will contribute towards more efficient and reliable research with the ultimate goal of translating promising discoveries into improved population health. To this end, multidisciplinary team science plays an important role in translational cancer research, which has been endorsed by the National Cancer Institute through a number of funding mechanisms, and by the American Association of Cancer Research's Team Science Award. Team science has also been cited as a driver of translational research.[21] Our work demonstrating the quantitative and inferential benefit achieved through the practice of multidisciplinary team science provides direct evidence of how the practice of team science can overcome barriers in translational research, further supporting such initiatives promoting and rewarding the practice of multidisciplinary team science.

Through the application of both the fail-safe number and conditional power analysis to meta-analyses of observational studies of biomarkers and cancer risk, we described an approach that stakeholders engaged in translational research including researchers, journal editors, grant reviewers, and funding agencies can use to assess the impact of continued study of a specific biomarker-outcome relationship. This approach can be easily implemented into journal's instructions to authors for submitting meta-analyses to include these metrics in their report. This will also provide a method for journal editors to evaluate the impact of a primary analysis of biomarker-cancer outcome association against the current evidence base. Our work further highlights additional considerations when planning future studies, and we showed how such considerations

can lead to improved biological understanding. Overall this process is designed to inform more efficient and productive use of resources in prioritizing research that fills important evidence gaps.

The public health impact of the third aim, revealing a strong association between elevated levels of glycemia and prostate cancer mortality speaks to the overall importance of preventing pre-diabetes and diabetes. With the rising prevalence of obesity, pre-diabetes, and type 2 diabetes in the US,[22-24] our work provides important evidence of the relationship between metabolic perturbations related to obesity with other important health outcomes. Furthermore, this work revealed a modifiable risk factor for prostate cancer – elevated glycemia, which could be a target for cancer prevention efforts.

5.4 Directions for future research

Based on the results of aims 1 and 2, two areas emerged regarding directions for future research. The first relates to evaluating if the strategies we discussed in aims 1 and 2, that improve research practices to overcome specific barriers in the T1 stage of the translational continuum, can be used to catalyze the progress of a promising discovery through the subsequent stages of translational continuum (e.g., T2 – T4). For example, the practice of multidisciplinary team science, including representation of both individuals and disciplines, including epidemiology, participating in earlier stages of translation may provide a valuable contribution in the subsequent stages of translation. Similar meta-research based approaches could be developed to test the impact of the practice of multidisciplinary team science on the trajectory of promising discoveries in the translational continuum. Similarly, the application of the fail-safe number and conditional power analysis could be developed into an approach for constructing an

evidence map across the spectrum of types of biomarker and cancer types, with consideration of the full complement of important outcomes and populations, as a way to prioritize the deployment of resources to move promising discoveries through the next phases of translation.

With respect to the third aim, the primary outcome was prostate cancer mortality and further research is needed to evaluate our joint classification strategy with additional outcomes including prostate cancer incidence and incidence of lethal prostate cancer (e.g., an incident prostate cancer that was metastatic or dying from prostate cancer). A formal mediation analysis of elevated glycemia as a mediator of between obesity and prostate cancer would further clarify the link obesity and prostate cancer.

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Curriculum Vitae

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PROFESSIONAL EXPERIENCE

2016 – Present **Health Data Analyst**
US News and World Report, Washington DC

2013 – 2014 **Cancer Research Training Award Fellowship**
Epidemiology and Genomics Research Program
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2011 – 2013 **Methodologist**
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2006 – 2007 **US Peace Corps Volunteer**
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HONORS & AWARDS

2016	**Distinguished Epidemiology Scholars Program** Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
2014	**The Harvey M. Meyerhoff Fellowship in Cancer Prevention** Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
2010	**Charles C. Shepard Award Finalist** Rollins School of Public Health, Emory University, Atlanta, GA
2010	**Outstanding Public Health Practicum Experience** Rollins School of Public Health, Emory University, Atlanta, GA
2010	**Travel Grant** 14th Annual Conference of the International Union against TB and Lung Disease – North America Region, Orlando, FL
2007	**Volunteer Activity Support & Training Grant** US Peace Corps, Swaziland

PROFESSIONAL ACTIVITIES

2017 – Present	American Association for Cancer Research, Associate Member
2011 – Present	Cochrane Prognosis Methods Group, Member
2008 – 2010	Student Outbreak and Response Team, Emory University, Member
2006 – 2007	Volunteer Action Committee, US Peace Corps, Swaziland, Member

EDITORIAL ACTIVITIES

EDITORIAL BOARD MEMBERSHIP:

1. PLoS Currents: Evidence on Genomic Tests

PEER REVIEWER:

1. Annals of Epidemiology
2. Annals of Oncology
3. Cochrane Eyes and Vision Group
4. Ophthalmology

EXTERNAL REVIEWER:

1. WHO/Europe: consensus paper on the role of surgery in the treatment of pulmonary tuberculosis and multidrug- and extensively drug-resistant tuberculosis.

TEACHING ACTIVITIES

TEACHING ASSISTANT:

1. Principles of Epidemiology (EPI 340.601.01): Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD: Summer 2016
2. Epidemiologic Inference in Public Health (EPI 340.721.60): Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD: 1st Term 2016

INSTRUCTOR:

1. Completing a Cochrane Systematic Review Workshop, Baltimore, MD: July 2013
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PUBLICATIONS

PEER-REVIEWED JOURNAL ARTICLES:

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3. Khosropour CM, Eldridge R, Moline HL, Link-Gelles R, **Marrone M**, Berkelman RL. Students for Surge: Participation of Emory University's Student Outbreak and Response Team in Novel H1N1 Investigation. Public Health Preparedness Summit 2010. Atlanta, GA, February 16 – 19, 2010.

PRESENTATIONS

INVITED PRESENTATIONS:

1. **MARCH 2013:** Clinical utility of gene-expression profiling in women with early breast cancer: an overview of systematic reviews. Presentation to the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group.

SCIENTIFIC MEETINGS:

1. **JUNE 2017:** Clinical utility When is enough, enough? Adapting the fail-safe number for deciding whether more research is needed on biomarker-cancer associations. Society for Epidemiologic Research Annual Meeting., Seattle, WA.

2. **APRIL 2017:** Glycemia is positively associated with prostate cancer mortality in white and black men without diabetes when better classifying hyper- and normo-glycemia using 3 biomarkers. American Association for Cancer Research Annual Meeting. Washington DC.

3. **DECEMBER 2010:** Priorities in genomic applications in practice and prevention: an evidence-based approach to moving forward with translation and implementation of genomic technologies in clinical and public health practice. Presented at the 4th National Conference on Genomics and Public Health. Bethesda, MD.