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THE HEALTH AND RETIREMENT STUDY-AN EVALUATION AND
SCIENTIFIC IDEAS FOR THE FUTURE

The State and Future of Blood-Based Biomarkers in the Health and Retirement Study

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Abstract

The Health and Retirement Study (HRS) is an important national resource for policy makers and investigators across a wide range of disciplines, and it is critical that the study collects the best information possible on the health status of its participants within the constraints of the survey design, and without compromising the integrity of the sample. Potential directions for the collection and analysis of biomarker data in future waves of HRS are discussed, with a primary focus on blood-based biomarkers. Advantages and disadvantages of various methods for collecting blood in the home are considered, with particular attention given to the strengths and weaknesses of dried blood spot (DBS) sampling. DBS sampling has been widely applied in recent biosocial surveys due to the low cost and burden associated with sample collection, but these benefits need to be weighed against challenges associated with quantification in the laboratory. Attention is also given to additional biomarkers that may be of relevance to HRS, and that would expand the survey's current focus on obesity and metabolic syndrome. Measures of inflammation, pathogen exposure, reproductive function, stress, and epigenetic modifications are suggested as potentially productive future directions for the study. In addition, the analysis concludes with the following recommendations for HRS: Continue to collect DBS samples, but consider alternatives; implement enhanced procedures for quality control; calibrate DBS results against plasma values, and invest in methods development.

KEYWORDS: Health and Retirement Study

INTRODUCTION

The Health and Retirement Study (HRS) was launched in 1992 to promote research into the challenges and opportunities of aging in America. It includes a large, nationally representative sample of adults over the age of 50, and focuses on physical and mental health, financial and employment status, retirement planning, social/family environments, health care access and usage, and insurance coverage. HRS represents a critically important data source on these issues that is frequently used by policy makers and investigators across a wide range of disciplines.

Given that health is a defining feature of HRS, it behooves the study to continually evaluate its options for collecting the best information possible on the health status of its participants. At its inception, the study relied on participant reports of mental and physical health across various domains, using methods and instruments typically applied in surveys with a health component. In 2001 HRS began considering the possibility of collecting biological specimens from participants, reflecting—and ultimately contributing to—the growing trend toward integrating biological measurement into population-based, social science surveys (Weir 2008).

Biomarkers are directly measured traits that provide insight into the functioning of biological systems. They often involve the collection and subsequent analysis of biological specimens (e.g., blood, saliva, urine), but they also include physical and functional measures (e.g., blood pressure, anthropometry, grip strength). Biomarkers provide information on normal biological processes, as well as pathogenic processes that may result in clinically identifiable disease. With the rapid development of “field-friendly” methods for collecting biological data outside the clinic (McDade et al., 2007; Lindau and McDade 2008), biomarker measurement is becoming an increasingly important part of surveys like HRS. This report discusses the relevance of biomarkers to HRS, and considers potential directions for the collection and analysis of biomarker data in future waves. The report focuses primarily on blood-based biomarkers, with an analysis of the advantages and disadvantages of various methods for collecting blood in the home. The focus on biomarkers in blood is justified by the fact that many analytes of interest to HRS are accessible only in blood, and not through other fluids like saliva or urine. Particular attention is given to the strengths and weaknesses of dried blood spot (DBS) sampling as a method for collecting blood since HRS has used this method in prior waves.

THE RELEVANCE OF BIOMARKERS TO HRS

There are several ways in which biomarkers have the potential to make significant contributions to HRS. First, biomarkers provide objective measures of disease status, as well as direct information on predisease pathways that are causally proximate to a wide range of important health outcomes. Self-reports rely on subjective, conscious experience, whereas biomarkers tap into physiological processes that may be below the threshold of perception, but are nonetheless predictive of current or future disease. Often, the utility of biomarker measurement does not lie in the discovery of a clinical disease state, but in the identification of individuals most at risk for the future development of disease. This is particularly true for prospective aging studies like HRS. Conversely, biomarkers may reveal the extent to which aspects of health shape individual life course trajectories and selection into various environments. HRS is in an excellent position to make innovative contributions in this area by considering whether health status—defined by self-reports, or underlying biological states—is a major determinant of decision-making among older Americans.

Second, biomarkers can shed light on the multiple physiological pathways—neuroendocrine, cardiovascular, metabolic, immune/inflammatory—through which contextual factors exert their influence on health. Such knowledge facilitates our understanding of how social, economic, and policy environments “get under the skin,” and may be useful for informing interventions that improve population health. Along these lines, the implementation of objective, “hard science” data may be particularly effective in mobilizing the attention of policy makers around important social issues.

Third, biomarkers are not susceptible to many of the shortcomings associated with self-reported health measures. Since they represent objective indicators of health that are beyond the conscious control of research participants, they do not depend on the participant’s ability to recall relevant health information, or their willingness to share this information. This aspect may be particularly advantageous for research on aging, where cognitive declines may alter an individual’s ability to report on their own health status. Biomarkers also facilitate comparisons across demographic groups that may vary in their perception, experience, and/or reporting of health. Although biomarkers may be seen as an improvement over self-report for these reasons, they raise a distinct set of challenges and potential limitations (more on this below). As such, biomarkers and self-reports are best viewed as complementary sources of information on health, each of which has its own set of strengths and weaknesses.

Lastly, biomarkers position HRS at the cutting edge of an emergent biosocial approach to survey research. This approach takes advantage of the development of a rapidly expanding toolkit for collecting biological samples in

non-clinical settings, and it represents a meaningful integration of models and methods from the social and biological sciences that answers the call for a more integrative, transdisciplinary approach to research in human health (National Research Council 2008). By drawing larger, more diverse, and representative samples, population-based studies greatly increase the generalizability of research findings, facilitate inclusion of groups (e.g., urban poor, rural populations) often excluded from clinical evaluations of health, and provide opportunities for investigating the population-level implications of biological processes. Biomarkers in HRS also facilitate comparisons with other studies of aging (e.g., English Longitudinal Study of Ageing, Mexican Health and Aging Study, China Health and Retirement Longitudinal Survey).

Despite the benefits of biomarkers, and current high levels of enthusiasm for adding them to social science surveys, it is important to evaluate their potential costs. A balanced consideration is particularly critical for multi-disciplinary, observational, and longitudinal studies like HRS where maintaining the integrity of the sample is essential, and where many researchers use the dataset to address questions not directly related to health. Biomarkers add to respondent burden, and may have implications for sample recruitment and retention.¹ Biomarkers also add logistical complications to data collection, and the shipment and analysis of biological specimens adds substantial costs to survey budgets. And like all research involving potentially sensitive information, biomarkers raise important ethical and confidentiality issues that require careful consideration. Some of these issues are unique to biological measures, and studies thus far have pursued a range of strategies for dealing with these issues.

Several large, population-based social science surveys have incorporated biomarkers over the past five years, and experience suggests that the challenges associated with biological measurement in the home are tractable, particularly since new “minimally-invasive” methods have substantially reduced the costs and burdens associated with the large-scale application of biomarkers. Regardless, the costs are real, and biomarkers should only be applied when the scientific pay-off is clear. According to the HRS online bibliography, data from the study have been featured in over 600 publications in the area of “health conditions and status.” Such a high level of interest in issues related to health suggests that the biomarker data will be widely used by HRS investigators, and that the impact of their analyses will reach across a wide range of disciplines.

¹ Although we often think of biological measures as inherently more invasive than self-report measures, it is worth noting that for many respondents, slight physical discomfort may be preferable to the psychological discomfort associated with disclosure of embarrassing or otherwise sensitive information (for example, a recent survey of young adults found that participants were more willing to provide a dried blood spot sample than they were to report income).

BIOMARKERS AND HRS: EXPERIENCE THUS FAR

In 2003 HRS initiated its first effort to measure biomarkers in blood with the “Diabetes Study.” The study focused on glycosylated hemoglobin (HbA1c) as a measure of diabetes risk. HbA1c provides an integrated measure of glucose control over the two to three months prior to blood collection, and it does not require fasting or collection at a specific time of day. As such, HbA1c is a good candidate for measurement in the home. HRS focused on individuals reporting a diagnosis of diabetes, and identified 2,581 eligible cases for the study. Since HRS had relied on phone interviews up to this point (the ADAMS substudy being a small exception), the decision was made to send participants a self-administered questionnaire, followed by a kit for the self-collection of a DBS sample. Samples were then sent to Flexsite Diagnostics and analyzed for HbA1c. Questionnaires were received from 1,897 participants, and 1,233 participants returned a DBS sample, representing a 65% rate of compliance with DBS sampling among willing participants (52% of the overall surviving eligible sample). This rate of compliance is unacceptably—and not surprisingly—low, and is substantially lower than other studies using face-to-face interviews. The National Longitudinal Study of Adolescent Health, for example, recently completed the wave IV survey and collected DBS samples from 94% of contacted participants. In 2005-6, the National Social Life, Health, and Aging Project—perhaps a better benchmark for HRS—collected DBS samples from 84.5% of eligible participants (Williams and McDade 2007).

The 2003 HRS experience with DBS provided the impetus for the “enhanced face-to-face interview” implemented in 2006 with a random one-half of the sample (N=8,392), followed by the other half of the sample in 2008. The interview format allowed for a more ambitious biomarker collection plan, including DBS samples, saliva for DNA, blood pressure, anthropometrics, and a series of physical performance tests (e.g., grip strength, balance, peak flow, timed walk). DBS samples were collected by interviewers on two cards: one for immediate analysis, and another for storage and future analysis as additional biomarkers of interest come online. Samples were analyzed for HbA1c, total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), C-reactive protein (CRP), and cystatin C (Crimmins et al. 2008). Samples in 2006 were originally sent to Biosafe Laboratories for analysis, but bankruptcy proceedings forced a change mid way through the survey. Samples are now being analyzed by Heritage Labs, and by Dr. Russ Tracy’s lab at the University of Vermont.

In the 2006 survey, 82.7% of participants consented to providing a DBS sample. In 2008, 87% consented, bringing the rate of compliance up to a point where it compares favorably with other surveys. In 2006, 91.4% of the DBS samples contained four or more drops of blood. In 2008, 96.9% of the samples

contained four or more drops, and 84.3% of the samples had the maximum number of six drops. These yields are exceptional, and they bode well for the analyses currently planned, as well as for future analyses using banked samples.

LOOKING TO THE FUTURE: CHALLENGES AND OPPORTUNITIES FOR HRS

As a large, prospective, nationally-representative study that serves as a critical resource for investigators and policymakers across a wide range of interests and disciplines, HRS is not in a position to experiment boldly with unproven biomarker technologies. However, if HRS is to continue as a leading biosocial survey, it needs to consider what steps can be taken to ensure that future biomarker data are of the highest possible quality, and highest possible relevance to the objectives of the study. Comments below are organized around two themes: 1) The logistics of blood collection and laboratory analysis, with a focus on DBS sampling and potential alternatives; and 2) Expanding the scope of biomarkers for future waves of HRS.

COLLECTING BLOOD IN THE FIELD: DRIED BLOOD SPOTS AND POTENTIAL ALTERNATIVES

Two waves of HRS—along with several other NIA-funded surveys—have focused on DBS sampling as a way to collect blood from participants in the home. The current clinical gold standard is to pull several milliliters of plasma or serum, but the costs, participant burden, and logistics associated with venipuncture have served as major impediments to integrating blood-based biomarkers into survey-based research. Most surveys will likely continue to focus on finger-stick blood sampling methods (although home-based phlebotomy, separate from collection of interview data, may represent a viable, albeit costly, alternative for at least a subset of participants).

The procedure for collecting whole blood from finger stick is relatively painless and non-invasive, low cost, and can be conducted by non-medically trained interviewers in the participant's home (or, as HRS has shown, by participants themselves) (McDade et al. 2007). The participant's finger is cleaned with isopropyl alcohol, and then pricked with a sterile, disposable lancet of the type commonly used by diabetics to monitor blood glucose. The first drop of blood is wiped away to stimulate blood flow, and for DBS sampling, multiple drops (~50 uL per drop) are applied to filter paper (Whatman #903), allowed to dry, and then stored at room temperature prior to shipment to the laboratory. The ease of finger stick blood sampling may be a particular advantage for research with the elderly for whom venipuncture may be especially difficult, and for

research in remote or underserved communities where the logistics of venipuncture prove to be insurmountable barriers to biomarker collection. The low burden of sampling also increases the feasibility of collecting multiple blood samples from the same individual over time. As discussed below, the utility of whole blood from finger stick is not limited to DBS samples.

DRIED BLOOD SPOTS: ADVANTAGES AND DISADVANTAGES In addition to the low burden and cost of sample collection, an advantage of DBS sampling is that requirements for sample handling, storage, and shipping are relatively minimal (Table 1). Unlike methods using liquid blood samples, DBS samples do not need to be centrifuged, separated, or immediately frozen following collection. A cold chain from the point of sample collection to receipt in the laboratory is not required: Drops of whole blood are simply applied to filter paper, allowed to dry, and then stacked and stored. Most analytes remain stable at room temperature for a week or more, providing considerable flexibility in procedures for sample collection and transport. In addition, DBS samples are stable in laboratory freezers for long periods of time, and can be analyzed in the future as new biomarkers of interest emerge. A typical drop of blood will contain approximately 50 μ L of whole blood, and will result in a dried blood spot approximately 12 mm in diameter. Such a spot will yield seven 3.2 mm discs of blood that can then be used in laboratory analyses. A full card of five blood spots will therefore contain enough sample to assay 35 analytes requiring one 3.2 mm disc, or 17 analytes requiring two such discs. However, in practice, five perfect blood spots are rarely obtained, and sufficient sample for 10 to 20 3.2 mm discs is a more reasonable expectation for a single finger prick. Lastly, the filter papers used for DBS sampling have played a central role in nationwide neonatal screening programs for a wide range of treatable metabolic disorders since the 1960s (Mei 2001). As a result, the papers are widely available and will continue to be available in the future, and they are certified to meet performance standards for sample absorption and lot-to-lot consistency by the Clinical and Laboratory Standards Institute.

Dried blood spots have been incorporated into several major data collection efforts in the United States, as well as internationally. Over the past five years, over 35,000 DBS samples have been collected by NIH funded studies in the US², and major data collection efforts have been, or are currently being, implemented in China, Indonesia, Mexico, and several nations in Europe and

² These studies include, in addition to HRS, Los Angeles Family and Neighborhood Survey (LA FANS), Moving to Opportunity (MTO), National Longitudinal Study of Adolescent Health (Add Health), National Social Life, Health, and Aging Project (NSHAP), the Oregon Health Study, and the Work, Family, and Health Network.

Africa. The opportunity for comparison of biomarkers across these studies represents an additional advantage of DBS sampling for HRS.

Disadvantages of DBS sampling relate to issues with quantification in the laboratory. First, the volume of collected blood is very small (up to 250 uL), particularly in comparison to the large volumes of blood collected with venipuncture (at least 2,500 uL and up). This constraint places a premium on the efficient use of DBS sample, and may limit the number of analytes that can be quantified in a given sample. Second, the vast majority of standard clinical laboratory methods require serum or plasma³, and assay protocols must therefore be developed specifically for blood spots and validated for accuracy, precision, reliability, and limits of detection. This is a methodical process that requires concerted effort, but it is essential if results from the analysis of DBS are to be taken seriously, and if biosocial surveys are to keep pace with developments emerging from the clinical and laboratory sciences. Third, from a clinical perspective, dried blood spots are considered a “non-standard” diagnostic substance, and results derived from DBS may not be directly comparable to those derived from venipuncture blood. This is not a concern for within-study analyses with large surveys, but it poses challenges for comparisons with clinical or epidemiological datasets using serum or plasma (e.g., NHANES), and to the calculation of clinically relevant cut points for biomarkers like CRP or TC that are based on the analysis of serum or plasma. However, it is possible to overcome this limitation by collecting a set of matched plasma and DBS samples to derive conversion factors that allow the calculation of “plasma equivalent” values, based on the fact that the correlation between results derived from plasma and DBS samples tends to be very high (Worthman and Stallings 1997; McDade et al 2007).

The relative ease of collecting, shipping, and storing DBS samples needs to be weighed against challenges in the lab. In particular, the number of labs that can currently analyze large numbers of DBS samples for surveys like HRS is severely limited, and quality of results produced by these labs can be mixed. There are currently less than 10 labs in the country with this kind of DBS expertise, and none has emerged as the go-to lab that can meet all the needs of the survey research community (there are many other labs focused on neonatal screening of DBS samples, but they use different m. In many cases, labs focus on one or two biomarkers only, requiring samples to be sent from one lab to another, adding costs to the survey and increasing the risk of sample loss. There is reason for optimism, though, as a handful of labs have recognized the demand (and

³ Serum and plasma are similar in that they comprise the liquid fraction of whole blood, and both are obtained through separation from white and red blood cells that are suspended in whole blood. Plasma includes clotting factors (typically inactivated by anti-coagulants following venipuncture blood collection) while clotting factors are not present in serum.

opportunity) presented by studies like HRS, and are ramping up their infrastructure to support high throughput analysis of biomarkers of relevance to HRS and other population-based surveys.

Method of collection	Type of sample	Processing	Collection costs	Analysis costs
Venipuncture	Serum or plasma	Centrifugation and separation in the lab	\$\$\$	\$-\$\$\$
Finger stick	Dried blood spot (whole blood, dried)	Apply directly to filter paper onsite; air dry	\$	\$-\$\$\$
	Whole blood (liquid)	Apply directly to device for onsite analysis	\$	\$-\$\$
	Serum (dried)	Apply directly to serum separator card; air dry	\$	?
	Serum or plasma (liquid, collected in microtainer or capillary tube)	Centrifugation and separation in the lab	\$\$	\$-\$\$\$
	Plasma (liquid, collected in Demecal device)	Apply directly to device; filter-based separation	\$\$	\$-\$\$\$

Table 1. Options for blood sample collection, processing, and analysis. Costs for collection and analysis are highly variable, and are estimated here only in relative terms.

ALTERNATIVES TO DBS: LIQUID WHOLE BLOOD, SERUM SEPARATORS, AND POINT-OF-CARE ASSESSMENTS The advantages of collecting whole blood through venipuncture are many (plenty of sample, increased options for analysis in the lab, comparability with clinical values) and any study that can reasonably manage the financial costs, logistical complications, and respondent burden should give venipuncture serious consideration. For large, national surveys like HRS, convincing participants to travel to a clinical setting for a blood draw is not likely to be an option. But it might be possible to send a professionally certified phlebotomist to a participant's home to draw blood as a follow up to the in-home or phone interview. The costs of such an effort would be high, and logistical issues regarding sample handling, transport, and storage would need to be carefully considered, but this option might be worth considering for at least a subset of HRS participants in future waves.

Alternatively, whole blood from a finger stick can be collected and transported in a liquid state in a capillary tube (a thin tube open on both ends) or a microtainer device (a small vial with a screw top). Like DBS, sample collection

is minimally-invasive and low cost since it relies on a simple finger stick. However, handling and transport are complicated by the fact that the samples remain in a liquid state. A cold chain from the point of collection to receipt in the lab is necessary to maintain the integrity of most analytes, and the blood has to be centrifuged and separated in the lab prior to analysis or freezing. The transportation of liquid blood also poses greater biohazard risk, and is therefore more costly and subject to a higher degree regulation than the transportation of dried blood samples.

An advantage of these methods is that they produce serum/plasma, which can then be analyzed using gold-standard clinical protocols. As such, it will be easier to find a high quality lab to implement the analyses, and comparisons with prior research will be more straightforward (although it cannot be assumed that results based on the analysis of plasma from finger stick will be exactly the same as results based on plasma from venipuncture—some additional validation may be required to confirm this association). Like DBS, liquid blood collection methods produce very small volumes of sample. Most clinical laboratory protocols are not designed for the efficient use of sample, since quantities of plasma from venipuncture are so large. Therefore, laboratory protocols for finger stick plasma samples will likely have to be optimized for use with small sample volumes.

Two classes of devices collect whole blood from finger stick, but separate cells from serum/plasma immediately following collection. Serum separator cards are very similar to DBS cards currently used by HRS, but they apply a diffusion gradient or membrane that isolates red and white blood cells while capturing serum in filter paper. Separation at the point of collection is an advantage in that it eliminates the need for centrifugation and separation of samples in the lab, and it removes red and white blood cells from the sample matrix which for some analytes may improve quantification. Drying serum also stabilizes analytes and simplifies sample handling, transport, and storage, similar to typical DBS samples. However, serum separator cards have yet to be developed that allow for precise quantification of analytes contained on the cards. This is a promising area for future innovation, but current technology has limited utility for studies like HRS.

An additional blood separation device has recently been introduced by the Demecal Corporation. Finger stick whole blood is absorbed into a sponge, which is then dropped into a small collection tube. The blood is then pressed through a filter that separates plasma from cells without the use of a centrifuge. Samples are then sent to a central facility for analysis of a wide range of clinical biomarkers, including many of current interest to HRS (e.g., CRP, TC, HDL, glucose). The method has recently been applied in Malawi and evaluated for its utility in community-based research settings (Soldo and Anglewicz 2009). Advantages of the device include the ease of collecting plasma samples in the

field, and the production of biomarker results of high clinical relevance from a small volume of sample. However, as with other liquid-blood based methods, samples must be stored frozen soon after collection and during transport to maintain sample integrity. An additional disadvantage is that the methods for sample analysis are proprietary, and the use of the collection device therefore entails a commitment to the Demecal labs. Flexibility for the analysis of additional biomarkers may also be constrained since the Demecal platform is optimized for a specific set of analytes, and little sample may be preserved for future use.

A final alternative to DBS is the onsite assessment of biomarkers using “point-of-care” instruments. Affordable, portable instruments for the analysis of hemoglobin, HbA1c, lipid profiles, and CRP are currently available that essentially bring the lab to the point of sample collection (e.g., Cholestech LDX System, CardioChek). Using the same finger stick sampling procedure detailed above, a drop of blood is placed into a cartridge that is inserted into the instrument, which provides a result in a minute or two. The result can be recorded manually, or downloaded directly into a data capture file on a laptop. With real-time analysis, there is no need to transport or store samples, and no need to engage the services of a central lab. For some studies, the ability to immediately share results with participants may provide a valuable health screening service and act as an incentive for research participation. Disadvantages of this approach include the costs associated with equipping each survey team with instrumentation, and the importance of monitoring consistency of results across instruments, and across time. Also, since samples are analyzed on-site, it is not possible to bank samples for future analyses (although a single finger stick can be used for point-of-care analysis, as well as DBS collection).

BEYOND METABOLIC SYNDROME: EXPANDING THE RANGE OF BIOMARKERS IN HRS

To date, HRS has focused on obesity and metabolic syndrome as key health domains motivating the selection of biomarkers for analysis in DBS samples. High rates of overweight/obesity, cardiovascular disease, and diabetes in the U.S. justify this emphasis, but for future waves HRS may want to consider expanding its set of biomarker measures to draw on recent discoveries in the biomedical sciences, and to address other health domains of relevance to aging.

MEASURES OF INFLAMMATION AND INFECTIOUS DISEASE Inflammation plays an important role in many chronic degenerative diseases associated with aging, and social, economic, and behavioral factors that contribute to inflammation may lead to differential burdens of morbidity and mortality in later life. HRS, along with several other surveys, has measured high sensitivity CRP as a key biomarker of

inflammation that predicts increased risk for cardiovascular disease, declines in cognitive and physical performance in the elderly, as well as increased mortality risk (Pearson et al. 2003). Recent analysis of CRP results from the NSHAP dataset reveal significant economic and race/ethnic differences in levels of inflammation (McDade et al. 2010).

Cytokines are low molecular weight peptides that regulate inflammation, and IL-6—a key pro-inflammatory cytokine, is currently of intense interest due to its association with diseases of aging. IL-1 β and TNF α are also central pro-inflammatory mediators, while IL-10 plays an important counter-regulatory role (Maggio et al. 2006; Krabbe et al. 2004). Aging is associated with two- to four-fold increases in circulating concentrations of inflammatory cytokines, as well as dysregulation in cytokine responses to stimulation. Depression, psychosocial stressors, infectious exposures, body fat, health behaviors, and multiple measures of socioeconomic status are all significant predictors of inflammatory cytokines (Ranjit et al. 2007). Inflammation may therefore be a key pathway through which contextual and behavioral factors shape trajectories of health and aging across the lifecourse.

The measurement of inflammatory cytokines above and beyond measuring CRP may be of interest to HRS for two reasons: 1) cytokines regulate the transcription and release of CRP as well as other acute phase reactants; having information on CRP in conjunction with key signaling pathways provides important data on the regulation, and potential dysregulation, of inflammation; and 2) unlike CRP, cytokines are small molecules that can cross the blood-brain barrier, where they may have direct neurological effects. The cytokine theory of depression is based on this mechanism, and experimental models indicate that peripheral cytokine release initiates sick behavior (fever, anorexia, etc.), induces social withdrawal, cognitive impairment, and anhedonia, and activates the hypothalamic-pituitary-adrenal axis (Dantzer et al. 2008). Cytokine measurement may therefore shed light on mind-body dynamics and behavioral/ emotional effects of inflammation and inflammation-related diseases.

In addition to measuring inflammatory cytokines, seropositivity to common pathogens may be of relevance to HRS (McDade and Hayward 2009). Blood samples can be used to detect prior/ongoing exposure to common viruses (e.g. Cytomegalovirus, herpes simplex virus -1 and -2, hepatitis A) and bacteria (e.g. *Helicobacter pylori*, *Chlamydia pneumoniae*), which have been positively associated with inflammation and other indicators of cardiovascular disease risk (Zhu et al. 2000). Although the mechanisms remain unclear, these associations suggest that social gradients in pathogen exposure may contribute to parallel gradients in cardiovascular disease (Aiello et al. 2009).

ALLOSTATIC LOAD The concept of allostatic load is currently a prominent paradigm in health-related stress research. Allostasis (“stability through change”) is the set of physiological and/or behavioral processes through which the body adjusts its internal milieu in order to meet the demands of the external environment, but when frequent or excessive demands push allostatic processes beyond their normal operating ranges, wear and tear on the soma follows. Allostatic load is the result, representing the cumulative impact of stressors on the body’s regulatory systems, with high allostatic load leading to poorer health outcomes (Seeman et al. 1997; McKewen 1998). An advantage of the allostatic load concept is its comprehensive, multidimensional approach to assessing physiological function. Measures of low socioeconomic status and poor interpersonal relationship histories have been associated with increased allostatic load in a number of community-based cohorts, and prospective research has associated allostatic load at baseline with increased risk for all-cause mortality, cardiovascular disease, and declines in cognitive and physical function. Recent definitions of allostatic load have used approximately 10 biomarkers representing key cardiovascular, metabolic, inflammatory, and endocrine systems. High-risk cut-points are identified for each marker, and the number of markers for which an individual is above the high risk value is summed and operationalized as allostatic load.

Many of the biomarkers currently collected by HRS are typically included in current formulations of allostatic load (e.g., TC, HDL, HbA1c, systolic and diastolic blood pressure). Lacking is measurement of hormones produced by the hypothalamic-pituitary-adrenocortical and sympathetic-adrenal-medullary axes, two key endocrine pathways mediating the impact of stress on health. Addition of these measures would allow HRS to construct a measure of allostatic load and to make comparisons with the growing number of studies using this concept. It would also allow HRS to investigate stress as a pathway through which social and economic factors shape behavior and health.

REPRODUCTIVE FUNCTION AND SENESENCE Menopause is a significant psychological, social, and biological event in the life course of women. Several endocrine measures of reproductive function and aging can be quantified in DBS samples, including estradiol, testosterone, luteinizing hormone, follicle stimulating hormone, and anti-mullerian hormone. These biomarkers may be of interest to HRS to gain insight into what factors predict the timing and pattern of reproductive aging, and how reproductive aging shapes mental and physical health, health behaviors, health-care access, and decision-making in other domains. Similar issues may be of relevance to men who also experience age-related declines in hormones associated with reproductive function.

EPIGENETICS: THE POTENTIAL FOR G X E X E There is currently intense scientific interest in investigating the extent to which experiential factors make durable, and potentially heritable, marks on the human genome. Epigenetics involves the study of changes in gene expression that are not the result of changes in DNA sequence. DNA methylation, in particular, has received substantial attention as a molecular process through which sequences of DNA are rendered inaccessible to transcription factors in specific sets of tissues. In effect, these genes become silenced. Methylation status of several genes has been shown to change with age, to be responsive to environmental exposures earlier in life, and to predict risk for several diseases of aging (Waterland and Michels, 2007). A recent study has documented epigenetic signatures of post-traumatic stress disorder in genes related to immune function (Uddin et al. 2010).

DBS samples contain plasma, as well as red and white blood cells which rupture as they dry on the filter paper. The presence of lysed cells in DBS samples is often seen as a negative, but with respect to epigenetics, their presence may represent an opportunity. DNA is released from lysed white blood cells and can be recovered from DBS with sufficient quality and quantity for epigenetic analyses. The possibility thus exists for population-based epigenetics, and HRS may be in a unique position to integrate epigenetic data with information on inherited DNA polymorphisms (based on analysis of DNA sequences from saliva samples collected in 2006) and rich contextual and behavioral data derived from multiple survey waves.

The scientific pay-off of gene x epigene x environment analyses is tremendous, and research in this area is progressing rapidly. The application of a population-level perspective will further catalyze discovery, but the high cost and time-intensive nature of current epigenetic methods will keep this out of reach for HRS for several years. In addition, important conceptual issues need to be addressed with respect to modeling epigenetic phenomena and clarifying the extent to which these processes are tissue-specific (e.g., epigenetic modifications in a subset of white blood cells may not be shared by other white blood cells, or by cells in the brain). However, HRS should take a forward-looking stance and consider what methodological as well as logistical steps it can take in the near term to be in a position to pursue future research in this area. One practical issue is informed consent: Can banked DBS samples be used for epigenetic analyses?

STATIC VS. DYNAMIC MEASURES OF PHYSIOLOGICAL FUNCTION AND HEALTH Biosocial surveys typically collect one set of biological measures during the in-home interview and interpret the results as indicators of stable, baseline functioning of select physiological systems. This approach is perfectly understandable given the costs and logistics associated with collecting biological specimens in the field, particularly in light of competing demands on survey time

and budgets for multi-disciplinary surveys like HRS. It also guides the selection of biomarkers, steering surveys toward measures like HbA1c and TC that have been shown to vary on the order of weeks or months, rather than hours or days, thereby enhancing the interpretability of a single measure.

However, physiological systems are dynamic, and in many cases, observing how a system responds to challenge may be more meaningful than observing baseline functioning. This approach may be particularly important for research on aging where the loss of dynamic responsiveness may be most predictive of adverse outcomes. Therefore, HRS may want to consider how it can integrate a quasi-experimental approach into future surveys without compromising the study's core objectives. One possibility is to use a part of the in-home interview as a psychological stressor, and to collect a saliva sample before and after the stressor. By measuring cortisol, and perhaps DHEA and alpha-amylase, investigators could identify individual differences in stress reactivity that may have implications for health, social relationships, or decision-making. A slightly more invasive approach would be to arrange for the delivery of a vaccine. Seasonal flu vaccine is currently recommended for older Americans, and by drawing a DBS sample before and a few days after the vaccine, CRP as well as pro- and anti-inflammatory cytokines could be quantified to assess individual differences in the inflammatory response. Antibody titers to the vaccine could also be quantified as a measure of immunocompetence and resistance to infectious disease. And lastly, laboratory protocols for assessing immune cell responsiveness could be adapted to the home-based sample collection setting. A couple of drops of blood from finger stick could be placed into a microtainer tube and incubated with antigen, as is done in the lab. The samples could then be analyzed for patterns of gene expression and/or cytokine production that provide insight into the regulation of inflammation and immune function. While a quasi-experimental approach may be logistically difficult, it is worth considering whether more dynamic biological measures could be profitably applied in future HRS surveys.

CONCLUSIONS AND RECOMMENDATIONS

In my opinion, HRS represents an excellent model for how a large, multi-purpose survey can productively incorporate biological measures in ways that expand and enrich the dataset without compromising its core objectives. While many studies have jumped on the biomarker bandwagon, HRS stands out for its thoughtful approach that has evolved in conversation with a wide range of experts and stakeholders over the past nine years. It is too soon to evaluate the scientific impact of this new direction for HRS, but it can be said with confidence that adding biomarkers has not compromised the integrity of the sample or the essence

of the survey. And there is every reason to believe that biomarker data will cast new light on issues of central interest to HRS data users.

Based on this experience HRS has positioned itself as the benchmark for large-scale biosocial surveys of aging. Looking to the future, what can we learn from HRS thus far, as well as from other studies collecting biomarkers? Recommendations are made in four areas where initiatives can be implemented to ensure that data are of the highest possible quality and that methods stay abreast of developments in the biomedical sciences.

CONTINUE TO COLLECT DBS SAMPLES, BUT CONSIDER ALTERNATIVES The low cost and low burden associated with DBS sampling has made this the method of choice for collecting blood in HRS, as well as other biosocial surveys. NHANES has recently initiated an effort to validate DBS methods, which will further facilitate the application of these methods. Based on the centrality of DBS to HRS thus far, and to several other studies of comparative value for HRS, it makes sense for HRS to continue to collect DBS samples as the foundation of its blood-based biomarker effort.

However, collecting the sample is the easy part—producing high quality results in the lab has proven to be the major challenge. If there are not substantial improvements in laboratory infrastructure nationally, then HRS should seriously consider alternative methods (note that Heritage Labs and the Tracy lab seem to be providing good results for HRS currently, but analyses are not yet complete).

Point-of-care devices represent the most viable option at this point, and have the added benefit of providing immediate feedback to respondents. Microtainers, capillary tubes, and the Demecal device all require a cold chain from the point of collection to the lab, placing these methods out of reach for national studies like HRS. A serum separator card that incorporates the advantages of DBS while facilitating quantitative analyses in the lab is an attractive alternative to current DBS methods, but such a card has yet to be developed. Even if HRS decides to pursue alternatives to DBS sampling in subsequent surveys, it should still continue to collect DBS as a back up, to have samples stored for future analyses, and to have samples that are directly comparable to prior rounds of data collection.

IMPLEMENT ENHANCED PROCEDURES FOR QUALITY CONTROL Laboratories routinely include “control” samples of known analyte concentration with their assays. The same set of controls is added along with samples to every assay to monitor day-to-day variation in results. Ideally, the same value will be produced every day for each control value. In reality, the coefficient of variation (CV; standard deviation/mean) ranges from 5 to 15 percent. Control values are used in the lab to identify sets of samples that may need to be re-analyzed, and they can

be used by investigators to assess the accuracy and reliability of assay results. The quality control (QC) process is managed internally by the lab, but a blind, external QC process can be implemented to provide HRS with a higher degree of confidence in lab results.

Consider the following example of what external QC monitoring might look like: 1) A single batch of QC materials is manufactured in a lab that will NOT be testing samples for HRS; 100 “low” controls and 100 “high” controls are made, with the same low and high concentrations of biomarkers of interest to HRS; controls are manufactured to look identical to HRS samples (same papers, ID codes, etc.); 2) Controls are stored frozen; 3) One “low” and one “high” control sample is included with each batch of samples that is sent to the lab for analysis; 4) Results for controls are returned along with sample results and extracted for analysis. Calculation of mean values provides a measure of the accuracy of lab results. Calculation of variation provides a measure of the reliability of the results across the entire period of sample collection and analysis. Having samples with known analyte concentrations also provides the ability to detect a “frame shift” in the delivery of results in, for example, an Excel spreadsheet.

The costs of an enhanced QC monitoring plan include the creation of the QC materials, and the reimbursement of the lab for analyzing additional samples. But this amount represents a small fraction of the overall cost of biomarker measurement in HRS, and will greatly increase confidence among users in the quality of the data. QC materials also provide an insurance policy for monitoring the integrity of samples and the quality of results if there is a delay in processing or a switch in labs and/or methods.

CALIBRATE DBS RESULTS AGAINST PLASMA RESULTS Analysis of DBS samples produces values that differ from values produced with plasma or serum. This difference is due to the presence of lysed red blood cells in DBS samples, and the use of whole blood eluate as the analysis matrix. The relationship between DBS and gold-standard plasma methods is linear and strong, so the use of DBS-based values for within-study analyses does not pose any challenges. However, HRS users may want to benchmark their analyses against other studies using plasma (e.g., NHANES, Framingham), and they may want to make use of commonly used clinical cut points for markers like TC and CRP. HRS users can rely on conversion factors that are typically published as part of the validation of DBS protocols, but these formulas provide a rough approximation of plasma-equivalent values at best. To the extent that comparisons with plasma are a priority for HRS, conversion formulas specific to HRS should be developed in order to ensure a high level of accuracy in the generation of plasma-equivalent values.

In order to make these comparisons, a matched set of plasma and finger-stick DBS samples should be collected from at least 50, and preferably 100, individuals. Participants do not have to be enrolled in HRS, but they should match study participants demographically to the extent possible. Plasma samples should be analyzed using gold-standard methods in a reliable clinical lab. DBS samples should be sent to the labs conducting analysis of HRS DBS samples. Analysis of these samples should be implemented during the period of time when HRS participant samples are being analyzed. Following confirmation of a linear relationship across the two sets of results, a study-specific linear regression formula can be used to calculate plasma equivalents based on DBS results. Note that this effort can be combined with the enhanced QC monitoring plan suggested above. This set of samples can also serve as a resource that facilitates comparison with other studies collecting DBS. For example, a small portion of the DBS samples could be sent to a lab conducting analyses for studies other than HRS. Results from that lab could then be calibrated against HRS plasma results, or HRS DBS results.

INVEST IN METHODS DEVELOPMENT The development of new methods for DBS (or other non-standard sample collection technologies) is a time-consuming and costly process. Many labs will use limited discretionary funds to support this work when they have identified biomarkers of interest in the biomedical literature. In some cases funds from NIH, other federal agencies, or industry may be available to support this work, but such opportunities are infrequent. Under these scenarios, HRS has to rely on a halting process to define the range of methodological options available for future waves of data collection. HRS might consider taking a more pro-active stance by setting aside funds for methods development as part of its biomarker budget. These funds could be directed toward the development of assays not previously validated for DBS, for the improvement of existing assays, for the development and/or evaluation of alternative sample collection technologies, for innovations related to sample handling, transport, and storage, or for pilot-testing quasi-experimental in-home data collection methods. Funds allocated to methods development in the near term should pay dividends in future waves of HRS by ensuring that the study has the methodological tools it needs to maximize the quality and impact of biomarker data.

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