Early Lessons from the Dallas Heart Study

From Proteins to Public Health

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Internal Medicine Grand Rounds
The University of Texas Southwestern Medical School

3/13/09

Dr. de Lemos would like to disclose grant support from Biosite, Inc, and Roche Diagnostics, consulting income from Astra Zeneca, Biosite, Roche, and Tethys, and speaker honoraria from Pfizer and Merck/Schering.

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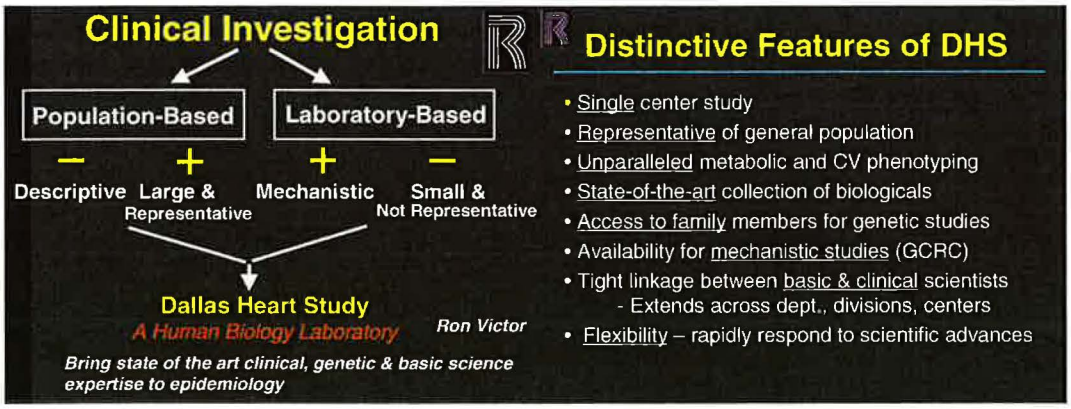
Although a number of highly effective preventive therapies have been developed for patients with coronary artery disease and heart failure, the typical initiation of treatment when disease becomes clinically evident is associated with only modest risk reduction. Preventive therapies initiated earlier in the disease process, or before disease develops at all, would almost certainly have a greater impact on long-term morbidity and mortality. Because universal treatment of the population with preventive medications may not be feasible, the challenge is to identify individuals with or at risk for cardiovascular disease before it becomes clinically event.

The focus of the Dallas Heart Study (DHS) is squarely on this issue of identifying individuals with or at risk for cardiovascular disease *before* clinical manifestations are present. The scope of the DHS is vast, and here I will focus on only a selected few topics of current investigation within the DHS.

Dallas Heart Study 101

History

In 1999, four senior UTSW investigators, R. Sanders (Sandy) Williams, Helen Hobbs, Ron Victor, and Eric Olson, responded to a request for proposal from the Donald W. Reynolds Foundation. This nationally competitive award was initially designed to select a single "Reynolds Center" that would be provided $24 million over 4 years (eventually expanded to almost $50 million over 10 years) to create broad-based programs in basic, translational, clinical, and population-based research focused on the improvement of morbidity and mortality due to atherosclerotic heart disease. In their original grant, the investigators used the *absence* of infrastructure and experience in clinical investigation at UTSW as a selling point, arguing that the grant would have a transformative effect on our institution. The application included expansion of highly successful basic science programs in cardiomyocyte biology and development, and the creation of the Dallas Heart Disease Prevention Project, or the DHPP. The DHPP (subsequently renamed as the Dallas Heart Study), was conceived by Ron Victor and Helen Hobbs, with critical input from Richard Cooper, an epidemiologist from Loyola University, as a "human biology laboratory" that would allow both population research as well as careful mechanistic study of highly phenotyped human subjects. The goal was to build on existing strengths in lipids and metabolism (Grundy, Vega, Cohen, Hobbs), hypertension and hypertrophy (Victor, Drazner, Olson, Williams), and genetics (Hobbs, Cohen); in addition, the study aimed to develop new programs in social sciences (Victor, Vaeth). Recognizing inherent limitations in traditional population and laboratory research (left figure), the investigators sought to create a study large enough and with sufficient granular detail in phenotypes to allow mechanistic study in the population. Other distinctive features of the DHS are shown in right hand panel below. UTSW was the only center selected in the initial round of funding, with Stanford funded the next year, followed by Harvard and Johns Hopkins.



The creation of the original cohort was outsourced to a private company (Research Triangle Institute (RTI), Ralaigh, NC) specializing in creation of grass-roots infrastructure for cohort collection and management. RTI assembled the cohort and performed the initial survey, but blood collection and the detailed clinic visit were handled by UTSW. Tom Andrews assumed initial operational responsibilities for managing the project, including creation of the state-of-the-art blood collection, processing and storage facilities, and management of the RTI field staff. Ron Peshock has directed all aspects of DHS imaging since the planning phases of the study: the DHS is one of the largest imaging studies ever performed in a single center and >10,000 imaging studies have been performed and interpreted under Dr. Pehsock's leadership. Duwayne Willett, leading a team of IT and database specialists, created a relational database and information management system to handle the enormous amounts of research data created by the study. This database now serves as a model for other clinical research relational databases on campus and beyond. Duwayne also assumed operational direction of the DHS from Tom Andrews, applying systems management techniques to the study operations, resulting in a project that completed on time and within budget. Ten months after the initial grant was funded, Sandy Williams left UTSW to become Dean of Duke University School of Medicine and Helen Hobbs became Principal Investigator of the study, and has remained in this position ever since. The total costs to date of the DHS are ~ $ 18 million.

Study Design

The Dallas Heart Study is a multiethnic, population-based, probability sample of residents from Dallas County.[1] A random probability sample of Dallas County residents age 18-65 was obtained from a pool of 841,943 eligible subjects using the U.S. Postal Service Delivery Sequence File, with deliberate oversampling of blacks so that they represented 50% of the cohort. From this eligible pool, a stratified random sample of addresses was selected (n=15,088) and screened (n=12,312) from which 8630 addresses were eligible. Among 7,586 eligible individuals from these addresses, 6101 participants (80%) completed a detailed in-home interview for demographic and health-related data, as well as measurements of weight, heart rate, and five sequential blood pressure measures. All subjects between the ages of 30-65 who completed the initial visit were invited to participate in a second in-home visit to collect fasting venous blood and urine samples. Importantly, demographics, medical history, blood pressure, and body mass index were similar between subjects participating in the home interview and the

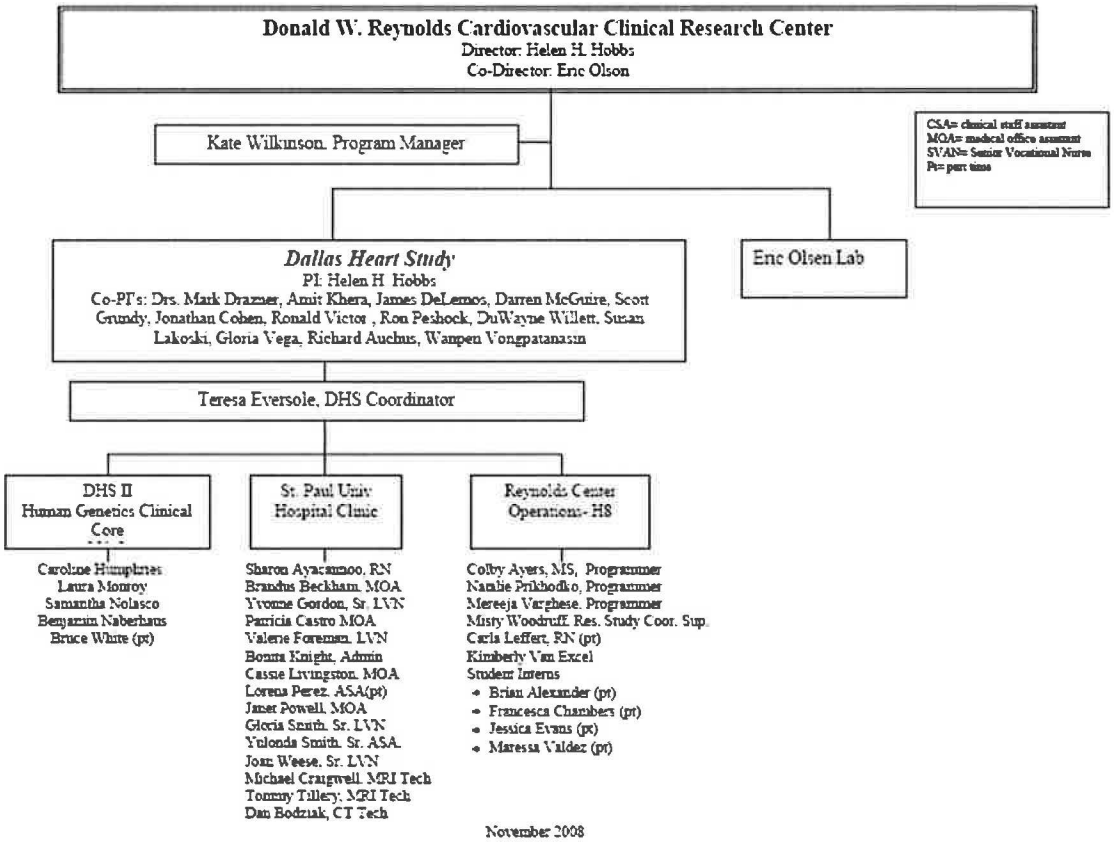
phlebotomy visit (n=3557).[1] Subjects completing visit 2 were invited to a 3rd visit, in which they underwent extensive imaging at USTW (n=2971), including coronary artery calcium (CAC) scanning by electron beam CT; MRI scanning of the heart (to determine LV size, structure and function) and aorta (to measure aortic plaque, wall thickness and compliance); DEXA scanning to assess body composition and bone density; abdominal MRI to measure subcutaneous and visceral fat; NMR spectroscopy to measure liver fat; and a 12-lead ECG.

Sampling weights, reflecting the different probabilities of selection for participants and sample attrition between visits, were constructed to generate unbiased estimates of population frequencies.[1]. When investigators use the sampling weights, it is possible to extrapolate results from the DHS back to the population from which the sample was collected (Dallas County, circa 2000). This is an important feature that distinguishes the DHS from many other population-based studies.

Unique features

In addition to the careful phenotyping described above, several other unique features of the DHS include the broad representation of ethnic minorities, the inclusion of a truly representative cohort with a very high prevalence of obesity and associated metabolic abnormalities, and equal representation of women. Moreover, a wealth of socio-economic and health beliefs data were collected.

Current Organization



The DHS Biomarker Program

Goals

The biomarker studies within the DHS have several aims. First, we have sought to better understand biomarkers in wide clinical use, including natriuretic peptides, C-reactive protein (CRP), and troponins. Second, we have created large development programs in which we are screening novel biomarkers against our cardiac and metabolic phenotypes. The ultimate clinical goal is to combine existing and novel biomarkers that represent non-redundant pathophysiological pathways into panels that can more effectively screen the population for cardiovascular disease. Finally, we also aim to exploit the careful phenotyping of the DHS so that we can use epidemiology to gain additional insight into the pathobiology of atherosclerosis and adverse ventricular remodeling.

Strategy

Our approach has been to limit infrastructure by creating partnerships with industry and other academic institutions for measurement of many of our biomarkers, rather than creating large core laboratory functions at UTSW (figure).

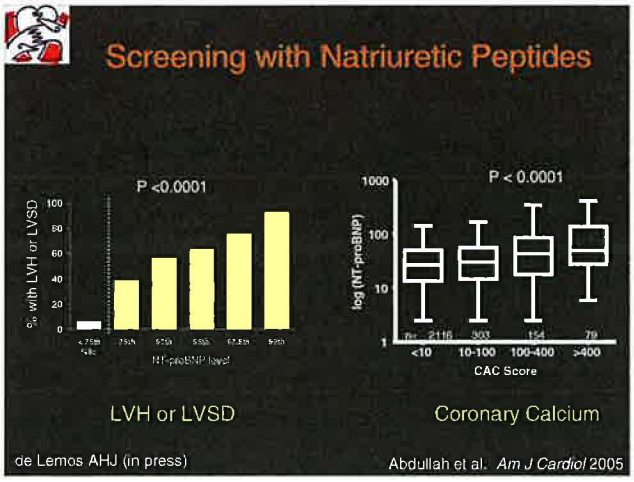
Discovery Strategies

- Limited infrastructure
- Industry core laboratories
  - Novel intellectual property relationships
  - Rapid throughput/high volume
  - Zero cost
- Extensive academic collaborations
- Recruit lots of smart young investigators and a great biostatistician

Unique challenges related to population screening

When biomarkers are used for diagnostic purposes in the clinical setting, noncardiac sources of variation are rarely accounted for. This approach may be reasonable in symptomatic individuals because the cardiac "signal" is typically robust, with biomarker levels rising many-fold over the upper reference range and dwarfing "noise" related to noncardiac factors such as gender, renal function, and body composition. In contrast, among asymptomatic individuals, the cardiac signal being screened for is typically more subtle, and the magnitude of elevation may be similar to that caused by noncardiac factors.

As one example, B-type natriuretic peptide (BNP) and N-terminal proBNP (NT-proBNP) are particularly attractive tools to screen for subclinical CVD because they rise in a linear fashion with increasing LV mass and coronary calcium and decreasing LV ejection fraction (figure)



Noncardiac sources of variation in natriuretic peptides

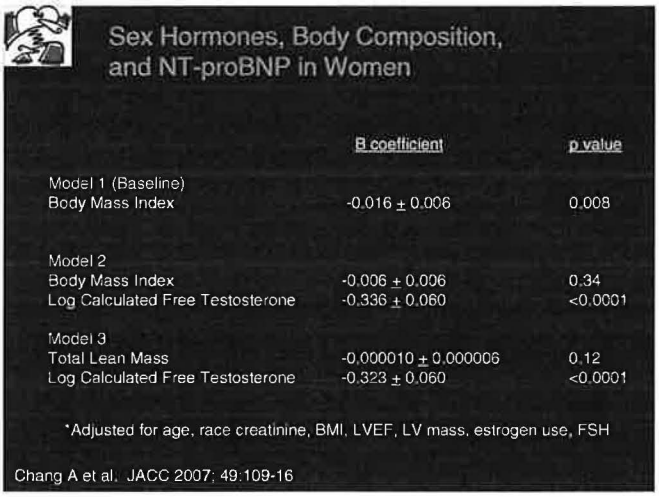
Several noncardiac factors have been identified that may influence BNP and NT-proBNP to a similar extent as do minor cardiac abnormalities, including renal function, sex and body composition. BNP levels are lower among obese than nonobese subjects,[2,3] a paradoxical observation that had previously been explained by the presence of natriuretic peptide clearance receptors on adipocytes. However, using the DHS, Sandeep Das reported the same association for NT-proBNP, which does not bind the clearance receptor.[4] Thus, the mechanism by which body composition influences natriuretic peptide levels must be via suppressing synthesis and/or release of BNP rather than increasing clearance. Using DEXA measurements of body composition, Sandeep surprisingly found that the lean component of body mass completely explained the inverse association between body mass and NT-proBNP levels. No association was seen between fat mass and NT-proBNP after accounting for lean mass.[4] (table) This finding suggested that either lean mass itself, or a substance associated with lean mass, suppresses natriuretic peptide synthesis and release.

Table. Logistic regression models for (BNP and N-terminal-proBNP *

	Men Odds Ratio (95% CI)	Women Odds Ratio (95% CI)
Odds of low BNP		
Model 1:		
Body mass index (per 5 kg/m^2)	1.316 (1.161-1.493)	1.142 (1.056-1.236)
Model 2:		
Total body fat mass (per 10 kg)	0.944 (0.785-1.135)	0.969 (0.840-1.118)
Total body lean mass (per 10 kg)	1.621 (1.315-2.000)	1.438 (1.124-1.841)
Odds of low N-terminal pro-BNP		
Model 1:		
Body mass index (per 5 kg/m^2)	1.311 (1.149-1.496)	1.203 (1.100-1.314)
Model 2:		
Total body fat mass (per 10 kg)	1.080 (0.873-1.337)	0.913 (0.772-1.079)
Total body lean mass (per 10 kg)	1.569 (1.227-2.007)	1.716 (1.271-2.315)

*Multivariable logistic regression models are stratified by sex and adjusted for age, race/ethnicity, diabetes, hypertension, prior MI, LV mass and end diastolic volume; low BNP is defined as <4 ng/L, low NT-proBNP is defined as in the lowest sex-specific quartile (<7.6 ng/L for men, < 20.4 ng/L for women).

Normal women have significantly higher BNP and NT-proBNP levels than do normal men. This difference has been thought to be mediated by estrogen, a hypothesis supported by the observation that women taking supplemental estrogens have slightly higher BNP levels than those not taking hormones.[5] However, recent evidence suggests that androgens rather than estrogens may mediate the gender-related differences in natriuretic peptide levels. Utilizing sex hormones collected among women enrolled in the DHS, Alice Chang surprisingly found no association between indirect measures of estrogen status and NT-proBNP levels, whereas a strong inverse association was observed between measures of free testosterone and NT-proBNP.[6] These findings, albeit indirect, suggest that androgen *suppression* of proBNP synthesis in men, rather than estrogen stimulation of synthesis in women, is most responsible for gender-related differences in natriuretic peptide levels. Moreover, when measures of testosterone status were added to multivariable models that contained assessments of body mass and body composition, the influence of BMI and lean mass was markedly attenuated, whereas testosterone remained inversely associated with NT-proBNP levels. (Figure)

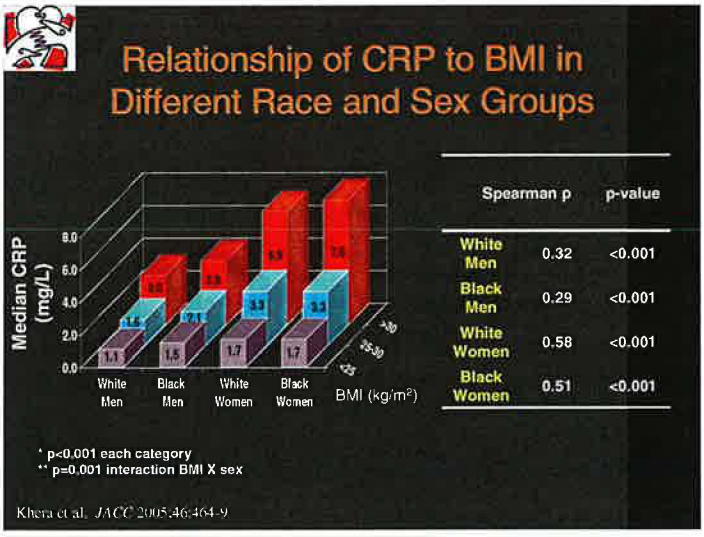


These findings indicate that androgens may mediate both the association between higher BMI and lower BNP levels as well as differences in BNP levels between men and women.[6] Recently, this finding has been confirmed by studies showing that NT-proBNP levels rise > 2-fold among men with prostate cancer who receive anti-androgen therapy.[7]

Renal function has an important influence on circulating concentrations of both NT-proBNP and BNP in the DHS. Within the normal range of GFR this effect is similar between NT-proBNP and BNP, but with declining GFR the effect appears to be slightly steeper for NT-proBNP. [8] Although this may not have a marked influence on the diagnostic or prognostic value of either peptide among patients with heart failure, in population based samples, where natriuretic peptide levels are typically much lower, the influence of even minor degrees of renal impairment may be important for both NT-proBNP and BNP.

CRP

Amit Khera has also observed important differences in C-reactive protein (CRP) levels according to race, gender, and body composition. In Dallas County, blacks have higher CRP than whites, independent of measured confounders, and more than twice as many women as men have CRP levels above the CDC-recommended high risk cutoff of 3 mg/L. The strongest association observed was between body mass index and CRP, with a particularly notable correlation seen in women (Spearman rho >0.5).[9] Interestingly, additional study from the DHS of this gender interaction between obesity and CRP suggests that it is at least partially mediated by leptin.[10]



These findings may be particularly relevant in light of the findings of the JUPITER trial,[11] which will lead many to suggest that CRP levels be used as a trigger for initiation of statin therapy. The DHS data would suggest that such a strategy would lead to more statin usage among women than men, despite markedly lower cardiovascular risk in women.

The DHS findings regarding noncardiac sources of variation in "cardiac" biomarkers suggests caution is required before implementing biomarker screening programs. Screening performance for a particular biomarker may differ by race and gender and according to body composition and renal function. In certain subgroups, results may not be reliable or thresholds may need to be adjusted. These noncardiac factors, unfortunately, will likely differ for each biomarker and thus full exploration of these sources of variation should be a prerequisite before clinical implementation.

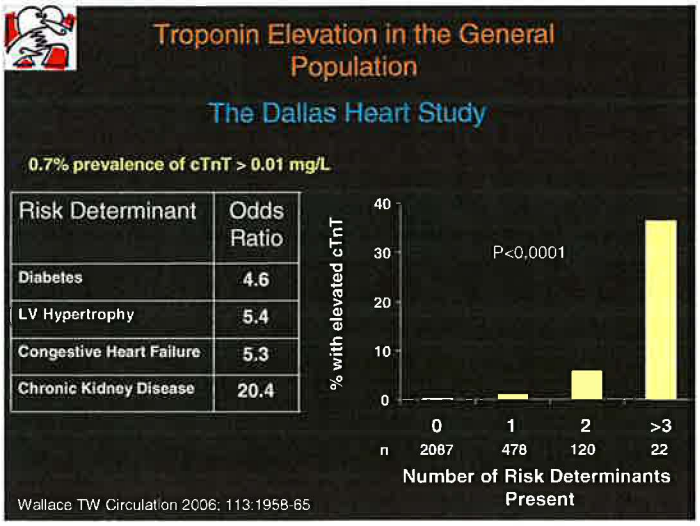
Established biomarkers: do they have a role in preclinical CVD detection?

Multiple observational studies have confirmed a modest association between CRP levels and the risk for future CVD events. In contrast, data from the DHS indicates that CRP does not associate with subclinical measures of atherosclerosis or LVH after multivariable adjustment,[12,13] suggesting that associations between CRP and incident

CVD events are mediated by processes distinct from the burden of atherosclerosis and hypertrophy.

As noted above, BNP and NT-proBNP are strongly associated with LVH and LVSD. However, despite these associations, operating characteristics (sensitivity, specificity, positive and negative predictive) and the area under the receiver operating characteristic curve (AUROC) remain suboptimal for detection of LVH or LVSD.[14] It is possible that when natriuretic peptides are used in combination with other biomarkers, or when testing is applied to a higher-risk population, screening performance may improve.

The cardiac troponins T (cTnT) and I (cTnI) are intriguing markers for consideration as screening tools for preclinical CVD. In the DHS, detectable cTnT elevations $\geq$ 0.01 ng/mL were found in only 0.7% of individuals, highlighting the very low sensitivity of troponins as screening tools using current assays. However, when cTnT elevation was present, a clear adverse cardiac phenotype was seen, with almost all individuals with detectable levels having one or more of the following four factors: diabetes, LVH, LVSD (or clinical evidence of heart failure), or an estimated GFR < 60 cc/min. In the absence of one of these factors troponin elevation only occurred in 2 individuals, but when 3 or more of these factors was present, the probability of cTnT elevation was > 30% (figure). [15]



These findings have implications both for clinical practice and population screening. From a clinical practice standpoint, they highlight that a) troponin elevation does not occur in individuals without CVD or major risk factors for CVD and thus should not be ignored and b) the high frequency of troponin elevation among ambulatory individuals with LVH, LVSD, diabetes, and CKD illustrates limitations of using a single troponin measurement to characterize the etiology of chest pain. For example, a person presenting to the Parkland Hospital ER with atypical chest symptoms and low-level troponin elevation who has diabetes, mild CKD, and LVH may well have *chronic* rather than acute troponin elevation, with vastly different clinical and therapeutic implications.

From a population screening perspective, the high specificity of troponins for pathological cardiovascular phenotypes is noteworthy. Although current assays are too insensitive for population screening, we are optimistic that assays in development, which

are up to 1000-fold more sensitive than conventional assays, may prove to be valuable tools to detect preclinical cardiac abnormalities such as LVH or LVSD.

Cystatin C is protein produced in all nucleated cells that has recently been characterized as a marker of renal function that overcomes a number of limitations with creatinine, as levels are independent of age, sex, and muscle mass. In many studies, serum cystatin C has performed better than creatinine and is at least comparable to creatinine-based equations to estimate GFR. Moreover, cystatin C has been associated with heart failure and cardiovascular disease mortality in a number of studies.[16,17] Parag Patel and David Markham have shown that increasing levels of cystatin C correlated with higher LV mass, concentricity, and wall thickness ($p < 0.001$) in the DHS, but not with LVESV, LVEDV, or LVEF. After adjustment with traditional covariates and estimated glomerular filtration rate, cystatin C remained independently associated with a specific concentric LVH phenotype.[18] These findings support the hypothesis that cystatin C may have utility for identifying individuals with preclinical cardiac structural abnormalities, particularly when combined with other biomarkers of LV structure and function (see below).

Novel Biomarkers

Through our collaborations with industry and academic partners, we have screened over 40 biomarkers in the DHS.

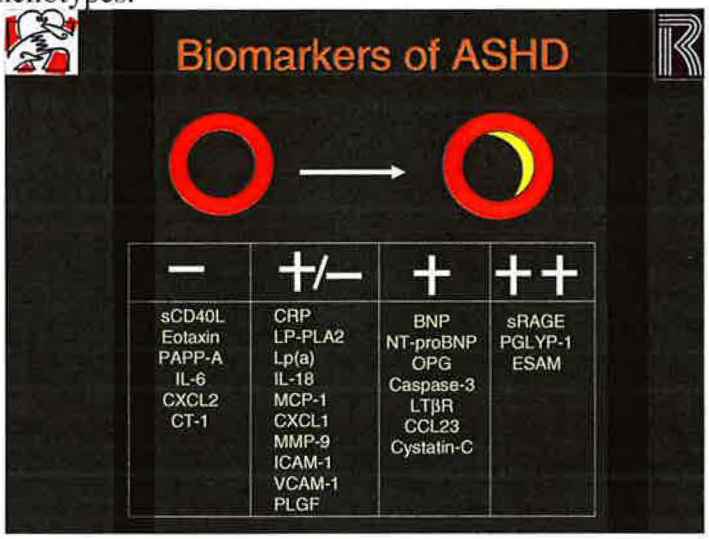
hs C-reactive protein	**Troponin-T**	**Fructosamine**
Monocyte chemoattractant protein-1[19]	**Brain Natriuretic Peptide**	**Soluble endothelial cell-selective adhesion molecule**
Soluble CD40 ligand[20]	**NT-proBNP**	**CXCL1**
Interleukin-6	**BNP 3-108**	**CXCL2**
Interleukin-18[21]	**Soluble receptor advanced glycation end-products (sRAGE)**	**CCL 23 (MIP3)**
Lipoprotein phospolipase-2[22,23]	**Cystatin C [18]**	**CCL11-Eotaxin**
Lipoprotein (a) [24]	**Soluble ICAM-1**	**Lymphotoxin Beta receptor**
Osteoprotegerin[25,26]	**Soluble VCAM-1**	**Caspase-3 [27]**
Adiponectin	**Uric acid**	**Neutrophil Gelatinase-Associated Lipocalin (NGAL)**
Leptin[10]	**Urine microalbumin**	**Peptidoglycan recognition protein-1 [28]**
Matrix metalloproteinase-9	**Cardiotrophin-1**	**Placental Growth Factor (PLGF)**
Myeloperoxidase	**Pregnancy associated plasma protein alpha**	**FSH**
Beta Crosslaps	**Lipoprotein subfractions (NMR)**	**LH**
Insulin	**Anti- Ox LDL (E06)**	**Testosterone**
ANA	**Chem 20**	**Sex Hormone Binding Globulin**

These markers have been selected either by DHS investigators or collaborators (or suggested by our industry partners) because either

1) Previous reports suggest associations with ASHD, LV remodeling or clinical events.

2) They represent pathways implicated in the pathogenesis of ASHD and heart failure from basic investigation.
3) A cardiac "signal" has been observed during evaluation of the biomarker for another purpose.

Colby Ayers, MS, has created a platform for high throughput, rapid biostatistical screening of candidate markers, allowing rapid prioritization of candidate markers. The figure below is a partial categorization of biomarkers based on their relative association with atherosclerosis phenotypes.

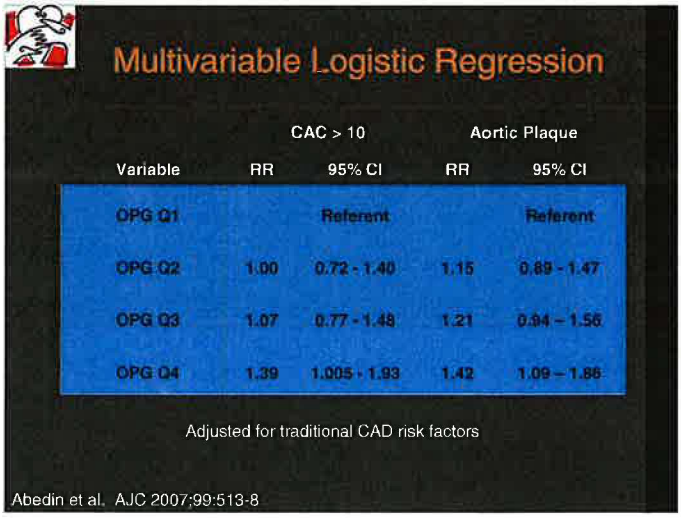


Review of more detailed findings from several selected biomarkers may help to illustrate our broad research approach.

Monocyte chemoattractant protein (MCP)-1 is a primary chemokine recruiting signal for monocytes, and contributes to the development, progression, and complications of atherosclerosis. This chemokine is both a potential biomarker and drug target in atherosclerosis and other inflammatory diseases. In patients with acute coronary syndromes, higher plasma levels of MCP-1 are associated with increased risk for adverse cardiovascular events over long-term followup.[29] In the DHS, MCP-1 correlated with CVD risk factors and with CAC in univariable analyses, but after multivariable adjustment the associations with CAC were attenuated and no longer statistically significant.[19] This finding suggests that MCP-1 may lie in the etiological pathways linking cardiac risk factors with atherosclerosis, and could have value as a soluble marker of the response to anti-atherosclerotic therapy. However, it is not likely to have value as a biomarker to screen for preclinical CAD.

Another marker of interest for detection of preclinical CVD is osteoprotegerin (OPG), a member of the TNF-α superfamily that acts as a decoy for receptor activator of nuclear factor kappa-B ligand (RANKL). Circulating levels of OPG may serve as a "readout" of activity of the RANK/RANKL system. In the DHS, plasma levels of OPG associate both with subclinical atherosclerosis, as measured by coronary calcium and

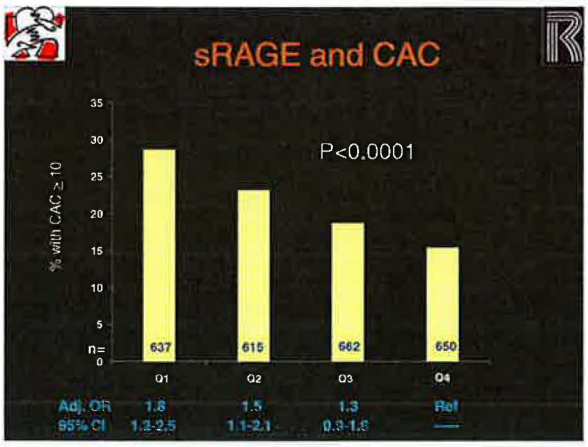
aortic plaque, as well as LVH as measured by MRI.[25,26] In contrast to MCP-1, the associations between OPG and the CV phenoytpes were independent of traditional risk factors.



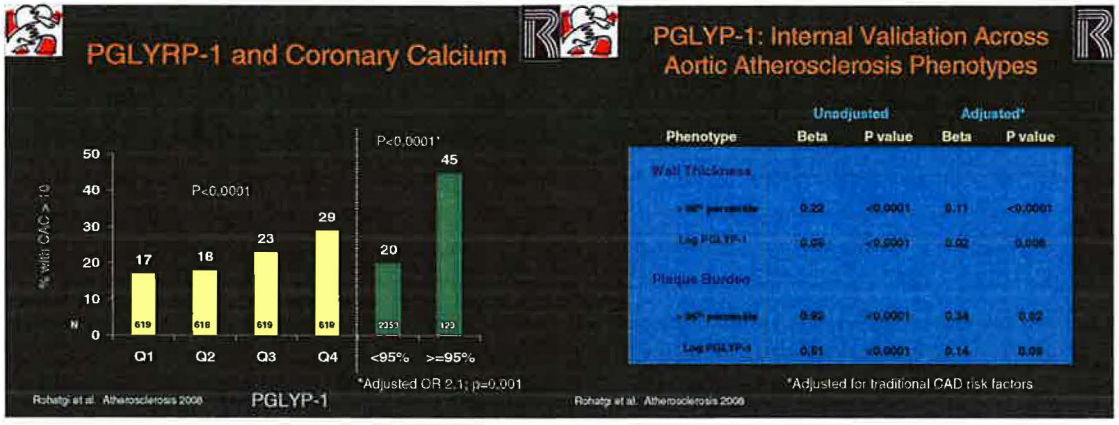
Although "independent" of traditional risk factors, the associations of OPG with CAC and aortic plaque are quantitatively modest, which is also the case for most of the other individual biomarkers that we (and others) have evaluated. A test that offers only a 40-50% increment of risk across the range of values will not improve discrimination of atherosclerosis in a meaningful way, as reflected in an increase in the area under the ROC curve. In our opinion, such modest predictors will provide limited value to clinicians as stand-alone tests. However, these modestly predictive biomarkers may still be useful additions to multiple biomarker panels, as described below.

The transmembrane receptor for advanced glycation end products (RAGE), expressed on a variety of cells including endothelial, smooth muscle, and mononuclear cells, binds advanced glycation end products and other pro-inflammatory ligands and modulates activity of several pro-thrombotic and pro-inflammatory mediators.[30] Results from animal models provide evidence supporting a role for ligand-RAGE binding in the development and progression of atherosclerosis. A circulating isoform of RAGE, soluble RAGE (sRAGE), has been identified and theorized to competitively inhibit transmembrane RAGE-ligand binding thereby attenuating atherosclerosis.[31] This hypothesis has been supported by several animal experiments, where administration of sRAGE to mouse models retarded the progression of atherosclerosis.[32]

Jason Lindsey and Darren McGuire correlated sRAGE with CAC in the DHS, reporting a robust, inverse association between sRAGE and CAC, which supports a *protective* role for this biomarker in ASHD. This association was independent of traditional CAD risk factors.



Peptidoglycan recognition protein-1 (PGLYRP-1) is one of four types of peptidoglycan recognition proteins in humans that is expressed primarily in polymorphonuclear leukocyte granules.[33] Of interest, there is not a single published paper relating this peptide to atherosclerosis in humans and animals and there are no reports describing circulating levels. We measured this peptide in the DHS based on recommendations from our industry collaborator: in the process of screening biomarkers for sepsis, they had noted that individuals with sepsis who had higher levels of PGLYRP-1 were at increased risk for cardiac complications. In the DHS, Anand Rohatgi has reported robust associations of PGLYRP-1 with multiple atherosclerosis phenotypes, including CAC, aortic wall thickness, and aortic plaque. These associations were particularly notable at extreme thresholds of the biomarker (figures).[28] Although the aortic wall and coronaries represent different vascular beds, confirmation across multiple phenotypes provides some internal validation of the exploratory findings.



Rohatgi has developed a collaboration with an investigative group from the Netherlands that has collected a tissue bank of atherectomy specimens. Staining of these specimens for PGLRYP-1 has confirmed presence of this protein in the atherosclerotic vascular wall (Rohatgi, personal communication).

These PGLYRP-1 observations highlight the potential power of a carefully designed epidemiological study to stimulate "backwards" translational research.

Although highly exploratory, such studies may identify novel mechanistic pathways that merit further study in appropriate animal and human models. A note of caution is warranted regarding these and other novel biomarkers, however. Development and evaluation of these markers is in its infancy, and extensive additional study and validation are required before they can be considered for clinical application.

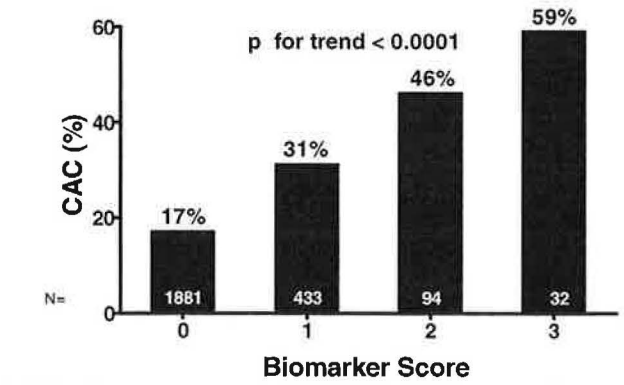
Combinations of Biomarkers—the clinical endgame

An emerging approach to improve upon the limitations of individual biomarkers is to combine multiple biomarkers for disease screening. Although early studies reported mixed results, several recent studies have reported much more favorable findings. For example, in a population of apparently healthy older community-living adults, both cTnT and NT-proBNP predicted mortality, with the combination providing incremental prognostic value.[34] Moreover, among 1135 elderly men , a multimarker panel combining cTnI, NT-proBNP, cystatin C, and CRP demonstrated a >16-fold gradient of risk for CV mortality.[35] A plausible explanation for the better results of these newer multiple marker panels compared to prior ones is that these panels included markers that reflect existing preclinical CVD or renal disease, such as troponin, NT-proBNP and cystatin C. These data would suggest that biomarkers and biomarker panels that identify existing subclinical disease may prove to be the best discriminators of future clinical event risk. [36]

Anand Rohatgi is leading DHS efforts for multiple-marker screening of ASHD while Parag Patel is taking a similar approach for heart failure phenotypes. Anand has completed proof-in-concept studies demonstrating that the use of a multiple marker panel is superior to a single marker in predicting atherosclerotic burden. He is focusing on extreme thresholds of biomarkers, with the hypothesis that highly specific markers (such as troponin) or extreme thresholds of less specific markers (such as CRP) may offer very high specificity. While individually these markers would be relatively insensitive, in aggregate, a panel of specific markers (or specific thresholds) may offer adequate sensitivity.

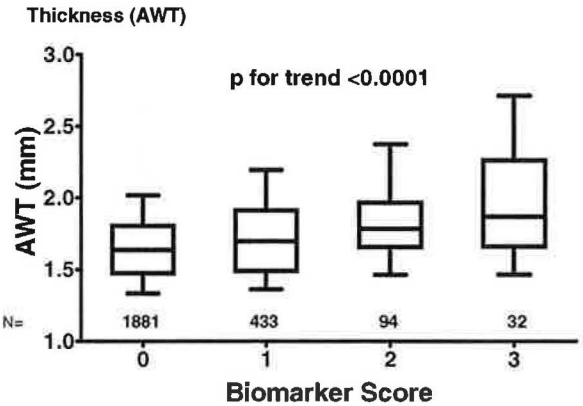
Established biomarkers were selected based on prior literature supporting their association with coronary atherosclerosis and/or cardiovascular events: hs-CRP, NT-proBNP, and osteoprotegerin (OPG). Three additional novel markers were included in the panel because they were independently associated with atherosclerosis in our study sample: sRAGE, PGLYRP-1, and soluble endothelial cell-selective adhesion molecule (sESAM).

All six markers were significantly associated with prevalent CAC in unadjusted analyses. An integer biomarker score determined by the number of markers above the 95th percent threshold (below 5th percentile for sRAGE) was significantly associated with CAC in both unadjusted and adjusted analyses (Figure, left panel). Furthermore, the associations with CAC were internally validated across a second atherosclerosis endpoint, abdominal aortic wall thickness measured by MRI (Figure, right panel), an endpoint qualitatively similar to carotid IMT. Finally, compared to the Framingham Risk Score, the gold standard for risk prediction in the population, the biomarker score improved the c statistic, other measures of model fit, as well as clinically relevant reclassification indices (Table).



Odds Ratio	—	1.9	3.8	5.1
95% CI	—	1.5-2.5	2.4-6.0	2.3-11.4
p value	—	<0.0001	<0.0001	<0.0001

* Odds ratios adjusted for Framingham risk categories



beta	Ref	0.05	0.16	0.24
p value	Ref	0.006	<0.0001	<0.0001

* beta and p values derived from linear regression models adjusted for Framingham risk categories

Table . Biomarker Score Improves Discrimination of CAC beyond Framingham Risk Score (FRS)

	c statistic	Likelihood χ^2	BIC	AIC	NRI	IDI
FRS	0.71*	373	2232	2180	—	—
FRS + Biomarker Score	0.75*	414	2195	2145	14.2%*	2.4%*

BIC = Bayesian Information Criterion (lower value indicates better model selection) Index
AIC = Akaike Information Criterion (lower value indicates better model selection) Index
NRI = Net Reclassification
IDI = Integrated Discrimination
* $p < 0.0001$ for comparisons between models

Although review of the statistical metrics for evaluating performance of novel screening tools is beyond the scope of this discussion, several points do merit emphasis. First, it is now clear that simply reporting adjusted relative risk or hazard ratio for a novel test often provides a misleadingly optimistic assessment of performance. On the other hand, over-reliance on the c-statistic or AUROC may create opposite problems as these tests have minimal clinical relevance and may be too insensitive. Novel metrics of reclassification, including the Net Reclassification Index (NRI) and Integrated Discrimination Index (IDI) provide complementary and potentially more clinically relevant information.[37] Investigators working in areas of screening and prediction, as well as clinicians reviewing papers in the future, will need to become facile with these emerging statistical tools.

These data provide proof-in-principle that combinations of biomarkers offer a viable approach for population screening. Iterative improvements to these panels are required, via testing of additional (and hopefully better) biomarkers and combinations. Moreover, the results require validation in other studies and with clinical endpoints.

These "early days" of multiple biomarker panels incorporate admittedly crude integrative strategies for the biomarkers, typically focusing on integer or weighted scores of individual biomarkers. Since each biomarker is selected based on unique pathobiology and independent contribution to prediction of the phenotype, it should eventually be possible to profile an individual based on relative contributions from biomarkers

reflecting specific biological pathways. Such information could be exploited to provide a more personalized assessment of dominant pathology and risk and open the door for targeted individual therapy.

Using the DHS to Model "What if" Questions (Implementation Research)

The evolving medical landscape includes a flood of recommendations and guidelines for screening and patient care. In no specialty is this more evident than cardiology. Increasingly, however, these guideline recommendations are derived from a modest evidence base, with recommendations increasingly based on expert opinion rather than solid clinical evidence.[38] Equally important, expert recommendations typically do not include an evaluation of the resource implications associated with implementation of the recommendations.

Because the DHS statistically represents Dallas County (and frequencies can be extrapolated using sampling weights) and because imaging studies and blood tests were performed in all subjects completing visits 2 and 3, the DHS has emerged as an excellent tool for modeling the implications of implementation of guideline recommendations or strategies in the population.

One example of such an approach is modeling of the current recommendations targeting the use of coronary artery calcium (CAC) screening to individuals deemed to be at moderately-high (also referred to as "intermediate") CHD risk, with a 10-year CHD risk estimate of 10-20% by the Framingham risk score (FRS).[39,40] Subjects identified with high risk CAC scores can be reclassified and promoted to high risk status, with accompanying changes in low-density lipoprotein (LDL) treatment goals. The rationale for restricting screening to the moderately-high risk (MHR) group is based upon Bayesian probability theory: post-test risks are more significantly influenced by test results among subjects whose pre-test risks are intermediate, rather than low or high. However, while theoretically sound, the utility and efficiency of this strategy when applied on a population basis remain unclear.

Given the uncertain implications of this strategy, Mahesh Patel and Amit Khera have evaluated the impact of applying CAC screening in the DHS. Importantly, only 1.0% of women and 15.4% of men were at intermediate risk and thus eligible for imaging, and only <0.1% and 1.1% respectively, were upgraded to high risk utilizing a CAC threshold ≥400. CAC imaging targeting intermediate risk subjects was also relatively inefficient (>100 women, 14.3 men scanned per subject reclassified). Restricting to an older age range (45-65) or expanding the scanned group to 6-20% risk had virtually no impact on risk assessment in women. Interestingly, they found that an imaging strategy targeting promotion of subjects from low risk to intermediate risk was more efficient and had greater yield than current recommendations targeting promotion from intermediate risk to high risk. They concluded that CAC screening strategies focusing on those at intermediate risk will have a negligible impact on risk assessment in women and a modest impact in men.

As a second example, recently, the Screening for Heart Attack Prevention and Education (SHAPE) Task Force proposed a broader application of atherosclerosis

imaging, in which all asymptomatic men (45-75 years) and women (55-75 years), except those already defined as at very high or very low risk, would undergo non-invasive imaging to detect subclinical atherosclerosis.[41] The imaging data would serve as the foundation for CHD risk stratification and assignment of LDL-C goals. It is important to note that the SHAPE Task Force does not represent an official or professional organization and that the recommendations are based on expert opinion. While the SHAPE authors predicted that implementation of these recommendations would substantially increase the population of individuals receiving cholesterol-lowering drugs, no formal assessment of the consequences of the SHAPE algorithm applied to a population has yet been reported.

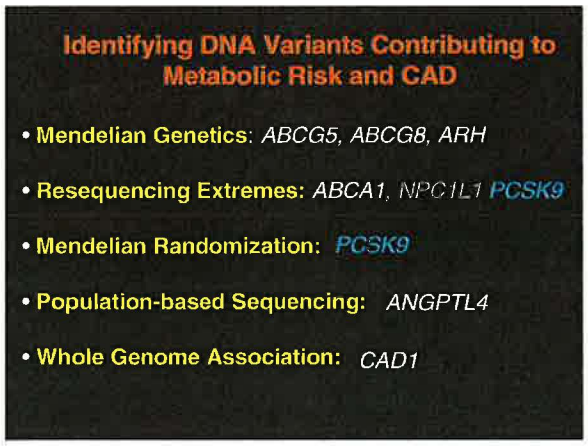
To estimate how implementation of the SHAPE recommendations would influence the proportion of subjects identified as failing to reach their respective LDL-C goals and therefore deserving consideration for cholesterol-lowering therapy, Raphael See and Jason Lindsey applied the NCEP-ATP III and SHAPE risk assessment algorithms to the DHS. Implementation of the SHAPE recommendations resulted in the reclassification of LDL-C goals in ~ 9.0% of subjects as compared with the NCEP-ATP-III guideline. Because subjects were reclassified into both higher and lower risk categories, the proportion of individuals identified as not meeting their individual LDL-C goal and thus potentially eligible for lipid-lowering intervention increased more modestly than had been predicted by the SHAPE authors.[41] The "efficiency" of CAC scanning as recommended by SHAPE was also evaluated. The Number Needed to Scan (NNS) to reclassify one individual as newly eligible (or no longer eligible) for lipid lowering therapy averaged 5.2.[42]

These data have potentially important implications, as the SHAPE guideline has received considerable attention; for example, the Texas Heart Attack Prevention Bill was recently proposed in the Texas House of Representatives to mandate private insurance reimbursement of non-invasive atherosclerosis imaging in asymptomatic, intermediate-risk individuals within SHAPE-defined age ranges. The DHS findings provide a starting point for discussion about the resource implications of such a bill.

Personal and Public Health

Implications of the PCSK9 Discovery for Prevention

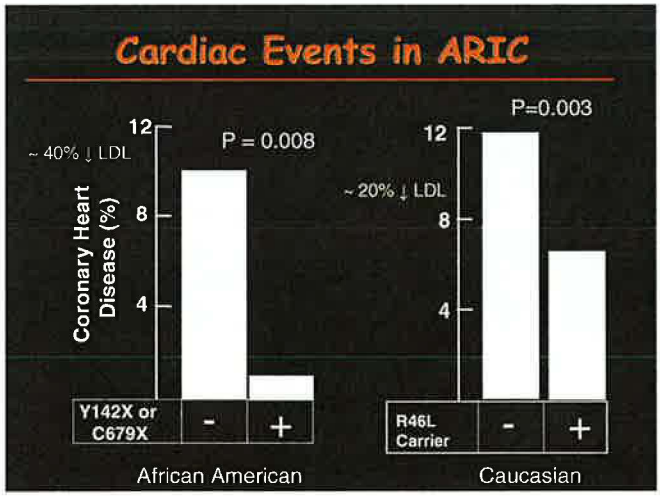
The most important discoveries from the DHS to date originate from the Hobbs and Cohen genetics program. They have taken a multifaceted approach to identifying genetic variants contributing to metabolic risk and CAD (figure), the details of which are beyond the scope of this discussion.



Hobbs and Cohen utilized the strategy of resequencing candidate genes in individuals with extreme phenotypes to discover the importance of *PCSK9.* By resequencing the *PCSK9* gene among the 5% of white and black subjects with the lowest levels of LDL cholesterol, they identified two sequence variants (Y142X and C679X) in African Americans. At least one of these alleles was present in ~2% of African Americans and was associated with a 40% lower LDL-C. Approximately 3% of white subjects carried a different allele (R46L) that was associated with a more modest effect on LDL-C (21% reduction).[43,44]

In collaboration with Eric Boerwinkle and the ARIC investigators, they utilized the emerging epidemiological tool of mendelian randomization to study the effects of lifelong reduction in LDL cholesterol on cardiovascular risk. Simply put, because the genetic variants are associated with **lifelong** LDL reduction and are not associated with other adverse cardiac risk factors, comparing cardiac risk over a lifetime between those with and without the allele would be analogous to performing a randomized trial of cholesterol lowering beginning at birth.

Among African American subjects from ARIC with the Y142X and C679X alleles (28% lower LDL-C), CV event rates were 88% lower than among the remainder of the population; among white subjects with the R46L allele (15% reduction in LDL), the reduction in CV risk was 46%.[45] (figure)



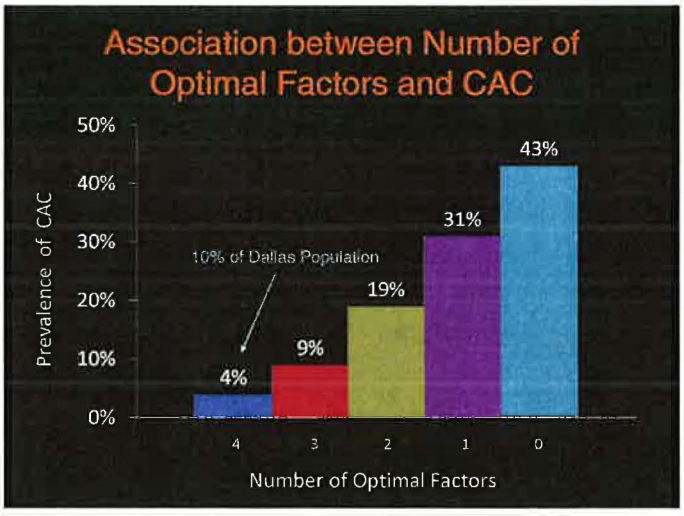
It is notable that the magnitude of relative risk reduction seen in this analysis is far greater than has been seen in primary and secondary prevention studies with statins. This observation has profound implications for cardiovascular prevention and cholesterol management, providing strong support that a strategy of earlier treatment with drugs and lifestyle factors that lower LDL cholesterol would have a greater impact than our current strategy of treating in middle age or later; moreover the findings also suggest that benefit will be proportional to the potency of the LDL lowering strategy.

Optimal CV Risk

Kammaki Banks, working with Amit Khera, has begun to explore the concept of optimal CV risk. This moves beyond traditional risk factor categories and instead targets **ideal** levels of each of the major risk factors. They have defined an optimal risk factor profile using 4 measured traditional risk factors (BP < 120/80 mmHg; LDL-C <100mg/dl; fasting blood glucose <100mg/dl; and lifetime non-smoking status).

In the DHS, the optimal risk factor profile was only present in 10-12% of individuals. Interestingly, when participants were characterized by the number of optimal risk factors present, the distribution was similar across race/ethnic groups; however, the composition of the individual factors differed. For example, blacks were less likely to have optimal blood pressure compared with other race/ethnic groups, hispanics were less likely to have optimal blood glucose and whites were less likely to have optimal smoking status but more likely than other racial groups to have optimal LDL.

The association between optimal risk factor categories and coronary calcium is shown in the figure below. After adjustment for age, sex, and race, the optimal risk factor profile was associated with a 90% reduction in coronary calcium prevalence compared to individuals with no optimal risk factors. A similar association was observed for aortic plaque.



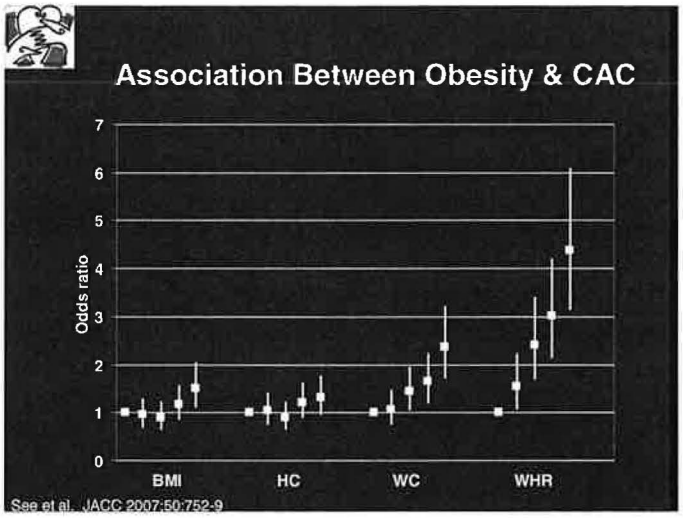
These findings suggest that the decreased CV morbidity and mortality for individuals with an optimal risk profile that has been reported in long-term cohort studies[46,47] is due at least in part to reduction in the burden of atherosclerosis. The observations also have

implications for personal health in that they provide strong supportive evidence that efforts to achieve an optimal CV risk profile will prevent atherosclerosis development.

Body Size and Shape

Prior studies evaluating the association between obesity and CV risk have reported varied results. Population-based studies measuring CV events have reported threshold-effects, [48] J-shaped relationships,[49] and linear relationships.[50] In most of these studies body mass index (BMI) was used as the primary measure of obesity rather than alternative measures such as waist circumference (WC) or waist-to-hip ratio (WHR), which have demonstrated stronger correlations with CV risk than BMI. [51]

Raphael See evaluated the association between different measurements of obesity and prevalent subclinical atherosclerosis in the DHS.[52] Prevalent CAC was strongly associated with BMI, WC, and WHR in univariable analysis in both men and women. This association was strongest for WHR and weakest for BMI. (figure)



After adjustment for traditional CV risk factors, only WHR was significantly and independently associated with CAC. A J-shaped relationship of prevalent CAC with BMI was observed in both men and women before and after multivariable adjustment. WHR was also significantly and independently associated with aortic plaque.

An important limitation of BMI is failure to differentiate between varied body compositions. It is poorly specific for excess adiposity, but more importantly does not discriminate between varying distributions of fat tissue. Centrally-distributed or abdominal obesity is specifically associated with adverse effects on metabolism, dyslipidemia, and insulin resistance.[53] In addition, BMI can be falsely elevated in the presence of increased lean body mass (such as in trained athletes), and low BMI values are associated with chronic conditions leading to loss of lean body mass. The present findings suggest that WC and WHR may be preferred measures of the cardiac risk associated with obesity.

A second possible explanation for the superior performance of WHR is that increased hip circumference may *protect* against atherosclerosis. Hip circumference has been observed to be inversely associated with CV risk factors. A recent study by Gloria Vega using the DHS cohort found increased lower body fat on DEXA scanning was

inversely associated with insulin resistance, dyslipidemia, C-reactive protein, and systolic blood pressure. These observations suggest that lower body fat may function as a protective reservoir against ectopic (visceral) adiposity.[54]

From a personal and public health standpoint, the most important implications of the DHS analyses are that the associations between WC and WHR are linear and *do not demonstrate a threshold effect.* This suggests that for most individuals, cardiovascular risk could be reduced by losing a few inches around the waist, even if body weight is considered "normal."

Looking to the Future

The DHS is currently transitioning from a cross sectional study to a cohort study as we are presently reassessing the cohort with clinical data collection and imaging, the "DHS-2". Directed again by Helen Hobbs, with Kathleen "Kate" Wilkinson as the program manager, the DHS-2 consists of a very detailed return visit to UTSW for participants from the original DHS exams 2 and 3. The goal of the DHS-2 is to assess ***progression of phenotypes over an 8-10 year time period.*** During the followup visit, participants undergo repeat CT scanning for coronary calcium, MRI scanning to assess LV structure and function, and MRI of the aorta to assess plaque and wall thickness. Several new imaging phenotypes are being collected as well, including MRI of the carotid artery to evaluate wall thickness, plaque prevalence, and plaque morphology; and MRI of the brain. Additional new assessments include assessment of ambulatory activity using an accelerometer, a limited exercise test to quantify fitness, and screening of cognitive function.

Moreover, under the direction of Amit Khera and Teresa Eversole, a comprehensive program has been implemented for detection and followup of clinical events. Given the young age of the DHS cohort, and the focus of the study on *preclinical disease* the study was not designed to have high statistical power for event prediction. However, the endpoint followup will serve as an important adjunct to assessments of changes in atherosclerosis and LV remodeling phenotypes over time.

Acknowledgements

I am grateful to Helen Hobbs for her tremendous leadership of this project, and the opportunities it has provide for me and so many others at UTSW. Beyond providing mentorship and study leadership, she has also devoted a huge amount of her time to the operational details of the study, which has freed up younger investigators to pursue their scientific interests. I would also like to thank Scott Grundy for his senior mentorship: his experience and guidance have been invaluable. I would like to thank my research partners, Mark Drazner, Darren McGuire, and Amit Khera, for creating such a fun and productive team, and Colby Ayers, our statistician, for his supreme competence and critical contributions to the productivity of our team. The credit for this research belongs to the junior faculty, research fellows, and residents who performed all the hard work for the individual projects. Finally, I would like to thank the DHS staff for their commitment and tireless efforts for the project, and of course all of the DHS participants.

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