

Ancillary Study Documentation

1) Genetic Variation in the Aldosterone Synthase Gene and Diastolic Heart Failure “DanielSNPs” Dataset

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Case-control study of diastolic heart failure (DHF) among patients from the Cardiovascular Health Study (CHS). Patients diagnosed with CHF who have a normal ejection fraction (>55%) will be considered DHF cases. Two controls without CHF from the CHS cohort would be matched to each case.

Aims include:

- SNPs in the aldosterone synthase gene and its promoter will be selected from public databases designed to adequately reflect common variants in the aldosterone synthase gene.
- The allele frequency of these SNPs, and the common haplotypes defined by these SNPs, will be determined after genotyping the selected SNPs in CHS subjects with DHF and a 2:1 sample of matched controls.

Sampling details:

- Used risk set sampling (aka incidence density sampling).
- Selected all eligible incident and prevalent cases of DHF (n=328).
- Matched 2 controls to each case (n=656 controls).
- These 984 cases & controls are represented by 905 participants:
- 828 were selected once, 75 were selected twice, and 2 were selected 3 times
- 22 participants were selected as controls (once) before they became DHF cases.
- Matched on sex, race, study clinic, and age at baseline. For age matching: started with age +/- 1 year, then widened the age band (to +/- 2, then +/- 3, etc.) as needed to get at least 2 matches per case.
- Matched controls had to be at risk (under CHS follow-up without incident CHF) at the equivalent time since baseline as the DHF event in the case. Participants were censored at the time of incident non-diastolic CHF.
- Prevalent DHF cases at baseline are included as cases, and are matched to controls who are at risk as of day 1 in CHS (i.e., all eligible participants).
- Excluded 1310 participants with (1) prevalent non-diastolic CHF at baseline, (2) valve disease at year 2 or 7, (3) race other than black or white, or (4) missing APOE data. Missing APOE indicates lack of DNA in the repository or lack of consent to genetic research.

2) “Role of NT-proBNP for detecting structural heart disease and predicting risk of new onset heart failure or cardiovascular death in an ambulatory elderly cohort: a Cardiovascular Health Study ancillary proposal.”

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Funding:

Roche Diagnostic Pharmaceuticals

Description:

NT-proBNP was measured from serum collections at years 2 (baseline) and 5 (first interval follow-up) for original cohort, and years 5 (baseline) and 7 (first interval follow-up) for African –American enhanced recruitment. All measurements were performed on serum samples stored at -70°C to -80°C and thawed just before testing by individuals blinded to participant data. NT-proBNP was measured on the Elecsys 2010 analyzer (Roche Diagnostics, Indianapolis, Indiana).

The coefficient of variation for the NT-proBNP assay was 2% to 5% during the testing period, and the analytical measurement range for NT-proBNP was 5 to 35,000 pg/ml. All samples were stored at -70°C to -80°C and were thawed before testing (maximum of 3 freeze-thaw cycles). Measurements of NT-proBNP using this assay do not change after 5 freeze-thaw cycles NT-proBNP assay is measured in pg/mL and we made our measurements 2007-2008

3) Soluble Protein C Receptor and Cardiovascular Disease

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Description:

CHD/stroke case control group.

Year 2 samples:

400 age/sex stratified random samples

Year 5 samples:

250 incident CHD cases
250 incident stroke cases
500 controls

10 ng DNA will be used to genotype the 1000 participants selected in year 5

4) “Subclinical Thyroid Dysfunction in the Elderly”

Principal investigator:

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Funding:

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Description:

Serum TSH, free T₄ (FT₄), total T₃, and antithyroid peroxidase antibody concentrations (TPOAb) were measured in 2010 from banked samples obtained in years 5, 7, 9 & 18

Thyroid function assays were performed at the CHS Central Blood Analysis Laboratory at the University of Vermont (Burlington, VT) using chemiluminescent immunoassays on the Elecsys 2010 analyzer (Roche Diagnostics, Indianapolis, IN). TSH concentrations were measured with a third-generation assay with a functional sensitivity of 0.005 mU/liter and 2.1% intraassay and 3.1% interassay coefficients of variation (CV). Two values of TSH reported as greater than 50 mU/liter were recoded as equal to 50 mU/liter and one suppressed TSH was recoded to 0.005 mU/liter for the purpose of analysis.

The FT₄ assay had a functional sensitivity of 0.23 ng/dl (2.96 pmol/liter) and 1.7% intraassay and 3.3% interassay CV. A reference range of 0.7–1.7 ng/dl (9–22 pmol/liter) was used. The total T₃ assay had a functional sensitivity of 19.5 ng/dl (0.3 nmol/liter), reference range of 84.4–201.3 ng/dl (1.3–3.1 nmol/liter), and 4.2% intrassay and 4.7% interassay CV. The TPOAb assay had a functional sensitivity of 5.0 U/liter, a threshold of 37 U/liter to define positivity ([10](#)) and 2.5% intrassay and 9.2% interassay CV.

5) Association Between Biomarkers of Calcium Metabolism and Cardiac Events Among Participants in CHS

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Funding:

NHLBI 1R01HL084443-01A2

Description:

The mineral metabolism and Vitamin D data used previously collected and stored serum samples from all CHS participants who completed the year 5 exam. The following were measured: 25-hydroxy vitamin D, parathyroid hormone, calcium, phosphate and bone alkaline phosphatase. The Laboratory for Clinical Biochemistry Research at the University of Vermont stored serum samples at -70°C using established methods, which have demonstrated long-term stability for serum markers of coagulation, fibrinolysis, and inflammation. The University of Washington Clinical Nutrition Research Unit performed mineral metabolism measurements from serum collected during the 1992 and 1993 CHS exams. Total 25-OHD ($25\text{-OHD}_2 + 25\text{-OHD}_3$) was measured using high-performance liquid chromatography and tandem mass spectrometry on a Waters Quattro micro mass spectrometer (Waters, Milford, Massachusetts).

The interassay coefficient of variation was $<3.4\%$. Intact serum PTH was quantified using a 2-site immunoassay on a Beckman UniCel DxI clinical analyzer (Beckman Coulter, Brea, California). The reference range is 17 to 66 pg/ml, as determined from the central 95% of values from 43 normal laboratory personnel with normal 25-OHD concentrations in March 2005. The interassay coefficient of variation for PTH was $<4.5\%$ at 37 pg/ml. Serum nonionized total calcium levels were measured using indirect potentiometry, and serum phosphorus levels were measured using a timed-rate colorimetric reaction method with ammonium molybdate on a Beckman DxC Synchron analyzer (Beckman Coulter).

6) Circulating Dietary & Metabolic Fatty Acids, Major CVD Outcomes, & Healthy Aging

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NIH R01 HL085710

Description:

Fatty acid concentrations were measured in stored blood samples obtained from 3630 participants in 1992 to 1993, our baseline for this analysis. Of these, 3130 participants were randomly selected from among those with available blood samples, and fatty acids had previously been measured in 500 participants as part of a nested case-control study of incident myocardial infarction in CHS (1). All analyses accounted for this sampling within the cohort by using inverse-probability-of-sampling weights. We excluded 214 persons with prevalent CHF and 681 with coronary heart disease at the time the samples were obtained, which left 2735 participants for this analysis. All fatty acid concentrations, other risk factors, and metabolic outcomes were assessed similarly in all participants by using data from the 1992 to 1993 visit and blood sample, except for dietary habits, which were assessed at enrollment 3 years earlier.

Plasma phospholipid fatty acids were measured at the Fred Hutchinson Cancer Research Center, which provided quantitative measurement of 45 fatty acids as a percentage of total fatty acids. Phospholipids represent a biomarker of longer-term (4- to 8-week) circulating fatty acid concentrations, with similar responses to those of concentrations in erythrocyte membranes (2). No degradation, lipolysis, or oxidation has been observed after 10 years under the blood storage conditions in CHS (3). Total lipids were extracted from plasma (4), and phospholipids were separated from neutral lipids by using 1-dimensional thin-layer chromatography. Samples of fatty acid methyl esters were prepared by direct transesterification (5) and separated by using gas chromatography (5890 gas chromatograph flame ionization detector, Agilent Technologies, Palo Alto, California; SP-2560 fused-silica 100-m capillary column, Supelco, Bellefonte, Pennsylvania) according to the following procedure: initial, 160 °C × 16 minutes; ramp, 3.0 °C/min to 240 °C; hold, 15 minutes. Identification, precision, and accuracy were continuously evaluated by using model mixtures of known fatty acid methyl esters and established in-house control samples. Laboratory coefficients of variation were less than 3% for major fatty acids and for EPA, DPA, and DHA.

We assessed the long-term reproducibility of ω -3 fatty acid concentrations by using serial blood samples (1992 to 1993, 1998 to 1999, and 2005 to 2006) in a subset of 100 participants, which would capture laboratory error, biological variability, and dietary changes over time. Six-year and 13-year correlations with baseline concentrations for EPA, DPA, and DHA were 0.55 and 0.50, 0.67 and 0.52, and 0.82 and 0.60, respectively; these are similar to within-person correlations over time for other common risk factors, such as blood pressure (6). In prospective studies of the relationships of risk factors to subsequent disease events, such normal biological fluctuation in levels of physiologic risk factors over time leads to underestimation of the strength of true associations. We used the reproducibility measurements to correct for this regression dilution bias and determine the association of usual fatty acid concentrations with risk for disease, using methods established in analyses of blood pressure and cholesterol levels and cardiovascular risk (7–8). Such methods correct the risk estimate and widen the CIs but do not change the statistical significance (*P* value).

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7) Low serum testosterone and risk of cardiovascular events, cardiovascular mortality and all-cause mortality in older men

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Funding:

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Description:

Testosterone data (serum testosterone) and SHBG were extracted from sera in year 7 (1994-1995) in only men who had no history of cardiovascular disease at that time.

We subsequently calculated “free” values of total T and DHT, using 2 different equations. PDFs of the papers and the R code and summary are provided for the calculations, as well as the freed data set.

8) Determinants and Cardiovascular Consequences of Diabetes in Older Adults

Principal Investigator

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Funding:

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Descriptions of data

A) Harmonization of Glucose and Insulin Variables in years 2, 5, 7, 9, 11 and 18

Fasting blood samples were obtained from participants during the annual clinic examinations in 1989-90, 1992-93, 1996-97, 1998-99, and 2005-06, and non-fasting blood samples were collected in 1994-95. Sera was available from all examinations except for 1998-99, for which EDTA plasma only was available. Glucose had been measured previously on the blood samples taken in 1989-90, 1992-93, 1996-97 and 2005-2006. In 2010, glucose was measured concurrently on the samples from the 1994-95 and 1998-99 examinations. All CHS glucose assays were performed at the Central Blood Analysis Laboratory at University of Vermont.

In order to minimize measurement error and misclassification of participants that may have resulted from differences in glucose measurement over time, we harmonized the measurements done prior to 2010 with the contemporary glucose measures. This was done by including sera samples from a subset of 48 participants who had serial blood samples from each of the previous examinations in the 2010 glucose assays. Additionally, in order to identify any differences in glucose measurements attributable to plasma, glucose was measured in 48 samples of both serum and plasma from stored specimens in 1996-97, when both specimen types were available. We then compared the new glucose measurements to the original ones among the subset of participants with paired measurements by evaluating correlation coefficients, regression lines, Bland-Altman plots and mean differences. In all years, the correlation between the original and new assays was high (.91-.99), and there was no statistical evidence of a multiplicative effect (regression slopes did not differ from 1). However, there were differences in the mean values, and adjustments were made based on these mean differences to align all glucose measurements to those from 1989-90. Harmonized glucose measurements were used to ascertain diabetes and in all analyses. Medication use was assessed at baseline and annually thereafter by medication inventory through 2007 (Psaty, 1992). We classified participants as having diabetes if fasting glucose was ≥ 126 mg/dL, casual glucose was ≥ 200 mg/dL, or individuals used insulin or oral hypoglycemic agents.

B) Year 5 Analytes

All analytes were measured in EDTA plasma among CHS participants at Year 5. The following analytes are included in the CHS dataset:

Analyte	Lower DL	Upper DL	Total No.	No. w/no sample	No. w/insuff. volume	No. <DL	No. >DL	Final N
Total adiponectin	130 ng/mL	45,000 ng/mL	4760	41	4	2	12	4715
NEFA	0.01 g/L	3.7 g/L	4760	41	4	0	0	4715
FABBP4	5 ng/mL	250 ng/mL	4760	41	12	0	1	4707
Fetuin-A	0.0156 mEq/L	~1.50 mEq/L	4760	41	5	0	0	4714

DL=Detection limit

9) CHS Year 18 All Stars Data Set

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CHS All Stars is an ancillary study of the Cardiovascular Health Study (CHS) with a focus on reexamining the long term survivors of CHS to determine the likelihood of maintaining function later in life. Functional aging was assessed in 2005-06 (CHS Year 18) by offering a clinic or in-home visit to surviving CHS participants who consented. The All Stars grant contributed to the support of subsequent six month interval telephone contacts, in order to continue to collect data on physical and cognitive functioning. A competing continuation was funded to continue this activity through the year 2014.

Any papers using CHS All Stars data should include the following acknowledgement:

‘CHS: The research reported in this article was supported by the National Institute on Aging AG-023629. CHS was supported by contract numbers N01- HC-85079 through N01-HC-85086, N01-HC-35129, N01 HC-15103, N01 HC-55222, N01-HC-75150, N01- HC-45133, grant number U01 HL080295 from the National Heart, Lung, and Blood Institute, with additional contribution from the National Institute of Neurological Disorders and Stroke. Additional support was provided through R01 AG-15928, R01 AG-20098, and AG-027058 from the National Institute on Aging, R01 HL-075366 from the National Heart, Lung and Blood Institute, and the University of Pittsburgh Claude. D. Pepper Older Americans Independence Center P30-AG-024827. A full list of principal CHS investigators and institutions can be found at <http://www.chs-nhlbi.org/pi.htm>.’

Funded by NIA Grant: R01 AG-023629: University of Pittsburgh (Anne Newman; Exceptional aging: 12 year trajectories to function (“All Stars”). CHS Year 18 visit.

10) Events Follow-up data

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The Events grant supports data collection on surviving CHS participants for cardiovascular events after year 18, including two phone calls per year to participants to identify potential events, data collection on potential events, and adjudication by the CHS Cardiac and Stroke Events Committees. The grant was originally funded in 2005, and a competing continuation was funded to continue this activity through the year 2014.

Any papers using CHS Events data from year 18 onward should include the following acknowledgement:

‘CHS: The research reported in this article was supported by the National Institute on Aging AG-023629. CHS was supported by contract numbers N01- HC-85079 through N01-HC-85086, N01-HC-35129, N01 HC-15103, N01 HC-55222, N01-HC-75150, N01- HC-45133, grant number U01 HL080295 from the National Heart, Lung, and Blood Institute, with additional contribution from the National Institute of Neurological Disorders and Stroke. Additional support was provided through R01 AG-15928, R01 AG-20098, and AG-027058 from the National Institute on Aging, R01 HL-075366 from the National Heart, Lung and Blood Institute, and the University of Pittsburgh Claude. D. Pepper Older Americans Independence Center P30-AG-024827. A full list of principal CHS investigators and institutions can be found at <http://www.chs-nhlbi.org/pi.htm>.’

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