Supplemental Methods

The Northern Sweden VIP, MONICA and MSP studies

Cases were identified by cross linkage between the MONICA myocardial infarction (MI) and stroke registries and the survey cohorts (VIP, MONICA and MSP), and controls from the survey cohorts were matched for sex, age, survey type and date of survey.

VIP is an ongoing community intervention program targeting cardiovascular disease and diabetes prevention.[1] Participants are asked to participate in a health survey at their primary health centre at the ages of 30, 40, 50, and 60 years. However, those aged 30 are no longer invited because of a lack of resources. The participation rate was initially 55% but has increased and is now approximately 65%. The total number of unique individuals surveyed in VIP was 99,268 as of 31 December 2014.

MONICA consists of randomly selected individuals aged 25–74 years from the counties of Västerbotten and Norrbotten who were invited to participate in a health study. The study has been repeated seven times at approximately 5-year intervals with new random samples of 2500 individuals each (the first two surveys invited 2000 individuals each).[2] For each survey 250 men and 250 women from each 10-year age group were randomly sampled from population registers, stratified for age and sex. The overall participation rate was 74%, and a total of 12,368 unique persons had participated by 31 December 2014.

Data for the MSP cohort, consisting of 28,778 women, were collected between 1995 and 2006 when the women attended their regular mammography examination and were asked to donate blood samples for research. In addition, anthropometric measurements were taken.

In all studies, participants were asked to donate blood to be stored at -80°C for future research. Participants were fasting before sampling for a minimum of 4 hours (extended to 8 hours in 1992).

Since 1985, all (in-hospital and out-of-hospital) cases with acute stroke (in the age group 25–74 years) and acute MI (in the age group 25–64 years) in the MONICA area (i.e., Västerbotten and Norrbotten) have been included in the Northern Sweden MONICA registries using WHO criteria and MONICA methodology. Possible CVD events (fatal and non-fatal CVD (MI or stroke)) were identified through screening of hospital discharge records, general practitioners' reports, and death certificates, with ICD 8 and 9 codes 410-413 and 430-438 corresponding to ICD 10 codes I20-I24 and I60-69. For death certificates, the codes 414 and 798–799 (ICD 8 and 9), and I25 and R96–99 (ICD 10) were also included. Data collection included information on medical history, symptoms, examinations, presenting electrocardiogram (ECG), and stroke subtypes. The number of subjects with MI and stroke included in the Northern Sweden MONICA registry not willing to participate in further studies has averaged two to six per year (0.2–0.6 %).

Detailed descriptions of criteria for diagnosis of stroke and classification of subtypes have been published.[3,4] In short, stroke cases were classified into one of the categories "definite stroke", "unclassified stroke", or "no stroke". Unclassified events were mostly fatal cases with a death certificate diagnosis of stroke where information on previous history of stroke or of the clinical event was not obtainable. In this study, only cases classified as "definite stroke" have been included in nonfatal events. In fatal events, the category "unclassifiable stroke" has also been included, in accordance with the agreed convention in the core MONICA project.

British Women's Heart and Health Study (BWHHS)

Women from the main cohort with CHD at baseline (N = 694 prevalent cases; 16.2%) were excluded. Incident cases of CHD (169 cases, 111 non-fatal and 58 fatal) were identified by two-yearly medical record reviews and through routine death registration until October 2016, defined as either of: (i) death with an underlying or contributing cause of CHD (ICD10 codes I20-I25, I51.6); or (ii) a MI (defined according to WHO criteria), first diagnosis of angina or coronary artery by-pass or angioplasty. For each case, two controls were randomly selected, within 5-year age groups of the cases, from women without CHD at the baseline assessment and who had been followed-up over the same time period as the cases without experiencing a CHD event. Additional controls were selected to replace those who subsequently died or experienced a CHD event within 1 year of the selection.

Additional analyses of prospective cohort studies

Additional analyses were performed to investigate whether the effect of cortisol on CVD differed by sex. Similarly, the outcome was stratified to investigate the effect of cortisol on CHD and stroke separately. The equality of coefficients from these stratified analyses were formally tested using the generalized Hausman specification test.[5]

Methods for dealing with missing data

In order to increase efficiency and minimise selection bias we used multivariate multiple imputation to impute missing data for potential confounders including all exposures, covariables, outcomes and potential predictors of missing data in the imputation equations.[6] This assumes the missing data can be explained by the observed data (missing at random assumption).[7] It is not possible to test this assumption, but we have included all exposures, outcomes, covariables and any variables that are predictive of missing data in our imputation

models in order to increase the plausibility that it is correct. Table S6 lists the variables included in these missing data prediction models and how they were entered into the models. In Stata, we carried out 20 cycles of regression switching as described by Royston[6] and generated 20 imputation datasets. The multiple imputation approach creates a number of copies of the data (in this case, 20 copies) in which missing values are imputed, with an appropriate level of randomness, by chained equations. The average estimate from each of these 20 datasets is obtained using Rubin's rules taking account of the uncertainty in the imputation so that the standard errors for any regression coefficients (used to calculate p-values and 95% confidence intervals) take account of uncertainty in the imputations as well as uncertainty in the estimate.

Multivariable regression of prospective studies meta-analysis

The Caerphilly study includes 2512 men (2323 with cortisol data) from the town of Caerphilly or surrounding villages examined between 1979 and 1983.[8] The majority of fasting blood samples were taken between 0700h and 0800h. The records of all men at the National Health Service Central Registry were flagged so that notification of death was automatic and a copy of the death certificate was received. Fatal ischaemic heart disease (IHD) events were classified as deaths with International Classification of Diseases 9 (ICD-9) codes 410 to 414. Non-fatal IHD events were ascertained through follow-up clinics and discharges from local hospitals with a diagnosis code of ICD-9 410 to 414.

The Vietnam Experience study consists of 18313 male former military personnel (4255 with cortisol data)[9]. All fasting blood samples were taken in the morning. Mortality due to CVD was classified using ICD-9 codes: 390–434 and 436–448, and ICD-10 codes: I00–I78. The majority of deaths were from IHD.

Sensitivity analyses of the multivariable regression of prospective studies meta-analysis

We undertook a leave one out analysis, in which the meta-analysis was repeated four times with one study removed each time, to explore whether any differences between study results importantly influenced the pooled estimate. To assess potential small study bias, a funnel plot was prepared of ln*OR* against the standard error ln*OR*[10] and analysed using Egger's test.[11]

One-sample Mendelian randomization

The causal estimate was derived using the two-stage method comprising a first-stage regression of the exposure on the SNP, and a second-stage regression of the outcome on the fitted values of the exposure from the first stage. As cortisol measurements were taken at baseline (prior to onset of CVD) then the first stage (SNP-cortisol) association was obtained using cases and controls.[12] We included covariates in the first-stage and second-stage regressions. This increases efficiency and hence the precision of the causal estimate. However, it may lead to bias in the causal estimate if a covariate is on the causal pathway between exposure and outcome or is a collider or causally downstream of a collider.[13]

Two-sample Mendelian randomization

This approach assumes that the gene-exposure and gene-outcome associations are estimated in non-overlapping samples and are representative of the same population (similar age, sex distribution and the same ethnic group).[18] For our analyses there are no studies that contributed to both CORNET and CARDIoGRAM and so we can rule out a large overlap. A proportion of participants of the ORCADES study, which contributed to CORNET, were eligible to participate in UK Biobank. We are unable to rule out the possibility that individuals may have participated in multiple studies and so may have contributed to CORNET and to CARDIoGRAM or UK Biobank. If this is the case then estimates from the two-sample Mendelian randomization analyses may be biased towards the estimate obtained from conventional methods (e.g. multivariable regression).[19]

We ran three additional analyses: first, a weighted median approach[14] which is consistent even when up to half of the information comes from invalid instrumental variables; second, a maximum likelihood[15] approach which uses linear relationship between the risk factor and outcome and a bivariate normal distribution for the genetic association estimates; finally, inverse variant weighting (IVW) to combine each of the three SNPs which is a linear regression analysis through the mean SNP-exposure and SNP-outcome results that is forced to go through zero (i.e. constrained to have intercept zero).[16] As a sensitivity analysis to explore horizontal pleiotropy we used MR-Egger regression[16], which is similar to IVW but does not constrain the regression line to go through zero. A non-zero intercept in MR-Egger suggests possible horizontal pleiotropy; the slope can be interpreted as the effect having relaxed the horizontal pleiotropy assumption. To investigate how pleiotropy might be influencing our estimates we performed multivariable Mendelian randomization[17] which uses multiple genetic variants associated with more than one risk factor to simultaneously estimate the causal effect of each of the risk factors on the outcome.

Supplemental References

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Supplemental Figure Legends

Figure S1. Funnel plot of log odds ratio (logOR) against the standard error of log odds ratio (se logOR) to assess potential small study bias in the meta-analysis of prospective studies