Associations of Serum Cortisol with Cardiovascular Risk and Mortality in Patients referred to Coronary Angiography

Stefan Pilz1*, Verena Theiler-Schwert1*, Christian Trummer1, Martin H. Keppel2, Martin R. Grübler1,3, Nicolas Verheyen1, Balazs Odler5, Andreas Meinitzer6, Jakob Voelkl7,9, Winfried März5,10,11

1Department of Internal Medicine, Division of Endocrinology and Diabetology, Medical University of Graz, Graz, Austria; 2University Institute for Medical and Chemical Laboratory Diagnostics, Paracelsus Medical University, Salzburg, Austria; 3Department of Geriatrics and Aging Research, University Hospital Zurich and University of Zurich, Switzerland; 4Department of Cardiology, Medical University of Graz, Graz, Austria; 5Department of Internal Medicine, Division of Nephrology, Medical University of Graz, Austria; 6Clinical Institute of Medical and Chemical Laboratory Diagnostics Medical, University of Graz, Graz, Austria; 7Institute for Physiology, Johannes Kepler University Linz, Linz, Austria; 8Departments of Nephrology and Medical Intensive Care and Internal Medicine and Cardiology, Charité University Medicine, Campus Virchow-Klinikum, Berlin, Germany; 9DZHK (German Centre for Cardiovascular Research), partner site Berlin, Berlin, Germany; 10Synlab Academy, Mannheim, Germany; 11Medical Clinic V (Nephrology, Hypertensiology, Rheumatology, Endocrinology, Diabetology), Medical Faculty Mannheim, University of Heidelberg, Heidelberg, Germany

*equal contribution as first authors

Correspondence:
Stefan Pilz, MD, PhD
Medical University of Graz
Department of Internal Medicine
Division of Endocrinology and Diabetology
Auenbruggerplatz 15, 8036 Graz, Austria
Tel: +43 316 385 81143
Fax: +43 316 385 13428
Email: stefan.pilz@medunigraz.at

Disclosure: The authors report no conflicts of interest.
Abstract

Context: Serum cortisol may be associated with cardiovascular risk factors and mortality in patients undergoing coronary angiography, but previous data on this topic are limited and controversial.

Objective: We evaluated whether morning serum cortisol is associated with cardiovascular risk factors, lymphocyte subtypes and mortality.

Design and Setting: This is a prospective cohort study performed at a tertiary care centre in southwest Germany between 1997 and 2000.

Participants: We included 3052 study participants who underwent coronary angiography.

Main Outcome Measures: Primary outcome measures were cardiovascular risk factors, lymphocyte subtypes and all-cause and cardiovascular mortality.

Results: Serum cortisol was associated with an adverse cardiovascular risk profile, but there was no significant association with coronary artery disease or acute coronary syndrome. In a subsample of 2107 participants, serum cortisol was positively associated with certain lymphocyte subsets including CD16+CD56+ (natural killer) cells (p<0.001). Comparing the fourth versus the first serum cortisol quartile, the crude Cox proportional hazard ratios (with 95% confidence intervals) were 1.22 (1.00 to 1.47) for all-cause and 1.32 (1.04 to 1.67) for cardiovascular mortality, respectively. After adjustments for various cardiovascular risk factors, these associations were attenuated to 0.93 (0.76 to 1.14) for all-cause, and 0.97 (0.76 to 1.25) for cardiovascular mortality, respectively.

Conclusions: Despite significant associations with classic cardiovascular risk factors and natural killer cells, serum cortisol was no significant and independent predictor of mortality in patients referred to coronary angiography. These findings might reflect that adverse cardiovascular effects of cortisol could be counterbalanced by some cardiovascular protective actions.

Keywords: Cortisol, NK cells, inflammation, cardiovascular, mortality, prospective
Introduction

The adrenal derived hormone cortisol and related glucocorticoids are important to maintain homeostasis in certain stress conditions, and exert a variety of effects with relevance for e.g. inflammation, metabolism and cardiovascular health [1-3]. Patients with Cushing’s syndrome, caused by either exogenous or endogenous cortisol excess, are at significantly increased risk of cardiovascular disease and mortality [4-7]. Interestingly, accumulating evidence derived from epidemiological studies and Mendelian Randomization studies suggests that morning cortisol levels are also a causal cardiovascular risk factor in the general population [8].

In patients with acute cardiovascular diseases, there is a stress induced increase in cortisol levels, but data on the clinical significance of morning cortisol concentrations in this setting are limited and controversial [9-14]. Clinical studies in patients with acute coronary syndromes (ACS) or in patients undergoing coronary angiography showed either a positive, no, or a negative association of morning cortisol concentrations and cardiovascular disease or mortality [10-14]. From a pathophysiological point of view, glucocorticoids are important modulators of several processes with relevance for cardiovascular health, and can, depending on the micro-environment and the nature of the stress stimulus, exert detrimental as well as beneficial effects [1-4, 10, 15-18]. In epidemiological studies, it must, of course, be considered that activation of the hypothalamic-pituitary-adrenal axis (HPA) resulting in high cortisol levels may merely reflect disease severity, so that it is challenging to disentangle the cause and effect relationship of cortisol and clinical outcomes [10, 16]. Nevertheless, given the central role of glucocorticoids in modulating inflammation, cardiovascular risk factors and the cardiovascular system itself, data on cortisol levels in patients undergoing coronary angiography are of interest, as cortisol and its metabolism and signaling represent promising targets for diagnostic and therapeutic approaches in cardiovascular medicine [10, 16-20].

In the present study, we aim to evaluate in patients referred to coronary angiography derived from the LUDwigshafen RIsk and Cardiovascular Health (LURIC) study, whether morning serum cortisol concentrations are associated with common cardiovascular risk factors, lymphocyte subtypes (in particular natural killer cells), and mortality [19-21]. A special focus will be on subgroup analyses.
of patients with ACS, as this patient group is of particular clinical interest and might have a pronounced HPA activation compared to patients with stable coronary artery disease (CAD).

**Methods**

**Study Design and Participants**

The LURIC study is a prospective cohort study in patients undergoing coronary angiography [21]. Main inclusion criteria were clinical stability except for ACS, availability of a coronary angiogram, and German ancestry (to limit genetic heterogeneity). Exclusion criteria were any acute illness other than ACS, any chronic disease where non-cardiac disease predominated, and a history of malignancy within the past five years. Design and methods of this study have been published elsewhere [21]. Written informed consent was obtained from all study participants and the study was approved by the ethics committee of the “Ärztekammer Rheinland-Pfalz” (Mainz, Germany). From July 1997 to January 2000, 3316 participants were enrolled in the LURIC study from the Cardiac Centre Ludwigshafen in Southwest Germany.

**Baseline Examination**

Angiographic CAD was diagnosed in participants with a visual lumen narrowing of ≥ 20% in at least one out of 15 coronary segments. ACS was classified in patients with unstable angina, non-ST elevation myocardial infarction (NSTEMI) and ST-elevation myocardial infarction (STEMI) as described previously [21]. Diabetes mellitus was defined as plasma glucose ≥ 126 mg/dL in the fasting state and/or plasma glucose ≥ 200 mg/dL 2 h after a 75 g oral glucose tolerance test, and/or HbA1c ≥ 6.5% and/or when participants were receiving oral anti-diabetics or insulin. Arterial hypertension was diagnosed if the systolic and/or diastolic blood pressure was 140 and/or 90 mmHg or higher or if patients had a significant history of arterial hypertension.
Laboratory Measurements

Venous blood sampling was performed after overnight fasting in the morning before coronary angiography. Details of laboratory measurements and study procedures have been published elsewhere [21, 22]. Serum cortisol was measured by a solid-phase chemiluminescence enzyme immunoassay (Cortisol-Immulite®, DPC Biermann GmbH, Bad Nauheim, Germany) on a DPC Immulite autosampler with intra-assay and inter-assay coefficients of variation (CV) of 6.8 to 9.0%, and 9.9 to 10.3%, respectively. Leucocytes were prepared by a whole blood lyse no-wash method according to manufacturer instructions (Becton-Dickinson) and were further analysed by using a four-colour flow cytometer (FACSCalibur, Becton-Dickinson) [22]. CD3, CD4, CD8, CD16/CD56 and CD 19 surface markers were used to identify main lymphocyte subsets and their respective counts with fluorescent-labelled antibodies (Becton-Dickinson, Heidelberg, Germany) [22].

Follow-up

Information on vital status was prospectively obtained from local registries. Participants were followed-up until death or until June 30, 2009 (censoring date). Two experienced clinicians who were blinded to any patient characteristics classified the causes of death based on review of death certificates, medical records of local hospitals and autopsy data. Cardiovascular mortality was classified in deaths due to cardiac and/or cerebrovascular causes. In cases of disagreement or uncertainty concerning the coding of a specific cause of death, the decision was made by a principal investigator of the LURIC study (W.M.).

Data analysis

Baseline characteristics are presented according to serum cortisol quartiles. Continuous data following a normal distribution are shown as means with standard deviations, while parameters with a skewed distribution are shown as medians with interquartile ranges. Categorical data are presented as percentages. Where appropriate, skewed variables are log(e) transformed before they are used in parametric analyses. Group differences across cortisol quartiles are calculated by analysis of variance.
(ANOVA) with $p$ for trend for continuous parameters or by chi-square test with $p$ for linear-by-linear test for categorical parameters. In addition, we use linear regression analyses with serum cortisol as the continuous explanatory (independent) variable and cardiovascular risk factors or lymphocyte subtypes (total counts) as continuous outcome (dependent) variables. These analyses are performed as crude analyses as well as with adjustment (forced entry) for various potential confounders.

Hazard ratios (HRs) with 95% confidence intervals (95% CI) for all-cause and cardiovascular mortality are calculated with Cox proportional hazard models using the first cortisol quartile as the reference. These analyses are performed without any adjustment (crude model), with adjustment for age (in years) and sex (women/men), and with additional adjustments for various cardiovascular risk factors. Cox proportional hazard analyses were adjusted for several a priori selected potential confounders including among lipids only triglycerides as we aim to avoid significant collinearity in our statistical models. Mortality analyses are performed in the entire cohort as well as in the subgroup of patients with ACS, and stratified by gender. A $p$-value <0.05 is considered statistically significant. Statistical analyses are performed by using SPSS Version 25.0 (IBM SPSS Inc., Chicago, IL, USA).

**Results**

After exclusion of participants with systemic glucocorticoid therapy and/or oral contraceptives and/or bronchodilators (all of these drugs significantly alter cortisol levels) and/or missing data of serum cortisol (n=264), 3052 individuals were eligible for analyses. In the study cohort, the mean age was 62.5 ± 10.6 years, 30% were women and median serum cortisol was 21.8 (17.6 to 26.4) µg/dL.

Baseline characteristics stratified by serum cortisol quartiles of all eligible study participants are presented in Table 1. In general, higher cortisol concentrations were associated with an adverse cardiovascular risk profile but there was no significant association with CAD and ACS. Linear regression analyses of serum cortisol and selected cardiovascular risk factors are shown in Table 2 and yield a significant association of serum cortisol with higher systolic blood pressure (BP), heart rate, fasting glucose, free fatty acids, triglycerides, N-terminal pro B-type natriuretic peptide (NT-proBNP) and lower glomerular filtration rate according to the abbreviated Modification of Diet in
Renal Disease (GFR-MDRD) formula. In a subsample of 2107 participants, data on lymphocyte subtypes were available showing a significant positive association of serum cortisol with CD16+CD56+ (natural killer cells) and CD3+CD8+ (T-suppressor) cell counts (see Table 3).

After a mean follow-up time of 8.95 ± 2.93 years, 856 participants (28%) deceased, and no participant was lost with respect to vital status. In 21 participants, we could not obtain sufficient data for classification of the cause of death. These participants were thus excluded for analyses on cardiovascular mortality which included 532 (17.4%) deaths due to cardiovascular causes. Data on Cox proportional HRs (with 95% CI) for all-cause and cardiovascular mortality according to cortisol quartiles with the first quartile as the reference in the entire study cohort are shown in Table 4. Crude analyses showed a moderately increased mortality in the fourth compared to the first quartile, but this effect was completely abrogated after adjustment for various cardiovascular risk factors (see Table 4).

Respective mortality data for the subgroup of patients with ACS are shown in Table 5 and show similar results as in the entire study cohort. All of our results remained materially unchanged when males and females were analysed separately and when we additionally adjusted for time of blood sampling, plasma aldosterone concentrations, or arterial hypertension as a binary variable using the BP cut-offs of 130/80 mm Hg or 140/90 mm Hg (data not shown).

**Discussion**

In this large cohort of patients referred to coronary angiography we have shown that morning serum cortisol is associated with an adverse cardiovascular risk profile and certain lymphocyte subtypes including natural killer cells. The moderate significant associations of serum cortisol with all-cause and cardiovascular mortality were completely attenuated after adjustment for cardiovascular risk factors.

Associations of serum cortisol with common cardiovascular risk factors such as BP, glucose levels and diabetes mellitus or dyslipidemia in our study confirm and extend previous investigations by new data on these issues in a large cohort of patients referred to coronary angiography [23-25]. Although we cannot prove causality from cross-sectional associations, there is compelling evidence
from clinical and mechanistic studies that cortisol excess may causally increase BP by e.g. altering vascular smooth muscle cell contractility and endothelial function with impaired nitric oxide synthesis, disturb glucose homeostasis by e.g. increased gluconeogenesis and insulin resistance and increase blood lipids by e.g. enhanced lipolysis [2, 5, 23-25]. The associations of cortisol with these cardiovascular risk factors may, however, also be partially driven by other pathways of the human stress response such as e.g. increased sympathetic activity or by other potential mediators or confounding factors, so that we must be cautious with claiming causality for our findings [26].

Given the various proposed effects of cortisol that may adversely affect cardiovascular risk and that include modification of cardiovascular risk factors as well as direct effects on the cardiovascular system, we evaluated whether these actions translate into increased mortality in patients with high cortisol levels [7-9]. Previous data on this topic are sparse, controversial and often limited by small sample sizes and insufficient adjustments for potential confounders [10-14]. Whereas Crawford et al. reported that higher morning cortisol concentrations were associated with a moderately significantly increased risk of incident cardiovascular events (fatal and non-fatal) in a meta-analyses of general population studies (696 cases and 6680 controls; odds ratio per SD of cortisol 1.18; 95% CI: 1.06 to 1.31), we extend these data by investigating data on all cause and cardiovascular mortality in patients referred to coronary angiography [9]. Despite an adverse cardiovascular risk profile in patients with high cortisol concentrations at baseline, there were only moderate significant associations of serum cortisol with increased all-cause and cardiovascular mortality in the LURIC study. Moreover, these associations were completely attenuated after adjustments for various cardiovascular risk factors. These findings could either be interpreted in the way that cortisol is simply associated with adverse cardiovascular outcomes by adversely affecting cardiovascular risk factors, so that adjustments for them diminishes any association of cortisol with mortality. Alternatively, our findings may also raise the hypothesis that cortisol may exert certain cardiovascular-protective actions. This might explain why we did not observe significant and independent associations of cortisol with mortality outcomes despite a significant association of cortisol with an adverse cardiovascular risk profile. It should also be considered, that associations of cortisol with cardiovascular risk could be partially driven by confounding factors of e.g. other stress
response pathways, thus even leading to an overestimation of the cortisol effect on mortality [27-29]. In support of cardiovascular protective actions of cortisol, accumulating evidence suggests that glucocorticoid receptor (GR) signaling in cardiomyocytes is critical for normal heart function, and cardiomyocyte-specific GR knock out mice die prematurely from cardiac hypertrophy that progressed to dilated cardiomyopathy and heart failure [2, 27-29]. In this context, there are also several reports of patients with adrenal insufficiency who suffered from heart failure and responded to hydrocortisone treatment with rapid improvement of ventricular function [30]. Various other potentially beneficial effects of cortisol on cardiovascular diseases such as certain anti-atherosclerotic or anti-inflammatory properties have been described [9, 16]. By contrast, glucocorticoids can also activate the mineralocorticoid receptor (MR), the target receptor for aldosterone, and this MR activation is considered to be largely detrimental for cardiac health, as evidenced by the survival benefit of MR antagonists such as spironolactone in patients with heart failure [27-31]. While the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) converts cortisol to its inactive metabolite cortisone in many organs such as e.g. the kidney, 11β-HSD2 expression is almost undetectable in cardiomyocytes [18]. Therefore, cortisol, that circulates at 100- to 1000-fold higher concentrations compared to aldosterone, is not inactivated in cardiomyocytes allowing signaling through both receptors, i.e. GR and MR in the heart [18]. Interestingly, there was no significant association of cortisol with CAD or ACS in the LURIC cohort supporting the hypothesis that adverse cardiovascular effects of cortisol on e.g. classic cardiovascular risk factors might hypothetically be counterbalanced by some cardioprotective actions of cortisol translating in an overall neutral effect on cardiovascular diseases. Therefore, and according to the allostasis concept, in patients with adrenal insufficiency and hydrocortisone replacement therapy as well as in patients suffering from cardiovascular diseases, deviations from an optimal hydrocortisone dose or optimal HPA activation in either direction might be detrimental if the cortisol level is inadequately high or low with reference to the prevailing stress condition [25]. A better characterization of the role of cortisol in cardiovascular diseases may thus have diagnostic and therapeutic implications to optimize glucocorticoid actions [18, 25, 29].

Considering the crucial role of cortisol in modulating the immune system we also evaluated associations of cortisol with circulating lymphocyte subtypes, and observed significant positive
associations with counts of CD16+CD56+ (natural killer) cells and CD3+CD8+ cells, that were formerly termed T-suppressor cells [19, 20, 28]. Natural killer cells are of particular importance as their function is significantly decreased in primary adrenal insufficiency and because they are considered to play an important immunoregulatory role in atherogenesis with potential proatherogenic but also some anti-atherosclerotic actions [19, 20, 32]. It is therefore challenging to interpret and discuss the clinical significance of the association between cortisol and natural killer cell counts. Nevertheless, this relationship was highly significant and clearly deserves further in-depth studies to confirm and characterize the association between cortisol and natural killer cells and their functions in the context of cardiovascular diseases [32, 33]. Apart from this, the link between cortisol and natural killer cells may also be relevant for infectious diseases such as COVID-19 [34].

Our findings are limited because we investigated a specific patient cohort so that our findings may not be generalizable to other patient groups or the general population. Moreover, the observational nature of our study design precludes definite conclusions regarding causality. We are well aware that morning cortisol concentrations are not an optimal tool for assessment of cortisol status as there are e.g. significant fluctuations, but previous investigations have indicated that morning serum cortisol concentrations are an acceptable diagnostic parameter, and in some studies plasma morning cortisol was even more closely associated with cardiovascular risk factors than 24h urinary cortisol. [9, 35]. We also have to acknowledge that the lymphocyte subtyping in our study is lacking some advanced tools for further characterizations of phenotypes and activity, thus limiting our conclusions on this topic [19, 20, 33, 34]. Furthermore, serum cortisol was measured by an immunoassay and not by a gold standard method, but given that we observed well-established associations of serum cortisol with parameters such as classic cardiovascular risk factors or leucocytes suggests an accurate validity of our measurements and methods. Classification of the causes of death by reviewing death certificates may be another limitation of our investigation. Main strengths of our study are the well characterized and large patient cohort allowing for multivariate adjusted models and novel data on e.g. endogenous serum cortisol and lymphocyte subtypes in patients referred to coronary angiography.
In conclusion, we have shown in a large cohort of patients referred to coronary angiography that serum cortisol concentrations are associated with an adverse cardiovascular risk profile including classic cardiovascular risk factors and natural killer cells. There was a moderate significant association of serum cortisol with higher mortality that was completely attenuated after adjustment for cardiovascular risk factors. Whether these findings reflect that adverse cardiovascular effects of cortisol are counterbalanced by beneficial actions of cortisol on cardiovascular health deserves further in-depth studies.
Acknowledgements

We thank all study participants.

Data availability

The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.
References

1. Timmermans S, Souffriau J, Libert C. A General Introduction to Glucocorticoid Biology. *Front Immunol.* 2019;10:1545.

2. Liu B, Zhang TN, Knight JK, Goodwin JE. The Glucocorticoid Receptor in Cardiovascular Health and Disease. *Cells.* 2019;8:1227.

3. Cruz-Topete D, Oakley RH, Cidlowski JA. Glucocorticoid Signaling and the Aging Heart. *Front Endocrinol (Lausanne).* 2020;11:347.

4. Fantidis P. The role of the stress-related anti-inflammatory hormones ACTH and cortisol in atherosclerosis. *Curr Vasc Pharmacol.* 2010;8:517-25.

5. Pivonello R, De Martino MC, Iacuaniello D, Simeoli C, Muscogiuri G, Carlomagno F, De Leo M, Cozzolino A, Colao A. Metabolic Alterations and Cardiovascular Outcomes of Cortisol Excess. *Front Horm Res.* 2016;46:54-65.

6. Neary NM, Booker OJ, Abel BS, Matta JR, Muldoon N, Sinaii N, Pettigrew RI, Nieman LK, Gharib AM. Hypercortisolism is associated with increased coronary arterial atherosclerosis: analysis of noninvasive coronary angiography using multidetector computerized tomography. *J Clin Endocrinol Metab.* 2013;98:2045-52.

7. Pivonello R, Isidori AM, De Martino MC, Newell-Price J, Biller BM, Colao A. Complications of Cushing's syndrome: state of the art. *Lancet Diabetes Endocrinol.* 2016;4:611-29.

8. Pimenta E, Wolley M, Stowasser M. Adverse cardiovascular outcomes of corticosteroid excess. *Endocrinology.* 2012;153:5137-42.

9. Crawford AA, Soderberg S, Kirschbaum C, Murphy L, Eliasson M, Ebrahim S, Davey Smith G, Olsson T, Sattar N, Lawlor DA, Timpson NJ, Reynolds RM, Walker BR. Morning plasma cortisol as a cardiovascular risk factor: findings from prospective cohort and Mendelian randomization studies. *Eur J Endocrinol.* 2019;181:429-438.

10. Nijm J, Jonasson L. Inflammation and cortisol response in coronary artery disease. *Ann Med.* 2009;41:224-33.
11. Aladio JM, Costa D, Matsudo M, Pérez de la Hoz A, González D, Brignoli A, Swieszkowski SP, Pérez de la Hoz R. Cortisol-Mediated Stress Response and Mortality in Acute Coronary Syndrome. *Curr Probl Cardiol.* 2020:100623.

12. Jutla SK, Yuyun MF, Quinn PA, Ng LL. Plasma cortisol and prognosis of patients with acute myocardial infarction. *J Cardiovasc Med (Hagerstown).* 2014;15:33-41.

13. Reynolds RM, Walker BR, Haw S, Newby DE, Mackay DF, Cobbe SM, Pell AC, Fischbacher C, Pringle S, Murdoch D, Oldroyd K, Macintyre P, O’Rourke B, Pell JP. Low serum cortisol predicts early death after acute myocardial infarction. *Crit Care Med.* 2010;38:973-5.

14. Reynolds RM, Ilyas B, Price JF, Fowkes FG, Newby DE, Webb DJ, Walker BR. Circulating plasma cortisol concentrations are not associated with coronary artery disease or peripheral vascular disease. *QJM.* 2009;102:469-75.

15. Drechsler C, Ritz E, Tomaschitz A, Pilz S, Schönfeld S, Blouin K, Bidlingmaier M, Hammer F, Krane V, März W, Allolio B, Fassnacht M, Wanner C. Aldosterone and cortisol affect the risk of sudden cardiac death in haemodialysis patients. *Eur Heart J.* 2013;34:578-87.

16. Cruz-Topete D, Cidlowski JA. One hormone, two actions: anti- and pro-inflammatory effects of glucocorticoids. *Neuroimmunomodulation.* 2015;22:20-32.

17. Richardson RV, Batchen EJ, Denvir MA, Gray GA, Chapman KE. Cardiac GR and MR: From Development to Pathology. *Trends Endocrinol Metab.* 2016;27:35-43.

18. Burford NG, Webster NA, Cruz-Topete D. Hypothalamic-Pituitary-Adrenal Axis Modulation of Glucocorticoids in the Cardiovascular System. *Int J Mol Sci.* 2017;18:2150.

19. Bonaccorsi I, De Pasquale C, Campana S, Barberi C, Cavaliere R, Benedetto F, Ferlazzo G. Natural killer cells in the innate immunity network of atherosclerosis. *Immunol Lett.* 2015;168:51-7.

20. Bancos I, Hazeldine J, Chortis V, Hampson P, Taylor AE, Lord JM, Arlt W. Primary adrenal insufficiency is associated with impaired natural killer cell function: a potential link to increased mortality. *Eur J Endocrinol.* 2017;176:471-480.
21. Winkelmann BR, März W, Boehm BO, Zotz R, Hager J, Hellstern P, Senges J; LURIC Study Group (Ludwigshafen RIsk and Cardiovascular Health). Rationale and design of the LURIC study--a resource for functional genomics, pharmacogenomics and long-term prognosis of cardiovascular disease. *Pharmacogenomics*. 2001;2(1 Suppl 1):S1-73.

22. Ó Hartaigh B, Bosch JA, Thomas GN, Lord JM, Pilz S, Loerbroks A, Kleber ME, Grammer TB, Fischer JE, Boehm BO, März W. Which leukocyte subsets predict cardiovascular mortality? From the LUdwigshafen RIsk and Cardiovascular Health (LURIC) Study. *Atherosclerosis*. 2012;224:161-9.

23. Scaroni C, Zilio M, Foti M, Boscaro M. Glucose Metabolism Abnormalities in Cushing Syndrome: From Molecular Basis to Clinical Management. *Endocr Rev.* 2017;38:189-219.

24. Ortiz R, Kluwe B, Odei JB, Echouffo Tcheugui JB, Sims M, Kalyani RR, Bertoni AG, Golden SH, Joseph JJ. The association of morning serum cortisol with glucose metabolism and diabetes: The Jackson Heart Study. *Psychoneuroendocrinology*. 2019;103:25-32.

25. Walker BR. Glucocorticoids and cardiovascular disease. *Eur J Endocrinol.* 2007;157:545-59.

26. Russell G, Lightman S. The human stress response. *Nat Rev Endocrinol.* 2019;15:525-534.

27. Oakley RH, Cruz-Topete D, He B, Foley JF, Myers PH, Xu X, Gomez-Sanchez CE, Chambon P, Willis MS, Cidlowski JA. Cardiomyocyte glucocorticoid and mineralocorticoid receptors directly and antagonistically regulate heart disease in mice. *Sci Signal.* 2019;12:eaau9685.

28. Oakley RH, Cidlowski JA. Glucocorticoid signaling in the heart: A cardiomyocyte perspective. *J Steroid Biochem Mol Biol.* 2015;153:27-34.

29. Hadoke PW, Iqbal J, Walker BR. Therapeutic manipulation of glucocorticoid metabolism in cardiovascular disease. *Br J Pharmacol.* 2009;156:689-712.

30. Devareddy A, Sawicki KT, Choundhury L, Wilcox JE. Reversible biventricular heart failure due to adrenal insufficiency. *JACC Case Rep.* 2020;2:411-413.

31. Tomaschitz A, Pilz S, Ritz E, Meinitzer A, Boehm BO, März W. Plasma aldosterone levels are associated with increased cardiovascular mortality: the Ludwigshafen Risk and Cardiovascular Health (LURIC) study. *Eur Heart J.* 2010;31:1237-47.
32. **Getz GS, Reardon CA.** Natural killer T cells in atherosclerosis. *Nat Rev Cardiol.* 2017;14:304-314.

33. **Capellino S, Claus M, Watzl C.** Regulation of natural killer cell activity by glucocorticoids, serotonin, dopamine, and epinephrine. *Cell Mol Immunol.* 2020;17:705-711.

34. **Alrubayyi A.** NK cells in COVID-19: protectors or opponents? *Nat Rev Immunol.* 2020;20:520.

35. **Reynolds RM, Walker BR, Syddall HE, Andrew R, Wood PJ, Whorwood CB, Phillips DI.** Altered control of cortisol secretion in adult men with low birth weight and cardiovascular risk factors. *J Clin Endocrinol Metab.* 2001;86:245-50.
Table 1. Baseline characteristics of the LURIC study population according to serum cortisol quartiles

| Serum cortisol quartiles | Variable                         | 1st quartile | 2nd quartile | 3rd quartile | 4th quartile | \( P \)-value |
|--------------------------|----------------------------------|--------------|--------------|--------------|--------------|---------------|
| Numbers                  | 779                              | 757          | 760          | 756          |              |               |
| Serum cortisol (µg/dL)   | 14.8 (12.7 to 16.3)              | 19.8 (18.8 to 20.8) | 24.1 (22.9 to 25.2) | 30.0 (27.9 to 33.2) | <0.001       |
| Age (years)              | 61.7 ± 10.5                      | 62.7 ± 10.5  | 62.0 ± 11.4  | 63.5 ± 10.1  | 0.010        |
| Females (%)              | 26.6                             | 28.0         | 29.5         | 37.2         | <0.001       |
| Sampling time (h:min ± min) | 7:25 ± 35                      | 7:24 ± 35    | 7:21 ± 30    | 7:19 ± 28    | <0.001       |
| Body mass index (kg/m²) | 27.8 ± 3.9                       | 27.7 ± 4.3   | 27.2 ± 3.8   | 27.3 ± 4.0   | 0.002        |
| Systolic BP (mm Hg)     | 138.8 ± 22.5                     | 140.0 ± 22.8 | 141.6 ± 24.1 | 144.4 ± 24.8 | <0.001       |
| Diastolic BP (mm Hg)    | 80.7 ± 11.4                      | 80.4 ± 11.0  | 81.0 ± 11.4  | 81.9 ± 11.7  | 0.024        |
| Resting heart rate (beats/min) | 66.1 ± 10.8                    | 67.6 ± 11.0  | 69.3 ± 11.5  | 70.0 ± 12.7  | <0.001       |
| Arterial hypertension (%) | 69.2                             | 68.8         | 74.3         | 77.4         | <0.001       |
| Diabetes mellitus (%)   | 34.0                             | 37.1         | 40.9         | 45.0         | <0.001       |
| Fasting glucose (mg/dL) | 89 (80 to 102)                   | 91 (83 to 104) | 91 (83 to 108) | 95 (85 to 115) | <0.001       |
| HbA1c (%)                | 5.9 (5.5 to 6.5)                 | 6.0 (5.5 to 6.5) | 6.0 (5.6 to 6.6) | 6.1 (5.6 to 6.8) | <0.001       |
| Current smoker (%)       | 20.2                             | 21.5         | 20.4         | 16.8         | 0.085        |
| HDL-cholesterol (mg/dL) | 37.7 ± 10.0                      | 38.6 ± 10.1  | 38.3 ± 10.8  | 39.8 ± 11.4  | 0.001        |
| LDL-cholesterol (mg/dL) | 115 ± 33                         | 118 ± 35     | 116 ± 33     | 117 ± 36     | 0.557        |
| Triglycerides (mg/dL)   | 146 (105 to 201)                 | 144 (111 to 196) | 141 (108 to 197) | 155 (115 to 211) | 0.001        |
| Free fatty acids (mmol/L) | 0.55 (0.39 to 0.78)            | 0.59 (0.41 to 0.83) | 0.65 (0.44 to 0.89) | 0.72 (0.51 to 1.04) | <0.001       |
| Plasma aldosterone (ng/dL) | 6.2 (3.8 to 9.6)               | 7.4 (4.6 to 11.6) | 8.4 (5.1 to 12.9) | 9.7 (6.0 to 15.1) | <0.001       |
| White blood cells (10³/µL) | 6.50 (5.49 to 7.80)            | 6.60 (5.56 to 7.99) | 6.80 (5.77 to 8.14) | 7.00 (5.85 to 8.30) | <0.001       |
| Serum potassium (mmol/L) | 4.2 ± 0.3                       | 4.2 ± 0.3    | 4.2 ± 0.3    | 4.2 ± 0.3    | 0.139        |
| Serum sodium (mmol/L)   | 141.4 ± 2.8                      | 141.3 ± 2.8  | 141.0 ± 2.7  | 141.0 ± 2.9  | 0.001        |
| GFR-MDRD (ml/min/1.73m²) | 84.5 ± 18.7                     | 82.0 ± 18.7  | 81.6 ± 19.5  | 80.0 ± 18.5  | <0.001       |
| C-reactive protein (mg/L) | 2.45 (1.08 to 5.68)            | 2.51 (1.11 to 7.05) | 2.68 (1.14 to 7.32) | 2.96 (1.08 to 8.10) | 0.053        |
| NT-proBNP (pg/mL)       | 247 (101 to 701)                | 262 (97 to 658) | 292 (109 to 915) | 347 (129 to 1042) | <0.001       |
| Coronary artery disease (%) | 78.4                             | 76.6         | 80.5         | 77.9         | 0.697        |
| Acute coronary syndrome (%) | 30.3                             | 30.4         | 32.1         | 31.9         | 0.387        |
| Medication use (%)      |                                  |              |              |              |               |
|                     | 66.8 ± 6.9 | 62.9 ± 7.2 | 65.4 ± 6.9 | 66.4 ± 6.3 |
|---------------------|------------|------------|------------|------------|
| **Beta-blockers**   | 66.8       | 62.9       | 65.4       | 66.4       |
| **ACE-inhibitors**  | 48.9       | 52.3       | 54.6       | 55.2       |
| **Statins**         | 47.4       | 46.6       | 48.7       | 47.2       |
| **Diuretics**       | 23.1       | 27.2       | 28.9       | 29.4       |
| **Insulin treatment** | 4.5        | 5.2        | 5.5        | 6.2        |

BP: blood pressure; GFR-MDRD: glomerular filtration rate according to the abbreviated Modification of Diet in Renal Disease formula; NT.proBNP: N-terminal pro B-type natriuretic peptide

Continuous data are presented as means ± standard deviation or as medians with 25th to 75th percentile and categorical data are presented as percentages.
Table 2. Linear regression analyses of cortisol* (explanatory variable) with cardiovascular risk factors (outcome variable) showing standardized Beta coefficients, their p value and the R square

|                      | Systolic BP | Heart rate | Fasting glucose* | Free fatty acids* | Triglycerides* | GFR-MDRD | NT-proBNP* |
|----------------------|-------------|------------|------------------|-------------------|----------------|----------|------------|
| **Crude**            | 0.075; 0.114; 0.127; 0.162; 0.060; -0.116; 0.087; | p<0.001; p<0.001; p<0.001; p<0.001; p<0.001; p<0.001; | R²=0.006 R²=0.013 R²=0.016 R²=0.026 R²=0.004 R²=0.014 R²=0.007 |
|                      | 0.061; 0.113; 0.123; 0.142; 0.068; -0.084; 0.068; | p<0.001; p<0.001; p<0.001; p<0.001; p<0.001; p<0.001; | R²=0.136 R²=0.018 R²=0.035 R²=0.082 R²=0.016 R²=0.181 R²=0.147 |
| **Model 1**          | 0.065; 0.106; 0.127; 0.142; 0.077; -0.084; 0.055; | p<0.001; p<0.001; p<0.001; p<0.001; p<0.001; p<0.001; | R²=0.161 R²=0.040 R²=0.083 R²=0.105 R²=0.072 R²=0.191 R²=0.229 |
|                      | 0.056; 0.080; 0.090; 0.101; 0.040; -0.061; 0.039; | p<0.001; p<0.001; p<0.001; p<0.001; p<0.001; p<0.001; | R²=0.175 R²=0.067 R²=0.148 R²=0.168 R²=0.133 R²=0.241 R²=0.278 |
| **Model 2**          | 0.068; 0.111; 0.123; 0.142; 0.068; -0.084; 0.068; | p<0.001; p<0.001; p<0.001; p<0.001; p<0.001; p<0.001; | R²=0.136 R²=0.018 R²=0.035 R²=0.082 R²=0.016 R²=0.181 R²=0.147 |
|                      | 0.061; 0.113; 0.123; 0.142; 0.068; -0.084; 0.068; | p<0.001; p<0.001; p<0.001; p<0.001; p<0.001; p<0.001; | R²=0.136 R²=0.018 R²=0.035 R²=0.082 R²=0.016 R²=0.181 R²=0.147 |
| **Model 3**          | 0.068; 0.111; 0.123; 0.142; 0.068; -0.084; 0.068; | p<0.001; p<0.001; p<0.001; p<0.001; p<0.001; p<0.001; | R²=0.136 R²=0.018 R²=0.035 R²=0.082 R²=0.016 R²=0.181 R²=0.147 |
|                      | 0.061; 0.113; 0.123; 0.142; 0.068; -0.084; 0.068; | p<0.001; p<0.001; p<0.001; p<0.001; p<0.001; p<0.001; | R²=0.136 R²=0.018 R²=0.035 R²=0.082 R²=0.016 R²=0.181 R²=0.147 |
| **Model 2 adjusted for age and sex** | 0.068; 0.111; 0.123; 0.142; 0.068; -0.084; 0.068; | p<0.001; p<0.001; p<0.001; p<0.001; p<0.001; p<0.001; | R²=0.136 R²=0.018 R²=0.035 R²=0.082 R²=0.016 R²=0.181 R²=0.147 |
| **Model 3 additionally adjusted for body mass index, current smokers and C-reactive protein** | 0.068; 0.111; 0.123; 0.142; 0.068; -0.084; 0.068; | p<0.001; p<0.001; p<0.001; p<0.001; p<0.001; p<0.001; | R²=0.136 R²=0.018 R²=0.035 R²=0.082 R²=0.016 R²=0.181 R²=0.147 |
| **Model 2 additionally adjusted for fasting glucose, triglycerides, systolic blood pressure (BP), heart rate, N-terminal pro-B-type natriuretic peptide (NT-proBNP) and glomerular filtration rate according to the abbreviated Modification of Diet in Renal Disease (GFR-MDRD) formula** | 0.068; 0.111; 0.123; 0.142; 0.068; -0.084; 0.068; | p<0.001; p<0.001; p<0.001; p<0.001; p<0.001; p<0.001; | R²=0.136 R²=0.018 R²=0.035 R²=0.082 R²=0.016 R²=0.181 R²=0.147 |

*p*logarithmically transformed variables (natural logarithm)
Table 3. Linear regression analyses of cortisol* (explanatory variable) with lymphocyte subsets (outcome variable) showing standardized Beta coefficients, their p value and the R square

|                | CD3+ (total T cells) | CD3+CD4+ (T helper cells) | CD3+CD8+* (T suppressor cells) | CD16+CD56+* (natural killer cells) | CD19+* (B-cells) |
|----------------|----------------------|----------------------------|---------------------------------|------------------------------------|------------------|
| Crude          | 0.029; p=0.184; R²=0.001 | 0.000; p=0.989; R²=0.000    | 0.067; p=0.002; R²=0.004        | 0.138; p<0.001; R²=0.019           | -0.014; p=0.532; R²=0.000 |
| Model 1        | 0.032; p=0.140; R²=0.048 | -0.003; p=0.890; R²=0.047   | 0.075; p=0.001; R²=0.029        | 0.138; p<0.001; R²=0.031           | -0.011; p=0.589; R²=0.064 |
| Model 2        | 0.032; p=0.134; R²=0.049 | 0.000; p=0.991; R²=0.047    | 0.076; p=0.001; R²=0.029        | 0.141; p<0.001; R²=0.039           | -0.013; p=0.558; R²=0.061 |

Model 1 adjusted for age and sex
Model 2 additionally adjusted for C-reactive protein
*logarithmically transformed variables (natural logarithm)
Table 4 Hazard ratios with 95% confidence intervals for all-cause and cardiovascular mortality according to serum cortisol quartiles in the entire study cohort

| Cortisol quartiles | 1st quartile | 2nd quartile | 3rd quartile | 4th quartile |
|--------------------|--------------|--------------|--------------|--------------|
| Range of values (µg/dL) | < 17.7 | 17.7 to 21.8 | 21.9 to 26.4 | > 26.4 |

### All-cause mortality

| Study participants at risk | 779 | 757 | 760 | 756 |
|----------------------------|-----|-----|-----|-----|
| Number of deaths           | 200 (25.7%) | 204 (26.9%) | 229 (30.1%) | 223 (29.5%) |

**Crude model**

- 1.0 reference
- 1.08 (0.89 to 1.31)
- 1.24 (1.02 to 1.50)
- 1.22 (1.00 to 1.47)

**Model 1**

- 1.0 reference
- 1.02 (0.84 to 1.24)
- 1.23 (1.02 to 1.49)
- 1.17 (0.96 to 1.41)

**Model 2**

- 1.0 reference
- 0.98 (0.81 to 1.20)
- 1.17 (0.97 to 1.42)
- 1.14 (0.94 to 1.38)

**Model 3**

- 1.0 reference
- 0.93 (0.76 to 1.14)
- 1.02 (0.84 to 1.25)
- 0.93 (0.76 to 1.14)

### Cardiovascular mortality

| Study participants at risk | 775 | 750 | 753 | 753 |
|----------------------------|-----|-----|-----|-----|
| Number of deaths           | 127 (16.4%) | 117 (15.6%) | 134 (17.8%) | 154 (20.5%) |

**Crude model**

- 1.0 reference
- 0.98 (0.76 to 1.26)
- 1.14 (0.89 to 1.45)
- 1.32 (1.04 to 1.67)

**Model 1 **

- 1.0 reference
- 0.92 (0.72 to 1.19)
- 1.13 (0.89 to 1.44)
- 1.26 (1.00 to 1.60)

**Model 2 **

- 1.0 reference
- 0.91 (0.71 to 1.17)
- 1.07 (0.84 to 1.37)
- 1.23 (0.97 to 1.56)

**Model 3**

- 1.0 reference
- 0.85 (0.65 to 1.10)
- 0.90 (0.70 to 1.17)
- 0.97 (0.76 to 1.25)

Model 1 adjusted for age and sex
Model 2 additionally adjusted for body mass index, current smokers and C-reactive protein
Model 3 additionally adjusted for fasting glucose, triglycerides, systolic blood pressure, heart rate, N-terminal pro B-type natriuretic peptide (NT-proBNP) and glomerular filtration rate according to the abbreviated Modification of Diet in Renal Disease (GFR-MDRD) formula
**Table 5** Hazard ratios with 95% confidence intervals for all-cause and cardiovascular mortality according to serum cortisol quartiles in patients with acute coronary syndromes

| Cortisol quartiles | 1st quartile | 2nd quartile | 3rd quartile | 4th quartile |
|--------------------|--------------|--------------|--------------|--------------|
| Range of values (µg/dL) | < 17.7 | 17.7 to 21.8 | 21.9 to 26.4 | > 26.4 |

### All-cause mortality

| Study participants at risk | 236 | 230 | 244 | 241 |
|----------------------------|-----|-----|-----|-----|
| Number of deaths           | 64 (27.1%) | 68 (29.6%) | 69 (28.3%) | 83 (34.4%) |

- **Crude model**: 1.0 reference<br>1.13 (0.80 to 1.59) 1.06 (0.76 to 1.49) 1.38 (0.99 to 1.91)
- **Model 1**: 1.0 reference<br>0.99 (0.70 to 1.39) 1.02 (0.72 to 1.43) 1.22 (0.88 to 1.70)
- **Model 2**: 1.0 reference<br>0.96 (0.68 to 1.35) 0.97 (0.69 to 1.38) 1.19 (0.85 to 1.65)
- **Model 3**: 1.0 reference<br>0.93 (0.65 to 1.33) 0.90 (0.63 to 1.28) 1.03 (0.73 to 1.46)

### Cardiovascular mortality

| Study participants at risk | 234 | 227 | 241 | 241 |
|----------------------------|-----|-----|-----|-----|
| Number of deaths           | 36 (15.4%) | 41 (18.1%) | 39 (16.2%) | 60 (24.9%) |

- **Crude model**: 1.0 reference<br>1.22 (0.78 to 1.91) 1.07 (0.68 to 1.69) 1.77 (1.17 to 2.68)
- **Model 1**: 1.0 reference<br>1.08 (0.69 to 1.69) 1.02 (0.65 to 1.61) 1.57 (1.04 to 2.38)
- **Model 2**: 1.0 reference<br>1.06 (0.67 to 1.66) 0.98 (0.62 to 1.55) 1.51 (0.99 to 2.31)
- **Model 3**: 1.0 reference<br>1.02 (0.64 to 1.63) 0.86 (0.54 to 1.39) 1.28 (0.82 to 2.00)

Model 1 adjusted for age and sex<br>Model 2 additionally adjusted for body mass index, current smokers and C-reactive protein<br>Model 3 additionally adjusted for fasting glucose, triglycerides, systolic blood pressure, heart rate, N-terminal pro B-type natriuretic peptide (NT-proBNP) and glomerular filtration rate according to the abbreviated Modification of Diet in Renal Disease (GFR-MDRD) formula.