European Journal of Preventive Cardiology

Original scientific paper

European Journal of Preventive Cardiology
2014, Vol. 21(3) 291–298

DOI: 10.1177/2047487312467903
ejpc.sagepub.com
SAGE

Dehydroepiandrosterone sulfate levels and risk of atrial fibrillation: The Rotterdam Study

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Abstract

Background: High plasma dehydroepiandrosterone sulfate (DHEAS) levels have been associated with a reduced risk of cardiovascular disease and atherosclerosis. To our knowledge, no previous follow-up study has investigated the association between DHEAS and the development of atrial fibrillation. Our objective was to investigate the association between DHEAS levels and incident atrial fibrillation.

Methods and results: The study was based on a random sample within the prospective population-based Rotterdam Study. The study population comprised 1180 participants without atrial fibrillation at baseline for whom baseline levels of DHEAS were measured in plasma. Atrial fibrillation was ascertained from centre visit electrocardiogram (ECG) assessments as well as medical records. During a mean follow-up period of 12.3 years, 129 participants developed atrial fibrillation. DHEAS levels were inversely associated with the risk of atrial fibrillation (hazard ratio (HR) per standard deviation (SD): 0.74, 95% confidence interval (CI): 0.58–0.94). Subjects in the highest DHEAS quartile had an almost three times lower risk of atrial fibrillation during follow-up, compared to those in the lowest DHEAS quartile (HR: 0.34, 95% CI: 0.18–0.64) adjusted for age, sex and cardiovascular risk factors.

Conclusion: DHEAS can be regarded as an important indicator of future atrial fibrillation in both men and women, independent of known cardiovascular risk factors.

Keywords
Atrial fibrillation, dehydroepiandrosterone sulfate, epidemiology

Received 15 August 2012; accepted 24 October 2012

Introduction

Dehydroepiandrosterone sulfate (DHEAS) is a precursor in the biosynthetic pathway of androgenic and estrogenic sex steroids. It is sulfated from dehydroepiandosterone (DHEA) by sulfotransferase in the adrenal glands and, in smaller quantities, by the liver and small intestine. In the blood, most of the DHEA is found as DHEAS which, due to its long plasma half-life, has a much higher concentration than any other sex steroid that shows little diurnal variation.[1] Previous studies indicated that higher DHEAS levels are associated with a lower risk of cardiovascular disease[2–3] and lower all-cause and cardiovascular mortality.[2,4–7] These associations might in part be explained by the results of later studies that suggested that high DHEAS levels are associated with a reduced risk of atherosclerosis.[8–10]

Both a history of cardiovascular disease as well as atherosclerosis are important independent risk factors for atrial fibrillation.[11,12] Atrial fibrillation is the most common sustained arrhythmia in the older population.

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Atrial fibrillation is associated with increased morbidity such as heart failure[13] and stroke[12] and also with increased total mortality as well as cardiovascular mortality.[12,14] Whether DHEAS levels are associated with the risk of atrial fibrillation is unknown.

The objective of this study was to investigate the association between DHEAS and incident atrial fibrillation in a population-based setting. As sex-specific effects may arise from DHEAS we also investigated the association in men and women separately.

Methods

Study population

The current study was performed within the Rotterdam Study, a population-based prospective cohort study, designed to examine the onset of and risk factors for disease in older adults, which started with a baseline visit between 1990 and 1993.[15] All participants aged 55 years and over in the Ommoord district of Rotterdam, The Netherlands were invited to participate ($n = 10{,}275$). Of them 7983 (78%) participated in the study. At baseline, participants were interviewed at home and were examined at the research centre and this examination included a 10 s, 12-lead electrocardiogram (ECG). Since then, participants have been followed continuously and were re-examined during three follow-up examination rounds (1993–1995, 1997–1999 and 2002–2004). Medical information is available on all participants through collaboration with general practitioners (GPs) and with the pharmacies in the area of Ommoord. The medical ethics committee of Erasmus University, Rotterdam, The Netherlands, approved the study, and all participants gave written informed consent.

DHEAS assessment

DHEAS levels were measured in plasma. Blood samples were drawn by venipuncture from non-fasting participants and collected in 5 ml tubes containing 0.5 ml sodium citrate solution. All tubes were stored on ice before and after blood sampling. Plasma levels of DHEAS were estimated using coated tube radioimmunoassays purchased from Diagnostic Systems Laboratories, Inc., Webster, Texas, USA.

Atrial fibrillation assessment

Prevalent and incident atrial fibrillation was ascertained using three methods.[16] At baseline and during follow-up examinations 12-lead ECGs were recorded, stored digitally, and analyzed by the Modular ECG Analysis System (MEANS).[17,18] To verify the diagnosis of atrial fibrillation, all ECGs with a diagnosis of atrial fibrillation, atrial flutter or any other rhythm disorder were recoded independently by two research physicians who were blinded to the MEANS diagnosis. The judgment of a cardiologist was sought and taken as decisive in those cases in which disagreement persisted between the coding physicians. Additionally, medical information was obtained from GPs which included their own results as well as results from physicians practising in hospitals and outpatient clinics. A patient was considered as a case of atrial fibrillation after diagnosis by a medical specialist or by a general practitioner, with ascertainment from an ECG. Finally, information was obtained from the national register of all hospital discharge diagnoses. Cases of atrial fibrillation occurring during a serious disease resulting in death, and cases during myocardial infarction or during cardiac operative procedures with recovery during the hospital admission were not included. We did not distinguish between atrial fibrillation and atrial flutter when we identified cases because both conditions are very similar with respect to risk factors and consequences.[19,20]

Vital status

Information on vital status was obtained on a regular basis from the Central Register of Population of the municipality of Rotterdam, from collaborating GPs and by collecting information during follow-up rounds. For the participants for whom information remained missing, the Central Registry of Genealogy of The Netherlands was consulted. This national institute receives population registry records of all inhabitants of The Netherlands who have died.

Covariable assessment

Body mass index (BMI) was calculated from weight in kilograms and height in meters. Blood pressure was measured twice at the right upper arm with a random zero mercury sphygmomanometer in the sitting position. Systolic and diastolic blood pressure were calculated as the average of two consecutive measurements. Data on blood pressure-lowering medication were obtained from questionnaires and from the participating pharmacies. Information on sex hormone therapy (ATC code: G03) was obtained in a similar way. Serum total cholesterol and high-density lipoprotein (HDL) levels were measured with an automated enzymatic method. Information on smoking status and alcohol use was obtained during a home interview. Smoking status was coded as never, former or current smoker. Alcohol use was calculated in grams per day. A history of myocardial infarction was defined as a self-reported myocardial infarction with hospital admission or the

presence of a myocardial infarction on the ECG.[21] The presence of heart failure at baseline was assessed using a validated score based on the definition of heart failure of the European Society of Cardiology.[22] Information on cardiovascular events during follow-up, including myocardial infarction and heart failure, was obtained from the GPs and was coded independently by two research physicians.[21,22] Prevalent diabetes mellitus was defined as the pre- or post-load serum glucose level of >11.1 mmol/l or the use of glucose-lowering medication. Carotid plaques were visualized by ultrasonography, as described in detail elsewhere.[11]

Population for analysis

Levels of DHEAS were assessed in a random sample of the Rotterdam Study participants. This random sample was drawn from participants who attended the baseline visit (1990–1993), and consisted of 1284 participants. The sample included 68 participants with prevalent atrial fibrillation at baseline who were excluded from the analysis. Also, 36 participants with missing baseline ECG data, due to technical problems or lack of qualified personnel, were excluded. This resulted in a study population of 1180 participants. All participants were followed from the day of blood sampling in the Rotterdam Study (1990–1993) to the date of onset of atrial fibrillation, the date of death or loss to follow-up or until 1 January 2008.

Statistical analysis

DHEAS levels were analyzed continuously (per standard deviation (SD) increment) and categorized into quartiles. Baseline characteristics are presented for the total group and for DHEAS quartiles. Age- and sex-adjusted hazard curves for atrial fibrillation were computed using Cox proportional hazards analyses, comparing the DHEAS quartiles. Furthermore, to assess the association of DHEAS levels with atrial fibrillation risk, adjusted for several well-known risk factors, we used Cox proportional hazards analyses. The proportional hazards assumption was tested using time-dependent interaction terms. We decided to use two models, firstly adjusting for age and sex, and secondly, additionally adjusting for other cardiovascular risk factors (systolic blood pressure, diastolic blood pressure, blood pressure lowering therapy, BMI, total and HDL cholesterol, smoking status, alcohol use, sex hormone therapy, prevalent myocardial infarction, heart failure and diabetes mellitus at baseline, and carotid plaque score). In secondary analyses, we first checked whether the additional adjustment for incident heart failure and/or myocardial infarction (time-dependent), added separately in one model, could modify our results. We included an interaction term of DHEAS levels*sex in the multivariate models. Second, we stratified analyses by sex, and used sex-specific DHEAS quartiles. Finally, because DHEAS levels are associated with smoking, we performed an analysis in non-smokers (never and past smokers). Data were analyzed using SPSS PASW statistical software, version 17.0 (IBM corporation, Armonk, New York, USA).

Results

Baseline characteristics

Follow-up included 129 participants with incident atrial fibrillation during a mean follow-up period of 12.3 years (SD = 4.7). Table 1 shows the baseline characteristics of the study population in the full sample and stratified by DHEAS quartiles. Participants in the higher DHEAS quartiles were significantly younger, and were more likely to be male compared to those in the lower quartiles. Also participants in the higher DHEAS quartile were more likely to smoke or to have a history of smoking compared to subjects in the lower quartiles, after adjustment for age and sex.

DHEAS levels and risk of atrial fibrillation

Figure 1 displays the age- and sex-adjusted hazard curves for risk of atrial fibrillation across DHEAS quartiles. The incidence rate of atrial fibrillation was lowest in the highest quartile of DHEAS. By 18 years of follow-up, the cumulative incidence in the highest quartile was 5.6% compared to 13.5% in the lowest quartile. After adjustment for age and sex, DHEAS levels were significantly associated with incident atrial fibrillation (hazard ratio (HR) per standard deviation (SD) = 0.76, 95% CI: 0.60–0.95; Table 2). Additional adjustment for cardiovascular risk factors only slightly changed this estimate (HR per SD = 0.74, 95% CI: 0.58–0.94). Participants in the highest quartile had a 66% lower risk of developing atrial fibrillation compared to the lowest quartile (HR on atrial fibrillation: 0.34, 95% CI: 0.18–0.64). Of the 1180 participants included in the analyses, 196 developed heart failure ($n = 137$) and/or myocardial infarction ($n = 91$) during follow-up of whom 21 participants later developed atrial fibrillation. However, the inclusion of a time-dependent covariate for incident heart failure and/or myocardial infarction in the multivariable model adjusted for age, sex and cardiovascular risk factors, resulted in only a minor change of the risk estimate.

Table 1. Baseline characteristics of study population

Characteristic	Full sample ($n = 1180$)	DHEAS quartiles			
		Q1 0.01–1.73 μmol/l ($n = 295$)	Q2 1.74–2.95 μmol/l ($n = 295$)	Q3 2.96–4.74 μmol/l ($n = 295$)	Q4 4.75–23.08 μmol/l ($n = 295$)
Age, years	69(8.4)	72(8.4)	70(8.3)[a]	68(8.0)[a]	66(7.1)[a]
Sex, male	547(46.3)	63(21.4)	124(42.0)[b]	154(52.0)[b]	206(69.8)[b]
Systolic blood pressure, mm Hg	138(21)	138(22)	138(21)	138(21)	136(21)
Diastolic blood pressure, mm Hg	73(11)	73(12)	73(11)	73(11)	74(10)
Use of antihypertensive medication	291(24.7)	81(27.5)	72(24.5)	75(25.3)	63(21.4)
BMI, kg/m^2	26.1(3.4)	26.2(3.6)	26.2(3.4)	26.4(3.4)	25.7(3.2)
Total cholesterol, mmol/l	6.7(1.2)	6.8(1.2)	6.7(1.2)	6.7(1.2)	6.6(1.2)
HDL cholesterol, mmol/l	1.4(0.4)	1.4(0.4)	1.4(0.4)	1.3(0.3)	1.3(0.4)
Smoking status:					
Current	279(23.6)	51(17.3)	50(16.9)	62(20.9)	116(39.3)[c]
Past	490(41.5)	101(34.2)	127(43.1)	139(47.0)	123(41.7)[c]
Alcohol use, g/day	11.9(15.5)	7.2(10.3)	10.5(14.3)	13.6(16.5)	16.3(18.2)
Myocardial infarction	148(12.5)	33(11.2)	34(11.5)	42(14.2)	39(13.2)
Heart failure	15(1.3)	4(1.4)	4(1.4)	4(1.4)	3(1.0)
Diabetes mellitus	84(7.1)	21(7.1)	27(9.2)	18(6.1)	18(6.1)
Carotid plaques:					
No	465(39.5)	113(38.3)	106(36.1)	116(39.3)	130(44.1)
Mild	143(12.1)	44(14.9)	29(9.9)	40(13.6)	30(10.2)
Moderate	182(15.3)	43(14.6)	53(17.7)	43(14.6)	43(14.6)
Severe	242(20.5)	58(19.7)	70(23.8)	57(19.3)	57(19.3)
Missing	148(12.5)	37(12.5)	37(12.6)	39(13.2)	35(11.9)
Use of steroid hormone therapy	14(1.2)	6(2.0)	4(1.4)	3(1.0)	1(0.3)

BMI: body mass index; DHEAS: dehydroepiandrosterone sulfate; HDL: high-density lipoprotein; Values are number of participants (%) or means (standard deviation (SD)); [a]$p < 0.05$, compared to lowest DHEAS quartile, adjusted for sex; [b]$p < 0.05$, compared to lowest DHEAS quartile, adjusted for age; [c]$p < 0.05$, compared to lowest DHEAS quartile, adjusted for age and sex.

DHEAS levels in the highest quartile were associated with a reduced risk of atrial fibrillation with an HR of 0.34 (95% CI: 0.19–0.64) for the participants in the highest quartile compared to those in the lowest quartile.

DHEAS levels and atrial fibrillation by gender

Mean DHEAS levels were 4.52 μmol/l (SD: 2.99) in men and 2.74 μmol/l (SD: 2.06) in women ($p < 0.01$; Table 2). However, there was no statistically significant interaction between DHEAS levels and sex in the association with atrial fibrillation (p for interaction = 0.17 in age-adjusted model, p for interaction = 0.08 after additional adjustment for cardiovascular risk factors). The association of DHEAS levels with risk of atrial fibrillation was slightly more apparent in women than in men in the stratified analyses. The HR for atrial fibrillation of participants in the highest quartile of DHEAS levels compared to the those in the lowest quartile was 0.42 (95% CI: 0.19–0.96) in men. In women, this association was even stronger with an HR of 0.33 (95% CI: 0.14–0.80).

DHEAS levels and atrial fibrillation in non-smokers

After exclusion of smokers, the study population included 901 participants of whom 113 developed atrial fibrillation during follow-up. In non-smokers, DHEAS levels were associated with atrial fibrillation (HR per SD increment: 0.62, 95% CI: 0.47–0.84). Also in this subsample, participants in the highest quartile of DHEAS levels had a 68% lower risk of developing atrial fibrillation during follow-up (HR: 0.32, 95% CI: 0.16–0.64).

Discussion

Our results suggest that high DHEAS levels are associated with a lower risk of atrial fibrillation, even after adjustment for age, sex, and known cardiovascular risk factors. Participants in the highest quartile of DHEAS levels were three times less likely to develop atrial fibrillation during follow-up when compared with participants in the lowest quartile of DHEAS levels. Although DHEAS levels varied significantly between men and women, the association of DHEAS levels and atrial

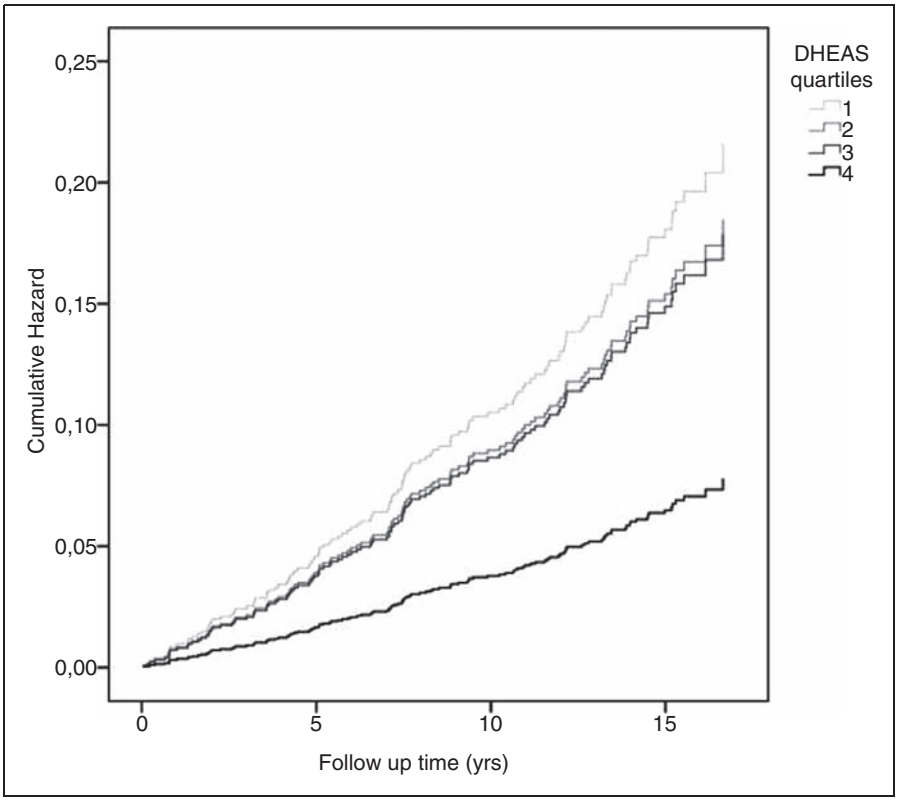

Figure 1. Age and sex adjusted hazard curves on risk for atrial fibrillation.
DHEAS: dehydroepiandrosterone sulfate.

fibrillation was not significantly different between men and women.

A previous cross-sectional study in 436 men of 65–94 years showed that subjects with atrial fibrillation had significantly lower DHEAS levels, even after adjustment for cardiovascular risk factors.[23] As far as we know, no previous population based follow-up study described the association of DHEAS levels with the development of atrial fibrillation. Similar to the inverse association of DHEAS levels with risk of atrial fibrillation that we report here, previous longitudinal studies suggested that higher DHEAS levels are associated with lower cardiovascular mortality.[2, 4–7] Trivedi and Khaw[5] showed that male participants in the highest DHEAS quartile were at lower risk for cardiovascular mortality compared to those in the lowest quartile (multivariate adjusted HR: 0.56, 95% CI: 0.32–0.97) in a community based study of 963 men and 1171 women aged 65–76 years. In women this study found no significant association but the point estimate suggested a similar magnitude of association (HR: 0.63, 95% CI: 0.29–1.34). Similar results have been presented by Ohlsson et al.,[7] in a multicentre study in 2644 men aged 69–81 years, showing that participants in the highest quartile had a lower risk of cardiovascular mortality (HR: 0.60, 95% CI: 0.44–0.83). Shufelt et al.[4] showed similar results in a study of 270 postmenopausal women, in which participants in the lowest tertile of DHEAS levels had a higher cardiovascular mortality (HR: 2.43, 95% CI: 1.06–5.56) compared to those in the highest tertile, after adjustment for cardiovascular risk factors.

Our results show that DHEAS levels varied significantly between men and women. Whereas men receive a continuous, albeit decreasing, supply of sex steroids from the testes during their whole postpubertal life,[24] DHEA is the quantitatively most significant source of sex steroids in postmenopausal women.[24] However, our results did not suggest any significant difference in the strength of the association of DHEAS levels with risk of atrial fibrillation between men and women. Previous studies suggested DHEAS levels to be associated with several health outcomes in both men and women. Several studies described DHEAS levels to be associated with mortality,[2,5–7] cardiovascular disease,[2,3] and atherosclerosis[10] in men and not in women, whereas other studies suggested DHEAS levels to be associated with mortality[4] and atherosclerosis[8,25] only

Table 2. Hazard rates of the association of dehydroepiandrosterone sulphate (DHEAS) levels with incident atrial fibrillation

			Model 1[a]	Model 2[b]
	N	*n*	HR (95% CI)	HR (95% CI)
			All	
DHEAS per SD (μmol/l)	1180	129	0.76 (0.60–0.95)	0.74 (0.58–0.94)
DHEAS quartiles:				
Q1. 0.01–1.73 μmol/l	295	39	Ref.	Ref.
Q2. 1.74–2.95 μmol/l	295	37	0.85 (0.55–1.40)	0.79 (0.49–1.25)
Q3. 2.96–4.74 μmol/l	295	36	0.82 (0.51–1.32)	0.74 (0.46–1.19)
Q4. 4.75–23.08 μmol/l	295	17	0.36 (0.19–0.66)	0.34 (0.18–0.64)
			Men	
DHEAS per SD (μmol/l)	547	67	0.83 (0.61–1.11)	0.79 (0.58–1.08)
DHEAS quartiles:				
Q1. 0.10–2.44 μmol/l	136	22	Ref.	Ref.
Q2. 2.45–3.87 μmol/l	137	20	0.90 (0.48–1.69)	0.88 (0.46–1.70)
Q3. 3.88–6.03 μmol/l	137	15	0.71 (0.36–1.40)	0.68 (0.34–1.39)
Q4. 6.04–23.08 μmol/l	137	10	0.46 (0.21–1.00)	0.42 (0.19–0.96)
			Women	
DHEAS per SD (μmol/l)	633	62	0.81 (0.68–0.96)	0.80 (0.67–0.95)
DHEAS quartiles:				
Q1. 0.01–1.29 μmol/l	158	22	Ref.	Ref.
Q2. 1.30–2.19 μmol/l	159	18	0.79 (0.42–1.47)	0.74 (0.39–1.32)
Q3. 2.20–3.81 μmol/l	158	15	0.74 (0.38–1.42)	0.67 (0.34–1.34)
Q4. 3.82–13.56 μmol/l	158	7	0.34 (0.14–0.82)	0.33 (0.14–0.80)

CI: confidence interval; HR: hazard ratio; Ref.: reference; SD: standard deviation; [a]Adjusted for age and sex; [b]Adjusted for age, sex, systolic blood pressure, diastolic blood pressure, blood pressure lowering therapy, body mass index (BMI), total and high-density lipoprotein (HDL) cholesterol, smoking status, alcohol use, sex hormone therapy, prevalent myocardial infarction, heart failure and diabetes mellitus at baseline, and carotid plaque score.

in women. The reason for these differences in results is unclear but is most likely due to differences in study populations and methodological approaches.[26]

Our data indicate that participants with high DHEAS levels were more likely to smoke. Previous studies also described smoking, which is a known risk factor for atrial fibrillation,[27] to be associated with higher DHEAS levels.[5,28,29] It is possible that these higher DHEAS levels in smokers are just a consequence of smoking but it is also possible that higher DHEAS levels play a protective role in the association of smoking with atrial fibrillation. To exclude the possible influence of smoking on the association of DHEAS levels with development of atrial fibrillation, we performed an additional analysis only in non-smokers. This analysis showed similar results compared to the results in the full sample.

Several explanations for the association of DHEAS levels with atrial fibrillation can be put forward. A previous study suggested that the zona reticularis of the adrenal gland, responsible for most DHEAS production, is highly susceptible to vascular damage.[30] It was therefore suggested that a low DHEAS level is only reflecting underlying vascular disease.[31] This might suggest that DHEAS levels are a non-etiological biomarker rather than a step in the causal pathway.

It has also been suggested that DHEAS could have tissue-specific effects, either directly or indirectly, by conversion to biologically-active androgens and estrogens.[1] Ii et al.[9] recently suggested that DHEAS inhibits vascular remodeling by reducing neointima formation after arterial injury. The observation that DHEAS has an inhibitory effect on cell growth and proliferation was supported in several recent studies that suggested DHEAS to be inversely associated with atherosclerosis.[8–10] Moreover, several studies suggested that DHEAS may have also an anti-inflammatory role.[32–34] Recent studies indicated that inflammation is associated with atrial fibrillation and plays a role in its etiology.[35]

The strengths of this study are its population-based design, with follow-up of up to 18 years. The study included extensive information on clinical details and multiple covariables. This allowed proper adjustment

and minimized the risk of false-positive misclassification, especially as diagnoses were unrelated to DHEAS levels and made prospectively and without knowledge of the research hypothesis. This study is limited in that we were not able to distinguish between paroxysmal and persistent atrial fibrillation. Also, as atrial fibrillation may occur without symptoms, false-negative misclassification may have occurred. However we used three different methods for the case-gathering and assessment, and included every clinically recognized case from two different sources of medical records. In addition, we included repeated screening ECG assessments of the study population at the research centre. Moreover, any false-negative misclassification is likely to be random and therefore will have led to an underestimation of the true risk estimate.

Since atrial fibrillation is highly prevalent in the elderly, and is associated with serious morbidity and mortality, it is a major public health problem.[36] Effective prevention strategies may arise from studies on biomarkers that are associated with the risk of atrial fibrillation. Our results show that DHEAS can be regarded as an important indicator for the risk of atrial fibrillation. Although further causal evidence is required, future measures aimed to prevent the occurrence of atrial fibrillation and improve the detection and management of this condition, should take into account the potential role of DHEAS.

Acknowledgements

The authors thank all the participants and staff of the Rotterdam Study, as well as the general practitioners and pharmacists of the Ommoord district for help with data collection and validation.

Funding

The Rotterdam Study is supported by the Erasmus Medical Centre and Erasmus University Rotterdam; The Netherlands Organization for Scientific Research (NWO); The Netherlands Organization for Health Research and Development (ZonMw); the Research Institute for Diseases in the Elderly; The Netherlands Heart Foundation; the Ministry of Education, Culture and Science; the Ministry of Health Welfare and Sports; the European Commission; and the Municipality of Rotterdam. This study was further supported by a grant from NOW/ZonMw (918.76.619).

Conflict of interest

None declared.

References

1. Labrie F. Adrenal androgens and intracrinology. *Semin Reprod Med* 2004; 22: 299–309.
2. Barrett-Connor E, Khaw KT and Yen SS. A prospective study of dehydroepiandrosterone sulfate, mortality, and cardiovascular disease. *N Engl J Med* 1986; 315: 1519–1524.
3. Mitchell LE, Sprecher DL, Borecki IB, et al. Evidence for an association between dehydroepiandrosterone sulfate and nonfatal, premature myocardial infarction in males. *Circulation* 1994; 89: 89–93.
4. Shufelt C, Bretsky P, Almeida CM, et al. DHEA-S levels and cardiovascular disease mortality in postmenopausal women: Results from the National Institutes of Health–National Heart, Lung, and Blood Institute (NHLBI)-sponsored Women's Ischemia Syndrome Evaluation (WISE). *J Clin Endocrinol Metab* 2010; 95: 4985–4992.
5. Trivedi DP and Khaw KT. Dehydroepiandrosterone sulfate and mortality in elderly men and women. *J Clin Endocrinol Metab* 2001; 86: 4171–4177.
6. Mazat L, Lafont S, Berr C, et al. Prospective measurements of dehydroepiandrosterone sulfate in a cohort of elderly subjects: relationship to gender, subjective health, smoking habits, and 10-year mortality. *Proc Natl Acad Sci U S A* 2001; 98: 8145–8150.
7. Ohlsson C, Labrie F, Barrett-Connor E, et al. Low serum levels of dehydroepiandrosterone sulfate predict all-cause and cardiovascular mortality in elderly Swedish men. *J Clin Endocrinol Metab* 2010; 95: 4406–4414.
8. Kanazawa I, Yamaguchi T, Yamamoto M, et al. Serum DHEA-S level is associated with the presence of atherosclerosis in postmenopausal women with type 2 diabetes mellitus. *Endocr J* 2008; 55: 667–675.
9. Ii M, Hoshiga M, Negoro N, et al. Adrenal androgen dehydroepiandrosterone sulfate inhibits vascular remodeling following arterial injury. *Atherosclerosis* 2009; 206: 77–85.
10. Yoshida S, Aihara K, Azuma H, et al. Dehydroepiandrosterone sulfate is inversely associated with sex-dependent diverse carotid atherosclerosis regardless of endothelial function. *Atherosclerosis* 2010; 212: 310–315.
11. Heeringa J, van der Kuip DA, Hofman A, et al. Subclinical atherosclerosis and risk of atrial fibrillation: The rotterdam study. *Arch Intern Med* 2007; 167: 382–387.
12. Levy S, Breithardt G, Campbell RW, et al. Atrial fibrillation: Current knowledge and recommendations for management. Working Group on Arrhythmias of the European Society of Cardiology. *Eur Heart J* 1998; 19: 1294–1320.
13. Wang TJ, Larson MG, Levy D, et al. Temporal relations of atrial fibrillation and congestive heart failure and their joint influence on mortality: The Framingham Heart Study. *Circulation* 2003; 107: 2920–2925.
14. Miyasaka Y, Barnes ME, Bailey KR, et al. Mortality trends in patients diagnosed with first atrial fibrillation: A 21-year community-based study. *J Am Coll Cardiol* 2007; 49: 986–992.
15. Hofman A, van Duijn CM, Franco OH, et al. The Rotterdam Study: 2012 objectives and design update. *Eur J Epidemiol* 2011; 26: 657–686.

16. Heeringa J, van der Kuip DA, Hofman A, et al. Prevalence, incidence and lifetime risk of atrial fibrillation: The Rotterdam study. *Eur Heart J* 2006; 27: 949–953.
17. Kors JA, van Herpen G, Wu J, et al. Validation of a new computer program for Minnesota coding. *J Electrocardiol* 1996; 29: S83–S88.
18. Van Bemmel JH, Kors JA and Van Herpen G. Methodology of the modular ECG analysis system MEANS. *Methods Inf Med* 1990; 29: 346–353.
19. Halligan SC, Gersh BJ, Brown Jr RD, et al. The natural history of lone atrial flutter. *Ann Intern Med* 2004; 140: 265–268.
20. Lelorier P, Humphries KH, Krahn A, et al. Prognostic differences between atrial fibrillation and atrial flutter. *Am J Cardiol* 2004; 93: 647–649.
21. De Torbal A, Boersma E, Kors JA, et al. Incidence of recognized and unrecognized myocardial infarction in men and women aged 55 and older: The Rotterdam Study. *Eur Heart J* 2006; 27: 729–736.
22. Bleumink GS, Knetsch AM, Sturkenboom MC, et al. Quantifying the heart failure epidemic: prevalence, incidence rate, lifetime risk and prognosis of heart failure. The Rotterdam Study. *Eur Heart J* 2004; 25: 1614–1619.
23. Ravaglia G, Forti P, Maioli F, et al. Dehydroepiandrosterone-sulfate serum levels and common age-related diseases: Results from a cross-sectional Italian study of a general elderly population. *Exp Gerontol* 2002; 37: 701–712.
24. Labrie F. DHEA, important source of sex steroids in men and even more in women. *Prog Brain Res* 2010; 182: 97–148.
25. Creatsa M, Armeni E, Stamatelopoulos K, et al. Circulating androgen levels are associated with subclinical atherosclerosis and arterial stiffness in healthy recently menopausal women. *Metabolism* 2012; 61: 193–201.
26. Glei DA, Goldman N, Weinstein M, et al. Dehydroepiandrosterone sulfate (DHEAS) and health: Does the relationship differ by sex? *Exp Gerontol* 2004; 39: 321–331.
27. D'Alessandro A, Boeckelmann I, Hammwhoner M, et al. Nicotine, cigarette smoking and cardiac arrhythmia: An overview. *Eur J Prev Cardiol* 2012; 19: 297–305.
28. Khaw KT, Tazuke S and Barrett-Connor E. Cigarette smoking and levels of adrenal androgens in postmenopausal women. *N Engl J Med* 1988; 318: 1705–1709.
29. Field AE, Colditz GA, Willett WC, et al. The relation of smoking, age, relative weight, and dietary intake to serum adrenal steroids, sex hormones, and sex hormone-binding globulin in middle-aged men. *J Clin Endocrinol Metab* 1994; 79: 1310–1316.
30. Angeli A, Masera RG, Magri F, et al. The adrenal cortex in physiological and pathological aging: issues of clinical relevance. *J Endocrinol Invest* 1999; 22: 13–18.
31. Sanders JL, Boudreau RM, Cappola AR, et al. Cardiovascular disease is associated with greater incident dehydroepiandrosterone sulfate decline in the oldest old: The cardiovascular health study all stars study. *J Am Geriatr Soc* 2010; 58: 421–426.
32. Arlt W and Hewison M. Hormones and immune function: Implications of aging. *Aging Cell* 2004; 3: 209–216.
33. Dillon JS. Dehydroepiandrosterone, dehydroepiandrosterone sulfate and related steroids: Their role in inflammatory, allergic and immunological disorders. *Curr Drug Targets Inflamm Allergy* 2005; 4: 377–385.
34. Chen CC and Parker Jr CR. Adrenal androgens and the immune system. *Semin Reprod Med* 2004; 22: 369–377.
35. Schoonderwoerd BA, Smit MD, Pen L, et al. New risk factors for atrial fibrillation: Causes of 'not-so-lone atrial fibrillation'. *Europace* 2008; 10: 668–673.
36. Clark AM and Hsu ZY. Addressing the growing burden of atrial fibrillation: Evidence, sustainability and accessibility more important than territory. *Eur J Cardiovasc Prev Rehabil* 2012; October 2012: 1089–1090.