# Physicians' lifestyle advice on primary and secondary CVD prevention in Germany: A comparison between the STAAB cohort study and the German subset of EUROASPIRE IV 

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#### Abstract

Background: We assessed prevalence and determinants on appropriate physician-led lifestyle advice (PLA) in a population-based sample of individuals without cardiovascular disease (CVD) compared to a sample of CVD patients.

Methods: PLA were assessed via questionnaire in a subsample of the population-based Characteristics and Course of Heart Failure Stages A-B and Determinants of Progression (STAAB) cohort free of CVD (primary prevention sample), and the German subset of the $4^{\text {th }}$ EUROASPIRE Survey (EA-IV) comprising CVD patients (secondary prevention sample). "PLA" was fulfilled if the participant reported on having ever been told by a physician to: stop smoking (current/former smokers), reduce weight (overweight/obese participants), increase physical activity (physically inactive participants), or keep a healthy diet (all participants). Factors associated with receiving at least $50 \%$ of PLA were identified using logistic regression: including in the first step age, sex, education, hypertension (HT), diabetes mellitus (DM), hyperlipidemia (HPL) and, in a second step, also lifestyle factors such as smoking, $\mathrm{BMI}>25 \mathrm{~kg} / \mathrm{m}^{2}$, and physical inactivity.

Results: Information on PLA was available in 665 STAAB participants ( $55 \pm 11$; $55 \%$ females) and in 536 EA-IV patients ( $67 \pm 9$; 18\% females). Except for smoking, appropriate PLA was more frequently given in the secondary compared to the primary prevention sample. Determinants associated with appropriate PLA in primary prevention were: DM (OR 5.61; 95\%CI 2.40-13.08), HPL (OR 2.92; 95\%Cl 2.03-4.21), and HT (OR 1.85; 95\%Cl 1.29-2.66); and in secondary prevention: age (OR per year $0.95 ; 95 \% \mathrm{Cl} 0.93-0.98$ ), and DM (OR 2.43; 95\%CI 1.43-4.12).

Conclusions: In primary prevention, PLA was mainly determined by the presence of vascular risk factors, whereas in secondary prevention the level of PLA was higher in general, but the association between CVD risk factors and PLA was less pronounced.


Key words: cardiovascular risk factors, primary prevention, secondary prevention, populationbased, primary care, lifestyle advice

## Introduction

Implementing and maintaining good lifestyle habits constitutes a central recommendation of guidelines on primary and secondary prevention of cardiovascular disease (CVD). ${ }^{1,2}$ CVD prevention aims to improve quality of life and life expectancy, both in primary and secondary care. ${ }^{3}$ The strong relationship between the risk of developing CVD and behavioural risk factors including lack of regular physical activity, smoking, and poor diet, is well described. ${ }^{3}$ Favourable adoption of lifestyle factors was shown to significantly reduced incident and recurrent CVD events as well as CVD mortality risk. ${ }^{3-8}$

The EUROASPIRE (EUROpean Action on Secondary and Primary prevention In order to Reduce Events) surveys iteratively report on the magnitude of and secular trends regarding lifestyle and risk factor management according to European guidelines in individuals at high CVD risk or with established CVD. ${ }^{6}$ For example, in EUROASPIRE IV (EA-IV), only half of coronary patients were advised to attend a CVD prevention and rehabilitation program. ${ }^{6,9,10}$ Time trends in lifestyle risk factors of the EUROASPIRE II, III and IV surveys of 9 European countries over a period of 14 years showed that the prevalence of adverse lifestyle factors such as obesity and central obesity increased by $7 \%$ and $6 \%$, respectively, and one out of five coronary patients still smoked at the time of evaluation, 6 months to three years after a cardiac event. ${ }^{11}$

To positively impact on cardiovascular risk, a multidisciplinary approach is advocated emphasizing lifestyle modification and risk factor management in both primary and secondary prevention. ${ }^{6,12,13}$ The importance of the treating physician promoting a healthy lifestyle in routine care has been underscored by various reports. ${ }^{6,14,15}$ Treating physicians play an important role in addressing good lifestyle habits and supporting patients to implement lifestyle into their everyday life using health education, health promotion, and behavioral counselling in primary and secondary prevention. ${ }^{13,16,15}$

However, little is known about the frequency of appropriate physician-led lifestyle advices (PLA) according to recent CVD prevention guidelines as well as factors determining, which patient will receive PLA in different health care settings. Therefore, we compared the prevalence and determinants of PLA between participants from the general population without CVD (i.e. primary prevention setting) and CVD patients (i.e., secondary prevention setting).

## Methods

## Primary prevention

Information regarding primary prevention sample was derived from a subset of the population-based Characteristics and Course of Heart Failure Stages A-B and Determinants of Progression (STAAB) cohort study. STAAB aims to examine the prevalence and natural course of early heart failure stages in a randomly selected representative sample of 5000 inhabitants of the City of Würzburg, aged 30-79 years. The study design and rationale have been published in detail previously. ${ }^{17}$ For the current analysis, we excluded participants with established cardiovascular disease (CVD) defined by a self-reported history of coronary artery disease, peripheral artery disease, or stroke.

## Data collection

Information on sociodemographic status (sex, age, education), smoking, recommended lifestyle advice given by physicians, and physical activity, was obtained via face-to-face interview. Weight, height, and blood pressure were obtained applying standard operative procedures. Blood pressure was measured on the dominant upper arm up to three times in sitting position. Self-reported history of CVD and cardiovascular risk factors (diabetes, high blood pressure, hyperlipidemia) were obtained by study physicians. Blood samples were collected in fasting participants. All examinations were performed according to standardized operation procedures.

## Secondary prevention setting

Information regarding secondary prevention sample was derived from the German subset of the "hospital-arm" of the EA-IV Survey recruited from the University Hospital Würzburg and the Dept. of Medicine, Klinik Kitzinger Land. Study subjects were approached six months to three years after hospitalization for a coronary event (index). EA-IV was conducted between 2012 and 2013 in 24 European countries to investigate the quality of cardiovascular risk factor control and guideline implementation in clinical practice. ${ }^{9}$ Patients were aged between 19 and 79 years and suffered from coronary heart disease (acute myocardial infarction, unstable angina, percutaneous coronary intervention, or coronary bypass grafting). The study design and rationale of the German subset of EA-IV have been published in detail previously. ${ }^{18}$

## Data collection

Medical records were reviewed to collect information about details of the index event. Sociodemographic status (sex, age and education), self-reported CVD risk factors (diabetes, high blood pressure, hyperlipidemia) and information on smoking were obtained in a personal patient interview 6 to 36 months after hospitalization for the index event. Weight, height, and blood pressure were assessed by physical examination. Blood pressure was measured twice on the dominant upper arm in sitting position. Provided lifestyle advice was collected by self-administered questionnaire. All examinations were performed according to EA-IV standards at the study visit.

For both prevention samples, body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Physical activity ( $\geq 150 \mathrm{~min} /$ week of moderate activity or $\geq 75 \mathrm{~min} /$ week of strenuous activity) was operationalized by the International Physical Activity Questionnaire (IPAQ). ${ }^{19}$

Due to the differences in the assessment of the individual's educational level in STAAB (school leave qualification) and EA-IV (vocational qualification), we harmonized the educational level as follows: no
formal schooling, less than primary schooling, primary school completed, secondary school completed, high school completed, intermediate between secondary level and university (e.g. technical training) to low school-leaving qualification. College/University completed and postgraduate degree were allocated to graduate of a higher school.

## Definition of recommended lifestyle advice

Physician-led appropriate lifestyle advice (PLA) was defined according to recent European Guidelines on CVD Prevention in Clinical Practice (version 2016) ${ }^{3}$ if the study participant reported on having ever been told by a physician: to stop smoking in current/former smoking participants; to reduce weight in participants with a $\mathrm{BMI}>25 \mathrm{~kg} / \mathrm{m}^{2}$; to increase physical activity in participants with less than $<150 \mathrm{~min} /$ week moderate activity; or to keep a healthy diet was recommended for all participants. The rate of adequately received lifestyle advices was calculated as the percentage of lifestyle advices divided by the number of all lifestyle advices a patient was potentially eligible for. For example, if a patient met criteria for three, but had received only two lifestyle advices, then he was considered having received $67 \%$ of adequate lifestyle advices.

## Determinants for receiving lifestyle advice

A priori, we identified a set of covariables potentially related to receiving PLA including sociodemographic factors (age, sex, education), self-reported CVD risk factors (diabetes, high blood pressure, hyperlipidaemia), and lifestyle risk factors (smoking, overweight, physical inactivity). ${ }^{6,20-22}$

## Data analysis

For categorical variables, we reported proportions (\%). For univariable analysis, $\chi^{2}$ - test for categorical and binary variables or Fisher's exact were used, as appropriate. Percentage of PLA was calculated and determinants of receiving at least 50\% of PLA in either study sample were identified. We calculated in a
sensitivity analysis proportions for PLA in STAAB participants at "high CVD risk" according to the recent definition for EA-IV patients from the "primary care arm". This definition based on the prescription of blood pressure lowering and/or lipid-lowering medication, and/or diabetes on treatment (diet or medications). ${ }^{23}$ In a first multivariable logistic regression analysis we calculated odds ratios (OR) with 95\% confidence intervals (CI) to assess whether sociodemographic status or self-reported CVD risk factors predicted the chance to receive $\geq 50 \%$ of PLA. In a second model, we adjusted also for CVD lifestyle risk factors. P-values $<0.05$ were considered statistically significant. Analyses were performed with IBM SPSS Statistics 23 (IBM ${ }^{\circledR}$ SPSS $^{\circledR}$ Statistics Version 23).

## Ethics and data protection

For the STAAB cohort study and the EA-IV study, approvals of the Ethics Committee of the Medical Faculty of the University of Würzburg (votes $98 / 13$ and $58 / 12$, respectively) and the data protection officer of the University of Würzburg (J-117.605-09/13) were obtained. All participants provided written informed consent prior to study examinations.

## Results

## Characteristics of the study participants in primary and secondary prevention

For the primary prevention sample, we assessed recommended lifestyle advice in a subsample of 707 participants via face-to-face interview. All 707 participants received and completed the lifestyle questionnaire. Of those, 42 (5.9\%) reported a history of CVD. Therefore, 665 participants (mean age $54.9 \pm 11.4$ years; $55.3 \%$ women) were included in the present analyses.

For the secondary prevention sample, recommended lifestyle advice was assessed in the entire 536 participants (mean age $67.4 \pm 8.9$ years; $17.7 \%$ women) from the German subsample of the EA-IV survey. Characteristics of both study populations are shown in Table 1.

In general, frequency of receiving PLA was higher in patients from the secondary compared to the primary prevention sample; "keep healthy diet": EA-IV 73.1\%, STAAB 43.9\% (p <0.001); "reduce weight": EA-IV 69.2\%, STAAB 43.8\% ( $p<0.001$ ); "increase physical activity": EA-IV: 71.4\%, STAAB: 52.1\% (p <0.01), except for the advice for smoking cessation: STAAB: 44.0\%, EA-IV: 36.7\% (p 0.08). According to the recent definition for EA-IV patients at high CVD risk from the "primary care arm" we identified 200 (31.0\%) STAAB participants at high CVD risk with higher proportions for PLA (reduce weight: 56.4\%; increase physical activity: 54.7\%), except for smoking with $42.7 \%$ compared to the entire STAAB primary prevention sample.

Significantly higher proportions for receiving $\geq 50 \%$ of PLA were observed in participants with selfreported hypertension (63.5\%, $\mathrm{p}<0.001$ ), high LDL-C levels ( $67.6 \%, \mathrm{p}<0.001$ ) and diabetes mellitus ( $85.4 \%, \mathrm{p}<0.001$ ) in the primary prevention sample.

In the secondary prevention sample, patients with self-reported diabetes mellitus (84.4\%, p 0.01) more frequently received $\geq 50 \%$ PLA; no statistically significant differences were observed for the other risk factors. Age, sex and education were not associated with receiving $\geq 50 \%$ PLA in both primary and secondary prevention settings (Table 1).

## Determinants for receiving $\mathbf{\geq 5 0 \%}$ appropriate lifestyle advices

In multivariable analyses, the chance for receiving $\geq 50 \%$ PLA in primary prevention was significantly higher in patients with self-reported diabetes mellitus (OR $5.61 ; 95 \% \mathrm{CI} 2.40-13.08$ ), hyperlipidemia (OR 2.92; $95 \% \mathrm{Cl} 2.03-4.21$ ), and hypertension (OR 1.85; 95\%CI 1.29-2.66). In secondary prevention setting, patients with diabetes mellitus was independently associated with a higher chance for receiving $\geq 50 \%$ PLA (OR 2.43 ; $95 \% \mathrm{Cl} 1.43-4.12$ ), whereas a decrease of receiving $\geq 50 \%$ PLA with advancing age (OR per year $0.95 ; 95 \% \mathrm{Cl} 0.93-0.98$ ) was observed in the total secondary prevention sample (Table 3a).

Adding lifestyle risk factors as potential determinants to the multivariable models did not substantially alter these results, except for $\mathrm{BMI}>25 \mathrm{~kg} / \mathrm{m}^{2}$ in the primary prevention sample (OR $1.80,95 \% \mathrm{Cl} 1.22$; 2.65) [Table 3b]. A relevant number of missing values occurred in the second multivariable model (18.2\% in STAAB and $28.7 \%$ in EA-IV) therefore we compared the distribution of the dependent as well as the independent variables between the total data set and the selected data set, and observed no variations. Consequently, we assume that the selected data set is representative for the whole sample and that values are missing at random (Supplement Table 3 a-b).

## Discussion

Our study assessed the prevalence and determinants for receiving PLA recommended in current clinical guidelines for CVD prevention in primary and secondary prevention settings. We observed that individuals in secondary prevention setting received significantly more often PLA compared to individuals without established CVD, except for smoking cessation. In the primary prevention setting, established CVD risk factors such as hypertension, hyperlipidemia and diabetes mellitus were strongly related to a higher chance of receiving $\geq 50 \%$ PLA. In secondary prevention settings, the only association between CVD risk factors and PLA was found for diabetes mellitus. In addition, we observe a decrease of PLA with increasing age in secondary prevention.

## Prevalence of adequate lifestyle advice in primary prevention samples

A direct comparison of lifestyle risk factor management with previous studies is limited due to varying target populations as well as due to differences in the assessment of lifestyle risk factors. In addition, most of the previous studies on this topic were conducted in primary care settings in patients with high CVD risk. The "primary care arm" from the EA-IV survey included 4579 patients at high risk of CVD in 14 European countries (mean age 58.8 years, $57.8 \%$ women) observed more frequently lifestyle advice (smoking cessation: $73.5 \%$, reduce weight: $65.2 \%$ and increase physical activity: $59.0 \%$ ) compared to the
entire STAAB primary prevention sample with $44.0 \%, 43.8 \%$ and $52.1 \%$, respectively. ${ }^{23}$ In addition, we observed still lower proportions in the predefined STAAB participants at high CVD risk. The higher recommendation rates in EA-IV could result from the fact, that also recommendations made by other healthcare professionals were considered. Another international cross-sectional observational study from the European Practice Assessment of Cardiovascular Risk Management (EPA Cardio) project ${ }^{24}$ in 9 European countries including 3723 individuals at high risk of CVD (mean age 66 years, $29.5 \%$ women; without established diabetes) from 268 general practices recorded lifestyle counselling by general practitioners documented within the medical records within the last 15 month. ${ }^{25}$ This study also observed higher proportions of advice for smoking cessation (65.5\%) compared to STAAB, but lower proportions for physical activity advice (38.8\%) were reported. ${ }^{25}$ Advice for a healthy diet was comparable with our findings (EPA Cardio: 42.9\%; STAAB: $43.8 \%) .{ }^{25}$

There may be a stronger tendency of physicians to recommend lifestyle advice in patients at high CVD risk compared to individuals with lower CVD risk as were present in the healthier STAAB subsample with less comorbidity, considering that the STAAB sample with a mean age 54.9 was overall younger than patients from the EA-IV survey (mean age 58.8 years) and EPA cardio project (mean age 66). ${ }^{23,25}$

## Prevalence of adequate lifestyle advice in secondary prevention samples

The EUROASPIRE "hospital arm" from the EA-IV survey with 7998 coronary patients (mean age at interview 64.0 years [ $\pm$ SD 11.3], 24.4\% women) observed higher rates of recommendation over all forms of advice in smoking cessation. Having received verbal advice was reported in $88.5 \%$, written information material in $42.6 \%$ and attended to a smoking cessation clinic in $18.6 \%$ which was more frequently when compared to the German EA-IV subsample with $34.2 \%, 13.1 \%$ and $8.2 \%$, respectively. Similarly, higher rates of smoking cessation advice compared to our data were observed in the New Zealand SNAPSHOT ACS study, a large prospective audit of 2299 patients (mean age 69 years [ $\pm$ SD 1.3 ], $35 \%$ women)

## Page 10 of $\mathbf{2 8}$

hospitalized with an acute coronary syndrome. ${ }^{26}$ In this survey, almost two-thirds of smoking patients received the advice to stop smoking. The higher recommendation rates regarding smoking advice in current studies underlines the insufficient recommendation to stop smoking advice in our German secondary prevention sample. The world-wide considerable potential of the improvement of healthy lifestyle in secondary prevention is also emphasized by the Prospective Urban Rural Epidemiology (PURE) cohort study in 153,996 adults, aged 35-70 years from 628 urban and rural communities of whom 7519 individuals reported CVD or stroke. From a total of three investigated healthy lifestyle behaviours (healthy eating, smoking cessation and physical activity) only $4.3 \%$ ( $95 \% \mathrm{Cl} 3.1-5.8 \%$ ) of the participants had received all healthy life style behaviours. ${ }^{27}$

## Comparison of lifestyle advice in primary and secondary prevention setting

We found PLA more frequently given in secondary compared to primary prevention sample, except for smoking. To the best of our knowledge, no comparable data was found with regard to directly analyzing recommended lifestyle advice between primary and secondary prevention settings based on comparable data collection and risk factor definitions. However, there are few studies focussing on differences in frequency of healthy lifestyle behaviours in patients using comparable data. A cross-sectional study by Wang et al. from the PURE-China study compared healthy lifestyle behaviours in individuals with and without CVD or stroke among 40,490 participants. Four healthy lifestyle habits were assessed (smoking cessation in current/former smokers, physical activity, healthy diet, self-reported alcohol consumption) and participants with CVD, stroke and diabetes were allocated to disease group, whereas participants without report any disease were allocated to control group. Overall, they reported significantly higher rates of smoking cessation, quit drinking alcoholic products in participants with disease compared to the healthy control group, whereas equal proportions were found for physical activity, and dietary intake was depended from income and rural vs. urban communities. Further, less than $10 \%$ had all four healthy lifestyle habits and the adoption of two or more lifestyle habits, increased with the number of CVD
events. ${ }^{28}$ In line with the present PURE study, there is evidence, that participants with CVD might be aware of their CVD related mortality and recurrent events and, therefore, more likely to follow healthy lifestyle habits. ${ }^{29,30}$ Our result showed that also at the physician's side, lifestyle advice in secondary prevention settings are more frequently recommend compared to primary prevention. However, considering that in our primary and secondary prevention samples, still a substantial number of patients are overweight/obese or physically inactive, there is a clear demand of improvement of increasing physicians' lifestyle advice in both, primary and secondary prevention settings.

## Determinants for adequate lifestyle advice

## Primary prevention setting

CVD risk factors (self-reported blood pressure, high LDL-C levels and diabetes) were strongly associated with a higher chance of receiving $\geq 50 \%$ PLA. A study from the EPA cardio project focused on determinants regarding recording rates of single lifestyle advice (stop smoking, diet and physical activity) from medical records in participants with high cardiovascular risk. ${ }^{25} \mathrm{~A}$ healthy diet was less often recorded with increasing age, but more often recorded in women as well as the advice for increasing physical activity compared to men. Furthermore, with increasing number of risk factors including CVD risk factors (elevated blood pressure, high total cholesterol and raised blood glucose) such as lifestyle risk factors (weight or BMI , physical activity and smoking), the documentation rates increased for all three lifestyle advice. ${ }^{25}$ This is in line with our finding from the STAAB primary prevention sample, where CVD risk factors also strongly related to receive PLA.

## Secondary prevention setting

We observed a lower probability of PLA with increasing age in our study. This is in line with results from previous studies. The SNAPSHOT ACS study assessed optimal preventive care defined by the patients having received at least one of exercise or healthy diet advice or advice for smoking cessation. ${ }^{31}$ The
probability of receiving in-hospital lifestyle advice was significantly lower ( p 0.012 ) in patients being older than 70 years compared to younger ones. ${ }^{31}$ The Survey of Health, Ageing and Retirement in Europe (SHARE) also showed improvable behavioural lifestyle habits in the elderly. ${ }^{32}$ However, as older patients benefit significantly from lifestyle interventions, the reasons for the lower frequency of adequate lifestyle recommendations by physicians in older age groups should be identified and amended. Individual comorbid conditions and the ability to adopt new lifestyle habits might be specifically considered in this patient group, e.g. by recommending light to moderate physical activities instead of reaching official recommended targets. ${ }^{33,34}$ A recent study examined the relationship between health beliefs and behavioural changes among younger (50-69 years) and older adults (70-89 years) and pointing out that compared to younger participants, older participants were more confused about healthy eating habits or how to stay healthy in general. ${ }^{35}$ This highlights that lifestyle interventions should be adapted according to the needs of older aged individuals including close guidance to avoid confusion with regard to health behaviours.

Furthermore, diabetes was the only cardiovascular risk factor, associated with a higher chance for lifestyle recommendations $\geq 50 \%$ in our secondary prevention sample. There is evidence, that especially patients with diabetes do not achieve treatment targets for secondary prevention. In addition, health care providers might specifically focus on this high-risk population because of their higher risk of allcause and CVD-related mortality. For example, the EA-III survey assessed determinants for risk factor control (failing recommended targets in total cholesterol $<4.5 \mathrm{mmol} / \mathrm{l}$, blood pressure $<149 / 90 \mathrm{mmHg}$ and non-smoking) in coronary subjects and observed a tree time higher risk for patients diagnosed with diabetes to failing secondary prevention targets compared to non-diabetics. ${ }^{36}$ The National Health Interview Survey (NHIS) examine the epidemic of diabetes in 22,305 subjects aged $\geq 18$ years from the United States. In male patients with diabetes, they observed a 1.56 times higher risk of death from allcause, a 1.72 times higher risk from heart disease, a 1.48 times higher risk from cerebrovascular disease

## Page 13 of $\mathbf{2 8}$

and 1.67 times risk higher from CVD than subjects without diabetes, respectively with no difference from female patients with diabetes. ${ }^{37}$ Further studies reported an increased mortality especially in patients with ACS and the Health Professional Follow up study shows that an overall healthy lifestyle is significantly associated with a $27 \%$ ( $p<0.001$ ) lower risk of CVD mortality in patients with type 2 diabetes. ${ }^{38,39,40,41}$

## Limitations

There might be a selection bias of invited participants. Healthier subjects from the primary prevention setting might tend to participate more frequently in the study, whereas present secondary prevention setting might represent a "healthier" CVD population, which survived long enough after index event and therefore able to undergo physical examinations and interviews. Therefore, our results might overestimate the level of PLA. The samples were derived from our local Würzburg population, thus, our results may not generalizable to other German regions due to different risk profiles, age structure, and distribution of lifestyle factors. We did not assess reasons for the individual lifestyle recommendations by the physician. Furthermore, the prevalence of self-reported lifestyle recommendations could be subject to recall bias. Due to the different assessment of the school-leave certificate in the primary and secondary sample with the resulting harmonization, a misclassification regarding this marker of socioeconomic status may have occurred. Finally, the multivariable model only accounted for a number of a priori defined factors leaving room for residual confounding.

## Conclusion

The present study demonstrates opportunities to improve the implementation of guideline-recommend lifestyle advice, in both primary and secondary prevention settings. Whereas presence of cardiovascular risk factors was strongly associated with the probability of receiving at least $50 \%$ of adequate PLA in primary prevention, such associations were absent or less pronounced in secondary prevention. This

## Page $\mathbf{1 4}$ of $\mathbf{2 8}$

might indicate that physicians are more focused on clinical risk factors instead of lifestyle risk factors when recommending lifestyle advice in the general population. In our secondary prevention sample, older patients with CVD had a lower chance of receiving PLA. Therefore, a closer guidance of lifestyle counselling with respect to the needs and quality of life should be considered.

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## Authors' contributions

TT contributed to conception and design of the STAAB cohort study and drafted the manuscript. CMo contributed to data acquisition and interpretation. CM contributed to analysis. GG contributed to study conception, analysis, and interpretation. MW contributed to study conception and data acquisition. SS and PUH designed the STAAB cohort study and the German subset of EUROASPIRE IV, acquired funding and drafted the manuscript. VW, KK, DW, GE, RL and WK contributed to data acquisition of the EA-IV survey. All authors critically revised and approved the final manuscript.

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