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CLINICAL RESEARCH
Prevention and epidemiology

Duration and life-stage of antibiotic use and risk of cardiovascular events in women

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Aims Growing data suggest that antibiotic exposure is associated with a long-lasting alteration in gut microbiota, and may be related to subsequent cardiovascular disease (CVD). We investigated associations of life-stage and duration of antibiotic exposure during adulthood with subsequent CVD events.

Methods and results This study included 36 429 women initially free of CVD and cancer from the Nurses' Health Study. We estimated hazard ratios (HRs) for CVD (a composite endpoint of coronary heart disease or stroke) according to duration of antibiotic use in young (age 20–39), middle (age 40–59), and late (age 60 and older) adulthood. During an average of 7.6 years of follow-up, 1056 participants developed CVD. Women with long-term use of antibiotics (for ≥2 months) in late adulthood had a significantly increased risk of CVD (HR 1.32, 95% confidence interval 1.03–1.70) after adjustment for covariates (such as demographic factors, diet and lifestyle, reasons for antibiotic use, overweight or obesity, disease status, and other medication use), as compared to women who did not use antibiotics in this life-stage. Longer duration of antibiotic use in middle adulthood was also related to higher risk of CVD (P trend = 0.003) after controlling for these covariates. There was no significant relationship between the use in young adulthood and the risk of CVD.

Conclusion In this study which examined the antibiotic use in different life-stages, longer duration of exposure to antibiotics in the middle and older adulthood was related to an increased risk of future CVD events among elderly women at usual risk.

Keywords Risk factors • Cardiovascular disease • Antibiotics

Introduction

A recent study suggests that a substantial proportion of antibiotics are not prescribed appropriately in an outpatient clinical setting.[1] Specific antibiotic classes have been linked to increased risk of prolongation of the QT interval and the potentially deadly rhythm, Torsades de Pointes,[2] and some antibiotics also stimulate proliferation and activity of macrophages[3,4] which may induce atherosclerosis.[5] Also, antibiotic exposure has been found to affect balance and composition of gut microbiota,[6–8] e.g. increasing gut pathogens[9–11] and decreasing the abundance of probiotic bacteria,[12,13] which are related to cardiometabolic abnormalities.[14]

Some, though not all, previous studies have reported positive associations between macrolide antibiotics and cardiovascular and sudden cardiac death[15–26] particularly among patients with a pre-existing disease such as coronary heart disease (CHD), peripheral artery

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disease, infections, or pneumonia. Studies have examined associations for antibiotics of this class with cardiovascular disease (CVD) events.[15,17,21,26–32] A meta-analysis has shown that macrolide antibiotic use (for 3 days to 1 year) is associated with sudden cardiac death, ventricular tachyarrhythmias, and cardiovascular death.[15] A study reported that clarithromycin use was associated with myocardial infarction (MI) incidence during 2 weeks after the first antibiotic prescription, as compared to amoxicillin; however, there was no significantly increased risk of MI for 2 weeks to 3 years after the start of antibiotic treatment.[26] Another recent meta-analysis also showed that use of macrolide antibiotics was related to increased risks of cardiovascular outcomes for a limited follow-up period (<30 days); however, there was no significant relationship between macrolide antibiotics and cardiovascular outcomes among studies with a longer follow-up time (>30 days to >3 years).[17] These observations suggest that the adverse cardiovascular effect of antibiotics may be weakening with time; however, associations of antibiotic use and long-term cardiovascular events has yet to be established. In addition, an evaluation of duration of antibiotic exposure for subsequent risk of CVD would be important. According to a study of patients with respiratory tract infections, a 7-day-or-more treatment of clarithromycin was associated with more cardiovascular events than a shorter-term (<3-day) treatment.[21] To the best of our knowledge, no longitudinal study has investigated associations of duration of antibiotic use in different phases of adulthood (young, middle, and late adulthood) with the CVD incidence in a population at usual risk.

Therefore, in the Nurses' Health Study (NHS) which has collected detailed information on cumulative antibiotic use during adulthood, we investigated associations of duration and life-stage of antibiotic use with CVD risk over 8 years.

Methods

Study participants

The NHS is an ongoing cohort study established in 1976 of 121 701 female registered nurses in the USA who were 30–55 years of age at enrolment. Information on demographics, lifestyle factors, medical history, and disease status was collected through a self-administered questionnaire in 1976 and has been updated every 2 years through follow-up questionnaires.

The baseline year for the present analysis was set as 2004 when information on antibiotic use was assessed; a total of 90 853 women returned the 2004 questionnaire[33] (*Figure 1*). Of the 90 853 women, 57 726 reported data on antibiotic usage during young (age 20–39), middle (age 40–59), and late (age 60+) adulthood in life. We excluded women with prior histories of MI, angina pectoris, or stroke ($n = 8127$) or cancer ($n = 11\,311$) in 2004, and those with no available data on demographic factors, such as body weight, height, smoking habit, or physical activity ($n = 2469$). Women who only returned the 2004 questionnaire were also excluded, leaving 36 851 women for our analyses. Among the 36 851 participants, 422 were aged <60 y in 2004, and they did not have the ability to provide information on antibiotics during late adulthood. After we excluded these 422 women to reduce the potential for detection bias, a total of 36 429 women aged ≥60 years were included in this study. Additional information on the study design and participants are described in Supplementary material online, *Methods*. The study was approved by the institutional review board at the Brigham and Women's Hospital.

Assessment of duration and life-stage of antibiotic use

In the 2004 questionnaire, women were asked to indicate the total time using antibiotics with eight categories ranging from none to 5+ years (excluding skin creams, mouthwash, or isoniazid) for time periods between age 20–39, 40–59, and age 60+. We combined participants with 2 months or more use, and categorized participants into four groups (none, <15 days, 15 days to <2 months, 2 months or more) to have a reasonable sample size for each category. Antibiotic use for long-term was defined as ≥2 months based on the previous analysis in the NHS.[33] The most common reason for the antibiotic use (respiratory infection, urinary tract infection, acne/rosacea, chronic bronchitis, dental, or other) was also assessed. Information on specific type of antibiotics or daily dosage was not available.

Ascertainment of cardiovascular disease

Incident CVD was defined as a composite endpoint of CHD (non-fatal MI or fatal CHD) and total stroke (non-fatal or fatal).[34,35] Details about outcome ascertainment are fully described in Supplementary material online, *Methods*. Deaths were reported by the next of kin or the postal system or identified by searching the National Death Index. Causes of death were primarily confirmed by review of autopsy reports, medical records, and death certificates.[36]

Statistical analysis

We calculated person-years of follow-up from the return date of the 2004 questionnaire until date of CVD diagnosis, date of death, or end of follow-up (30 June 2012), whichever occurred first. Cox proportional hazard regression, including age as the time scale and stratified by calendar time in 2-year intervals, was performed to calculate hazard ratios (HRs) for CVD according to categories of duration of antibiotic use (none, <15 days, 15 days to <2 months, and ≥2 months) in each young, middle, or late adulthood. Tests of linear trend (P_{trend}) across categories of duration of antibiotic use were conducted with the use of the Wald test of a continuous variable on the basis of midpoint day for each category; the highest category (≥2 months) conservatively set to 60 days. We tested the proportional hazard assumption using interaction terms between antibiotic use and follow-up time, and the assumption was unlikely violated ($P > 0.05$). Covariates of multivariate-adjusted Model 1 included reasons for antibiotic use and traditional risk factors for CVD [demographic, diet, lifestyle factors, and body mass index (BMI)] based on previous publications of the NHS and guidelines on CVD prevention.[37,38] Multivariate-adjusted Model 2 included additional covariates to examine whether other metabolic risk factors (hypercholesterolaemia, hypertension, and diabetes) and medication use [aspirin, non-aspirin non-steroidal anti-inflammatory drugs (NSAIDs) or COX-2 inhibitors, calcium channel blockers, statin, H2 blocker, proton pump inhibitors, and steroid][39,40] influenced the associations.

The antibiotic use in each adulthood was treated separately; sensitivity analyses were performed considering usage in prior life-stages. Other sensitivity analyses were also conducted to examine whether competing risks[41] of non-cardiovascular events (i.e. all cancers) were present. We also compared HRs for CVD during the first 4 years vs. the last 4 years of the follow-up period to investigate whether associations were different over 8 years of total follow-up. We performed stratified analyses to assess potential effect modification according to disease status and indications for antibiotic use. The *P*-values for interactions between antibiotic use and these factors were calculated by the log likelihood ratio test which compared models with and without cross-product interaction terms. Further, being obesity is one of important modifiable risk factors for CVD,[37,38] and obese individuals may have altered gut microbiome

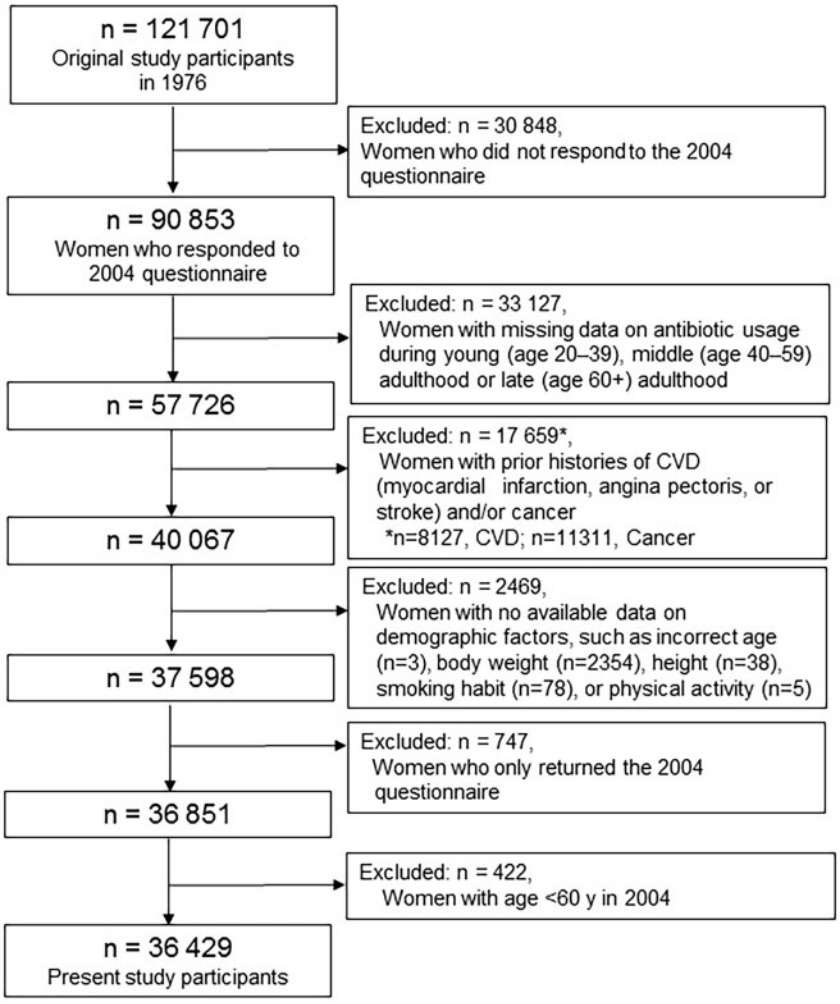


Figure 1 Selection of study participants.

compared with lean individuals.[42] To examine whether the presence of obesity may augment the effect of antibiotic exposure for CVD, we calculated HRs for CVD according to a joint classification of antibiotics and BMI. Statistical analyses were performed with SAS version 9.4; *P*-value <0.05 was considered statistically significant.

Results

Women with longer duration of antibiotic use were more likely to have unfavourable cardiovascular risk profiles including having a family history of MI, higher BMI, metabolic abnormalities (such as hypertension, hypercholesterolaemia, and diabetes); also they were more likely to use other medications (Supplementary material online, *Table S1*). The most common indication for using antibiotics was for a respiratory infection, with urinary tract infections, and dental indications also being common indications. Study participant characteristics were similar according to categories of use during middle- (40–59 years) and young adulthood (20–39 years) (Supplementary material online, *Table S2*).

Over 276 409 person-years of follow-up [average 7.6 (SD 1.0) years], 1056 participants developed CVD. A longer duration of exposure to antibiotics in late- (P_{trend} = 0.03) and middle adulthood (P_{trend} = 0.001) was significantly associated with higher risk of CVD in age-adjusted model (*Table 1*). The association remained significant after adjustment for covariates in the multivariate-adjusted Model 1 (P_{trend} = 0.03 for the use in late adulthood; P_{trend} < 0.001 for the use in middle adulthood). In the Model 1, as compared to women did not use antibiotics, those with long-term use (for ≥2 months) in late- or middle adulthood had an adjusted HR of 1.44 [95% confidence interval (CI) 1.13–1.85] or HR 1.39 (95% CI 1.04–1.85) for CVD, respectively. After additional adjustment for covariates in Model 2, the long-term use in late adulthood was significantly associated with an increased risk of CVD (HR 1.32, 95% CI 1.03–1.70). Women with long-term use in middle adulthood showed a multivariate-adjusted HR of 1.28 (95% CI 0.95–1.70) for CVD in the Model 2 (P_{trend} = 0.003). Antibiotic use in young adulthood was not significantly associated with the CVD incidence. Results were similar when we performed sensitivity analysis excluding women with a prior history

Table 1 Hazard ratios for CVD according to duration of antibiotic use in late-, middle-, and young adulthood

	Total time using antibiotics				P_{trend}
	None	<15 days	15 days to <2 months	2 months or more	
Late-adulthood (60 years and older) use					
Incident cases/person-years	141/52 312	515/134 255	264/65 720	136/24 123	
Age-adjusted HR	1.00	1.14 (0.94–1.37)	1.11 (0.90–1.37)	1.42 (1.12–1.81)	0.03
Multivariate-adjusted HR, Model 1	1.00	1.15 (0.95–1.40)	1.14 (0.92–1.41)	1.44 (1.13–1.85)	0.03
Multivariate-adjusted HR, Model 2	1.00	1.14 (0.93–1.38)	1.09 (0.88–1.36)	1.32 (1.03–1.70)	0.17
Middle-adulthood (during age 40–59) use					
Incident cases/person-years	104/23 523	565/155 872	284/72 309	103/24 705	
Age-adjusted HR	1.00	0.95 (0.77–1.17)	1.13 (0.90–1.42)	1.28 (0.97–1.69)	0.001
Multivariate-adjusted HR, Model 1	1.00	0.99 (0.80–1.24)	1.20 (0.95–1.52)	1.39 (1.04–1.85)	<0.001
Multivariate-adjusted HR, Model 2	1.00	0.96 (0.77–1.20)	1.15 (0.90–1.46)	1.28 (0.95–1.70)	0.003
Young-adulthood (during age 20–39) use					
Incident cases/person-years	203/43 502	601/157 044	187/55 733	65/20 131	
Age-adjusted HR	1.00	1.07 (0.90–1.26)	1.03 (0.84–1.27)	1.07 (0.80–1.42)	0.91
Multivariate-adjusted HR, Model 1	1.00	1.10 (0.93–1.29)	1.09 (0.88–1.34)	1.13 (0.84–1.51)	0.60
Multivariate-adjusted HR, Model 2	1.00	1.07 (0.91–1.27)	1.05 (0.85–1.30)	1.06 (0.79–1.42)	0.94

Model 1: age, menopausal status and postmenopausal hormone use, race, family history of myocardial infarction, reasons for using antibiotics, smoking (never, former, or current), physical activity (quintiles), alcohol consumption (none, 0–4.9, 5–14.9, or ≥15.0 g/day), Alternate Healthy Eating Index without alcohol (quintiles), and BMI (<25, 25–<30, or 30 kg/m^2). Model 2: Model 1 + hypertension, hypercholesterolaemia, diabetes, aspirin, NSAIDs or COX-2 inhibitors, calcium channel blockers, statin, H2 blocker, proton pump inhibitors, and steroid.

of major diseases before the baseline (2004) (Supplementary material online, *Table S3*). There was no significant effect modification by follow-up time on associations of antibiotics use with the CVD incidence (Supplementary material online, *Table S4*). Also, results of the competing risk analyses considering risks of non-CVD events (Supplementary material online, *Table S5*) were identical to results presented in *Table 1*.

Figure 2 shows results when outcomes of stroke and CHD were examined separately. Women who used antibiotics for <15 days (HR 1.56, 95% CI 1.03–2.34) or 15 days to <2 months (HR 1.65, 95% CI 1.07–2.55) during middle adulthood (*B*) showed an increased risk of CHD as compared to those who did not use in this period. Furthermore, we assessed risks of total exposure to antibiotics for stroke and CHD based on the sum of average days using antibiotics after age 40. Since the use of antibiotics during young adulthood was not significantly associated with the outcomes, we assessed the total exposure of antibiotics especially after age 40. Compared with non-users, women who used antibiotics for average 15 days to <2 months, and average ≥2 months had an adjusted HR of 2.30 (95% CI 1.21–4.38) and HR 2.00 (95% CI 1.05–3.79) for CHD (*Figure 3*). The total exposure of antibiotics after age 40 did not show a significant association with stroke (data not shown).

We did not observe significant interactions of the antibiotic use with overweight or obesity, dyslipidaemia, emphysema or chronic bronchitis, postmenopausal hormone use, or reasons of antibiotic use for respiratory infections on the CVD risk ($P_{interaction}$ > 0.05) (Supplementary material online, *Tables S6* and *S7*). Associations of antibiotic use in late adulthood with CVD appeared to be stronger in women without diabetes ($P_{interaction}$ = 0.08); those who reported the common indication of using antibiotics for urinary tract infections ($P_{interaction}$ = 0.02); not for dental indications ($P_{interaction}$ = 0.048) (Supplementary material online, *Table S6*). The positive association of antibiotic use in middle adulthood with CVD appeared to be stronger in non-hypertensive than in hypertensive participants ($P_{interaction}$ = 0.06) (Supplementary material online, *Table S7*). If we calculated HRs for CVD considering the use in prior life-stages, the positive relationship between antibiotic use in middle- or late adulthood and CVD risk was observed for women who also used antibiotics in prior life-stages (Supplementary material online, *Figures S1* and *S2*).

Figure 4 shows results assessing joint associations of antibiotic use in late or middle adulthood and BMI with CVD. When compared with a reference group of women with 'BMI <25.0 kg/m^2 and antibiotic use <2 months', long-term antibiotic use in late adulthood (≥2 months) was not associated with an elevated risk for CVD among women with BMI <25 kg/m^2 (*A*); the risk was lower than that of obese (BMI ≥30 kg/m^2) women without long-term antibiotic use. We observed a similar joint association of BMI categories with antibiotic use during middle adulthood for CVD events (*B*).

Discussion

We found that longer duration of antibiotic use in middle adulthood was significantly associated with subsequent risk of CVD, independent of traditional risk factors for CVD. Also, women with long-term (≥2 months) use of antibiotics in late adulthood had a significantly increased risk of CVD. Our findings were not influenced by the presence of major diseases, or other medication use.

Several previous studies found that antibiotic use was related to increased risks of cardiovascular deaths and MI,[15] especially during a limited period (<30 days) after the use.[17] Nonetheless, inconsistent results were also reported, probably due to heterogeneous study

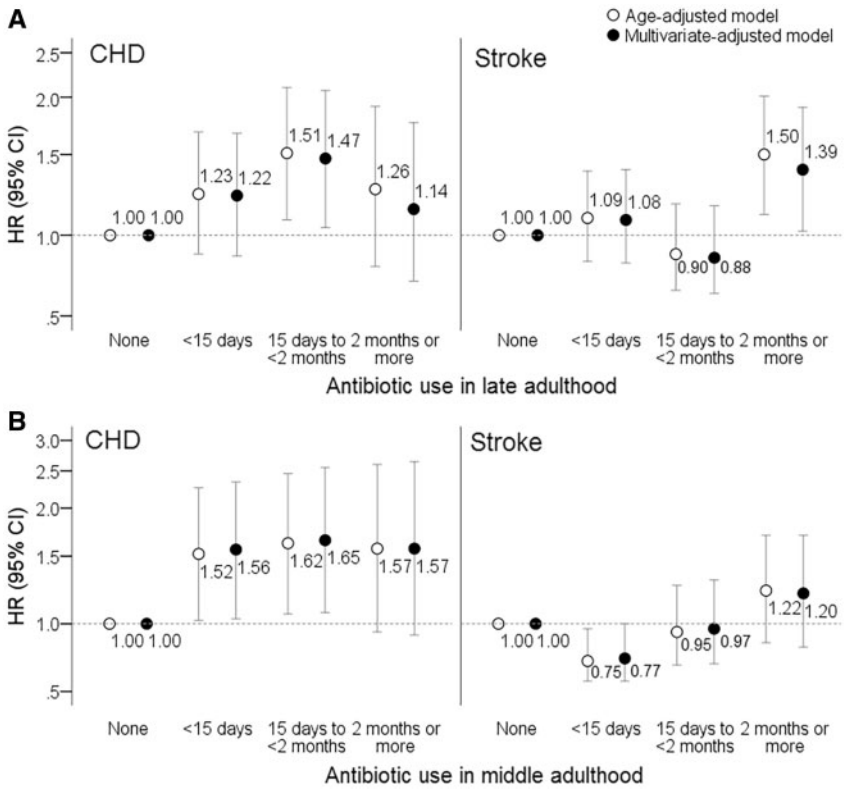


Figure 2 Hazard ratios for coronary heart disease or stroke according to antibiotic use in late- (*A*) or middle adulthood (*B*). Multivariate-adjusted model included covariates of Model 2 in *Table 1*.

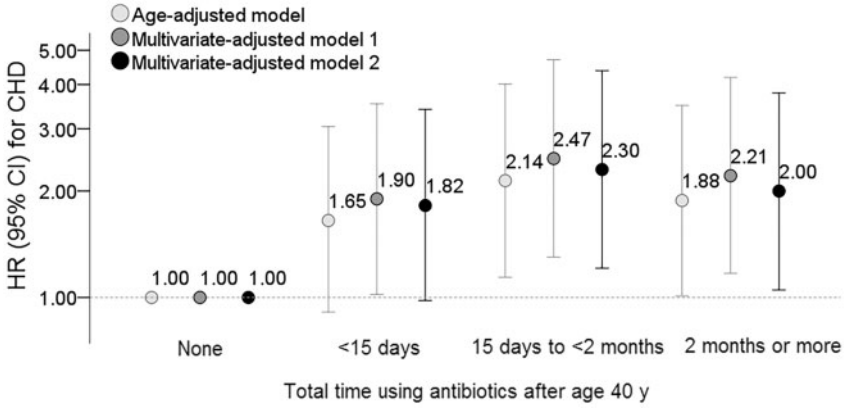


Figure 3 Hazard ratios for coronary heart disease according to total time using antibiotics after age 40 years. Multivariate-adjusted models included the same covariates in *Table 1*.

designs and differences in study populations, duration of antibiotic exposure, antibiotic classes examined, outcome definitions, and follow-up time. A retrospective study of older patients hospitalized with pneumonia showed that azithromycin use was significantly associated with 90-day MI event risk.[31] In a study among antibiotic users, clarithromycin use was associated with an increased risk of MI compared with amoxicillin, although no long-term effect of clarithromycin for MI was observed.[26] According to follow-up analyses of a randomized clinical trial of patients with stable CHD, a 2-week treatment of clarithromycin was related to a risk of cerebrovascular disease over 10 years after the treatment.[32] On the other hand, meta-analyses showed no increased risk of stroke.[15,17] We introduced a

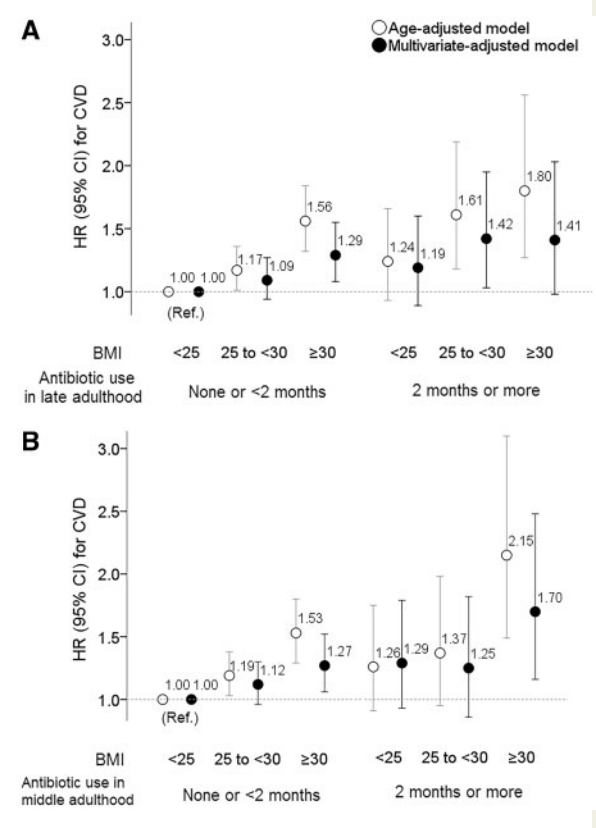
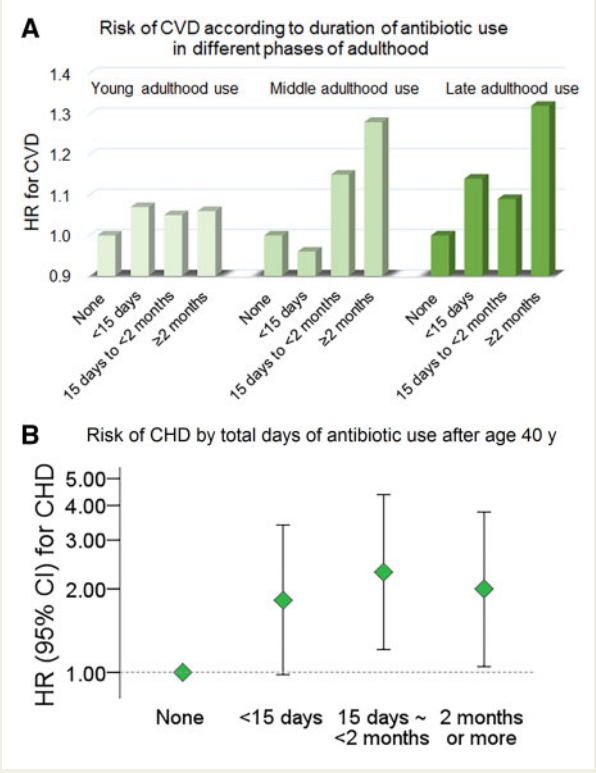


Figure 4 Joint associations of body mass index and antibiotic use in late- or middle adulthood with the risk of cardiovascular disease. Participants were categorized into six groups in each panel. The category with 'body mass index <25.0 kg/m^2' and 'none or <2 months antibiotic use' in late- (*A*) or middle adulthood (*B*) was used as a reference. Multivariate-adjusted model included covariates of Model 2 (except for body mass index) in *Table 1*.



Take home figure Associations of longer duration of exposure to antibiotics in middle and older adulthood with increased risks of cardiovascular events.

different approach and investigated the duration of antibiotic use in various stages of adulthood with the incidence of CHD and stroke over 8 years. Our results are in line with a study of patients with respiratory tract infections which reported that longer clarithromycin use (for ≥7 days) might be related to more cardiovascular events during the next year than shorter-term use (<3-day).[21] Our results, together with findings from other studies, suggest that use of antibiotics especially long-term use during more recent adulthood may adversely affect the CVD incidence in later life.

There are several potential explanations for the observed associations. Antibiotic treatment may induce prolongation of the QT interval and the Torsades de Pointes, and sudden cardiac death.[2] Antibiotics can stimulate proliferation and activity of macrophages[3,4] which may induce accumulation of lipids and atherosclerosis in long-term.[5] A recent animal study suggests an unexpected effect of antibiotics for promoting inflammation.[43] Also, antibiotic exposure might affect cardiovascular risk by influencing the abundance and composition of gut microbiota, which has been associated with atherosclerotic CVD in humans.[14] Evidence has shown that effects of a single course of antibiotics on the specific microbial populations can persist for years.[44–46] The gut microbe-related metabolites may also have roles in increasing platelet hyperreactivity and propensity to thrombosis that are risk factors for CVD.[47] Microbiota disruption caused by antibiotics may also lead to weight gain and greater adiposity by increasing energy harvest or altering metabolic signals and inflammation.[48,49] The presence of obesity augmented the association of long-term antibiotic exposure with CVD risk in this study.

Our study has several important strengths. We used a well-established cohort with high follow-up rates and well-validated assessment of CVD events, which minimized selection and ascertainment biases. A large sample size and a long-term follow-up provided the statistical power to detect relevant associations. Comprehensive information on demographics, disease status, lifestyle and diet minimized the potential for residual confounding. Our study has also several potential limitations. First, information on antibiotic use was self-reported, leading to potential misclassification particularly for exposure to antibiotics during early life periods. Nonetheless, all participants were health professionals who were able to provide more accurate information on medication use than general populations. Second, we did not have information on specific types of antibiotics, precluding assessment of whether our findings were specific to certain types of antibiotics. On the other hand, the most common type of prescription largely depends on the cause, and we considered this information in our analysis. Indeed, if the CVD risks were attributable to only specific antibiotics, our analysis of antibiotic use might have underestimated the magnitude of the association. Furthermore, men

were not included in this study, and our study population consisted of midlife and elderly women. Also, the present analysis only included 30% of the original study participants. Whether our results would apply to other populations needs to be further investigated. Lastly, our observational study cannot determine a causal effect for our observations. Women who reported antibiotic use might be sicker in other unmeasured ways, and there might be residual or unmeasured confounding factors in the associations, although we carefully considered traditional risk factors, lifestyles, other medication use, indications for antibiotic use, and disease status.

Conclusion

In this study which examined antibiotic use in different life-stages, duration of antibiotic use in the middle- and older adulthood, but not in young adulthood, showed significant associations with the development of CVD in later life. Cumulative antibiotic use during different stages of adulthood may be linked to CVD incidence among elderly women.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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Conflict of interest: none declared.

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