Circulation Research

ORIGINAL RESEARCH

Duration and Life-Stage of Antibiotic Use and Risks of All-Cause and Cause-Specific Mortality

Prospective Cohort Study

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RATIONALE: The overuse of antibiotics has been an important clinical issue, and antibiotic exposure is linked to alterations in gut microbiota, which has been related to risks of various chronic diseases such as cardiovascular disease and cancer. Also, duration of antibiotic exposure may be a risk factor of premature death.

OBJECTIVE: We investigated associations of life-stage and duration of antibiotic use during adulthood with risks of all-cause and cause-specific mortality.

METHODS AND RESULTS: This prospective cohort study included 37 516 women aged ≥60 years who were free of cardiovascular disease or cancer from the Nurses' Health Study. Participants reported a total amount of time they used antibiotics (none, <15 days, 15 days to <2 months, or ≥2 months) in the middle- (age, 40–59) and late adulthood (age, 60 or older). We estimated hazard ratios for all-cause mortality and deaths from cardiovascular disease or cancer over 10 years according to duration of antibiotic use. During 355 918 person-years of follow-up, we documented 4536 deaths from any cause (including 728 cardiovascular deaths and 1206 cancer deaths). As compared with women who did not use antibiotics, those who used them for ≥2 months in late adulthood had increased risks of all-cause mortality (hazard ratio, 1.16 [95% CI, 1.01–1.33]) and cardiovascular mortality (hazard ratio, 1.49 [95% CI, 1.04–2.13]), but not cancer mortality (hazard ratio, 0.85 [95% CI, 0.65–1.12]) after adjustment for chronic metabolic diseases, antibiotic use during middle adulthood, indication for use, demographic factors, and lifestyle/dietary factors. The association was more evident among women who also used antibiotics in middle-adulthood than among those who did not use during this life-stage.

CONCLUSIONS: Long-term use of antibiotics in late adulthood may be a risk factor for all-cause and cardiovascular mortality. The unfavorable effect of antibiotic exposure for subsequent risks of deaths due to chronic diseases needs to be considered.

VISUAL OVERVIEW: An online visual overview is available for this article.

Key Words: cardiovascular diseases ■ chronic disease ■ metabolic diseases ■ mortality ■ risk factors

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A recent study suggests that a substantial proportion of antibiotics are not prescribed appropriately in an outpatient clinical setting in the United States,[1] and patients who are hospitalized may also receive excess antibiotic therapy.[2] Thus, the potential effect of antibiotics for subsequent risks of chronic diseases[3–5] and mortality[6–18] is considered as an important clinical issue. Antibiotic exposure affects balance and composition of the gut microbiome[19–22] even after cessation of the use.[23] Gut microbiota alterations have been related to a variety of life-threatening disorders such as cardiovascular diseases and certain types of cancer.[24–29]

In previous studies that examined associations between antibiotic use and risk of death from any cause

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Novelty and Significance

What Is Known?

- It has been known that a substantial proportion of antibiotics have not been appropriately prescribed in clinical settings, and the overuse of antibiotics is an important clinical issue.
- Antibiotic use is linked to gut microbiota alterations even after cessation of the use, and alterations in gut microbiota are associated with various life-threatening diseases, such as cardiovascular disease and cancer.
- Duration of antibiotic use may differentially affect the risk of premature death; however, associations of duration of antibiotic use in different phases of adulthood with risks of all-cause and cause-specific mortality over years have not been clarified in a population at usual risk.

What New Information Does This Article Contribute?

- Long-term antibiotic use (for 2 months or more) in late adulthood was associated with increased risks of all-cause mortality and cardiovascular mortality, independently of traditional risk factors among women.
- The increased risk of mortality associated with long-term antibiotic use in late adulthood was more evident among women who also used antibiotics in middle adulthood than among those who did not use during this life stage, suggesting the importance of the cumulative antibiotic use in middle and late adulthood for mortality.
- The cumulative antibiotic use in different life stages of adulthood may be related to a higher risk of all-cause mortality.

Antibiotic exposure affects the abundance and composition of the gut microbiome, and gut microbiota alterations have been associated with various life-threatening diseases. The potential effect of antibiotics on risks of chronic diseases and mortality is an important clinical issue to be examined. This prospective cohort study investigated associations of life-stage and duration of antibiotic use during adulthood with risks of all-cause and cause-specific mortality. Our results show that long-term use of antibiotics for 2 months or more in late adulthood was associated with an increased risk of all-cause mortality and deaths due to cardiovascular diseases. The associations were independent of traditional risk factors, such as demographic and lifestyle factors, dietary habits, obesity, metabolic disorders, and other medication use. Further, the adverse relationship between long-term use of antibiotics in late adulthood and mortality was more evident among women who also used antibiotics in middle-adulthood than among those who did not use during this life stage, suggesting the cumulative antibiotic use in different stages of adulthood may be related to a higher risk of mortality.

Nonstandard Abbreviations and Acronyms

CVD	cardiovascular disease
HR	hazard ratio
NHS	Nurses' Health Study

or cardiovascular death,[6,10,30–32] some types of antibiotics were found to be related to subsequent risk of death from cardiovascular causes[10,11,32] although the findings are not entirely consistent across studies.[15,18,30,31] Of note, many of the previous studies were conducted among patients with specific diseases, or with a limited follow-up period.[8,30,31] Additionally, previous studies did not control for detailed information on several known risk factors for cardiovascular disease (CVD) and death that would influence the association of antibiotic use with mortality. We recently investigated associations of life-stage and duration of antibiotic exposure during adulthood with subsequent cardiovascular events and found that longer duration of exposure to antibiotics in the middle and older adulthood was related to an increased risk of the development of CVD among women at usual risk.[33] Duration of antibiotic exposure may also affect the risk of premature death; however, associations of duration of antibiotic use in different phases of adulthood with risks of all-cause and cause-specific mortality over years have not been clarified in a population at usual risk.

In the present study, we investigated whether duration of antibiotic use was associated with higher risks of all-cause and cause-specific mortality independent of traditional risk factors among participants in the NHS (Nurses' Health Study), which has collected detailed information on lifestyle factors, diet, and duration of antibiotic use.

METHODS

The data, analytical methods, and study materials will be available to other researchers from the corresponding author on reasonable request for purposes of reproducing the results or replicating the procedure.

Study Participants

The NHS is a prospective cohort study established in 1976 of 121 701 female registered nurses aged 30 to 55 years in the United States. 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. Dietary intake was assessed using validated food frequency questionnaires every 4 years.[34] The follow-up rate was high, with ≈90% participation in each 2-year follow-up cycle. Details of the study have been described elsewhere.[35,36]

The present analysis included women returned the 2004 questionnaire (n=90 853) when the information on antibiotic exposure was first collected[4,33] (Online Figure I); a total of 60 296 had available data on antibiotic use during the middle (age, 40–59) and late (after age 60) adulthood. We did not include women (n=1926) who only returned the baseline (2004) questionnaire, leaving 58 370 participants. We excluded women who had a history of CVD (myocardial infarction, angina pectoris, stroke, or coronary artery bypass grafting) or cancer at baseline (n=18 039). We considered that antibiotics might be prescribed for people with these diseases, and exclusions of prevalent cardiovascular and cancer cases at baseline also affected the number of death events during the follow-up time, which might be a potential selection bias. Thus, we carried out sensitivity analyses without excluding people with CVD or cancer at baseline, and we observed that such exclusions did not appreciably change results on the primary outcome (data available upon request). After additional exclusion of women with missing data on some covariates (age, height, body weight, or physical activity), a total of 37 936 women remained for the analyses. As women <60 y in 2004 (n=420) did not have the ability to provide information on antibiotics during late adulthood, the present study only included women aged 60 or older at baseline. Subsequently, the final analytical cohort comprised 37 516 women with a follow-up from 2004 to 2014 (Online Figure I). The study was approved by the institutional review board at the Brigham and Women's Hospital.

Assessment of Antibiotic Use and Covariates

In the baseline 2004 questionnaire, women were asked to indicate the total time using antibiotics with 8 categories ranging from none to 5+ years (none, <15 days, 15 days to 2 months, 2–4 months, 4 months to 2 years, 2–3 years, 3–5 years, and 5+ years) excluding skin creams, mouthwash, or isoniazid for the time periods during between age 40 and 59, and age 60 to the baseline. Participants added up the total amount of time they used antibiotics for each period. The sum of all days of exposure to antibiotics was reported as total days, regardless of consecutive or not consecutive days. The assessment of antibiotic use has also been described elsewhere.[4,33] The participants reported the most common reason that they used antibiotics among the following 6 indications (respiratory infection, urinary tract infection, acne/rosacea, chronic bronchitis, dental, or other). Information on the specific type of antibiotics or daily dosage was not available.

Data on height, body weight, medical history, smoking habit, physical activity habit, physician-diagnosed diseases, medication use, and other characteristics were collected at baseline and on biennial validated questionnaires. Self-reported body weight was highly correlated with technician-measured weight (r=0.97) in a subsample.[37] Body mass index was calculated as body weight in kilograms divided by height squared in meters. Alcohol consumption and dietary intake were assessed using validated food frequency questionnaires.[34] The Alternate Healthy Eating Index score was calculated based on intakes of foods and nutrients predictive of chronic disease risk, including vegetables, fruits, nuts and legumes, whole grains, red or processed meat, sugar-sweetened beverages, alcohol, sodium, trans fat, long-chain ω-3 fatty acids, and other polyunsaturated fats.[38] The healthy lifestyle score was calculated using data on diet (top 2 quintiles of Alternate Healthy Eating Index scores, yes)+smoking status (no smoking habit, yes)+physical activity habit (moderate or high physical activity which was equivalent 150 minutes/week, yes)+body mass index (<25 kg/m^2, yes) with ranges 0 to 4 points.[39]

Ascertainment of Death

Our primary outcome was all-cause mortality, and secondary outcomes were deaths from cardiovascular diseases (*International Classification of Diseases, Eighth Revision*), codes 390 to 458) or cancer (*International Classification of Diseases, Eighth Revision*, 140–209). We also assessed other death, which was due to other than CVD or cancer. We identified deaths from the next of kin, the postal system, and the National Death Index. We previously estimated that at least 98% of deaths were identified using these approaches.[40] We attempted to obtain the death certificate of each participant who had died, and when appropriate, we requested permission from the participant's next of kin to review medical records. Causes of death were primarily confirmed by review of autopsy reports, medical records, and death certificates.

Statistical Analysis

Cox proportional hazards regression was performed to calculate hazard ratios (HRs) and 95% CIs for the outcomes across categories of duration of antibiotic use. Follow-up time was calculated from the return date of the 2004 questionnaire until the date of death, or end of follow-up (June 30, 2014), whichever occurred first. To minimize confounding and investigate which factors might attenuate associations of antibiotics with all-cause mortality, we performed 5 different multivariate-adjustment models including covariates of demographic factors (such as age, race, family history of myocardial infarction, menopausal state and postmenopausal hormone use, and marital status [married or not married]), aspirin use, diet, and lifestyle factors (such as smoking status [5 categories: never, former, current 1–14, 15–24, or ≥25 cigarettes], physical activity [quintiles], the Alternate Healthy Eating Index score without alcohol consumption [quintiles], and alcohol consumption [none, 0–4.9, 5–14.9, or ≥15.0 g/day]), body mass index (<25, 25 to <30, or ≥30 kg/m^2), and metabolic diseases (such as hypertension, hypercholesterolemia, and diabetes mellitus) in model 1. We included additional covariates of antibiotic use during middle adulthood in model 2; reasons for antibiotic use as respiratory infection (yes), urinary tract infection (yes), acne/rosacea (yes), chronic bronchitis (yes), or dental (yes) in model 3; other medication use (such as other anti-inflammatory medication including non-aspirin nonsteroidal anti-inflammatory drugs/COX-2 inhibitors/steroid use, statin use, H2 blockers use, and proton pump inhibitors use)[7,41–44] in model 4; and other disease status in model 5 (such as congestive heart failure, chronic renal failure, pneumonia, and emphysema/chronic bronchitis). We also constructed additional models adjusting for other dietary factors, such as

multivitamin supplement use, total energy intake, dietary fiber intake, and dietary phosphatidylcholine intake; these adjustments did not influence the primary results, so these dietary factors were not included in the main analysis. Tests of linear trend (P_{trend}) across antibiotic use categories 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 or more) conservatively set to 60 days.[33]

To assess potential effect modification and confounding, we performed stratified analyses according to indications for antibiotic use, participants' characteristics and prevalent diseases at baseline (including overweight or obese, hypertension, dyslipidemia, diabetes mellitus, pneumonia, and emphysema/chronic bronchitis). The *P* for interactions between antibiotic use and these factors for mortality were calculated by the log-likelihood ratio test, which compared models with and without cross-product interaction terms. To control of potential confounding, we also performed sensitivity analyses in restricted cohorts, such as by excluding major disease cases (myocardial infarction, angina pectoris, stroke, coronary artery bypass grafting, or cancer) occurring within the first 2 years of follow-up time. We also performed a sensitivity analysis by excluding people with a history of gastrointestinal bleeding that requires hospitalization or a transfusion to examine whether a disorder in the digestive tract may alter our findings considering. Further, healthy lifestyles are important modifiable factors for health outcomes and death; therefore, we compared HRs for mortality according to a joint classification of antibiotic use and categories of the healthy lifestyle score (low: 0–1 point, middle: 2 points, and high: 3–4 points).

RESULTS

Our primary analysis included a total of 37 516 women; characteristics of study participants according to duration of antibiotic use in late adulthood are shown in Online Table I. Women with longer duration of antibiotic use were more likely to be older and to have lower Alternate Healthy Eating Index scores, less physical activity, higher body mass index, hypertension, hypercholesterolemia, diabetes mellitus, and were more likely to use other medications. A total of 9% (n=3351) of the participants reported long-term (≥2 months) use of antibiotics in late adulthood. The most common reason for antibiotic use differed, with respiratory infections, urinary tract infections, and dental indications also being common indications. Women with long-term use in late adulthood were also more likely to use antibiotics in middle adulthood (age, 40–59).

During 355 918 person-years of follow-up, we documented 4536 deaths from any cause (including 728 cardiovascular deaths and 1206 cancer deaths). More details on the causes of death in a present study population are shown in Online Table II. A long duration of antibiotic use in late adulthood was significantly associated with an increased risk of all-cause mortality in age-adjusted model as compared with no use (HR, 1.27 [95% CI, 1.14–1.42]; $P_{trend}=6\times10^{-6}$; Table 1). The association was not materially changed after adjusted for demographic, dietary, and lifestyle factors, metabolic diseases (model 1), antibiotic use in middle adulthood (model 2), reasons for antibiotic use (model 3), and other medication use (model 4). As compared with women who did not use antibiotics, those who used ≥2 months in late adulthood had a significantly increased risk of all-cause death with an adjusted HR of 1.29 (95% CI, 1.13–1.47, model 4; $P_{trend}=0.001$). When we further adjusted for disease status in model 5, the trend was attenuated but the elevated risk of long-term use remained significant ($P_{trend}=0.14$; HR of the long-term use, HR 1.16 [95% CI, 1.01–1.33]).

For specific causes of death, we found a positive association between the long-term use and CVD mortality (HR, 1.49 [95% CI, 1.04–2.13]), but not for cancer mortality (HR, 0.85 [95% CI, 0.65–1.12]; Figure 1). Interestingly, we also found an increased risk of cardiovascular

Table 1. Hazard Ratios for All-Cause Mortality According to Total Time Using Antibiotics in Late Adulthood

Models	Total Time Using Antibiotics in Late Adulthood				P_{Trend}
	None	<15 D	15 D to <2 Mo	2 Mo or More	
Incident cases/person-years	651/66 399	2143/174 153	1120/84 453	622/30 912	…
Age-adjusted model	1.00 (Ref.)	0.91 (0.83–0.998)	0.91 (0.82–1.00)	1.27 (1.14–1.42)	6×10^{-6}
MV-adjusted model 1: demographic, dietary, and lifestyle factors, and metabolic diseases	1.00 (Ref.)	0.93 (0.85–1.01)	0.93 (0.85–1.03)	1.28 (1.14–1.43)	3×10^{-6}
MV-adjusted model 2: antibiotic use in middle adulthood	1.00 (Ref.)	0.98 (0.89–1.07)	0.98 (0.87–1.09)	1.33 (1.16–1.51)	0.0001
MV-adjusted model 3: reasons for antibiotic use	1.00 (Ref.)	0.98 (0.89–1.08)	0.97 (0.87–1.08)	1.31 (1.15–1.50)	0.0003
MV-adjusted model 4: other medication use	1.00 (Ref.)	0.97 (0.88–1.07)	0.96 (0.86–1.07)	1.29 (1.13–1.47)	0.001
MV-adjusted model 5: full	1.00 (Ref.)	0.96 (0.87–1.05)	0.90 (0.81–1.01)	1.16 (1.01–1.33)	0.14

Model 1: age, race, family history of myocardial infarction, menopausal state and postmenopausal hormone use, aspirin, marital status (married or not married), smoking status (never, former, current 1–14, 15–24, ≥25 cigarettes), physical activity (quintiles), Alternative Healthy Eating Index score (quintiles), alcohol consumption (none, 0–4.9, 5–14.9, or ≥15.0 g/d), body mass index (<25, 25 to <30, or ≥30 kg/m^2), hypertension, hypercholesterolemia, and diabetes mellitus. Model 2: model 1+antibiotic use during middle adulthood (during age 40–59). Model 3: model 2+reasons for antibiotic use as respiratory infection (yes), urinary tract infection (yes), acne/rosacea (yes), chronic bronchitis (yes), or dental (yes). Model 4: model 3+other anti-inflammatory medication (nonaspirin nonsteroidal anti-inflammatory drugs, COX-2 inhibitors, or steroid use), statin use, H2 blockers use, proton pump inhibitors use. Model 5: model 4+congestive heart failure, chronic renal failure, pneumonia, and emphysema/chronic bronchitis. MV indicates multivariate-adjusted.

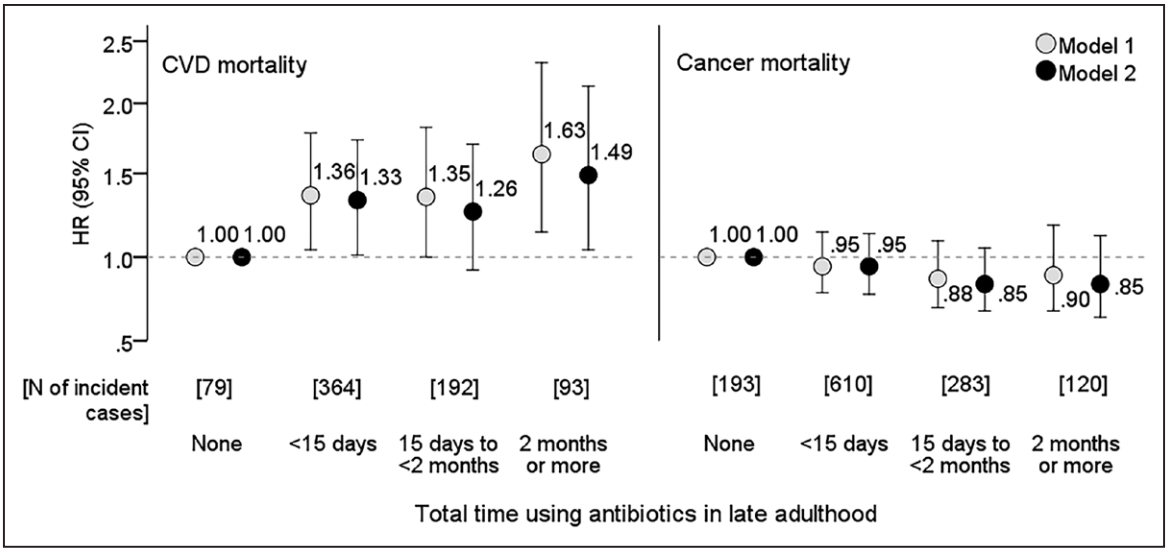

Figure 1. Hazard ratios (HRs) for deaths from cardiovascular disease (CVD) or cancer according to total time using antibiotics in late adulthood.

Model 1: age, race, family history of myocardial infarction, menopausal state and postmenopausal hormone use, aspirin, marital status (married or not married), smoking status (never, former, current 1–14, 15–24, or ≥25 cigarettes), physical activity (quintiles), Alternative Healthy Eating Index score (quintiles), alcohol consumption (none, 0–4.9, 5–14.9, or ≥15.0 g/day), body mass index (<25, 25 to <30, or ≥30 kg/m^2), hypertension, hypercholesterolemia, diabetes mellitus, antibiotic use in middle adulthood, reasons for antibiotic use as respiratory infection (yes), urinary tract infection (yes), acne/rosacea (yes), chronic bronchitis (yes), or dental (yes), other anti-inflammatory medication (nonaspirin nonsteroidal anti-inflammatory drugs, COX-2 inhibitors or steroid use), statin use, H2 blocker use, proton pump inhibitors use. Model 2: model 1+congestive heart failure, chronic renal failure, pneumonia, and emphysema or chronic bronchitis. Please see in Table 1 for person-years in each category.

mortality among women who used antibiotics for <15 days with a fully adjusted HR of 1.33 (95% CI, 1.01–1.73) as compared with nonusers. If we analyzed the risk of other death (incident cases=2602), there was a significantly increased risk of mortality among the long-term users; fully adjusted HRs for death across the duration of antibiotic use were HR, 0.89 (95% CI, 0.78–1.01) for <15 days use, HR 0.86 (95% CI, 0.74–0.99) for 15 days to <2 months use; HR, 1.23 (95% CI, 1.03–1.47) for ≥2 months use; P_{trend}=0.015 (Online Table III).

When we calculated HRs of antibiotic use in middle adulthood after adjustment for the same covariates, we did not find an association between increased risk of all-cause mortality and long-term (>2 months) use of antibiotics in middle adulthood. Instead, women with the reported use in middle adulthood showed slightly decreased HRs for all-cause mortality (HR, 0.81 [95% CI, 0.73–0.90]) for <15 days use; HR, 0.82 (0.72–0.92) for 15 days to <2 months use, and HR, 0.82 (0.70–0.96) for 2 months or more use, respectively). We then calculated HRs of the use in late adulthood for all-cause mortality after we stratified participants who used or did not use antibiotics in middle adulthood (Figure 2). The association between all-cause death and the use in late adulthood was found to be significant among women who also used antibiotics during middle adulthood (HR of ≥2 months use: 1.24 [95% CI, 1.08–1.42]).

In stratified analyses to assess potential effect modification according to characteristics or disease status at the baseline year 2004 (Table 2), we did not observe significant interactions of the antibiotic use with smoking, overweight/obesity, metabolic diseases, respiratory diseases (emphysema/chronic bronchitis or pneumonia), or indications for the antibiotic use (respiratory infections, urinary tract infections, dental indication, or chronic bronchitis) on all-cause death (*P* value for interaction >0.05).

When we performed a sensitivity analysis by excluding major disease cases (myocardial infarction, angina pectoris, stroke, coronary artery bypass grafting, or cancer) occurring during the first 2 years of follow-up, overall results remained unchanged (Online Table IV). Also, we observed similar associations even after excluding people (n=1379) with a history of gastrointestinal bleeding that requires hospitalization or a transfusion (Online Table V).

Finally, we investigated whether a combination of healthy lifestyles modified the relation between antibiotic use and all-cause mortality (Figure 3). Compared with a reference group of women who did not use antibiotics in late adulthood and with healthier lifestyles (scores 3-4 points), women with the long-term antibiotic use and with high healthy lifestyle score had an elevated risk for all-cause death (HR, 1.22 [95% CI, 1.01–1.48]). Women with unhealthier lifestyles had an HR of 1.74 (95% CI, 1.40–2.18) of all-cause death among the long-term users.

DISCUSSION

In the present study, we found significant associations between long-term antibiotic use in late adulthood and

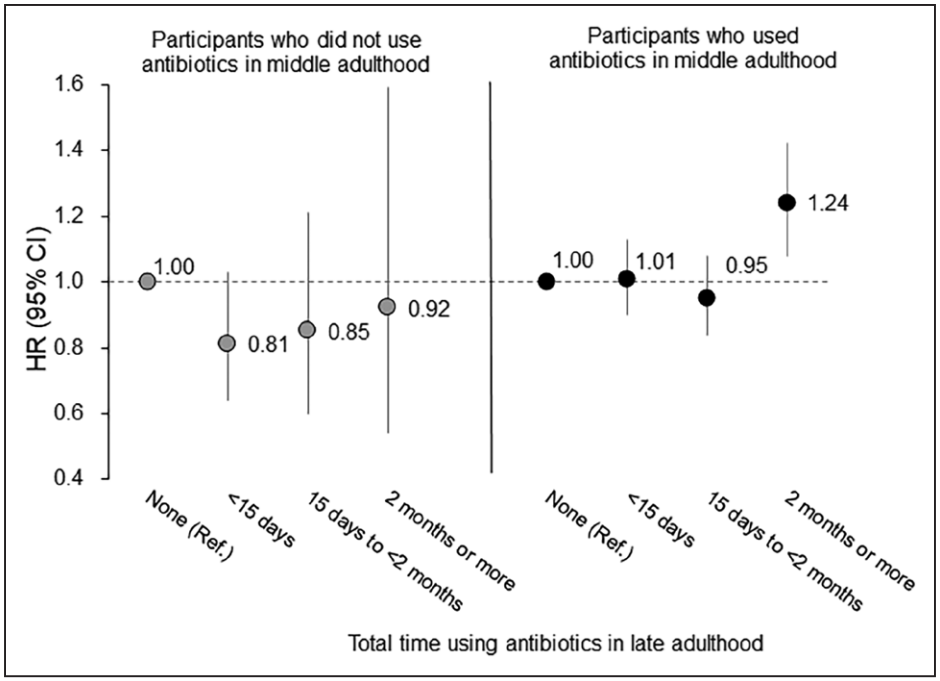

Figure 2. Hazard ratios (HRs) for all-cause death according to antibiotic use in late adulthood (after age 60 y) and middle adulthood (age, 40–59 y).

HRs after adjusted for age, race, family history of myocardial infarction, menopausal state and postmenopausal hormone use, aspirin use, reasons for antibiotic use, smoking habit, physical activity, Alternative Healthy Eating Index score, alcohol consumption, body mass index, hypertension, hypercholesterolemia, diabetes mellitus, other anti-inflammatory medication (nonaspirin nonsteroidal anti-inflammatory drugs, COX-2 inhibitors, or steroid use), statin use, H2 blocker use, proton pump inhibitors use, congestive heart failure, chronic renal failure, pneumonia, and emphysema or chronic bronchitis.

an increased risk of mortality, especially deaths due to cardiovascular diseases. The associations were independent of traditional risk factors, such as demographic and lifestyle factors, dietary habits, obesity, metabolic disorders, and other medication use.

Evidence on associations between antibiotic use and the risk of all-cause death are not consistent across studies,[9,10,16,18,30,31,45,46] possibly due to the heterogeneity of study populations, causes of deaths, limited follow-up time for mortality, or assessment of duration of antibiotic use. In a meta-analysis by Cheng et al,[10] there was no association of macrolide antibiotic use with all-cause mortality, and taking macrolide antibiotics was significantly associated with increased risks of sudden cardiac death and cardiovascular death. The meta-analysis also revealed stronger associations between macrolide antibiotics and cardiovascular mortality among studies that controlled for cardiovascular risk factors,[10] although these studies did not control for detailed information on lifestyle and dietary factors. In a more recent meta-analysis, fluoroquinolone antibiotics that are commonly used to treat certain bacterial infections have been related to a 71% increase in the risk of cardiovascular death.[32] A recent retrospective study of patients in the United Kingdom followed long-term mortality and showed that outpatient clarithromycin use was significantly associated with increased mortality over 10 years.[47] Our study examined associations of life-stage and duration of antibiotic use during adulthood and found that the long-term use of antibiotics in particular late adulthood was significantly associated with all-cause and cardiovascular mortality over 10 years after adjusted for lifestyle, diet, and other risk factors among elderly women who were initially free of CVD and cancer. These findings are in line with those of a recent meta-analysis on duration of antibiotic use for the treatment of community-acquired pneumonia,[48] which showed that short-course antibiotic treatment for <6 days was associated with fewer serious adverse events and lower mortality rate, as compared with longer-course therapy (ie, treatment for ≥7 days).[48]

Also in line with other studies, we observed that women with long-term antibiotic use and unhealthy lifestyles had a particularly elevated risk of mortality compared with those with healthy lifestyles. A study reported that azithromycin use (for 5 days) was associated with an increased risk of cardiovascular mortality, which was the most pronounced for patients with unfavorable cardiovascular risk profiles at the baseline, although their results might be confounded by other coexisting conditions, lifestyle risk factors (eg, smoking, obesity. poor diet, and low physical activity), and indication for antibiotic therapy.[8] We observed that the increased risk of mortality among long-term antibiotic users was attenuated but not eliminated by healthy lifestyle factors, suggesting that women who took antibiotics for long term during late adulthood may be a high-risk group to target for lifestyle modifications to reduce mortality in later life.

Table 2. HRs for All-Cause Mortality According to Total Time Using Antibiotics in Late Adulthood by Characteristics/Disease Status at Baseline

Status	Incident Cases/ Person-Years	Total Time Using Antibiotics					*P* Value for Interaction
		None	<15 D	15 D to <2 Mo	2 Mo or More	P_{Trend}	
Age							0.05
<70 y	331/121744	1.00 (Ref.)	1.17 (0.90–1.53)	1.06 (0.74–1.50)	2.19 (1.45–3.33)	0.016	
≥70 y	4205/234173	1.00 (Ref.)	0.88 (0.80–0.97)	0.88 (0.80–0.98)	1.21 (1.08–1.36)	6×10^{-5}	
Never smokers							0.31
Yes	1710/162815	1.00 (Ref.)	0.83 (0.72–0.96)	0.76 (0.65–0.89)	1.12 (0.93–1.34)	0.28	
No	2826/193102	1.00 (Ref.)	0.93 (0.83–1.05)	0.97 (0.85–1.11)	1.28 (1.10–1.48)	6×10^{-5}	
Overweight or obese							0.6
Yes	2326/194165	1.00 (Ref.)	0.92 (0.81–1.04)	0.89 (0.77–1.02)	1.21 (1.03–1.43)	0.02	
No	2210/161753	1.00 (Ref.)	0.91 (0.80–1.03)	0.92 (0.80–1.06)	1.33 (1.13–1.56)	0.0001	
Hypertension							0.57
Yes	3014/197915	1.00 (Ref.)	0.90 (0.80–1.01)	0.90 (0.79–1.02)	1.29 (1.12–1.48)	2×10^{-5}	
No	1522/158003	1.00 (Ref.)	0.92 (0.80–1.07)	0.88 (0.75–1.04)	1.14 (0.93–1.39)	0.35	
Dyslipidemia							0.89
Yes	3082/237275	1.00 (Ref.)	0.92 (0.82–1.03)	0.91 (0.80–1.03)	1.26 (1.09–1.45)	0.0003	
No	1454/118642	1.00 (Ref.)	0.92 (0.79–1.06)	0.92 (0.78–1.09)	1.37 (1.12–1.67)	0.004	
Diabetes							0.88
Yes	593/30284	1.00 (Ref.)	0.99 (0.76–1.30)	1.10 (0.82–1.48)	1.50 (1.09–2.07)	0.003	
No	3943/325634	1.00 (Ref.)	0.91 (0.82–1.00)	0.90 (0.81–1.00)	1.23 (1.09–1.39)	0.0003	
Emphysema/chronic bronchitis							0.26
Yes	645/21518	1.00 (Ref.)	0.75 (0.52–1.10)	0.85 (0.59–1.24)	0.94 (0.64–1.38)	0.12	
No	3891/334399	1.00 (Ref.)	0.90 (0.82–0.98)	0.82 (0.74–0.91)	1.14 (1.01–1.29)	0.20	
Pneumonia							0.11
Yes	642/34715	1.00 (Ref.)	0.86 (0.58–1.28)	0.88 (0.59–1.30)	1.46 (0.98–2.19)	6×10^{-5}	
No	3894/321202	1.00 (Ref.)	0.90 (0.82–0.99)	0.86 (0.78–0.96)	1.13 (0.99–1.28)	0.13	
Proton pump inhibitors use							0.54
Yes	428/36085	1.00 (Ref.)	0.90 (0.62–1.33)	1.04 (0.7–1.55)	1.51 (1.00–2.30)	0.001	
No	4108/319833	1.00 (Ref.)	0.91 (0.83–1.00)	0.89 (0.81–0.99)	1.24 (1.10–1.39)	0.0007	
H2 blockers use							0.83
Yes	404/25687	1.00 (Ref.)	0.88 (0.59–1.31)	0.88 (0.58–1.35)	1.04 (0.66–1.64)	0.49	
No	4132/330230	1.00 (Ref.)	0.91 (0.83–1.00)	0.90 (0.82–1.00)	1.28 (1.13–1.44)	2×10^{-5}	
Most common reasons for antibiotics use							
Respiratory infection							0.41
Yes	2453/194852	1.00 (Ref.)	0.95 (0.82–1.09)	0.97 (0.83–1.12)	1.41 (1.19–1.67)	5×10^{-6}	
No	2083/161066	1.00 (Ref.)	0.90 (0.80–1.02)	0.88 (0.76–1.01)	1.16 (0.99–1.36)	0.08	
Urinary tract infection							>0.99
Yes	769/63270	1.00 (Ref.)	0.96 (0.74–1.26)	0.96 (0.72–1.28)	1.36 (1.01–1.83)	0.0095	
No	3767/292647	1.00 (Ref.)	0.91 (0.83–1.00)	0.90 (0.81–1.01)	1.28 (1.13–1.45)	0.0001	
Dental							0.41
Yes	1021/73718	1.00 (Ref.)	0.97 (0.75–1.25)	0.90 (0.68–1.20)	1.23 (0.91–1.68)	0.21	
No	3515/282200	1.00 (Ref.)	0.90 (0.82–1.00)	0.92 (0.83–1.03)	1.29 (1.14–1.46)	7×10^{-6}	
Chronic bronchitis							0.46
Yes	335/16492	1.00 (Ref.)	0.90 (0.48–1.67)	0.83 (0.44–1.55)	1.44 (0.76–2.73)	0.026	
No	4201/339426	1.00 (Ref.)	0.89 (0.81–0.99)	1.20 (1.07–1.35)	1.34 (1.06–1.70)	0.001	

HRs (95% CIs) adjusted for age, race, and family history of myocardial infarction. HR indicates hazard ratio.

In contrast, the use of antibiotics in middle adulthood was associated with a slightly decreased risk of mortality among elderly women (aged 60 or more), although the use in late adulthood was positively associated with all-cause and cardiovascular mortality after we adjusted for each use in the multivariate analysis. Our findings

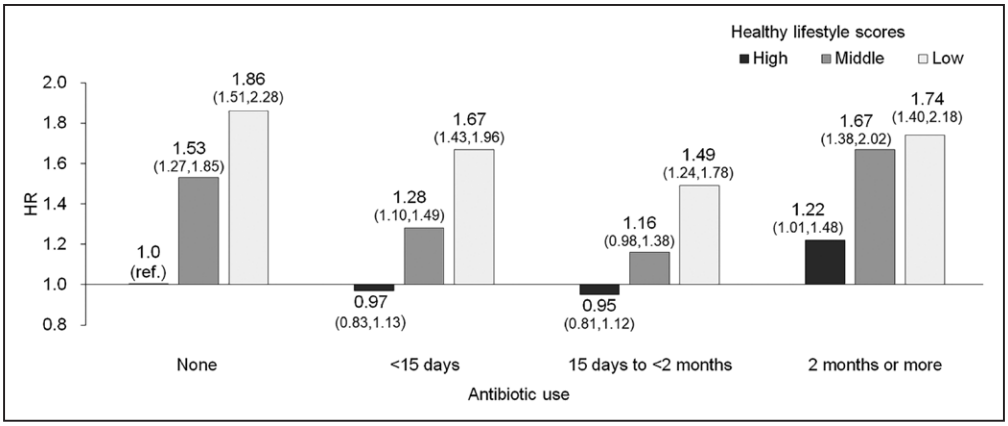

Figure 3. Join effect of healthy lifestyles and antibiotic use in late adulthood on all-cause mortality.
Data in blanket are 95% CIs. Hazard ratios (HRs; 95% CIs) after adjusted for age, race, family history of myocardial infarction, menopausal state and postmenopausal hormone use, aspirin use, reasons for antibiotic use, alcohol consumption, hypertension, hypercholesterolemia, diabetes mellitus, other anti-inflammatory medication (nonaspirin nonsteroidal anti-inflammatory drugs, COX-2 inhibitors, or steroid use), statin use, H2 blocker use, proton pump inhibitors use, congestive heart failure, chronic renal failure, pneumonia, and emphysema or chronic bronchitis. Healthy lifestyle score was calculated using data on diet (top 2 quintiles of Alternate Healthy Eating Index scores, yes)+smoking habit (nonsmoker, yes)+physical activity (moderate or high physical activity habit equivalent 150 min/wk, yes)+body mass index (nonoverweight or nonobese, body mass index <25.0 kg/m^2, yes) according to the American Heart Association guideline; ranges 0 to 4 points. Low scores: 0 or 1 points, middle score: 2 points, and high scores: 3 or 4 points.

are consistent with results of the previous meta-analysis in which different associations between antibiotics and mortality were observed according to age groups, and there was no association between azithromycin use and a higher risk of death in younger populations (mean age, <40 years); however, in older populations (mean age ≥40 years), there was an increased risk of death associated with current use of the antibiotic use.[18] Another meta-analysis has also reported that macrolide antibiotic use (except for roxithromycin) was found to be associated with an increased risk of cardiac death, particularly in older adults (aged >48 years).[30] Nonetheless, we found that the increased risk of mortality associated with long-term antibiotic use in late adulthood was more evident among women who also used antibiotics in middle adulthood than among those who did not use during this life stage, suggesting the importance of the cumulative antibiotic use in middle and late adulthood for mortality among elderly women.

As for the biological mechanisms, it is known that antibiotic treatment may induce a prolongation of QT interval and the Torsades de Pointes[49,50] and may stimulate proliferation and activity of macrophages,[51,52] which may induce atherosclerosis.[53] Previous studies have suggested that antibiotic use may induce alterations in microbiota composition persistently (over 2 months) even after cessation of the treatment[23] and that gut microbe-dependent metabolites may increase platelet hyperreactivity and propensity to thrombosis.[54] Our previous meta-analysis of prospective cohorts showed that circulating gut microbial metabolites were associated with risks of major adverse cardiovascular events independently of traditional risk factors.[55] In a prior study of women in the NHS, long-term antibiotic use in early-to-middle adulthood was associated with increased risk of colorectal adenoma.[4] Our study examined total cancer mortality as an outcome event, and further investigations would be needed about risks of deaths from different types of cancer.

Our study has several strengths and limitations. We used a well-established prospective cohort with a reasonably large sample size with high follow-up rates over extended follow-up. Our results were controlled for comprehensive information on demographics, lifestyle habits, and diet, which minimized the potential for residual confounding. However, similar to other observational studies, we could not determine a causal effect for our observations, and residual and unmeasured confounding could not be completely ruled out. Individuals who reported antibiotic use might be less healthy in other unmeasured ways. Also, information on antibiotic use was self-reported and potential misclassification was inevitable; thus, we could not deny the possibility that the risks of outcomes might have been under- or overestimated. On the contrary, our study population consisted of health professionals who would be able to provide more accurate information on their medication use than general populations, which could minimize such a possibility. Last, we did not have information on specific types of antibiotics, precluding assessments whether the findings were specific to certain types of antibiotics. If the risks of all-cause and cause-specific deaths were attributable to only specific classes of antibiotics, our results might have underestimated the magnitude of associations of antibiotic use with the outcomes. On the contrary, the most common types of prescription largely depend on the cause; prevalent disease status and indications for the antibiotic use were considered in our analyses.

In conclusion, this prospective analysis of women suggested that women who take antibiotics for long periods during adulthood may be a high-risk group to target for risk-factor modification to prevent cardiovascular and all-cause deaths.

ARTICLE INFORMATION

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Author Contribution

Y. Heianza contributed to the study concept and design, statistical analysis and interpretation of data, drafting and revising the manuscript, and study supervision. W. Ma, X. Li, and Y. Cao contributed statistical analysis and interpretation of data and drafting and revising the manuscript. A.T. Chan, E.B. Rimm, F.B. Hu, K.M. Rexrode, and J.E. Manson contributed to acquisition of data, interpretation of data, drafting and revising the manuscript, and funding. L. Qi contributed to the study concept and design, acquisition of data, analysis and interpretation of data, drafting and revising the manuscript, funding, and study supervision. All authors contributed to the interpretation of results and critical revision of manuscript for important intellectual content and approved the final version of the manuscript. L. Qi had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of data analysis.

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Disclosures

None.

REFERENCES

1. Fleming-Dutra KE, Hersh AL, Shapiro DJ, Bartoces M, Enns EA, File TM Jr, Finkelstein JA, Gerber JS, Hyun DY, Linder JA, et al. Prevalence of inappropriate antibiotic prescriptions among US ambulatory care visits, 2010-2011. *JAMA*. 2016;315:1864–1873. doi: 10.1001/jama.2016.4151
2. Vaughn VM, Flanders SA, Snyder A, Conlon A, Rogers MAM, Malani AN, McLaughlin E, Bloemers S, Srinivasan A, Nagel J, et al. Excess antibiotic treatment duration and adverse events in patients hospitalized with pneumonia: a Multihospital Cohort Study. *Ann Intern Med*. 2019. doi: 10.7326/M18-3640.
3. Kilkkinen A, Rissanen H, Klaukka T, Pukkala E, Heliövaara M, Huovinen P, Männistö S, Aromaa A, Knekt P. Antibiotic use predicts an increased risk of cancer. *Int J Cancer*. 2008;123:2152–2155. doi: 10.1002/ijc.23622
4. Cao Y, Wu K, Mehta R, Drew DA, Song M, Lochhead P, Nguyen LH, Izard J, Fuchs CS, Garrett WS, et al. Long-term use of antibiotics and risk of colorectal adenoma. *Gut*. 2018;67:672–678. doi: 10.1136/gutjnl-2016-313413
5. Hansen MP, Scott AM, McCullough A, Thorning S, Aronson JK, Beller EM, Glasziou PP, Hoffmann TC, Clark J, Del Mar CB. Adverse events in people taking macrolide antibiotics versus placebo for any indication. *Cochrane Database Syst Rev*. 2019;1:CD011825. doi: 10.1002/14651858.CD011825.pub2
6. Svanström H, Pasternak B, Hviid A. Use of azithromycin and death from cardiovascular causes. *N Engl J Med*. 2013;368:1704–1712. doi: 10.1056/NEJMoa1300799
7. Ray WA, Murray KT, Meredith S, Narasimhulu SS, Hall K, Stein CM. Oral erythromycin and the risk of sudden death from cardiac causes. *N Engl J Med*. 2004;351:1089–1096. doi: 10.1056/NEJMoa040582
8. Ray WA, Murray KT, Hall K, Arbogast PG, Stein CM. Azithromycin and the risk of cardiovascular death. *N Engl J Med*. 2012;366:1881–1890. doi: 10.1056/NEJMoa1003833
9. Mortensen EM, Halm EA, Pugh MJ, Copeland LA, Metersky M, Fine MJ, Johnson CS, Alvarez CA, Frei CR, Good C, et al. Association of azithromycin with mortality and cardiovascular events among older patients hospitalized with pneumonia. *JAMA*. 2014;311:2199–2208. doi: 10.1001/jama.2014.4304
10. Cheng YJ, Nie XY, Chen XM, Lin XX, Tang K, Zeng WT, Mei WY, Liu LJ, Long M, Yao FJ, et al. The role of macrolide antibiotics in increasing cardiovascular risk. *J Am Coll Cardiol*. 2015;66:2173–2184. doi: 10.1016/j.jacc.2015.09.029
11. Wong AYS, Chan EW, Anand S, Worsley AJ, Wong ICK. Managing cardiovascular risk of macrolides: systematic review and meta-analysis. *Drug Saf*. 2017;40:663–677. doi: 10.1007/s40264-017-0533-2
12. Andersen SS, Hansen ML, Norgaard ML, Folke F, Fosbøl EL, Abildstrøm SZ, Raunsø J, Madsen M, Køber L, Gislason GH, et al. Clarithromycin use and risk of death in patients with ischemic heart disease. *Cardiology*. 2010;116:89–97. doi: 10.1159/000315394
13. Wong AY, Root A, Douglas IJ, Chui CS, Chan EW, Ghebremichael-Weldeselassie Y, Siu CW, Smeeth L, Wong IC. Cardiovascular outcomes associated with use of clarithromycin: population based study. *BMJ*. 2016;352:h6926. doi: 10.1136/bmj.h6926
14. Svanström H, Pasternak B, Hviid A. Use of clarithromycin and roxithromycin and risk of cardiac death: cohort study. *BMJ*. 2014;349:g4930. doi: 10.1136/bmj.g4930
15. Schembri S, Williamson PA, Short PM, Singanayagam A, Akram A, Taylor J, Singanayagam A, Hill AT, Chalmers JD. Cardiovascular events after clarithromycin use in lower respiratory tract infections: analysis of two prospective cohort studies. *BMJ*. 2013;346:f1235. doi: 10.1136/bmj.f1235
16. Little P, Stuart B, Smith S, Thompson MJ, Knox K, van den Bruel A, Lown M, Moore M, Mant D. Antibiotic prescription strategies and adverse outcome for uncomplicated lower respiratory tract infections: prospective cough complication cohort (3C) study. *BMJ*. 2017;357:j2148. doi: 10.1136/bmj.j2148
17. Jespersen CM, Als-Nielsen B, Damgaard M, Hansen JF, Hansen S, Helø OH, Hildebrandt P, Hilden J, Jensen GB, Kastrup J, et al; CLARICOR Trial Group. Randomised placebo controlled multicentre trial to assess short term clarithromycin for patients with stable coronary heart disease: CLARICOR trial. *BMJ*. 2006;332:22–27. doi: 10.1136/bmj.38666.653600.55
18. Bin Abdulhak AA, Khan AR, Garbati MA, Qazi AH, Erwin P, Kisra S, Aly A, Farid T, El-Chami M, Wimmer AP. Azithromycin and risk of cardiovascular death: a meta-analytic review of observational studies. *Am J Ther*. 2015;22:e122–e129. doi: 10.1097/MJT.0000000000000138
19. Ianiro G, Tilg H, Gasbarrini A. Antibiotics as deep modulators of gut microbiota: between good and evil. *Gut*. 2016;65:1906–1915. doi: 10.1136/gutjnl-2016-312297

20. Bäumler AJ, Sperandio V. Interactions between the microbiota and pathogenic bacteria in the gut. *Nature*. 2016;535:85–93. doi: 10.1038/nature18849
21. Sana TG, Monack DM. Microbiology: the dark side of antibiotics. *Nature*. 2016;534:624–625. doi: 10.1038/nature18449
22. Modi SR, Collins JJ, Relman DA. Antibiotics and the gut microbiota. *J Clin Invest*. 2014;124:4212–4218. doi: 10.1172/JCI72333
23. Reijnders D, Goossens GH, Hermes GD, Neis EP, van der Beek CM, Most J, Holst JJ, Lenaerts K, Kootte RS, Nieuwdorp M, et al. Effects of gut microbiota manipulation by antibiotics on host metabolism in obese humans: a Randomized Double-Blind Placebo-Controlled Trial. *Cell Metab*. 2016;24:63–74. doi: 10.1016/j.cmet.2016.06.016
24. Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, Almeida M, Arumugam M, Batto JM, Kennedy S, et al; MetaHIT consortium. Richness of human gut microbiome correlates with metabolic markers. *Nature*. 2013;500:541–546. doi: 10.1038/nature12506
25. Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, Liang S, Zhang W, Guan Y, Shen D, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature*. 2012;490:55–60. doi: 10.1038/nature11450
26. Jie Z, Xia H, Zhong SL, Feng Q, Li S, Liang S, Zhong H, Liu Z, Gao Y, Zhao H, et al. The gut microbiome in atherosclerotic cardiovascular disease. *Nat Commun*. 2017;8:845. doi: 10.1038/s41467-017-00900-1
27. Wang J, Jia H. Metagenome-wide association studies: fine-mining the microbiome. *Nat Rev Microbiol*. 2016;14:508–522. doi: 10.1038/nrmicro.2016.83
28. Feng Q, Liang S, Jia H, Stadlmayr A, Tang L, Lan Z, Zhang D, Xia H, Xu X, Jie Z, et al. Gut microbiome development along the colorectal adenoma-carcinoma sequence. *Nat Commun*. 2015;6:6528. doi: 10.1038/ncomms7528
29. Barrington WT, Lusis AJ. Atherosclerosis: association between the gut microbiome and atherosclerosis. *Nat Rev Cardiol*. 2017;14:699–700. doi: 10.1038/nrcardio.2017.169
30. Li X, Wang M, Liu G, Ma J, Li C. Association of macrolides with overall mortality and cardiac death among patients with various infections: a meta-analysis. *Eur J Intern Med*. 2016;28:32–37. doi: 10.1016/j.ejim.2015.09.009
31. Gorelik E, Masarwa R, Perlman A, Rotshild V, Muszkat M, Matok I. Systematic review, meta-analysis, and network meta-analysis of the cardiovascular safety of macrolides. *Antimicrob Agents Chemother*. 2018;62:e00438-18. doi: 10.1128/AAC.00438-18
32. Gorelik E, Masarwa R, Perlman A, Rotshild V, Abbasi M, Muszkat M, Matok I. Fluoroquinolones and Cardiovascular Risk: A Systematic Review, Meta-analysis and Network Meta-analysis. *Drug Saf*. 2019;42:529–538. doi: 10.1007/s40264-018-0751-2
33. Heianza Y, Zheng Y, Ma W, Rimm EB, Albert CM, Hu FB, Rexrode KM, Manson JE, Qi L. Duration and life-stage of antibiotic use and risk of cardiovascular events in women. *Eur Heart J*. 2019;pii:ehz231. doi: 10.1093/eurheartj/ehz231
34. Yuan C, Spiegelman D, Rimm EB, Rosner BA, Stampfer MJ, Barnett JB, Chavarro JE, Subar AF, Sampson LK, Willett WC. Validity of a dietary questionnaire assessed by comparison with multiple weighed dietary records or 24-hour recalls. *Am J Epidemiol*. 2017;185:570–584. doi: 10.1093/aje/kww104
35. Colditz GA. Nurses' Health Study: demonstrating the impact of research, and adapting new measures and approaches to increase relevance and effect of cohort studies. *Public Health Res Pract*. 2016;26:2631628. doi: 10.17061/phrp2631628
36. Colditz GA, Manson JE, Hankinson SE. The Nurses' Health Study: 20-year contribution to the understanding of health among women. *J Womens Health*. 1997;6:49–62. doi: 10.1089/jwh.1997.6.49
37. Rimm EB, Stampfer MJ, Colditz GA, Chute CG, Litin LB, Willett WC. Validity of self-reported waist and hip circumferences in men and women. *Epidemiology*. 1990;1:466–473. doi: 10.1097/00001648-199011000-00009
38. Chiuve SE, Fung TT, Rimm EB, Hu FB, McCullough ML, Wang M, Stampfer MJ, Willett WC. Alternative dietary indices both strongly predict risk of chronic disease. *J Nutr*. 2012;142:1009–1018. doi: 10.3945/jn.111.157222
39. Lloyd-Jones DM, Hong Y, Labarthe D, Mozaffarian D, Appel LJ, Van Horn L, Greenlund K, Daniels S, Nichol G, Tomaselli GF, et al; American Heart Association Strategic Planning Task Force and Statistics Committee. Defining and setting national goals for cardiovascular health promotion and disease reduction: the American Heart Association's strategic impact goal through 2020 and beyond. *Circulation*. 2010;121:586–613. doi: 10.1161/CIRCULATIONAHA.109.192703
40. Stampfer MJ, Willett WC, Speizer FE, Dysert DC, Lipnick R, Rosner B, Hennekens CH. Test of the national death index. *Am J Epidemiol*. 1984;119:837–839. doi: 10.1093/oxfordjournals.aje.a113804
41. Charlot M, Grove EL, Hansen PR, Olesen JB, Ahlehoff O, Selmer C, Lindhardsen J, Madsen JK, Køber L, Torp-Pedersen C, et al. Proton pump inhibitor use and risk of adverse cardiovascular events in aspirin treated patients with first time myocardial infarction: nationwide propensity score matched study. *BMJ*. 2011;342:d2690. doi: 10.1136/bmj.d2690
42. Shih CJ, Chen YT, Ou SM, Li SY, Chen TJ, Wang SJ. Proton pump inhibitor use represents an independent risk factor for myocardial infarction. *Int J Cardiol*. 2014;177:292–297. doi: 10.1016/j.ijcard.2014.09.036
43. Imhann F, Vich Vila A, Bonder MJ, Lopez Manosalva AG, Koonen DP, Fu J, Wijmenga C, Zhernakova A, Weersma RK. The influence of proton pump inhibitors and other commonly used medication on the gut microbiota. *Gut Microbes*. 2017:1–8. doi: 10.1080/19490976.2017.1284732
44. Blake Z. Study provides insufficient evidence of a link between clarithromycin and myocardial infarction. *BMJ*. 2016;352:i1342. doi: 10.1136/bmj.i1342
45. Berni E, de Voogd H, Halcox JP, Butler CC, Bannister CA, Jenkins-Jones S, Jones B, Ouwens M, Currie CJ. Risk of cardiovascular events, arrhythmia and all-cause mortality associated with clarithromycin versus alternative antibiotics prescribed for respiratory tract infections: a retrospective cohort study. *BMJ Open*. 2017;7:e013398. doi: 10.1136/bmjopen-2016-013398
46. Winkel P, Hilden J, Hansen JF, Kastrup J, Kolmos HJ, Kjøller E, Jensen GB, Skoog M, Lindschou J, Gluud C; CLARICOR trial group. Clarithromycin for stable coronary heart disease increases all-cause and cardiovascular mortality and cerebrovascular morbidity over 10years in the CLARICOR randomised, blinded clinical trial. *Int J Cardiol*. 2015;182:459–465. doi: 10.1016/j.ijcard.2015.01.020
47. Mosholder AD, Lee JY, Zhou EH, Kang EM, Ghosh M, Izem R, Major JM, Graham DJ. Long-term risk of acute myocardial infarction, stroke, and death with outpatient use of clarithromycin: a Retrospective Cohort Study. *Am J Epidemiol*. 2018;187:786–792. doi: 10.1093/aje/kwx319
48. Tansarli GS, Mylonakis E. Systematic review and meta-analysis of the efficacy of short-course antibiotic treatments for community-acquired pneumonia in adults. *Antimicrob Agents Chemother*. 2018;62:e00635-18. doi: 10.1128/AAC.00635-18
49. Lee KL, Jim MH, Tang SC, Tai YT. QT prolongation and torsades de pointes associated with clarithromycin. *Am J Med*. 1998;104:395–396. doi: 10.1016/s0002-9343(98)00059-x
50. Nachimuthu S, Assar MD, Schussler JM. Drug-induced QT interval prolongation: mechanisms and clinical management. *Ther Adv Drug Saf*. 2012;3:241–253. doi: 10.1177/2042098612454283
51. Kita E, Sawaki M, Mikasa K, Oku D, Hamada K, Maeda K, Narita N, Kashiba S. Proliferation of erythromycin-stimulated mouse peritoneal macrophages in the absence of exogenous growth factors. *Nat Immun*. 1993;12:326–338.
52. Xu G, Fujita J, Negayama K, Yuube K, Hojo S, Yamaji Y, Kawanishi K, Takahara J. Effect of macrolide antibiotics on macrophage functions. *Microbiol Immunol*. 1996;40:473–479. doi: 10.1111/j.1348-0421.1996.tb01097.x
53. Robbins CS, Hilgendorf I, Weber GF, Theurl I, Iwamoto Y, Figueiredo JL, Gorbatov R, Sukhova GK, Gerhardt LM, Smyth D, et al. Local proliferation dominates lesional macrophage accumulation in atherosclerosis. *Nat Med*. 2013;19:1166–1172. doi: 10.1038/nm.3258
54. Zhu W, Gregory JC, Org E, Buffa JA, Gupta N, Wang Z, Li L, Fu X, Wu Y, Mehrabian M, et al. Gut microbial metabolite TMAO enhances platelet hyperreactivity and thrombosis risk. *Cell*. 2016;165:111–124. doi: 10.1016/j.cell.2016.02.011
55. Heianza Y, Ma W, Manson JE, Rexrode KM, Qi L. Gut microbiota metabolites and risk of major adverse cardiovascular disease events and death: a systematic review and meta-analysis of Prospective Studies. *J Am Heart Assoc*. 2017;6:e004947. doi: 10.1161/JAHA.116.004947.