Annals of Hepatology 31 (2026) 102136

Contents lists available at ScienceDirect

Annals of Hepatology

journal homepage: www.elsevier.es/annalsofhepatology

Original article

Antibiotic consumption, genetic risk and incidence of metabolic dysfunction-associated steatotic liver disease: a prospective cohort study

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ARTICLE INFO

Article History:
Received 13 March 2025
Accepted 24 September 2025
Available online xxx

Keywords:
Antibiotics
Metabolic dysfunction-associated steatotic liver disease
Metabolic syndrome
Prospective cohort
UK Biobank

ABSTRACT

Introduction and Objectives: The association between antibiotic consumption and the risk of developing metabolic dysfunction-associated steatotic liver disease (MASLD) remains ambiguous. This study aimed to investigate this relationship within a large prospective cohort from the UK Biobank.
Patients and Methods: We conducted a prospective cohort study of 143,279 adults aged 40 to 70 years, among whom 1477 were diagnosed with MASLD for the first time. Multivariate Cox proportional hazards regression models were employed to assess the data. The genetic risk score (GRS) for MASLD was derived from five single-nucleotide variants, and mediation analysis was performed to evaluate the role of metabolic syndrome (MetS).
Results: Our findings demonstrated that individuals with antibiotic exposure during childhood or adolescence exhibited a significantly higher risk of developing MASLD compared to those without antibiotic exposure ($P < 0.001$, HR 1.39; 95 % CI 1.21−1.59). No significant interaction was observed between antibiotic consumption and genetic predisposition for MASLD. Mediation analysis revealed that MetS and central obesity accounted for 21.98 % and 13.55 % of the association between early-life antibiotic exposure and MASLD, respectively ($P < 0.001$), particularly in women (P for interaction = 0.031).
Conclusions: Long-term antibiotic exposure in early life was significantly associated with a higher risk of developing MASLD, and this association persisted after adjustment for genetic predisposition factors.

1. Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD), affecting 25 % of the global population, has become the leading cause of chronic liver disease and is anticipated to be the primary reason for liver transplants by 2030 [1]. Growing evidence suggests that MASLD represents a multisystem disorder, with clinical consequences extending beyond hepatic morbidity and mortality to include extrahepatic organ damage and metabolic dysregulation [2]. For example, MASLD increases the risk of type 2 diabetes mellitus (T2DM) [3], cardiovascular and cardiac diseases [4], and chronic kidney disease [5]. Currently, the pathogenesis of MASLD remains incompletely understood, and no drug is approved for the treatment of MASLD. Consequently, identifying key modifiable risk factors influencing MASLD development and progression is essential for developing effective prevention strategies.

Antibiotics are widely used in clinical practice for treating bacterial infections through their bacteriostatic or bactericidal effects [6]. The extensive consumption of antibiotics is frightening. By 2030, it is predicted that global antibiotic consumption will be 200 % higher than the 42 billion defined daily doses estimated in 2015 [7]. Antibiotic-induced alterations in microbial composition have been recently emphasized as they decrease microbial diversity and modify microbiota functions, disrupting ecological homeostasis and causing

Abbreviations: BMI, body mass index; GRS, genetic risk score; HDL, high-density lipoprotein; HDL-C, high-density lipoprotein cholesterol; HR, hazard ratio; MASLD, metabolic dysfunction–associated steatotic liver disease; MetS, metabolic syndrome; NASH, non-alcoholic steatohepatitis; SNPs, single nucleotide polymorphisms; UKB, UK Biobank; WHO, World Health Organization

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https://doi.org/10.1016/j.aohep.2025.102136

diseases [8]. These findings have raised increasing concerns about the potential health consequences of antibiotic exposure.

While epidemiological studies have paid limited attention to the association between antibiotic exposure and MASLD incidence, preliminary evidence from a cohort study suggests a potential increased risk of MASLD with antibiotic exposure [9]. Paradoxically, certain antibiotics including solithromycin, ampicillin, and gentamicin have shown therapeutic potential in clinical trials or experimental models of MASLD [10,11]. This apparent contradiction may be explained by the mechanism whereby oral antibiotics reduce intestinal bacterial load, subsequently reducing portal secondary bile acid levels and alleviating MASLD [12]. Thus, more authoritative real-world studies are needed to clarify whether antibiotic exposure exerts beneficial or detrimental effects on the occurrence and progression of MASLD.

To the best of our understanding, the usage of antibiotics has been associated with a higher risk of MetS in recent studies [13,14]. However, no previous studies have investigated the potential mediating role of metabolic syndrome (MetS) in the association between early-life antibiotic exposure and MASLD incidence, despite MetS containing several metabolic risk factors that are strong predictors of MASLD [15]. Therefore, this study investigated the association between extended antibiotic intake in early life and the risk of MASLD through a prospective cohort from the UK Biobank (UKB). Additionally, a genetic risk score (GRS) was used to evaluate the potential interaction and joint association. Furthermore, MetS and its five components were evaluated as potential mediators to elucidate the underlying mechanisms connecting early-life antibiotic exposure to MASLD development.

2. Patients and Methods

2.1. Study population and exposure definition

Between 2006 and 2010, the UKB recruited over 500,000 participants aged 37 to 69 from 22 assessment centers across England, Scotland, and Wales [16]. Participants provided informed consent before completing touchscreen questionnaires and verbal interviews, undergoing physical examinations, and donating biological samples.

In the present analysis, from this cohort, individuals who later withdrew from the study (n = 1298) and those diagnosed with MASLD or other liver diseases at baseline (n = 9705) were excluded. Furthermore, participants missing data on long-term antibiotic exposure during early life were also excluded (n = 319,298). Additionally, participants with incomplete genetic information (n = 5569), those with discrepancies between self-reported and genetic gender (n = 106), and those not of European descent based on self-reported ethnicity (UKB field ID: 21,000) were excluded (n = 23,237). After these exclusions, the main analysis included 143,279 participants (Fig. 1). Inclusion criteria and exclusion criteria are provided in Supplemental Table S2.

2.2. Outcome definition and follow-up

The outcome of MASLD was defined as either hospitalization or mortality attributable to MASLD, identified using the algorithm recommended by the UKB[17]. Detailed definitions are provided in Supplemental Table S1. Data related to the disease were sourced from

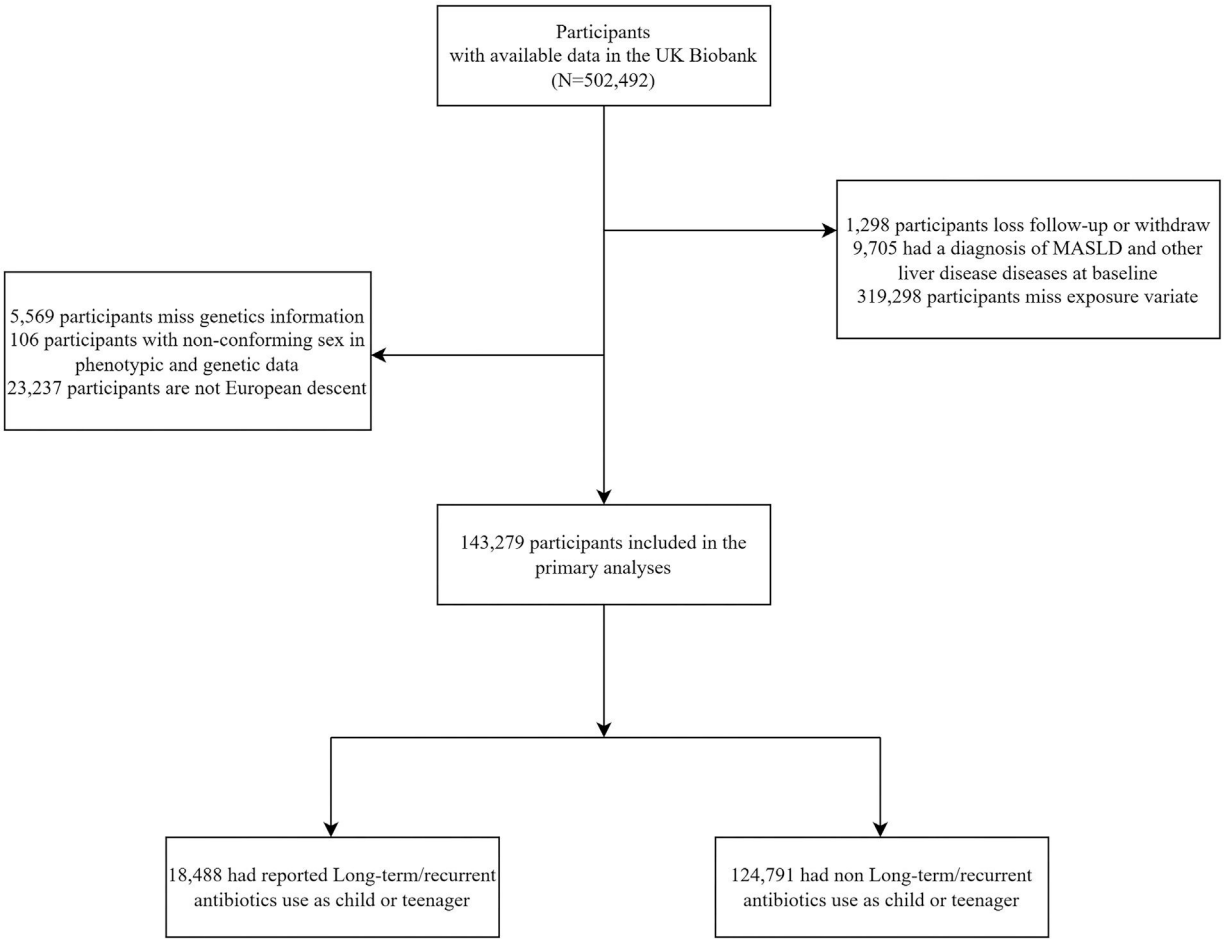

Fig. 1. Flowchart of participants in the study cohort.

electronic health records of hospital admissions and death registries, linked with the Hospital Episode Statistics for England, Scottish Morbidity Records for Scotland, and the Patient Episode Database for Wales. As of the time of analysis, the censoring dates for the Hospital Episode Statistics were 1 November 2021 for England, 25 September 2021 for Scotland, and 29 May 2021 for Wales. Follow-up time was calculated from baseline to the earliest occurrence of MASLD diagnosis, death, loss to follow-up, or censoring. In accordance with the latest Expert Panel Consensus Statement [18], MASLD was defined for the primary analyses of this study as ICD-9 code 578.9 and ICD-10 codes K76.0 and K75.8. A broader definition was employed for the sensitivity analysis, as detailed in the statistical analyses section.

2.3. Covariates assessment

Structured questionnaires were utilized to evaluate various potential confounding variables, including sociodemographic characteristics (age, sex, ethnicity, education, and occupation), socioeconomic status (as measured by the Townsend Deprivation Index), lifestyle factors (such as physical activity, smoking, alcohol consumption, and habitual diet), and comorbidities (notably cardiovascular disease and cancer). According to the World Health Organization (WHO), overweight and obesity in adults are classified as follows: overweight is defined by a body mass index (BMI) of 25 kg/m^2 or greater, while obesity is defined by a BMI of 30 kg/m^2 or greater. MetS was characterized in accordance with the Harmonized Criteria established by the International Diabetes Federation (IDF) and the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) in 2009 [19]. The components of metabolic syndrome, namely central obesity, elevated glycemia/diabetes, hypertension, low high-density lipoprotein (HDL) cholesterol, and elevated triglycerides, were identified using baseline data. Central obesity was defined as a waist circumference of 88 cm or greater in women and 102 cm or greater in men. Elevated glycemia/diabetes was defined as a fasting glucose level of 5.6 mmol/L or higher, or the use of medication for elevated blood glucose. Hypertension was defined as a systolic blood pressure of $\geq$ 130 mmHg, a diastolic blood pressure of $\geq$ 85 mmHg, or the use of antihypertensive medications. Hypertriglyceridemia was characterized by triglyceride levels of $\geq$ 150 mg/dL, while low HDL-cholesterol was defined as $<$ 1.3 mmol/L in women and $<$ 1.0 mmol/L in men, or the use of lipid-modifying medications. Additional information regarding the measurement protocols is available on the UKB website (http://www.ukbiobank.ac.uk).

2.4. Polygenic risk score

Study participants were genotyped for the following single nucleotide polymorphisms (SNPs): rs738409 (PNPLA3), rs58542926 (TM6SF2), rs641738 (MBOAT7), rs1260326 (GCKR), and rs72613567 (HSD17B13), as outlined in a previous study [20]. The GRS demonstrated an association between genotype and the risk of MASLD by assigning scores based on these five SNPs, which were associated with MASLD incidence in white participants. Detailed information about these SNPs is provided in Supplemental Table S3. Furthermore, details on genotyping procedures and quality control measures are available online [21].

2.5. Statistical analysis

In the analysis of descriptive statistics, categorical variables were represented by frequencies and percentages, whereas continuous variables were expressed as means with standard deviations (SD) or medians with interquartile ranges (IQR). Group differences were assessed using the Student's *t*-test, Wilcoxon test, or Chi-squared test, as appropriate.

Cox proportional hazards models were employed to estimate hazard ratios (HRs) and 95 % confidence intervals (CIs) for the association between antibiotic consumption and the incidence of MASLD. The proportional hazards assumption was evaluated using Schoenfeld residuals, with no violations detected. In Model 1, adjustments were made for age and sex. Model 2 included additional adjustments for education, the Townsend deprivation index, smoking status, alcohol consumption, dietary patterns, total physical activity, baseline cancer, cardiovascular disease, GRS, the first ten principal components of ancestry, and genotype measurement batch. Model 3, the full model, incorporated further adjustments for the five components of MetS based on Model 2. Missing covariate values were addressed using multiple imputation with five imputations, analyzed via SAS PROC MI and PROC MIANALYZE.

Stratified analyses were conducted to examine the association between early-life antibiotic consumption and the onset of MASLD, considering variables such as age ($<$ 60 and $\geq$ 60 years), sex, BMI ($<$ 25 and $\geq$ 25 kg/m^2), alcohol consumption, and genetic risk level (categorized as low and high risk, with the mean GRS serving as the threshold). Mediation analyses were performed to evaluate the mediated effects of MetS, central obesity, hypertension, elevated triglycerides, high blood glucose, and reduced HDL cholesterol on the relationship between antibiotic consumption and MASLD risk. These analyses also assessed mediation proportions across different sex groups using SAS PROC CAUSALMED. To investigate the combined association of antibiotic consumption and genetic risk on MASLD incidence, individuals with low GRS and no antibiotic consumption were designated as the reference group for a joint analysis. Additionally, the interaction between antibiotic consumption and GRS was evaluated on a multiplicative interaction scale. This was achieved by comparing statistical models with and without a cross-product interaction term for antibiotic consumption and genetic risk of MASLD, utilizing likelihood ratio tests to assess the effect of multiplicative interactions.

Sensitivity analyses were conducted to assess the robustness of the primary analysis results. To mitigate potential reverse causality, analyses were repeated after excluding individuals who either died or developed MASLD within two years of follow-up. The Fine-Gray competing risk model was employed to investigate possible competing risks associated with all-cause mortality. Additional potential broader incident MASLD cases, identified by ICD-10 codes K768, K769, K740, K741, K742, and K746, were considered, and participants with incomplete covariate data were excluded. To further ensure the validity of our findings and eliminate the possibility of results arising by chance, we evaluated the associations between our primary exposures and the incidence of head injuries as a falsification endpoint [22]. Inverse probability of treatment weighting was also applied to certain variables.

All statistical analyses were conducted using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) and R version 4.1.3 (R Foundation for Statistical Computing). Statistical significance was determined with two-sided *P* values, with a threshold set at $<$0.05.

2.6. Ethics statements

The UKB was approved by the North West Multi-Centre Research Ethics Committee. This research has been carried out using the UKB resource under application number 68,307 (https://www.ukbiobank.ac.uk/ethics/).

3. Results

3.1. Characteristics of the study population

This study included 143,279 participants, of whom 56.5 % were female, with a mean age of 56.2 years. Baseline demographics and characteristics, stratified by exposure to antibiotic consumption in early life, are presented in Table 1. Compared to individuals not

Table 1
Baseline characteristics of participants.

Characteristics	Antibiotic consumption in early life (Before PSM)					Antibiotic consumption in early life (After PSM)			
	Total	Exposure	Non-exposure	SMD	*P*	Exposure	Non-exposure	SMD	*P*
Patients, n	143,279	18,488	124,791			16,515	66,060		
Age (years), mean (SD)	56.2 ± 7.6	54.0 ± 7.4	56.5 ± 7.6	0.331	<0.001	55.0 ± 7.1	55.0 ± 7.4	0.011	0.226
Female (%)	80,982 (56.5)	13,002 (70.3)	67,980 (54.5)	0.332	<0.001	11,036 (66.8)	44,281 (67.0)	0.004	0.619
BMI (kg/m^2), mean (SD)	26.8 ± 4.5	27.2 ± 5.0	26.7 ± 4.4	0.113	<0.001	26.9 ± 4.7	26.7 ± 4.6	0.046	<0.001
Townsend deprivation index, median (IQR)	−2.6 (−3.9, −0.4)	−2.5 (−3.8, −0.1)	−2.6 (−3.9, −0.5)	–	<0.001	−2.5 (−3.9, −0.3)	−2.5 (−3.8, −0.4)	–	0.418
College or University degree (%)	61,810 (43.3)	7764(42.2)	54,046 (43.5)	0.065	<0.001	7130 (43.2)	28,393 (43.0)	0.005	0.854
Smoking status (%)				0.033	<0.001			0.005	0.851
Never	83,410 (58.3)	10,719 (58.1)	72,691 (58.4)			9758 (59.1)	38,874 (58.8)		
Previous	49,936 (34.9)	6355 (34.4)	34,581 (35.0)			5641 (34.2)	22,710 (34.4)		
Current	9671 (6.8)	1384 (7.5)	9532 (6.7)			1116 (6.8)	4476 (6.8)		
Alcohol consumption (%)				0.140	<0.001			0.003	0.943
Never/special occasions only	19,488 (13.6)	3100 (16.8)	16,388 (13.1)			2421 (14.7)	9623 (14.6)		
No >2 times/week	51,811 (36.2)	7133 (38.6)	44,678 (35.8)			6272 (38.0)	25,159 (38.1)		
>2 times/week	71,950 (50.2)	8249 (44.6)	63,701 (51.1)			7822 (47.4)	31,278 (47.3)		
Total physical activities (%)				0.043	<0.001			0.006	0.518
< 10 MET	25,559 (18.9)	3542 (20.4)	22,017 (18.7)			3203 (19.4)	12,962 (19.6)		
≥10 MET	109,653 (81.1)	13,823 (79.6)	95,830 (81.3)			13,312 (80.6)	53,098 (80.4)		
Dietary pattern (%)				0.051	<0.001			0.002	0.823
Unhealthy	61,993 (43.2)	7590 (41.1)	54,403 (43.6)			6887 (41.7)	27,482 (41.6)		
Healthy	81,286 (56.8)	10,898 (59.0)	70,388 (56.4)			9628 (58.3)	38,578 (58.4)		
Genetic risk score (%)				0.009	0.241			0.002	0.783
Low level	80,716 (56.3)	10,489 (56.7)	70,227 (56.3)			9361 (56.7)	37,363 (56.6)		
High level	62,563 (43.7)	7999 (43.3)	54,564 (43.7)			7154 (43.3)	28,697 (43.4)		
History of CVD (%)	8369 (5.9)	1084 (5.9)	7285 (5.8)	0.001	0.890	919 (5.6)	3708 (5.6)	0.002	0.823
History of cancer (%)	10,059 (7.1)	1335 (7.2)	8724 (7.0)	0.009	0.253	1178 (7.1)	4794 (7.3)	0.005	0.593
MetS	27,369 (19.1)	3627 (19.6)	23,742 (19.0)	0.015	0.056	3138 (19.0)	12,768 (19.3)	0.008	0.346
Central obesity (%)	40,924 (28.6)	6161 (33.3)	34,763 (27.9)	0.119	<0.001	4952 (30.0)	20,011 (30.3)	0.007	0.447
Hypertension (%)	71,245 (49.7)	8531(46.1)	62,714 (50.3)	0.082	<0.001	7773 (47.1)	31,152 (47.2)	0.002	0.841
Hyperglycaemia (%)	7452 (5.2)	926 (5.0)	7224 (5.1)	0.010	0.207	805 (4.9)	3318 (5.0)	0.007	0.446
Hypertriglyceridemia (%)	51,005 (35.6)	6475 (35.0)	44,530 (35.7)	0.014	0.080	5717(34.6)	22,941 (34.7)	0.002	0.797
Dyslipidemia (%)	18,572 (12.9)	2063 (11.2)	16,509 (13.2)	0.063	<0.001	1908 (11.6)	7765 (11.8)	0.006	0.480

MET, metabolic equivalent; SMD, standardized mean difference; PSM, propensity score matching.

taking antibiotics, those who used them long-term were generally younger, more likely to be women, had a higher BMI, smoked more often, had less education, and maintained healthier eating habits. To mitigate potential baseline differences between the exposed and unexposed groups, propensity score matching was employed. The study population was generally well-balanced post-matching, although the antibiotic-exposed group exhibited significantly higher baseline BMI than the non-exposed group (Table 1).

3.2. Association of antibiotic consumption in early life with MASLD

The median follow-up duration was 13.7 years (interquartile range: 13.2 to 14.2 years). During this period, 1466 participants developed MASLD, as indicated by hospitalization or mortality due to MASLD. A significantly higher cumulative hazard ratio for MASLD was observed in the antibiotic-exposed group compared to the non-exposed group (Fig. 2). Both the crude model and Model

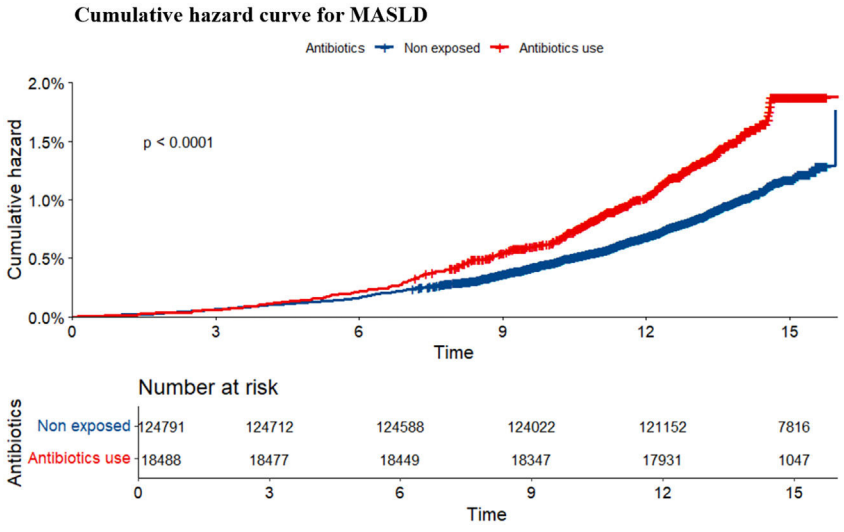

Fig. 2. Cumulative hazard curve of MASLD according to antibiotic consumption in early life.

Table 2
Association between long-term antibiotics consumption as a child or teenager and incident MASLD.

Antibiotic consumption	Events / Total	Model 1[a]		Model 2[b]		Model 3[c]	
		HR (95 % CI)	*P* Value	HR (95 % CI)	*P* Value	HR (95 % CI)	*P* Value
Nonexposed group	1194/ 124,791	1.00 (Ref.)		1.00 (Ref.)		1.00 (Ref.)	
Exposed group	272 / 18,488	1.52 (1.42, 1.85)	<0.001	1.52 (1.33, 1.74)	<0.001	1.39 (1.21, 1.59)	<0.001
After PSM							
Nonexposed group	636/66,060	1.00 (Ref.)		1.00 (Ref.)		1.00 (Ref.)	
Exposed group	218/16,515	1.38 (1.19, 1.61)	<0.001	1.39 (1.19, 1.62)	<0.001	1.39 (1.20, 1.63)	<0.001

HR, hazard ratio; 95 % CI, 95 % confidence interval; MASLD, metabolic dysfunction-associated steatotic liver disease.
a. Model 1 was adjusted for age and sex.
b. Model 2 was adjusted for age, sex, education, Townsend deprivation index, smoking status, alcohol consumption, dietary pattern, GRS, batch, and top 10 principal components.
c. Model 3 was adjusted for five components of metabolic syndrome (central obesity, high glycaemia/diabetes, high blood pressure/hypertension, low HDL, and high triglycerides) based on model 2.

2 demonstrated an elevated risk of MASLD in the exposed group, with HR of 1.52 (95 % CI: 1.42−1.85) and 1.52 (95 % CI: 1.33−1.74), respectively. Model 3, which was further adjusted for MetS, yielded similar results (HR 1.39, 95 % CI: 1.21−1.59). Following the application of propensity score matching, the association between antibiotic consumption and the incidence of MASLD remained significant (HR 1.39, 95 % CI: 1.20−1.63 in Model 3) as presented in Table 2.

3.3. Joint association between the weighted GRS for MASLD and antibiotic consumption

The joint association between the weighted GRS for MASLD, antibiotic consumption, and the incidence of MASLD was also analyzed. Participants who had antibiotic exposure in early life and a high GRS exhibited the highest risk for MASLD. A significant association between early-life antibiotic consumption and an increased risk of MASLD was observed across different strata of genetic susceptibility (P = 0.005 [low], P < 0.001 [high]), as illustrated in Supplemental Figure S1. No genetic multiplicative interactions were detected in the longitudinal association between antibiotic consumption and MASLD incidence, indicating that this association was not influenced by genetic susceptibility (P interaction = 0.474), as shown in Table 3.

Table 3
Stratified analysis for the association between long-term antibiotics consumption as child or teenager and incident MASLD.

Characteristics	*N*	Non exposure	Exposure	*P* interaction
BMI				0.309
< 25	55,541	1.00 (Ref.)	1.32 (1.08, 1.61)	
≥ 25	87,738	1.00 (Ref.)	1.39 (1.21, 1.60)	
Sex				0.031
Female	80,982	1.00 (Ref.)	1.49 (1.27, 1.75)	
Male	62,297	1.00 (Ref.)	1.16 (0.91, 1.49)	
Age				0.708
< 60	86,272	1.00 (Ref.)	1.39 (1.18, 1.63)	
≥ 60	57,007	1.00 (Ref.)	1.35 (1.05, 1.73)	
Drink				0.694
Never/special occasions only	19,488	1.00 (Ref.)	1.28 (0.99, 1.66)	
No >2 times/week	51,811	1.00 (Ref.)	1.45 (1.16, 1.80)	
>2 times/week	71,950	1.00 (Ref.)	1.50 (1.20, 1.87)	
GRS level				0.474
Low	80,716	1.00 (Ref.)	1.35 (1.11, 1.64)	
High	62,563	1.00 (Ref.)	1.50 (1.25, 1.81)	
Dietary				0.804
Healthy	81,286	1.00 (Ref.)	1.39 (1.16, 1.68)	
Unhealthy	61,993	1.00 (Ref.)	1.38 (1.13, 1.68)	

GRS, genetic risk score; MASLD, metabolic dysfunction-associated steatotic liver disease.
Adjusted for age, sex, education, Townsend deprivation index, smoking status, alcohol consumption, dietary pattern and five components of metabolic syndrome (central obesity, high glycaemia/diabetes, high blood pressure/hypertension, low HDL, and high triglycerides), GRS, batch and top 10 principal components.

3.4. Subgroup and mediation analysis results

In the stratified analyses, participants were categorized according to BMI, sex, age, alcohol consumption, dietary, and GRS levels. The interactions between early-life antibiotic consumption and MASLD were more pronounced in women (P interaction = 0.03). No significant associations were observed across different age groups, BMI categories (normal-weight or overweight/obese), levels of alcohol consumption, dietary patterns, and GRS levels among participants (all P interaction > 0.05) (Table 3). Prior to conducting mediation analysis, a correlation analysis was performed, which revealed no relationship between elevated blood glucose levels and early-life antibiotic consumption. The mediation analysis indicated that MetS and its four components, excluding high blood glucose, could mediate the association between early-life antibiotic consumption and the incidence of MASLD in all participants. The mediating effect of MetS was most significant (P < 0.001, 21.98 %, 95 % CI 16.31−27.65 %), while central obesity, elevated triglycerides, hypertension, and reduced HDL mediated the effects by 13.55 %, 2.64 %, 0.83 %, and 0.58 %, respectively. Furthermore, the mediation analysis conducted in different genders, MetS, and central obesity showed the association between antibiotic consumption and MASLD incidence only in females, not males (Table 4).

3.5. Sensitivity analysis results

We employed various methodologies to validate our findings. The associations between MASLD and antibiotic consumption during childhood or adolescence remained significantly correlated even after excluding individuals censored within the first two years of follow-up. Additionally, analyses using the Fine−Gray models and the exclusion of participants with missing covariates yielded highly consistent results (all P < 0.001). Furthermore, the application of inverse probability of treatment weighting in this cohort demonstrated similar associations between MASLD and early-life antibiotic consumption (Model 3: HR 1.33, 95 % CI 1.15−1.54, P < 0.001). To study whether the definition of broader MASLD yields consistent results, we incorporated additional criteria, which did not alter the results (Model 3: HR 1.31, 95 % CI 1.18−1.47, P < 0.001). Lastly, to mitigate potential spurious correlations inherent in large cohort databases, we employed head injury incidence as a falsification endpoint, finding no significant association between head injury incidence and early-life antibiotic consumption (P = 0.361) (Supplemental Table S4).

Table 4
Mediation analysis to evaluate whether MetS and each five components.

Antibiotic consumption during childhood or youth	MetS Proportion mediated, % (95 % CI)	*P* Value	Central obesity Proportion mediated, % (95 % CI)	*P* Value	Hypertension Proportion mediated, % (95 % CI)	*P* Value
Total	21.98 (16.31, 27.65)	<0.001	13.55 (8.83, 18.26)	<0.001	0.83 (0.07,1.59)	0.033
Female	21.05 (15.45, 26.66)	<0.001	15.49 (10.19, 20.80)	<0.001	0.33 (−0.21,0.86)	0.230
Male	15.81 (−0.73, 32.35)	0.060	13.09 (−4.85, 31.04)	0.153	3.81 (−2.61,10.22)	0.244

Continued.

Antibiotic consumption during childhood or youth	High blood glucose Proportion mediated, % (95 % CI)	*P* Value	High triglycerides Proportion mediated, % (95 % CI)	*P* Value	Reduced HDL Proportion mediated, % (95 % CI)	*P* Value
Total	NA	NA	2.64 (1.11, 4.17)	<0.001	0.58 (0.01, 1.15)	0.048
Female	NA	NA	2.45 (0.90, 4.01)	0.002	0.36 (−0.15, 0.88)	0.167
Male	NA	NA	5.25 (−3.15, 13.65)	0.221	1.69 (−1.57, 4.94)	0.310

mediated the associations of antibiotic consumption with MASLD risk.
Adjusted for age, sex, education, Townsend deprivation index, smoking status, alcohol consumption, dietary pattern and five components of metabolic syndrome (central obesity, high glycaemia/diabetes, high blood pressure/hypertension, low HDL, and high triglycerides), GRS, batch and top 10 principal components.

4. Discussion

To our knowledge, this is the first large prospective cohort study to reveal that long-term antibiotic consumption is connected with the risk of MASLD. This association remained significant after adjustment for genetic predisposition to MASLD, with a modest mediation by MetS and central obesity, especially in women.

MASLD has traditionally been regarded as a consequence of metabolic syndrome [23]. The diagnostic components of MetS include central obesity, increased triglycerides, increased blood pressure, increased fasting glucose, and decreased HDL-C levels [24]. Growing evidence indicates that antibiotic consumption is significantly associated with metabolic dysregulation, leading to obesity [25] and metabolic disorders [13]. However, it is still uncertain how MetS mediates the relationship between antibiotic consumption and the risk of MASLD. Our results showed that MetS and central obesity (21.98 % and 13.55 % respectively) partially mediated the association between antibiotic consumption and the risk of MASLD.

No previous prospective cohort studies have examined MASLD incidence associated with early-life antibiotic exposure. A single cross-sectional cohort study involving 2584 Swedish adults with histologically confirmed MASLD was carried out to investigate how antibiotic consumption affects the incidence rate of MASLD [9]. It was found that antibiotic intake could increase the incidence rate of MASLD in a dose-dependent manner, especially in individuals without metabolic syndrome. Our findings, demonstrating MetS-mediated associations between antibiotic consumption and MASLD risk, suggested that antibiotic exposure in the population without metabolic syndrome was more likely to increase the incidence rate of MASLD by causing metabolic disorders. In addition to a detailed mediation analysis, our study also explored whether genetic variation played a role in the connection between antibiotic intake and MASLD incidence.

MASLD susceptibility and progression are influenced by the interaction between environmental and genetic factors [26]. A significant amount of evidence indicates that hepatic fat content is highly heritable [27]. In recent years, multiple genome-wide associations and large-scale candidate gene studies have identified multiple genetic contributors to MASLD. However, our data showed that the association between antibiotic consumption and the incidence of MASLD was not affected by genetic susceptibility (*P* interaction = 0.474). This pointed out that in the population examined in this study, gene variants known to be genetic determinants of MASLD, such as PNPLA3 [28], TM6SF2 [29], and GCKR [30], showed less susceptibility to antibiotic intake. The main mechanism underlying antibiotic-induced MASLD may involve antibiotic-induced hepatocellular-cholestatic injury and gut microbiota dysbiosis. Antibiotics, such as amoxicillin-clavulanate [31], flucloxacillin [32], azithromycin [33], and ceftriaxone [34], could induce cholestatic liver injury in clinical practice. Some patients may not show symptoms such as jaundice, but potential cholestasis and bile acid (BA) metabolism disorders in hepatocytes may have already occurred.

As signaling molecules, BAs are capable of initiating cellular death pathways or releasing chemokines to recruit inflammatory cells [35]. It has been confirmed that BAs accumulation in hepatocytes may induce mitochondrial permeability changes and dysfunction through Fas receptor oligomerization and activation of TNF-related apoptosis-inducing ligand receptor or death receptor 5 [36,37]. Mitochondrial dysfunction results in reduced free fatty acid oxidation and increased reactive oxygen species (ROS) production, resulting in hepatic lipid accumulation and aggravated oxidative stress, which further accelerates the occurrence and progression of MASLD [38,39] Exposure of hepatocytes to elevated BA levels, such as in obstructive cholestasis, increases inflammatory cytokines, inflammatory cell adhesion molecules, and oxidase, thereby accelerating MASLD progression [40].

Interestingly, our results demonstrated a higher MASLD incidence rate in males not using antibiotics (Supplemental Figure S2). This finding aligns with epidemiological reports indicating significantly higher overall MASLD prevalence in men compared to women [41]. Conversely, among individuals with early-life antibiotic exposure, females showed higher MASLD incidence. Furthermore, mediation analysis stratified by gender revealed that MASLD associated with early-life antibiotic use in females was mediated by MetS and central obesity. Studies indicate that the risk of cholestasis caused by antibiotics (such as flucloxacillin) is higher in women [42], which partially explains why the interactions between antibiotic consumption in early life and MASLD are more pronounced in women.

Several studies have shown that patients with MASLD exhibit significant intestinal dysbiosis, featuring elevated abundances of *Proteus* and *Enterobacter* bacteria and reduced content of *Ruminococcus* and *Lactobacillus* [43,44]. A meta-analysis of gut microbiome alterations in MASLD patients revealed characteristic fecal microbiota composition changes, including increased *Escherichia, Prevotella*, and *Streptococcus* with concomitant reductions in *Coprococcus, Faecalibacterium*, and *Ruminococcus* [45]. These pieces of evidence indicate that gut microbiota dysbiosis plays an important role in MASLD pathogenesis. The changes in the composition of substances transported to the liver due to changes in microbial metabolites and increased intestinal permeability are the main damages caused by gut microbiota dysbiosis, leading to the development of MASLD through dysregulation of the gut liver axis [46]. Antibiotics alter the diversity and composition of the human gut flora, initiating a series of events that directly cause various metabolic changes, which ultimately result in the occurrence and progression of MASLD.

The UK Biobank enabled simultaneous examination of exposures within a large, well-characterized cohort of middle-aged and older

adults, permitting adjustment for numerous potential confounders, including established MASLD risk factors. Nonetheless, this research has some limitations. First, early-life antibiotic exposure assessment was limited by categorical data collection, making it impossible to account for details such as the specific types, dosages, duration of antibiotic use, exact age at exposure, or other therapeutic interventions. Second, the exclusive inclusion of Caucasian participants limits generalizability to other ethnicities. Third, the observational design precludes causal inferences and cannot exclude potential reverse causation. Fourth, despite including a long list of confounding factors in the analyses, unmeasured or residual confounding remains possible. Therefore, further investigation is essential to confirm the association between early-life antibiotic consumption and MASLD incidence.

5. Conclusions

In conclusion, long-term antibiotic consumption in early life is highly associated with the incidence of MASLD over a follow-up period of 13.4 years and is modestly mediated by MetS. These findings probably highlight the importance of detailed guidance on antibiotic intake for MASLD prevention.

Author contributions

YZ, YFL, and ZMZ were instrumental in developing the study concept. Statistical analyses were conducted by YMH, JJZ, and JCX. The initial draft of the manuscript was prepared by ZMZ and YMH. AP, ZMZ, YMH, JJZ, and JCX all contributed to the data interpretation. AP has confirmed access to and verification of the underlying data. YZ confirms that all authors listed fulfill the criteria for authorship and that no eligible authors have been overlooked. All authors had access to the data and share responsibility for the submission decision.

Data sharing statement

Researchers interested in accessing the data used in this study can apply for access to the UK Biobank by visiting their website (http://www.ukbiobank.ac.uk/register-apply) and submitting an application that includes a research protocol summary and requested data fields. Upon approval by the UK Biobank management team and payment of applicable fees, researchers will be granted access to the database. This research has been carried out using the UKB resource under application number 88,159.

Declaration of interests

None.

Funding

YFL was supported by the National Nature Science Foundation of China (82070830) and National Key Research and Development Program of Hubei province (2022BCA036). YZ was supported by the National Key Research and Development Program (2023YFF1205000).

Acknowledgments

We thank all participants in the UKB study and all those involved in the UKB study construction.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.aohep.2025.102136.

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