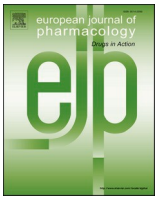
European Journal of Pharmacology 932 (2022) 175209

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar



Serum cholesterol increase in statin users associated with antibiotic use: Case-crossover study

Idit Lavi [a], Naomi Gronich [a,b,*]

[a] Clalit Department of Community Medicine and Epidemiology, Lady Davis Carmel Medical Center, Haifa, Israel
[b] Ruth and Bruce Rappaport Faculty of Medicine, Technion–Israel Institute of Technology, Haifa, Israel

ARTICLE INFO

Keywords:
Antibiotics
Gut microbiota
Statins
HMC-CoA reductase inhibitors
Cholesterol
LDL-C
Effectiveness

ABSTRACT

Purpose: 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors ("statins") reduce risk of atherosclerotic disease. However, statins need secondary bile acids, produced by the gut microbiota, for absorption. Our hypothesis was that a change in the gut microbiota induced by antibiotics might cause a decrease in statin absorption, and decreased statin effectiveness. Our objective was to study the association between antibiotic treatment and increased cholesterol level in statin users.

Methods: Case-crossover study, in which an individual serves as his own control, by comparing outcome risk among the same individual at different times, adjusting for time-dependent comorbidity index. The study is based on adherent statin users' cohort and two cohorts of patients not treated with statins, in Clalit Health Services. Exposure were antibiotic prescriptions dispensed in the 3 months prior to LDL-C measurements.

Results: There were 25,496 statin users and 72,638 time-points. A significant association was found between LDL-C increase and exposure to macrolides and clindamycin, OR = 1.237 (1.138–1.345), p = $6.5*10^{-7}$, number needed to harm (NNH) = 19. There was no association between LDL-C increase and negative control objects such as anti-viral treatments; nor between LDL-C and exposure to antibiotics in non-statin users. As a secondary outcome, we have found an association between LDL-C increase and a following atherosclerotic ischemic event.

Conclusion: An increase in LDL-C in highly adherent statin users is associated with precedent macrolides or clindamycin treatment.

Key messages

- Statins absorption depends on secondary bile acids, produced by the gut bacteria.
- Oral macrolides and oral clindamycin, known to induce changes in the gut microbiota composition, are associated with an increase in LDL-C, in statin users.

1. Introduction

Strong evidence indicates that 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMG-CoA reductase inhibitors) ("statins") reduce the risk and complications of atherosclerotic disease as primary and as secondary prevention. Each 1 mmol/l reduction in serum low-density lipoprotein-cholesterol (LDL-C) reduces the risk of atherosclerotic events by 22% after five years (Adhyaru and Jacobson, 2018). Statins activity depends on drug absorption using secondary bile acids, converted from primary bile acids by the gut bacteria (Chiang, 2013; Devlin and Fischbach, 2015; Sonnenburg and Bäckhed, 2016; Lynch and Pedersen, 2016).

Short-term exposure to antibiotics has been shown to change the quantity and composition of normal human flora bacteria (Arumugam et al., 2011; Ding and Schloss, 2014; Lynch and Pedersen, 2016; Zhernakova et al., 2016; Ferrer et al., 2017; Winston and Theriot, 2019), including depletion of bacterial 7α-dehydroxylation activity and decrease in the rate of conversion of primary bile acids to secondary forms (Samuel et al., 1973; Høverstad et al., 1986; Zhang et al., 2014; Wahlström et al., 2016; Zarrinpar et al., 2018).

In statin users, decreased secondary bile acids pool size might be manifested by a reduction in statins absorption, thus a decline in exposure to statins in serum, and, as a result, an increase in serum LDL-C. It has been shown in mice, that simvastatin effect in reducing lipids levels in high-fat fed mice was attenuated in a group receiving the antibiotic imipenem that altered gut microbiota composition (He et al.,

* Corresponding author. Department of Community Medicine and Epidemiology Carmel Medical Center, 7 Michal St. Haifa, 34362, Israel.
E-mail address: gronich@technion.ac.il (N. Gronich).

https://doi.org/10.1016/j.ejphar.2022.175209
Received 9 May 2022; Received in revised form 10 August 2022; Accepted 11 August 2022
Available online 17 August 2022

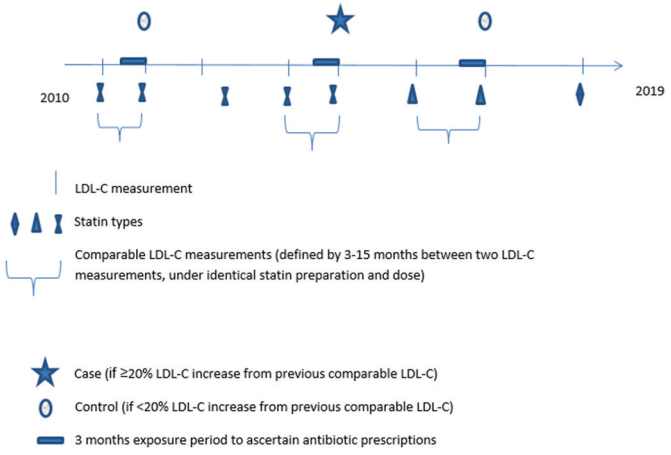


Fig. 1. An example of a single participant with a case time-point and two control time-points, in a case-crossover study of an association between antibiotics and serum LDL-C increase in stain users
Only patients with at least one case time-point enter the study, and an individual serves as his own control. Exposures immediately before all cases and control time-points within each individual are compared. An updated Charlson Comorbidity Index was obtained in each time-point (case and control time-points) to adjust for time-dependent potential confounders.

Table 1
Characteristics of study population at cohort entry, n (%)

	Persons N = 25,496
Age (years)	
Mean (SD)	66.9 (10.6)
Min, Max	20.6, 105.5
Sex	
Males	11,200 (43.9)
Females	14,296 (56.1)
Smoking history	
Ever	8202 (32.2)
Never	17,243 (67.6)
Missing	51 (0.2)
Body Mass Index (kg/m^2)	
Mean (SD)	29.2 (5.4)
Hypertension	17,073 (67.0)
Diabetes mellitus	14,810 (58.1)
Ischemic heart disease	8406 (33.0)
Chronic renal failure	3080 (12.1)
S/P Cerebrovascular accident	2649 (10.4)
Chronic obstructive pulmonary disease	1884 (7.4)
Peripheral vascular disease	1828 (7.2)
Congestive Heart Failure	1670 (6.6)
History of solid malignancy	3691 (14.5)
History of hematological malignancy	643 (2.5)
Dementia	505 (2.0)
Liver cirrhosis	138 (0.5)

A case-crossover study: only patients with an LDL-C increase enter the study, and an individual serves as his own control, by comparing risk among the same individual at different times. A case time-point was defined as an increase in serum LDL-C of at least 20% from LDL-C measured 3–15 months before. A control defined as no-increase/decrease/increase of <20% in LDL-C, from LDL-C measured 3–15 months before. There were 2.85 mean time points per patient (1.14, 1.71 case, control time points, respectively).

2017). The same has been shown with rosuvastatin-ceftriaxone treated mice (Wang et al., 2018). Duration of the effect following the antibiotics was four weeks in mice. Kaddurah-Daouk et al. (2011) have demonstrated in the Cholesterol and Pharmacogenetics study, contribution of bacterial-derived bile acids to the prediction of the magnitude of statin-induced LDL-C lowering in good responders. Liu et al. (2018) have shown that the complexity of fecal microbiome could be positively

Table 2
Association between antibiotics and serum LDL-C increase in a case-crossover study (total time-points n = 72,638, cases time-points: n = 29,103, control time-points: n = 43,535)

Antibiotic Group/ Active comparators	Antibiotic use			Adjusted risk for LDL-C increase OR (95% CI), p
	Total n	Cases n (%)	Controls n (%)	
Any antibiotic	15606	6489 (22.3)	9117 (20.9)	**1.062 (1.024–1.102), 0.001**
Penicillins	7817	3243 (11.1)	4574 (10.5)	1.028 (0.981–1.077), 0.250
Other Beta-lactams	4331	1839 (6.3)	2492 (5.7)	**1.080 (1.017–1.148), 0.013**
Sulfonamides and Trimethoprim	247	102 (0.4)	145 (0.3)	1.092 (0.851–1.401), 0.489
Fluroquinolones	2331	983 (3.4)	1348 (3.1)	**1.089 (1.004–1.181), 0.041**
Nitrofuran derivatives	745	300 (1.0)	445 (1.0)	1.023 (0.885–1.184), 0.757
Tetracyclines	701	288 (1.0)	413 (0.9)	0.995 (0.858–1.153), 0.943
Macrolides, Lincosamides	701	980 (3.4)	1159 (2.7)	**1.237 (1.138–1.345), 6.5*10^{-7}**
Metronidazole	360	166 (0.6)	194 (0.5)	**1.259 (1.028–1.542), 0.026**
Drugs for the treatment of tuberculosis	24	13 (0.04)	11 (0.03)	1.237 (0.574–2.663), 0.587
Drugs for treatment of lepra	3	1 (<0.01)	2 (<0.01)	0.960 (0.080–11.526), 0.974
Other antibacterials	326	121 (0.4)	205 (0.5)	0.966 (0.771–1.209), 0.760
Active comparators:				
Antivirals for influenza or herpes zoster	420	184 (0.6)	236 (0.5)	1.119 (0.924–1.354), 0.249
Upper respiratory tract infection	2750	1646 (3.8)	1104 (3.8)	0.993 (0.918–1.075), 0.864
Renal colic	185	108 (0.25)	77 (0.26)	1.016 (0.742–1.391), 0.921
Headache	31	18 (0.04)	13 (0.04)	1.185 (0.580–2.421), 0.641

We compared antibiotic prescription dispensed 3 months to 3 days before LDL-C measurement within the same patient. All analyses were adjusted for the updated Charlson Comorbidity Index at cases and control time-points.
In stratification by low/high CCI, there was an association between LDL-C increase and exposure to any antibiotic in both strata [1.338 (1.161–1.543), 6*10^{-5}; 1.171 (1.018–1.348), 0.028)]. In stratification by the time in the year in which the LDL-C measurement had been performed, association was found in both strata, with ORs for the association with macrolides and lincosamides of 1.298 (1.102–1.529) and 1.443 (1.148–1.815), in the cold and warm months, respectively.

correlated with rosuvastatin effectiveness. In a systematic review a conclusion was made that the higher diversity in microbiota composition is linked to atorvastatin and rosuvastatin hypolipidemic effects; however, it remained uncertain what kinds of microorganisms were involved and how they could exert this effect (Dias et al., 2020).

Many factors such as diet, travel, and where we live can affect the bacterial composition of our microbiota (Sullivan et al., 2001; Brook et al., 2013). For instance, secondary bile acids level measured in patients from Richmond, Virginia, was found to vary from <1% to >60% (believed to reflect level and activities of bile acid dehydroxylating gut bacteria, and colonic transit time)(Ridlon et al., 2016). Since there is considerable subject-to-subject variability in the composition of gut microbiota among humans, grouping of microbial data from several individuals results in loss of statistical significance or false-negative results (Turnbaugh et al., 2009; Lynch and Pedersen, 2016); investigation of the impacts of antibiotics on the microbiota is currently best assessed on an individual basis (Dethlefsen et al., 2008; Jernberg et al., 2010). Within-person comparisons are accomplished by measuring individual divergences from baseline levels after treatment

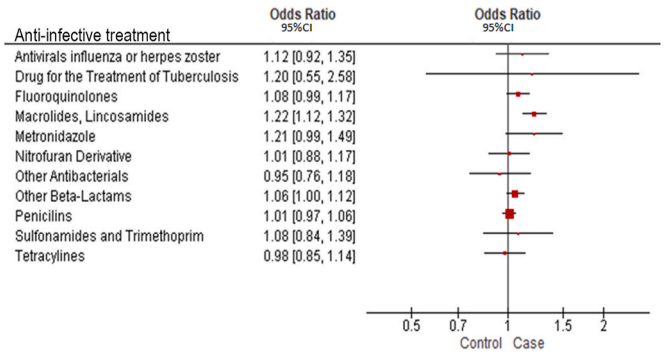


Fig. 2. Forest plot of the association between risk of LDL-C increase and antibiotics prescriptions, in statin users
Multivariable analysis for the association between exposure to any antibiotic in the community and a following LDL-C increase, in statin users. Case-crossover study, adjusted for a time-dependent Charlson Comorbidity Index. An association with exposure to macrolides and lincosamides (clindamycin) was the most prominent. *Drugs for the treatment of lepra (number of users: 11, 3, in cases and controls, respectively) were not included in the analysis due to low number of users.

(Engelbrektson et al., 2006).

We have hypothesized that antibiotic use (e.g., macrolides and clindamycin)(Orrhage et al., 1994; Sullivan et al., 2001; Maurice et al., 2013; Dias et al., 2020) might induce significant changes in gut composition and might be associated with the decrease of statins absorption in the gut, which in turn, may result in the increase of LDL-C in patients' serum.

In contrast, we hypothesized that antibiotic agents that generate milder effect on the microbiota diversity, such as nitrofurantoin, or amoxicillin, would not be associated with serum LDL-C increase (Vervoort et al., 2015); as so exposure to antiviral drugs having no impact on the gut bacteria.

We used a real-life setting approach to test the association between antibiotics and statins effects using a case-crossover design to measure individual divergences in LDL-C.

2. Materials and methods

2.1. Study design

This is a case-crossover study (Maclure, 2007; Hallas and Pottegård, 2014). In this study, only patients with an outcome enter the study, and an individual serves as his own control, by comparing exposure among the same individual before an outcome (case) to exposure before the control time-point (Fig. 1). This eliminates confounding by between-person characteristics that remain stable over time, and helps limit confounding other than for time. Potential time-varying characteristics are additionally adjusted.

For each outcome, we compared at least one control time-point within the same patient, which could occur before or after the outcome until death, end of study period, or end of registration in Clalit, whichever came first. Exposure were antibiotic prescriptions dispensed before each case and control time-points (Fig. 1).

2.2. Source of data

This study is based on data from the computerized database of Clalit Health Services. Clalit provides inclusive health care for more than half of the Israeli population. The electronic medical records of Clalit encompass data from multiple sources: records of primary care physicians, community specialty clinics, hospitalizations, laboratories and pharmacies. A registry of chronic diseases diagnoses is compiled from these data sources. High quality studies have been conducted based on data retrieved from Clalit database (Low et al., 2019; Dagan et al., 2021).

2.3. Participants

We chose from Clalit database all enrollees, age>18, that started treatment with statin (simvastatin, pravastatin, fluvastatin, atorvastatin, and rosuvastatin) between January 1, 2009 and November 25, 2019. The first year of treatment was defined as "the run-in period". We calculated medication adherence, through the percentage of days covered by dispensed prescriptions throughout the year. Participants with <80% in the run-year period were excluded. We also excluded patients with at least one of the following throughout each participant' run-in period: abnormal serum thyroid level; serum triglycerides (TG) level >400 mg/dL; active cancer (defined by any administration of an antineoplastic drug); or ≥2 hospitalizations, due to potential influence on cholesterol levels, and inability to search treatment administered during hospitalization. Next, we included only those who had at least two intervals of 3–15 months between two LDL-C measurements, with identical statin preparation (no generic replacement) and same dose administered (up to 3 months) before the two comparable LDL-C measurements (Fig. 1).

In order to distinguish native effects of the exposure factor (antibiotics), we reexamined associations using two additional cohorts. For the second cohort we chose from our source population those who had only one statin prescription dispensed during their entire follow up (and thus were excluded from the main study cohort due to low adherence). We started follow up one year after the sole statin prescription (after the "run-in" year). These patients were not exposed to statins during the study period. We used the same exclusion criteria (abnormal thyroid tests, hypertriglyceridemia, active cancer, and ≥2 hospitalizations) to define the cohort.

A third cohort was assembled from patients who have not been prescribed statins throughout the study period, and were prescribed bezafibrate or niacin. For the fibrates/niacin cohort we applied the same

Table 3
Association between antibiotics and serum LDL-C increase - analysis by statin type[a] n, OR (95% CI), p

Antibiotic Group	Atorvastatin (n = 28,249)	Pravastatin (n = 4,335)	Rosuvastatin (n = 15,166)	Simvastatin (n = 24,853)
Any antibiotic	**6134; 1.091 (1.029–1.156), 0.003**	949; 1.067 (0.919–1.240), 0.395	**3444; 1.130 (1.047–1.219), 0.002**	5075; 0.986 (0.925–1.050), 0.656
Penicillins	3085; 1.038 (0.963–1.119), 0.327	466; 1.048 (0.864–1.270), 0.634	1719; 1.053 (0.955–1.162), 0.301	2543; 0.994 (0.916–1.078), 0.885
Other beta-lactam antibacterials	**1678; 1.113 (1.009–1.228), 0.032**	256; 1.074 (0.834–1.385), 0.579	**918; 1.235 (1.087–1.403), 0.001**	1479; 0956 (0.860–1.062), 0.399
Macrolides, Lincosamides	**842; 1.207 (1.054–1.383), 0.007**	145; 0.908 (0.649–1.270), 0.573	**501; 1.319 (1.114–1.562), 0.001**	**651; 1.290 (1.110–1.500), 0.001**
Fluoroquinolones	950; 1.08 (0.95–1.23), 0.26	142; 1.36 (0.98–1.90), 0.07	504; 1.15 (0.97–1.37), 0.10	734; 1.01 (0.88–1.17), 0.86
Metronidazole	156; 1.256 (0.922–1.711), 0.149	18; 1.827 (0.755–4.421), 0.181	73; 1.084 (0.690–1.703), 0.727	113; 1.314 (0.913–1.892), 0.142

Multivariable analysis adjusted for Charlson Comorbidity Index.
[a] Low number of fluvastatin users (350) did not permit separate analysis.

Table 4
Studies of microbiota changes following oral antibiotics in humans

Antibiotic type	Bacterial types affected	Effect	Studied Population	Duration of the effect	Ref
Lincosamides:	*Enterococci, Enterobacteria*	↑	Healthy adults	1-4 months/	Orrhage et al.,1994; Sullivan et al., 2001; Löfmark et al., 2006; Zaura et al., 2015
Clindamycin	Total anaerobic, *Lactobacilli, Clostridia, Bacteroides Bifidobacteria*	↓↓		Significantly altered even after 18 months	
Macrolides:					
Azithromycin	33 gut bacteria species reduced	↓	Healthy children	Up to 12 months/ 24 months	Oldenburg et al., 2018; Wei et al., 2018; Doan et al., 2019
Clarithromycin	Anaerobic *Lactobacilli, Bifidobacteria Bacteroides*	↓	Healthy	35 days	Brismar et al., 1991; Edlund et al., 2000
Regimens for Helicobacter pylori (HP) infections:					
Clarithromycin + metronidazole + omeprazole	Gut microbiota dramatically perturbed	E	HP infected	Up to 4 years	Jernberg et al., 2010; Jakobsson et al., 2010
Clarithromycin + amoxicillin + lansoprazole	*Bacteroidetes, Firmicutes, Fusobacteria*	↓	HP infected	Up to 8 weeks	Liou et al., 2019
Clarithromycin + amoxicillin + lansoprazole + metronidazole	*Bacteroidetes, Firmicutes, Fusobacteria*	↓	HP infected	>1 year	Liou et al., 2019
Fluoroquinolones					
Ciprofloxacin	Gram-positive aerobes, *Bacteroidetes, Proteobacteria*	↑	Healthy	1 month/ A year	Zaura et al., 2015; Hertz et al., 2020
	Diversity	↓			
Moxifloxacin	Richness	↓	volunteers	>30 days	Edlund et al., 2000; de Gunzburg et al., 2018
	Lactobacilli, Bacteroides Fusobacteria.	→			
	Bifidobacteria Clostridia	↓		35 days	
Beta-lactams					
Cefuroxime axetil	*Enterococci, Staphylococci*	↑	Healthy	14 days	Edlund et al., 1993
	Eubacteria, Lactobacilli, Bacteroides	→			
	Bifidobacteria and Clostridia	↓			
Ampicillin/sulbactam, then cefazolin	Diversity affected	E	A patient	40 days	Perez-Cobas et al., 2013
	Active microbiota	→			
Amoxicillin +/- clavulanate	Aerobic gram-positive cocci	↓	Heathy children/ adults	1 week	Brismar et al., 1993; Floor et al., 1994; Edlund et al., 1994; Sullivan et al., 2001; Zaura et al., 2015; Oldenburg et al., 2018; Doan et al., 2019
	Enterobacteria, anaerobic Gram-positive rods, *Bacteroides*	↑ →			
	Diversity	→			
Nitrofurantoin	*Enterobacteria, Enterococci*	→	Women with recurrent urinary tract infections	≥2 weeks	Mavromanolakis et al., 1997

↑ - increase,
↓ - decrease,
→ - no change
E - some effect demonstrated

The gastrointestinal tract is a large microbial ecosystem that lives symbiotically with the host, housing several trillion microbial cells; including at least 5,000 different bacteria types. The two phyla firmicutes and bacteroidetes represent 90% of gut microbiota. Studies exploring effects on the microbiota by antibiotic classes were showing variable results. Bacteria with capability to produce secondary bile acid have been identified only within a few anaerobic *Clostridium* species and in *Eubacterium* (both genera belonging to the *Firmicutes* phylum) (Winston and Theriot, 2019; Wahlström et al., 2016).
Reduced diverse bacterial composition was observed in statin users compared to non-users, with lower abundance of *Proteobacteria, Enterobacteriaceae* and *Desulfovibrio* (Dias et al., 2020).

exclusion criteria, disregarding the TG < 400 criterion.

2.4. Study outcome and variables

We defined an outcome (case) as an increase in serum LDL-C of at least 20% from LDL-C measured 3–15 months before. We chose 20% increase as the minimum increase, because the full effect of low intensity statins is a decrease of less than 30% in LDL-C levels (Adhyaru and Jacobson, 2018). Full abolishment of the effect would be measured by an increase of the same magnitude. A control (for the case-control comparison) was defined as no-increase/decrease/increase of less than 20% in LDL-C, from LDL-C measured 3–15 months before. Importantly, each subject was compared only to himself.

We used the updated Charlson Comorbidity Index (CCI) (Charlson et al., 1987) in each case and control time-point to adjust for time-dependent factors. CCI was used to evaluate changes in chronic diseases, because they might be associated with gut microbiota (Claesson et al., 2012), with LDL-C, or with antibiotics use.

Clinical atherosclerotic event: As a secondary outcome we ascertained atherosclerotic ischemic diagnoses (defined as primary/secondary

discharge diagnoses of myocardial ischemia, or of stroke), that have occurred in the 3 months immediately following the LDL-C increase (meaning following the case time-point), to those occurring in the 3 months following a control time-point.

2.5. Exposure

Pharmacy dispensing of antibiotics in the 3 months prior to LDL-C measurement was the exposure of interest. An exposure was defined when one drug prescription was administrated, from the drug group.

Active comparators (negative controls) were: a) short-term anti-viral treatments (acyclovir or anti-influenza treatment), not influencing bacterial population; b) diagnoses of acute diseases (that could have been related to effects on LDL-C through various stress or inflammatory processes, or might have caused reduced adherence to chronic statin treatment, but were not associated with anti-infective treatment; to serve as comparators to the acute infection treated with antibiotics). For this purpose, we ascertained diagnoses of upper respiratory tract infection, renal colic, and headache in the 3 months' exposure period.

Because drug treatment of hospitalized patients was not available in the database, we excluded patients who have been hospitalized in the 3 months exposure period.

2.6. Statistical analysis

All comparisons were done within each person by conditional logistic regression, and included the updated CCI at cases and control time-points. Multivariable analysis with all antibiotic groups was performed to study the association of antibiotic combinations with the outcome.

For a first sensitivity analysis, we stratified the outcomes by CCI. In the second sensitivity analysis we stratified by season: to the 6 cold-weather months (October 1-March 31), and the 6 warm-weather months (April 1- September 30) separately, to take into account possible changes in LDL-C levels in winter months (for example due to increased weight), and the possible bias that might have occurred because exposure to antibiotics might have increased in the cold months. We performed a third sensitivity analysis in which we assessed exposure to antibiotics in different periods within the 3-months' exposure time before the outcome, by subdividing into different periods: a) 3–14; b) 15–30; c) 31–60: and d) 61–90 days before the outcome.

We performed multivariable logistic regression analysis to evaluate the risk for atherosclerotic disease associated with LDL-C increase. The model included CCI and atherosclerotic ischemic events at baseline, which was defined as myocardial infarction or stroke diagnoses in the 3–6 months before the "first" LDL-C in each comparison.

All analyses were done with the use of SPSS software (version 24). All p values are two-sided, and $p < 0.05$ was considered significant.

Ethics approval and consent to participate: Lady Davis Carmel Medical Center IRB approved the study (0086-20-CMC). Owing to the retrospective nature of the study, the institutional reviewed board granted a waiver of informed consent.

3. Results

Overall, 25,496 statin users, with 72,638 time points (29,103 cases, and 43,535 control time-points) were analyzed. A flow chart depicting study cohort selection is shown in Supplementary Fig. 1. Participant's characteristics on cohort entry date are shown in Table 1.

We found an association between antibiotic use and LDL-C increase (Table 2). In an analysis of each antibiotic group separately, and in a multivariable analysis to account for antibiotic combinations, we found significant associations with macrolides and lincosamides (clindamycin), after correction for multiple comparisons with Bonferroni test, with ORs = 1.237 (1.138–1.345), $6.5*10^{-7}$; 1.217 (1.118–1.325), $5.5*10^{-6}$, respectively (Table 2, Fig. 2); number needed to harm (NNH) = 19. This association was apparent in patients treated with atorvastatin, rosuvastatin, and simvastatin (Table 3).

There was no association between LDL-C increase and exposure to the active comparators: antiviral drugs; acute infectious diseases not treated with antibiotics (upper respiratory tract infection); and acute diagnoses of headache, or renal colic.

Interestingly, the effect of exposure to lincosamides was stronger in patients administered the antibiotic 60–90 days before LDL-C measurement, and the weakest effect was associated with the closest exposure (3–14 days) before LDL-C measurement [ORs 1.56 (1.09–2.22), 1.22 (0.76–1.95), respectively, $p = 0.048$]. This implies for late effect of antibiotic, which might have been mediated through the microbiota, as hypothesized. On the other hand, macrolide exposure that was 3–14 days, or 15–30 days before LDL-C measurement had stronger association with LDL-C increase than earlier exposure [ORs 1.38 (1.10–1.72), 1.088 (0.917–1.291), $p < 0.001$, for 3–14, and 60–90 days before LDL-C measurement, respectively] (Supplementary Table 2).

Association between LDL-C increase and use of any antibiotic (OR 0.953, 0.613–1.483) or use of macrolides/lincosamides (OR 0.908, 0.404–2.041), adjusted for CCI, was not apparent in the second cohort of patients not treated with statins. However, this group had 848 time-points only (cases and controls time-points), because regular serum LDL-C measurements were scarce in patients not treated with statins. In an analysis in which we allowed wider time interval between adjacent LDL-C measurements (up to 5 years), there were 2,749 time-points (cases and controls) within 453 persons. There was no association between LDL-C increase and exposure to any antibiotics (OR 1.05, 0.83–1.33), or to macrolides/lincosamides (OR 1.11, 0.68–1.82) in this analysis. In the third cohort of patients treated with bezafibrate or niacin (that were not treated with statins throughout their entire follow up), no association was found between LDL-C increase and exposure to any antibiotic (OR 0.946, 0.797–1.123) or to macrolides/lincosamides (OR 0.996, 0.454–2.185) (10,311 time-points within 3,153 patients; allowing 5 years interval between LDL-C measurements).

Acute atherosclerotic ischemic events (myocardial ischemia, or stroke) have been diagnosed following LDL-C increase in 1.7% of cases, and following a control time-point in 1.4%, adjusted OR 1.16 (1.03–1.31) $p = 0.017$ for an acute ischemic event associated with LDL-C increase.

4. Discussion

We demonstrate, in a real-life cohort of statin users, an association between treatment with macrolides and lincosamides (clindamycin) and an increase in serum LDL-C. An association was not demonstrated between LDL-C increase and anti-viral treatment, or acute non-infectious or infectious diseases that were not treated with antibiotics. Moreover, the association was not demonstrated in two additional cohorts of patients not treated with statins.

This association might be explained by a change in the gut microbiota induced by the antibiotics, as statins depend on secondary bile acids, produced by gut microbiota, for absorption (Devlin and Fischbach, 2015; Sonnenburg and Bäckhed, 2016). The gut microbiota is implicated in the metabolism of many medical drugs (Zimmermann et al., 2019). Due to major inter-individual variability, described almost as unique microbiota for each person (Lynch and Pedersen, 2016; Zhernakova et al., 2016; Claesson et al., 2012; Vich et al., 2020), there are consequences for interpersonal, as well as within personal variation in drug efficacy and toxicity. We designed a case-crossover study, to control for the enormous microbiota variation from one person to another. We included constantly updating CCI that incorporates multiple parameters (including age and chronic diseases) as time-dependent variable in this self–comparative analysis.

Following treatment with clindamycin, a relatively broad-spectrum antibiotic that primarily targets anaerobic bacteria, a substantial decrease in total anaerobic numbers was seen in healthy people

(Orrhage et al., 1994; Sullivan et al., 2001; Zaura et al., 2015), that was significant 18 months from treatment (Löfmark et al., 2006) (Table 4). The macrolide, azithromycin, affected the composition of pediatric intestinal microbiome at 24 months, in a randomized clinical trial (Oldenburg et al., 2018; Doan et al., 2019); yet in another trial of azithromycin given to children, the microbiota change was not apparent in the long-term (13–39 months after treatment)(Wei et al., 2018). In healthy volunteers, following the macrolide, clarithromycin, suppression of the anaerobic microflora *Lactobacilli*, *Bifidobacteria* and *Bacteroides* was described (Brismar et al., 1991; Edlund et al., 2000).Variable effects on the microbiota by other antibiotic classes are listed in Table 4.

Decreased (and not increased) LDL-C is reported in severe infections (Sharma et al., 2019). However, in order to contend with possible bias we used few active comparators: viral upper respiratory infection; acute events that might have caused reduced adherence to statins (renal colic, or headache); and exposure to anti-influenza treatment, or treatment for acute herpes zoster. We did not find an association between these exposures and LDL-C increase.

It should be noted that conversion of cholesterol to bile salts in the liver provides the major route for the elimination of excess cholesterol. As gut microbiota regulate expression of enzyme involved in bile acid production and conjugation (Sayin et al., 2013; Jones et al., 2013; Wahlström et al., 2016) it could impact serum LDL-C (Jones et al., 2013) without involvement of statins. However, antibiotic treatment had led to the depletion of colonic bacterial 7α-dehydroxylation activity, along with significant decrease and not increase in serum cholesterol level in 25 human subjects (Samuel et al., 1973). In addition, it was shown in 22 subjects that metronidazole (and less pronouncedly, ciprofloxacin), caused a reduction and not an increase in LDL-C (Jenkins et al., 2005). Nonetheless, we studied two additional cohorts of non-statin users in order to explore the association of antibiotic exposure and LDL-C (with no statin involvement) and did not find the association we have observed in patients treated with statin.

We chose three months for the period of exposure to antibiotics, although, in humans, wide range reported for the normal flora constitution (Table 4). In further stratification by different periods within the 3-months range before the outcome, there was difference between clindamycin - its use more than two months before LDL-C measurement was associated with LDL-increase, while the association with macrolides was most prominent when it had been administered up to one month before the LDL-C evaluation. Zaura et al. (2015) observed the lowest diversity in the gut after a month rather than immediately after treatment. Yet, a possible explanation might be temporary withholding of statin treatment during the macrolide course due to possible pharmacokinetic interaction. This statin interruption may directly affect the LDL-C serum levels. There is no concern for clindamycin-statins interaction, thus no statin interruption is expected and the association with LDL-C increase might be a result of a microbiota change. The pharmacokinetic interaction itself (inhibition of CYP3A4 by macrolides)(Kantola et al., 1998) would have caused the opposite effect because inhibition of statins metabolism would have caused higher exposure to statins (and thus lower LDL-C).

This study had several limitations. First, due to the retrospective nature of the study cause and effect could not be ascertained. In addition, use of some antibiotic types was infrequent, and type II error could have occurred. The second limitation was our inability to study antibiotic drugs administered during hospitalizations, such as third generation cephalosporins, aminoglycosides or carbapenems. A third limitation was the selected population finally included in the analysis after multiple exclusions as detailed (Supplementary Fig. 1), primarily due to our demand for an identical statin preparation type and dose for comparisons, and because of the case-only design of the study. Fourth, no data existed on the different statins' bile acid "dependency". Thus, we did not know if the stronger association we have observed with rosuvastatin and atorvastatin was due to various dependency on secondary bile acid, or due to rosuvastatin's and atorvastatin's higher potency. Additionally, from the scarce human data available, microbiota diversity seems to be reduced in statin users (Dias et al., 2020). This might serve as a predisposing factor, such that when certain antibiotics are added to treatment, microbiota abundance and diversity are further reduced. Strengths of the study include the self-controlled design enabling minimization of bias related to between-individuals differences in response to drugs and in microbiota composition, while exploiting the full clinical database to adjust for time-dependent possible confounders, and to determine exposure to antibiotics.

In conclusion, we demonstrate an increase in serum LDL-C in statin users, in association with macrolides and clindamycin antibiotics. Additional research is warranted to delineate the effects of short antibiotic courses on absorption of oral medications, and a probable physiological role of the microbiota in effectiveness of various drugs.

Consent for publication

Not applicable.

Funding

No funding received for this study.

CRediT authorship contribution statement

Idit Lavi: Methodology, Investigation. **Naomi Gronich:** Conceptualization, writing, Supervision.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

Acknowledgements

Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejphar.2022.175209.

References

Adhyaru, B.B., Jacobson, T.A., 2018. Safety and efficacy of statin therapy. Nat. Rev. Cardiol. 15 (12), 757–769. https://doi.org/10.1038/s41569-018-0098-5.

Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D.R., Fernandes, G.R., Tap, J., Bruls, T., Batto, J.M., 2011. Enterotypes of the human gut microbiome. Nature 473, 174–180.

Brismar, B., Edlund, C., Nord, C.E., 1991. Comparative effects of clarithromycin and erythromycin on the normal intestinal microflora. Scand. J. Infect. Dis. 23, 635–642.

Brismar, B., Edlund, C., Nord, C.E., 1993. Impact of cefpodoxime proxetil and amoxicillin on the normal oral and intestinal microflora. Eur. J. Clin. Microbiol. Infect. Dis. 12, 714–719.

Brook, I., Wexler, H.M., Goldstein, E.J.C., 2013. Antianaerobic antimicrobials: spectrum and susceptibility testing. Clin. Microbiol. Rev. 26 (3), 526, 46.

Charlson, M.E., Pompei, P., Ales, K.L., MacKenzie, C.R., 1987. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J. Chron. Dis. 40 (5), 373, 83.

Chiang, J.Y.L., 2013. Bile acid metabolism and signaling. Compr. Physiol. 3 (3), 1191–1212.

Claesson, M.J., Jeffery, I.B., Conde, S., Power, S.E., O'Connor, E.M., Cusack, S., Harris, H. M., Coakley, M., Lakshminarayanan, B., O'Sullivan, O., Fitzgerald, G.F., Deane, J., O'Connor, M., Harnedy, N., O'Connor, K., O'Mahony, D., van Sinderen, D., Wallace, M., Brennan, L., Stanton, C., Marchesi, J.R., Fitzgerald, A.P., Shanahan, F., Hill, C., Ross, R.P., O'Toole, P.W., 2012. Gut microbiota composition correlates with diet and health in the elderly. Nature 488 (7410), 178–184. https://doi.org/10.1038/nature11319.

Dagan, N., Barda, N., Kepten, E., Miron, O., Perchik, S., Katz, M.A., Hernán, M.A., Lipsitch, M., Reis, B., Balicer, R.D., 2021 Apr 15. BNT162b2 mRNA covid-19 vaccine in a nationwide mass vaccination setting. N. Engl. J. Med. 384 (15), 1412–1423. https://doi.org/10.1056/NEJMoa2101765.

de Gunzburg, J., Ghozlane, A., Ducher, A.1, Le Chatelier, E., Duval, X., Ruppé, E.2, Armand-Lefevre, L., Sablier-Gallis, F., Burdet, C., Alavoine, L., Chachaty, E., Augustin, V., Varastet, M., Levenez, F., Kennedy, S., Pons, N., Mentré, F., Andremont, A., 2018. Protection of the human gut microbiome from antibiotics. J. Infect. Dis. 217 (4), 628–636.

Dethlefsen, L., Huse, S., Sogin, M.L., Relman, D.A., 2008. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. PLoS Biol. 6 (11), e280 https://doi.org/10.1371/journal.pbio.0060280.

Devlin, A.S., Fischbach, M.A., 2015. A biosynthetic pathway for a prominent class of microbiota-derived bile acids. Nat. Chem. Biol. 11, 685–690.

Dias, A.M., Cordeiro, G., Estevinho, M.M., Veiga, R., Figueira, L., Reina-Couto, M., Magro, F., 2020. Gut bacterial microbiome composition and statin intake-A systematic review. Pharmacol Res Perspect 8 (3), e00601.

Ding, T., Schloss, P.D., 2014. Dynamics and associations of microbial community types across the human body. Nature 509, 357–360.

Doan, T., Hinterwirth, A., Worden, L., Arzika, A.M., Maliki, R., Abdou, A., Kane, S., Zhong, L., Cummings, S.L., Sakar, S., Chen, C., Cook, C., Lebas, E., Chow, E.D., Nachamkin, I., Porco, T.C., Keenan, J.D., Lietman, T.M., 2019. Gut microbiome alteration in MORDOR I: a community-randomized trial of mass azithromycin distribution. Nat. Med. 25 (9), 1370–1376. https://doi.org/10.1038/s41591-019-0533-0.

Edlund, C., Brismar, B., Sakamoto, H., Nord, C.E., 1993. Impact of cefuroxime-axetil on the normal intestinal microflora. Microb. Ecol. Health Dis. 6, 185–189.

Edlund, C., Stark, C., Nord, C.E., 1994 Jul. The relationship between an increase in beta-lactamase activity after oral administration of three new cephalosporins and protection against intestinal ecological disturbances. J. Antimicrob. Chemother. 34 (1), 127, 38.

Edlund, C., Beyer, G., Hiemer-Bau, M., Ziege, S., Lode, H., Nord, C.E., 2000. Comparative effects of moxifloxacin and clarithromycin on the normal intestinal microflora. Scand. J. Infect. Dis. 32, 81–85.

Engelbrektson, A.L., Korzenik, J.R., Sanders, M.E., Clement, B.G., Leyer, G., Klaenhammer, T.R., Kitts, C.L., 2006. Analysis of treatment effects on the microbial ecology of the human intestine. FEMS Microbiol Ecol 57 (2), 239. https://doi.org/10.1111/j.1574-6941.2006.00112.x, 50.

Ferrer, M., Méndez-García, C., Rojo, D., Barbas, C., Moya, A., 2017. Antibiotic use and microbiome function. Biochem. Pharmacol. 134, 114–126. https://doi.org/10.1016/j.bcp.2016.09.007.

Floor, M., van Akkeren, F., Rozenberg-Arska, M., et al., 1994. Effect of loracarbef and amoxicillin on the oropharyngeal and intestinal microflora of patients with bronchitis. Scand. J. Infect. Dis. 26, 191–197.

Hallas, J., Pottegård, A., 2014 Jun. Use of self-controlled designs in pharmacoepidemiology. J. Intern. Med. 275 (6), 581–589. https://doi.org/10.1111/joim.12186.PMID:24635348.

He, X., Zheng, N., He, J., Liu, C., Feng, J., Jia, W., Li, H., 2017. Gut microbiota modulation attenuated the hypolipidemic effect of simvastatin in high-fat/cholesterol-diet fed mice. J. Proteome Res. 16 (5), 1900–1910. https://doi.org/10.1021/acs.jproteome.6b00984.

Hertz, F.B., Budding, A.E., van der Lugt-Degen, M., Savelkoul, P.H., Løbner-Olesen, A., 2020. Frimodt-møller N effects of antibiotics on the intestinal microbiota of mice. Antibiotics 9 (4), 191.

Høverstad, T., Carlstedt-Duke, B., Lingaas, E., Midtvedt, T., Norin, K.E., Saxerholt, H., Steinbakk, M., 1986. Influence of ampicillin, clindamycin, and metronidazole on faecal excretion of short-chain fatty acids in healthy subjects. Scand. J. Gastroenterol. 21, 621–626. https://doi.org/10.3109/00365528609003109.

Jakobsson, H.E., Jernberg, C., Andersson, A.F., Sjölund-Karlsson, M., Jansson, J.K., Engstrand, L., 2010. Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. PLoS One 5 (3), e9836.

Jenkins, D.J.A., Kendall, C.W.C., Hamidi, M., Vidgen, E., Faulkner, D., Parker, T., Irani, N., Wolever, T.M.S., Fong, I., Kopplin, P., Connelly, P.W., Onderdonk, A., Rao, A.V., 2005. Effect of antibiotics as cholesterol-lowering agents. Metabolism 54 (1), 103, 12.

Jernberg, C., Löfmark, S., Edlund, C., Jansson, J.K., 2010. Long-term impacts of antibiotic exposure on the human intestinal microbiota. Microbiology 156 (Pt 11), 3216–3223. https://doi.org/10.1099/mic.0.040618-0.

Jones, M.L., Tomaro-Duchesneau, C., Martoni, C.J., Prakash, S., 2013. Cholesterol lowering with bile salt hydrolase-active probiotic bacteria, mechanism of action, clinical evidence, and future direction for heart health applications. Expet Opin. Biol. Ther. 13 (5), 631–642.

Kaddurah-Daouk, R., Baillie, R.A., Zhu, H., Zeng, Z.B., Wiest, M.M., Nguyen, U.T., Wojnoonski, K., Watkins, S.M., Trupp, M., Krauss, R.M., 2011. Enteric microbiome metabolites correlate with response to simvastatin treatment. PLoS One 6 (10), e25482. https://doi.org/10.1371/journal.pone.0025482.

Kantola, T., Kivistö, K.T., Neuvonen, P.J., 1998. Erythromycin and verapamil considerably increase serum simvastatin and simvastatin acid concentrations. Clin. Pharmacol. Ther. 64, 177–182.

Zhernakova, A., Kurilshikov, A., Bonder, M.J., Tigchelaar, E.F., Schirmer, M., Vatanen, T., Mujagic, Z., Vila, A.V., Falony, G., Vieira-Silva, S., Wang, J., Imhann, F., Brandsma, E., Jankipersadsing, S.A., Joossens, M., Cenit, M.C., Deelen, P., Swertz, M. A., , LifeLines cohort study, Weersma, R.K., Feskens, E.J., Netea, M.G., Gevers, D., Jonkers, D., Franke, L., Aulchenko, Y.S., Huttenhower, C., Raes, J., Hofker, M.H., Xavier, R.J., Wijmenga, C., Fu, J., 2016. Population based metagenomics analysis reveals markers for gut microbiome composition and diversity. Science 352 (6285), 565. https://doi.org/10.1126/science.aad3369, 9.

Liou, J.M., Chen, C.C., Chang, C.M., Fang, Y.J., Bair, M.J., Chen, P.Y., Chang, C.Y., Hsu, Y.C., Chen, M.J., Chen, C.C., Lee, J.Y., Yang, T.H., Luo, J.C., Chen, C.Y., Hsu, W. F., Chen, Y.N., Wu, Y.H., Lin, J.T., Lu, T.P., Chuang, E.Y., El-Omar, E.M., Wu, M.S., 2019. Taiwan gastrointestinal disease and Helicobacter consortium. Long-Term changes of gut microbiota, antibiotic resistance, and metabolic parameters after Helicobacter pylori eradication: a multicentre, open-label, randomised trial. Lancet Infect. Dis. 19 (10), 1109–1120.

Liu, Y., Song, X., Zhou, H., Zhou, X., Xia, Y., Dong, X., Zhong, W., Tang, S., Wang, L., Wen, S., Xiao, J., Tang, L., 2018. Gut microbiome associates with lipid-lowering effect of rosuvastatin in vivo. Front. Microbiol. 9, 530.

Löfmark, S., Jernberg, C., Jansson, J.K., Edlund, C., 2006. Clindamycin-induced enrichment and long-term persistence of resistant Bacteroides spp. Resistance Genes Antimicrob Chemother 58 (6), 1160. https://doi.org/10.1093/jac/dkl420, 7.

Low, M., Neuberger, A., Hooton, T.M., Green, M.S., Raz, R., Balicer, R.D., Almog, R., 2019. Association between urinary community-acquired fluoroquinolone-resistant Escherichia coli and neighbourhood antibiotic consumption: a population-based case-control study. Lancet Infect. Dis. 19 (4), 419–428.

Lynch, S.V., Pedersen, O., 2016. The human intestinal microbiome in health and disease. N. Engl. J. Med. 375 (24), 2369–2379.

Maclure, M., 2007 Aug. 'Why me?' versus 'why now?'–differences between operational hypotheses in case-control versus case-crossover studies. Pharmacoepidemiol. Drug Saf. 16 (8), 850–853. https://doi.org/10.1002/pds.1438.PMID:17636552.

Maurice, C.F., Haiser, H.J., Turnbaugh, P.J., 2013. Xenobiotics shape the physiology and gene expression of the active human gut microbiome. Cell 152, 39–50.

Mavromanolakis, E., Maraki, S., Samonis, G., Tselentis, Y., Cranidis, A., 1997. Effect of norfloxacin, trimethoprim-sulfamethoxazole and nitrofurantoin on fecal flora of women with recurrent urinary tract infections. J. Chemother. 9, 203–207.

Oldenburg, C.E., Sié, A., Coulibaly, B., Ouermi, L., Dah, C., Tapsoba, C., Bärnighausen, T., Ray, K.J., Zhong, L., Cummings, S., Lebas, E., Lietman, T.M., Keenan, J.D., Doan, T., 2018. Effect of commonly used pediatric antibiotics on gut microbial diversity in preschool children in Burkina Faso: a randomized clinical trial. Open Forum Infect. Dis. 5 (11), ofy289.

Orrhage, K., Brismar, B., Nord, C.E., 1994. Effect of supplements with Bifidobacterium longum and Lactobacillus acidophilus on the intestinal microbiota during administration of clindamycin. Microb. Ecol. Health Dis. 7, 17–25.

Perez-Cobas, A.E., Gosalbes, M.J., Friedrichs, A., et al., 2013. Gut microbiota disturbance during antibiotic therapy: a multi-omic approach. Gut 62 (11), 1591–1601.

Ridlon, J.M., Harris, S.C., Bhowmik, S., Kang, D.J., Hylemon, P.B., 2016. Consequences of bile salt biotransformations by intestinal bacteria. Gut Microb. 7 (1), 22–39.

Samuel, P., Holtzman, C.M., Meilman, E., Sekowski, I., 1973. Effect of neomycin and other antibiotics on serum cholesterol levels and on 7alpha-dehydroxylation of bile acids by the fecal bacterial flora in man. Circ. Res. 33, 393–402. https://doi.org/10.1161/01.res.33.4.393.

Sayin, S.I., Wahlstro, M.A., Felin, J., Jantti, S., Marschall, H.U., Bamberg, K., Angelin, B., Hyotylainen, T., Oresic, M., Backhed, F., 2013. Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. Cell Metabol. 17, 225–235.

Sharma, N.K., Ferreira, B.L., Tashima, A.K., Brunialti, M.K.C., Torquato, R.J.S., Bafi, A., Assuncao, M., Azevedo, L.C.P., Salomao, R., 2019. Lipid metabolism impairment in patients with sepsis secondary to hospital acquired pneumonia, a proteomic analysis. Clin. Proteonomics 16, 29.

Sonnenburg, J.L., Bäckhed, F., 2016. Diet-microbiota interactions as moderators of human metabolism. Nature 535 (7610), 56–64.

Sullivan, A., Edlund, C., Nord, C.E., 2001. Effect of antimicrobial agents on the ecological balance of human microflora. Lancet Infect. Dis. 1 (2), 101, 14.

Turnbaugh, P.J., Hamady, M., Yatsunenko, T., Cantarel, B.L., Duncan, A., Ley, R.E., Sogin, M.L., Jones, W.J., Roe, B.A., Affourtit, J.P., Egholm, M., Henrissat, B., Heath, A.C., Knight, R., Gordon, J.I., 2009. A core gut microbiome in obese and lean twins. Nature 457 (7228), 480–484. https://doi.org/10.1038/nature07540.

Vervoort, J., Xavier, B.B., Stewardson, A., Coenen, S., Godycki-Cwirko, M., Adriaenssens, N., Kowalczyk, A., Lammens, C., Harbarth, S., Goossens, H., Malhotra-Kumar, S., 2015. Metagenomic analysis of the impact of nitrofurantoin treatment on the human faecal microbiota. J. Antimicrob. Chemother. 70 (7), 1989, 92.

Vich, V.A., Collij, V., Sanna, S., Sinha, T., Imhann, F., Bourgonje, A.R., Mujagic, Z., Jonkers, D.M.A.E., Masclee, A.A.M., Fu, J., Kurilshikov, A., Wijmenga, C., Zhernakova, A., Weersma, R.K., 2020. Impact of commonly used drugs on the composition and metabolic function of the gut microbiota. Nat. Commun. 11 (1), 362. https://doi.org/10.1038/s41467-019-14177-z.

Wahlström, A., Sayin, S.I., Marschall, H.U., Bäckhed, F., 2016. Intestinal crosstalk between bile acids and microbiota and its impact on host metabolism. Cell Metabol. 24 (1), 41–50. https://doi.org/10.1016/j.cmet.2016.05.005.

Wang, L., Wang, Y., Wang, H., Zhou, X., Wei, X., Xie, Z., Zhang, Z., Wang, K., Mu, J., 2018. The influence of the intestinal microflora to the efficacy of Rosuvastatin. Lipids Health Dis. 17 (1), 151. https://doi.org/10.1186/s12944-018-0801-x.

Wei, S., Mortensen, M.S., Stokholm, J., Brejnrod, A.D., Thorsen, J., 2018. Morten Arendt Rasmussen MA, Trivedi U, Bisgaard H, Sørensen SJ. Short- and long-term impacts of azithromycin treatment on the gut microbiota in children: a double-blind, randomized, placebo-controlled trial. EBioMedicine 38, 265–272.

Winston, J.A., Theriot, C.M., 2019. Diversification of host bile acids by members of the gut microbiota. Gut Microb. 9, 1–14. https://doi.org/10.1080/19490976.2019.1674124.

Zarrinpar, A., Chaix, A., Xu, Z.Z., Chang, M.W., Marotz, C.A., Saghatelian, A., Knight, R., Panda, S., 2018. Antibiotic-induced microbiome depletion alters metabolic

homeostasis by affecting gut signaling and colonic metabolism. Nat. Commun. 9 (1), 2872.

Zaura, E., Brandt, B.W., Teixeira de Mattos, M.J., Buijs, M.J., Caspers, M.P.M., Rashid, M.-U., et al., 2015. Same exposure but two radically different responses to antibiotics: resilience of the salivary microbiome versus long-term microbial shifts in feces. mBio 6, e01693. https://doi.org/10.1128/mBio.01693-15. –15.

Zhang, Y., Limaye, P.B., Renaud, H.J., Klaassen, C.D., 2014. Effect of various antibiotics on modulation of intestinal microbiota and bile acid profile in mice. Toxicol. Appl. Pharmacol. 277 (2), 138. https://doi.org/10.1016/j.taap.2014.03.009, 45.

Zimmermann, M., Zimmermann-Kogadeeva, M., Wegmann, R., Goodman, A.L., 2019 Feb 8. Separating host and microbiome contributions to drug pharmacokinetics and toxicity. Science 363 (6427), eaat9931. https://doi.org/10.1126/science.aat9931. Epub 2019 Feb 7. PMID: 30733391; PMCID: PMC6533120.