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Effect of antibiotics as cholesterol-lowering agents

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Abstract

Antibiotics were once proposed as hypercholesterolemic agents although the mechanism is unclear, despite broad implications, including providing an alternative approach to cholesterol reduction, with potential relevance for current trials of antibiotics to reduce cardiovascular disease, and possible confounding of routine diagnostic cholesterol measurements. The effect on serum lipids of antibiotics against aerobes and anaerobes, together with possible mechanisms, was therefore explored. Twenty-two men and women took antibiotics for 10 days (either ciprofloxacin for 13 subjects or metronidazole for 10 subjects), with 10 days control in random order separated by 2-week washout periods. Subjects maintained low-fat diets throughout the study. Blood samples and blood pressure were obtained on days 0 and 10 of each phase with 3-day fecal collections and 12-hour breath gas collections at the end of each phase. The results indicated that metronidazole markedly reduced low-density lipoprotein cholesterol ($-14.0 \pm 4.0\%$, $P = .006$), oxidized low-density lipoprotein ($-23.0 \pm 5.1\%$, $P = .002$), and the apolipoprotein B/A-I ratio ($-18.0 \pm 2.8\%$, $P < .001$), whereas the reduction with ciprofloxacin was less pronounced (apolipoprotein B/A-I, $-5.0 \pm 1.8\%$, $P = .017$). Neither antibiotic altered C-reactive protein or blood pressure. The low-density lipoprotein cholesterol reduction related to an increase in bifidobacteria ($r = -0.46$, $P = .029$), but not to markers of colonic fermentation. We conclude that antibiotics can reduce serum lipids acutely. These effects may confound diagnostic measurements but indicate possible links between colonic microflora and blood lipids and the need to study ways of altering colonic microflora by nonantibiotic means as a potential therapeutic option.

1. Introduction

Thirty years ago certain antibiotics were proposed as hypocholesterolemic agents [1,2], but the mechanism was not clearly defined and the phenomenon was left relatively unexplored. Work at the time and subsequently pointed to the ability of antibiotics to alter colonic microbial events which in turn appear to influence cholesterol metabolism. Recently, there has been renewed interest in the health benefits of altering colonic bacterial numbers and metabolism through the use of prebiotics and probiotics [3-7]. Thus, for example, consumption of the prebiotic fructoligosaccharides, which increase the growth of bifidobacteria [4], has also been reported to lower serum cholesterol in some studies [6,7]. Yet the potential benefits, including the cholesterol reduction achieved by these means [8-10], have not been linked with previous similar metabolic advantages resulting from antibiotic use. These include the cholesterol-lowering effect of neomycin and metronidazole [8-10]. For

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at least 3 reasons we believe it is important now to reassess the metabolic effects of antibiotics. First, to determine whether antibiotic effects provide clues to new ways to lower serum lipids. Next, to determine whether antibiotic use may be a confounding factor preventing the diagnosis of hypercholesterolemia. Finally, to assess whether knowledge of the mechanisms of antibiotic effects may contribute to selection of antibiotics for large multicenter trials of coronary heart disease (CHD) risk reduction [11-13].

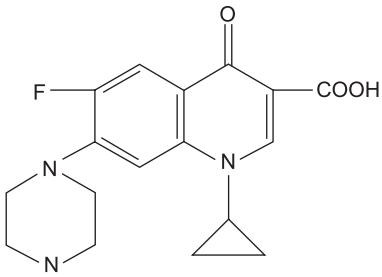
We have therefore assessed the effects of antibiotics on serum cholesterol, colonic microflora, and associated metabolic events using antibiotics against aerobes and anaerobes.

2. Materials and methods

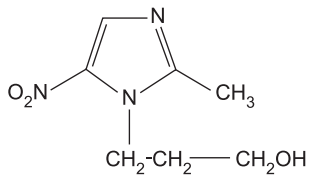
2.1. Study protocol

Twenty-two healthy middle-aged men and postmenopausal women previously diagnosed with raised cholesterol levels took 1 of 2 antibiotics, ciprofloxacin (13 subjects) or metronidazole (10 subjects) (Fig. 1) for study purposes at the standard therapeutic dose for 10 days. All participants also undertook a 10-day control period, the order of which was randomized with the test phase and separated by a 2-week washout period. One man took part in both studies. Ten-day periods were used to correspond with the length of time over which antibiotics might have bee prescribed for therapeutic purposes. Subjects were asked to follow the same low-fat diet (step 1 diet, <30% of energy, <10% saturated fat) during the control and test 10-day study phases to ensure the consistency of dietary intake and body weight (Table 1).

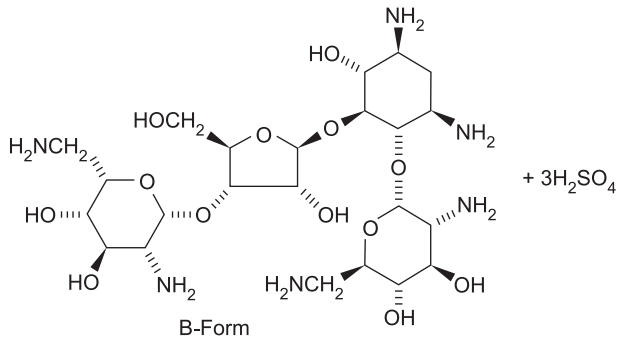
A 12- to 14-hour fasting blood sample, body weight, and blood pressure were obtained at the start and end of each study phase. Blood pressure was taken after sitting for 15 to 20 minutes in the nondominant arm by the same 3 observers throughout the course of the study using a standard 52 by 14 cm cuff. Three-day fecal collections were obtained from day 7 to 10 of each phase using under-seat frames lined with



ciprofloxacin
(1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[1-piperazinyl]-3-quinolinecarboxylic acid; $C_{17}H_{18}FN_3O_3 \cdot HCl \cdot H_2O$.; MW: 385.82)



metronidazole
(1-hydroxyethyl-2-methyl-5-nitroimidazole; $C_6H_9N_3O_3$; MW: 171.16)



neomycin B (trisulfate salt)
$C_{23}H_{46}N_6O_{13} \cdot 3H_2SO_4 \cdot xH_2O$; MW 908.88

Fig. 1. Chemical structure of ciprofloxacin, metronidazole, and neomycin.

Table 1
Daily dietary intakes in ciprofloxacin (n = 13) and metronidazole (n = 9) studies

	Control	Ciprofloxacin	$P<$	Placebo	Metronidazole	$P<$
Energy (MJ/d)	7.5	7.6	.756	7.7	8.1	.263
(kcal/d)	1806	1827		1844	1947	.263
Total protein (g/d)	82	80	.492	76	83	.262
(% of energy)	18.9	18.1	.383	16.5	17.1	.444
Available carbohydrates (g/d)	250	247	.826	246	238	.588
(% of energy)	55.2	54.1	.482	53.1	49.8	.038
Total dietary fiber (g/d)	29	27	.493	30	29	.359
(g/MJ)	3.9	3.7	.265	4.1	3.8	.147
(g/kcal)	16.3	15.4		17.0	15.8	.147
Total fat (g/d)	51	54	.365	59	72	.052
(% of energy)	24.7	26.7	.335	29.0	31.9	.081
SFA (g/d)	14	15	.581	18	20	.068
(% of energy)	6.9	7.4	.461	9.1	9.4	.378
MUFA (g/d)	20	23	.139	23	29	.047
(% of energy)	9.7	11.2	.121	11.3	12.9	.077
PUFA (g/d)	11	11	.974	14	16	.228
(% of energy)	5.5	5.4	.894	6.3	6.7	.370
Dietary cholesterol (mg/d)	165	160	.683	164	200	.098
Alcohol (g/d)	3	4	.699	3	14	.354

SFA indicates saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
Values are average of 10 days.

plastic bags. The bag was removed after use, sealed with an elastic band, and the sample was labeled and stored on frozen CO_2. Microbiological samples were also obtained by core suction biopsy of 1 to 2 mL of fresh fecal material and added to peptone water containing 0.03% cysteine hydrochloride to maintain anaerobic conditions and glycerol to improve storage [14,15]; a further sample was placed in a plastic vial for short-chain fatty acid (SCFA) analysis. Samples were stored on frozen CO_2 until transported to the laboratory where they were kept at $-70^{\circ}C$ until plating and enumeration. Colonies designated as bifidobacteria and lactobacilli were transferred to anaerobic transport media and shipped to Boston where the presence of bifidobacteria was confirmed by fermentation characteristics and chromatography.

Breath gases were obtained at hourly intervals for 12 hours over the course of the day (day 9) for H_2 and CH_4 using a modified Haldane-Priestly tube.

Symptoms were recorded, including flatulence, abdominal distension, or discomfort, during the last 3 days of each phase using a semantic scale of 0 to 6, where 0 was no effect and 6 was very distressing.

The study was approved by the Ethics Committee of the University of Toronto and St Michael's Hospital and informed consent was obtained from the subjects.

2.2. Subjects

Healthy men (n = 25) and postmenopausal women (n = 16) were recruited by newspaper advertisement and from patients attending the Risk Factor Modification Center, St Michael's Hospital. Two physicians also took part in the study. Of the original 41 subjects, 18 subjects withdrew either before or after randomization, but before starting the first phase due largely to scheduling conflicts and loss of interest, and 1 after completing one study phase. None quit for reasons directly related to the study. Twenty-two subjects completed both phases: for ciprofloxacin, 9 men and 4 women, age 63 ± 3 years, body mass index 25.9 ± 1.0 kg/m^2; for metronidazole, 6 men and 4 women, age 63 ± 3 years, body mass index 26.0 ± 0.9 kg/m^2 with 1 subject undergoing both studies. All subjects but one had elevated low-density lipoprotein cholesterol (LDL-C) levels on initial assessment (>4.1 mmol/L). Immediately before the start of the study, 11 subjects had elevated LDL-C and 3 subjects had elevated triglyceride levels (>2.3 mmol/L; range, 2.71-4.24 mmol/L). None had clinical or biochemical evidence of diabetes, liver, or renal disease. Five men were taking hypolipidemic agents (statins 10-20 mg/d). One woman was taking hormone replacement therapy and three were taking levothyroxine (0.1 mg/d). Two men were taking either one or both of the following: β-blocking agents (n = 2) and a combination of calcium channel blocker and diuretic (n = 1). Two women were taking a calcium channel blocker either with a diuretic (n = 1) or an angiotensin-converting enzyme inhibitor (n = 1). Dosages for all medications for all subjects were held constant throughout the study. Subjects were also asked to maintain their habitual level of physical activity throughout both study phases.

2.3. Antibiotics

Subjects were provided with test tablets on the ciprofloxacin study to be taken as 500 mg in the morning and 500 mg in the evening for a total dose of 1000 mg/d; no tablets were provided in the control phase. On the metronidazole study, two 250-mg metronidazole capsules were taken in the morning and evening, providing a dose of 1000 mg/d. Capsules containing lactose were provided on the control

Table 2
Effect of ciprofloxacin (1000 mg/d) on bodyweight, blood pressure, and serum measurements

n = 13	Control		Ciprofloxacin		Ciprofloxacin-control end of treatment	$P \leq$
	Day 0	Day 10	Day 0	Day 10	% Difference	
Body weight (kg)	72.3	72.0	72.5	72.3	0.3	.195
Lipids and apolipoproteins						
T-C (mmol/L)	6.43	5.99	6.28	5.98	0.2	.909
LDL-C (mmol/L)	4.27	3.86	3.93	3.78	−1.8	.391
HDL-C (mmol/L)	1.39	1.29	1.30	1.34	3.4	.216
Triglycerides (mmol/L)	1.69	1.84	2.32	1.88	3.5	.735
Apo A1 (mmol/L)	1.74	1.63	1.70	1.68	2.7	.174
Apo B (mmol/L)	1.32	1.23	1.27	1.20	−2.6	.153
T-C/HDL	4.94	4.90	5.22	4.84	−2.4	.406
LDL/HDL	3.32	3.17	3.22	3.07	−4.5	.081
Apo B/A1	0.78	0.77	0.76	0.73	−5.0	.017
Oxidized LDL-C						
LDL-conjugated dienes (mmol/L)	51.3	49.3	47.7	47.1	−2.8	.682
LDL-conjugated dienes/LDL	12.0	13.1	12.3	12.9	−1.0	.876
Nonlipid CHD risk factors						
Blood pressure (mm Hg)						
Systolic	116	117	117	118	1.2	.635
Diastolic	75	72	76	76	5.3	.114
Calculated CHD risk						
CHD risk (10 y, %)	10.1	10.2	10.6	10.1	−2.5	.608
Nitric oxide	17.9	17.4	18.6	18.7	21.5	.271
CRP (mg/L)	4.29	1.93	1.75	2.30	31.4	.098

% end difference = (antibiotics day 10−placebo day 10) × 100 / placebo day 10.

phase. Compliance was assessed from the number of tablets in antibiotic containers returned at the end of the study.

2.4. Analyses

Serum was analyzed according to the Lipid Research Clinics protocol [16] for total cholesterol (T-C), triglyceride, and high-density lipoprotein cholesterol (HDL-C) after dextran sulfate-magnesium chloride precipitation [17]. All samples from a given individual were analyzed in the same batch. Low-density lipoprotein cholesterol was calculated [18]. Serum apolipoprotein (apo) A-I and B were measured by nephelometry [19] and C-reactive

Table 3
Effect of metronidazole (1000 mg/d) on bodyweight, blood pressure, and serum measurements

n = 10	Placebo		Metronidazole		Metronidazole-placebo end of treatment	$P \leq$
	Day 0	Day 10	Day 0	Day 10	% Difference	
Body weight (kg)	79.0	79.0	79.0	78.6	−0.6	.0115
Lipids and apolipoproteins						
T-C (mmol/L)	6.10	5.80	6.49	5.38	−6.7	.077
LDL-C (mmol/L)	3.98	3.78	4.18	3.20	−14.4	.006
HDL-C (mmol/L)	1.41	1.39	1.50	1.42	3.6	.324
Triglycerides (mmol/L)	1.57	1.39	1.77	1.66	34	.506
Apo A1 (mmol/L)	1.79	1.73	1.90	1.80	6.0	.174
Apo B (mmol/L)	1.30	1.21	1.32	1.04	−13.2	.002
T-C/HDL	4.37	4.22	4.36	3.82	−8.8	.129
LDL/HDL	2.85	2.75	2.81	2.26	−17.2	.000
Apo B/A1	0.73	0.71	0.70	0.58	−17.6	.000
Oxidized LDL-C						
LDL-conjugated dienes (mmol/L)	53	59	64	47	−23	.002
LDL-conjugated dienes/LDL	14.0	15.5	15.7	14.6	−12	.056
Nonlipid CHD risk factors						
Blood pressure (mm Hg)						
Systolic	118	117	119	119	1.3	.693
Diastolic	73	72	70	69	−4.0	.285
Calculated CHD risk						
CHD risk (10 y, %)	7.7	8.3	7.3	6.3	−26.0	.019
CRP (mg/L)	2.65	3.72	1.24	1.48	58	.216

% end difference = (antibiotics day 10−placebo day 10) × 100 / placebo day 10.

Table 4
Change across treatments on the ciprofloxacin and metronidazole studies

	Ciprofloxacin study				Metronidazole study			
	Control	Ciprofloxacin	Control-ciprofloxacin change difference	$P \leq$	Control	Metronidazole day 10	Control-metronidazole change difference	$P \leq$
Body weight (kg)	−0.2	−0.3	0.0	.892	0.0	−0.3	−0.3	.339
Lipids and apolipoproteins								
T-C (mmol/L)	−0.44	−0.31	0.14	.648	−0.30	−1.11	−0.81	.025
LDL-C (mmol/L)	−0.41	−0.15	0.26	.227	−0.20	−0.98	−0.78	.044
HDL-C (mmol/L)	−0.10	0.05	0.14	.040	−0.02	−0.08	−0.06	.448
Triglycerides (mmol/L)	0.15	−0.44	−0.59	.410	−0.18	−0.11	0.07	.871
Apo A1 (mmol/L)	−0.10	−0.03	0.07	.265	−0.07	−0.10	−0.03	.744
Apo B (mmol/L)	−0.09	−0.08	0.02	.782	−0.09	−0.29	−0.19	.023
T-C/HDL	−0.05	−0.38	−0.34	.430	−0.15	−0.54	−0.39	.045
LDL/HDL	−0.15	−0.15	−0.01	.975	−0.10	−0.55	−0.45	.029
Apo B/A1	−0.01	−0.03	−0.02	.416	−0.02	−0.12	−0.10	.005

Values are means.

protein (CRP) by end-point nephelometry (Behring BN-100, N high sensitivity CRP reagent, Dade-Behring, Mississauga, Ontario).

Oxidized LDL was measured on serum stored at −70°C as conjugated dienes in the LDL fraction after isolation of LDL particles by precipitation with buffered heparin at their isoelectric point (pH 5.05) [20,21].

Bacterial populations were expressed as the log 10 of colony-forming units per gram of fresh sample (log CFU/g). Total aerobes, total anaerobes, bacteroides, bifidobacteria, and fusobacteria were determined on serially diluted fecal samples, plated onto Schaedler agar and incubated aerobically and anaerobically at 37°C for 72 hours for total aerobes and anaerobes, respectively. Kanomycin-vancomycin laked blood agar, Man Rogosa Sharpe agar plus Cysteine-HCl and neomycin blood agar were incubated under anaerobic conditions at 37°C for 72 hours for bacteroides, bifidobacteria, and fusobacteria, respectively [14]. Colonies were identified based on their morphology and confirmed through microscopic observation and fermentation characteristics [22].

Fecal SCFAs were measured on fecal homogenates, which had been stored at −20°C. Ultrafiltrates of these samples were vacuum distilled and SCFA concentrations were determined by gas chromatography using a flame injection detector (HP 5890 series II gas chromatograph, Hewlett Packard, Mississauga, Ontario) and HP FFAP bonded polyethylene glycol capillary columns (30 m, 0.53 mm i.d., 1 M df, Agilent Technologies, New Castle, DE) [23].

Breath CH_4 and H_2 were analyzed using a Quintron gas chromatograph (Quintron Microanalyzer Model DP, Quintron Co, Milwaukee, Wis).

Feces were analyzed using the Association of Official Analytical Chemists methods for fat, protein [24], and fiber [25] with available carbohydrate calculated by difference.

Diet histories were assessed using a computer program based on US Department of Agriculture data [26].

2.5. Statistical analysis

The results are expressed as means ± SE. Treatment differences were assessed by analysis of covariance (ANCOVA) using the General Linear Model Procedure

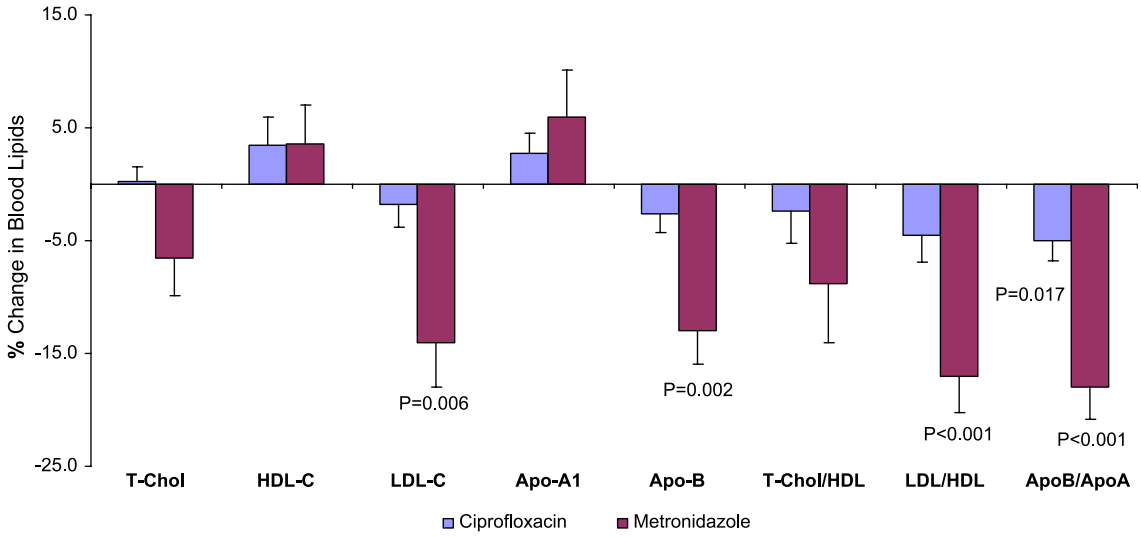


Fig. 2. Effect of ciprofloxacin/metronidazole (1000 mg/d) on blood lipids, expressed as percentage change from the respective control value.

Table 5
Effect of ciprofloxacin/metronidazole (1000 mg/d) on daily fecal nutrient losses

n = 13		Control	Ciprofloxacin	End difference	$P <$
Energy	kcal/d	225	262	37	.137
	MJ/d	0.94	1.10	0.16	0.137
Ash	g/d	6.0	6.0	0.2	.727
Protein	g/d	12	12	−0.6	.580
Fat	g/d	6.0	5.0	−0.7	.357
Fiber	g/d	15	19	4.7	.067
Available CHO	g/d	4.0	6.0	2.2	.023
Wet weight	g/d	204	271	67	.115
Dry weight	g/d	45	50	5.8	.213
Moisture	g/d	1.3	1.3	0.0	.989
n = 10		**Placebo**	**Metronidazole**	**End difference**	$P <$
Energy	kcal/d	153	171	18	.417
	MJ/d	0.64	0.72	0.08	0.417
Ash	g/d	6.0	6.0	−0.3	.609
Protein	g/d	13	14	1.0	.627
Fat	g/d	6.0	6.0	−0.7	.570
Fiber	g/d	13	19	5.3	.014
Available CHO	g/d	4.0	7.0	2.5	.039
Wet weight	g/d	256	277	22	.655
Dry weight	g/d	49	53	4.5	.464
Moisture	g/d	2.0	2.7	0.6	.298

CHO indicates carbohydrates.

(PROC GLM) in SAS. The statistical model had end of treatment as the response variable and antibiotic study, treatment, sequence (treatment order), and the interaction term of treatment-by-sex as main effects, a random term representing the subject nested within sex-by-sequence-by-study and baseline as a covariate. Whenever a significant difference of the type of antibiotic was observed, ANCOVA was run on data within each antibiotic study separately using an appropriately abbreviated specification based on the original model. Student *t* test for paired data (2-tailed) was used to determine the significance of changes across diets and to compare percent changes between test and control treatments. Coronary heart disease risk was calculated using the T-C/HDL-C ratio and systolic blood pressure in the Framingham cardiovascular disease risk equation (10 years) [27]. Although the equation also has terms for smoking, diabetes, and left ventricular hypertrophy, all the subjects were nonsmokers and nondiabetic and none had clinical evidence history of left ventricular hypertrophy. The SAS version 8.2 software was used throughout [28].

3. Results

The antibiotics were taken as prescribed. The compliance for ciprofloxacin was 99.2 ± 0.5% and 100% for metronidazole, with no significant difference between treatments. Both antibiotics produced a similar level of side effects and there were no large differences in body weight change across treatments (Tables 2 and 3) or between the antibiotic treatments: ciprofloxacin −0.3 ± 0.1 kg, $P = .062$; control −0.2 ± 0.1 kg, $P = .074$; and metronidazole −0.3 ± 0.3 kg, $P = .339$; control 0.0 ± 0.2 kg, $P = .893$; or between antibiotic treatments ($P = .418$).

3.1. Blood lipids and lipoproteins

Ciprofloxacin tended to lower the LDL/HDL-C ratio (−4.5 ± 2.4%, $P = .081$) compared to the control phase (Table 2), but only the reduction in the apo B/A-I ratio was significant (−5.0 ± 1.8%, $P = .017$) (Table 2, Fig. 2).

On metronidazole, however, substantial and significant reductions were seen in LDL-C (−14.4 ± 4.0%, $P = .006$), apo B (−13.2 ± 3.0%, $P = .002$), LDL/HDL (−17.2 ± 3.2%, $P < .001$), and apo B/A-I ratio (−17.6 ± 2.8%, $P < .001$) (Table 3) whereas HDL cholesterol and apo A-I tended to increase when compared to the control (Fig. 2 and Table 4).

Serum levels of oxidized LDL-C were also lower after metronidazole compared to control (23.2 ± 5.1%, $P = .002$). No effect was seen with ciprofloxacin. Calculated 10-year cardiovascular risk was reduced on metronidazole when compared to the control period (26.0 ± 9.1%, $P = .019$).

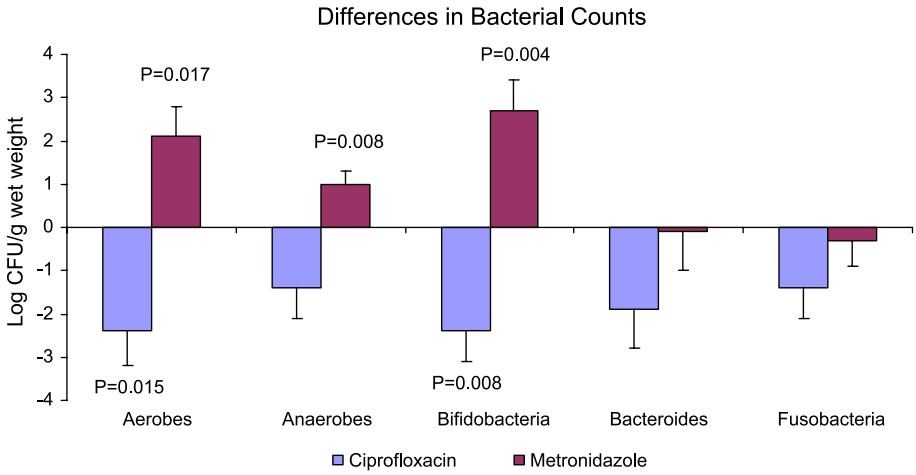


Fig. 3. Effect of ciprofloxacin/metronidazole (1000 mg/d) on bacterial concentrations expressed as differences between test and control treatments.

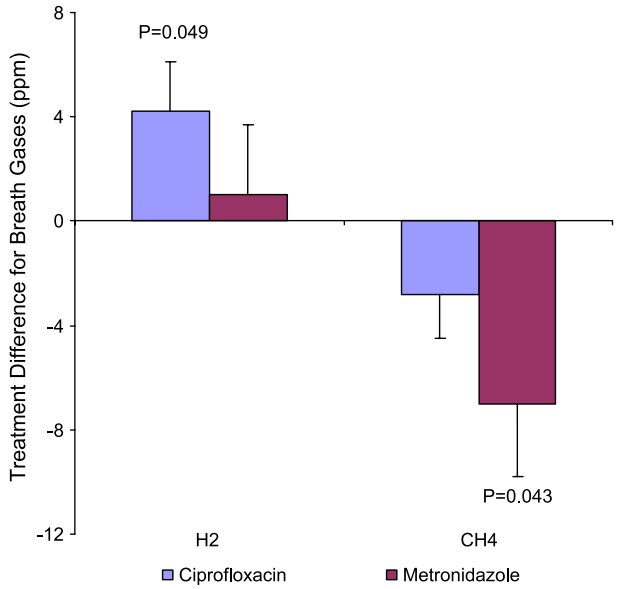


Fig. 4. Effect of ciprofloxacin/metronidazole (1000 mg/d) on breath gases expressed as differences between test and control treatments.

The significance of the percent differences in blood lipids and lipoprotein measurements were confirmed for the absolute differences by ANCOVA (Tables 2 and 3).

For ciprofloxacin, the statin users (n = 2) response data were very similar to the rest of the group (n = 11). For metronidazole, although there were no significant differences between the responses of statin users (n = 3) and nonusers (n = 7), the relative HDL-C increase following metronidazole tended to be greater in the users (7.7% vs 1.9%) as did the reduction in the T-C/HDL-C (13.0% vs 6.8%) and apo B/A-I ratios (23.0% vs 15.0%). On the other hand, the relative reduction in LDL-C tended to be greater in the nonusers (11.0% vs 16.0%).

3.2. C-reactive protein and blood pressure

No significant changes were seen in CRP or blood pressure across treatments, and there were no significant differences between test and control treatments for either of the antibiotic studies (Tables 2 and 3). Moreover, no differences with respect to treatment effect on the above measurements were observed between ciprofloxacin and metronidazole.

3.3. Fecal microbiology

Concentrations of aerobes and anaerobes were reduced on ciprofloxacin in contrast to metronidazole where aerobic and anaerobic counts increased (Fig. 3). The effect was seen most clearly with bifidobacteria which were reduced on ciprofloxacin (−2.38 ± 0.74 log CFU/g, P = .008) and increased on metronidazole (2.70 ± 0.69 log CFU/g, P = .004). The comparisons of the two antibiotics directly as differences from their respective controls indicated significantly higher counts on metronidazole for bifidobacteria, anaerobic, and aerobic bacteria (P < .001, P = .007, P = .001, respectively).

3.4. Fecal bulk, macronutrients, and fiber recovery

Neither antibiotic increased fecal bulk or fecal moisture significantly relative to the control. Both ciprofloxacin and metronidazole increased the fecal recovery of fiber (20.9 ± 7.2%, P = .013 and 28.4 ± 9.9%, P = .021, respectively) and available carbohydrate (1.0 ± 0.4%, P = .026 and 1.1 ± 0.05%, P = .053, respectively) (Table 5). No effect was seen on fecal fat and nitrogen recovery with either antibiotic.

3.5. Short-chain fatty acids

No treatment differences were seen in the output of total or individual SCFAs on either treatment. The concentration, as opposed to the output, was reduced on ciprofloxacin for total SCFA (P = .003), acetate (P = .004), propionate (P = .021), and butyrate (P = .010) (Table 6).

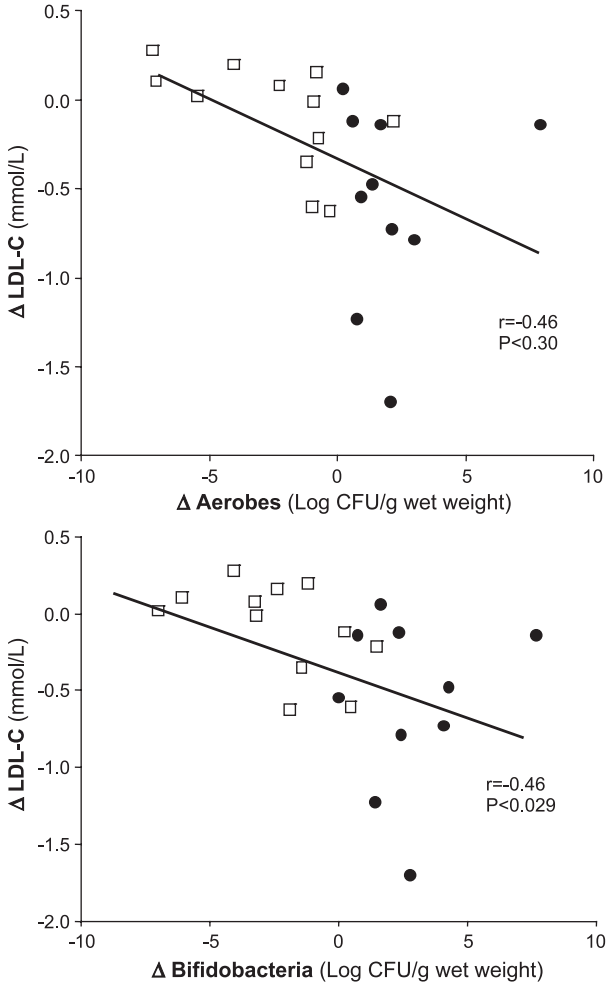


Fig. 5. Correlations for the difference between antibiotic and control treatments in bacterial counts compared to the difference between treatments in LDL-C.

3.6. Breath H_2 and CH_4

Ciprofloxacin was associated with an increase in H_2 production by comparison with the control (4.2 ± 1.9 ppm, $P = .049$), but there was no significant change in breath CH_4. Metronidazole reduced breath CH_4 (7.0 ± 2.8 ppm, $P = .043$) with no significant change in breath H_2 concentrations over the day (Fig. 4).

3.7. Relation of changes in blood lipids to microbial numbers

After combining data from both antibiotic studies, significant negative associations were seen between the treatment differences in blood lipids and fecal microbiology. The treatment differences in LDL-C and LDL/HDL-C ratio related to treatment differences in bifidobacteria ($r = -0.46$, $P = .029$ and $r = -0.49$, $P = .021$, respectively) and to total aerobes ($r = -0.46$, $P = .030$ and $r = -0.43$, $P = .044$, respectively) (Fig. 4), and not to total anaerobic bacteria ($r = -0.34$, $P = .126$ and $r = -0.34$, $P = .119$) (Fig. 5).

4. Discussion

In the present study, commonly used antibiotics reduced serum lipid risk factors for cardiovascular disease. On metronidazole the blood lipid reductions were substantial even by comparison with recognized dietary approaches to lowering serum cholesterol including increased intakes of viscous fiber, where 5% to 10% reductions in LDL-C are reported rather than the 14% reduction seen here [29-31]. Blood cholesterol measurements made after routine metronidazole use in clinical practice might therefore risk misclassifying hypercholesterolemic individuals as normal. Although the long-term effects of antibiotics on blood lipids are not addressed in this study, these findings may still be relevant to the ongoing trials of antibiotics aimed at preventing cardiovascular disease. However, of possibly greater importance, this study focuses attention on the colonic microflora, especially bifidobacteria and facultative anaerobes including lactobacilli, and their potential role in cholesterol metabolism. The potential benefits of changing the colonic microflora and their metabolic activities are not new, but have been an integral part of the dietary fiber hypothesis and the potential health advantages of high-fiber diets [32] and more recently the use of pre- and probiotics [3-5].

Furthermore, metronidazole treatment was associated with a reduced level of oxidized LDL although there was no effect on CRP or blood pressure as additional risk factors for CHD [33], which may be influenced by changes in microbial activity [34].

Metronidazole and ciprofloxacin were selected for study to assess the effects of reducing aerobic and anaerobic bacteria, respectively. Three studies with a duration of 1 to 3 weeks have reported a reduction in LDL-C with metronidazole (750-2250 mg/d) in both normolipidemic and hyperlipidemic subjects [8-10]. A later study of 7 normolipidemic and 5 hyperlipidemic subjects who took 1500 mg/d metronidazole for 1 week failed to detect an effect [35]. No studies have been carried out with ciprofloxacin or assessed the effect of these antibiotics on colonic fermentation.

It was proposed that antibiotics by reducing anaerobic bacterial numbers and activity in the colon would reduce colonic fermentation of carbohydrates and increase fecal fiber recovery. Short-chain fatty acid synthesis would therefore be reduced, resulting in less colonic acetate absorption and reduced hepatic cholesterol biosynthesis. In the present study both metronidazole and ciprofloxacin increased fecal fiber recovery equally but in neither case were daily SCFA outputs reduced although SCFA concentrations decreased significantly on ciprofloxacin.

In previous studies, subjects with reduced capacity to ferment resistant starch in the colon were observed to have reduced serum lipids [36]. Dietary supplementation with lactulose, which is largely fermented to acetate in the colon, has been shown to raise serum cholesterol [37]. It has also

Table 6
Effect of ciprofloxacin/metronidazole on fecal SCFAs daily outputs and concentrations

n = 13	Control	Ciprofloxacin	End difference	P<
Output (mmol/d)				
Acetate	10.12	9.53	−0.60	.872
Proprionate	1.77	1.63	−0.10	.791
Butyrate	2.14	1.72	−0.40	.470
Isobutyrate	0.17	0.15	0.00	.705
Valerate	0.15	0.10	−0.10	.309
Isovalerate	0.19	0.17	0.00	.724
Concentrations (mmol/L)				
Acetate	46.9	28.5	−18.0	.004
Proprionate	9.08	5.76	−3.3	.021
Butyrate	9.81	5.59	−4.2	.010
Isobutyrate	1.04	0.63	−0.4	.009
Valerate	0.90	0.31	−0.6	.002
Isovalerate	1.21	0.68	−0.5	.014
Acetate/propionate	6.98	5.88	−1.1	.409
n = 10	**Placebo**	**Metronidazole**	**End difference**	**P<**
Output (mmol/d)				
Acetate	13.1	15.7	2.6	.408
Proprionate	2.0	1.8	−0.3	.559
Butyrate	2.4	2.1	−0.4	.431
Isobutyrate	0.3	0.2	−0.1	.091
Valerate	0.3	0.2	−0.1	.099
Isovalerate	0.3	0.2	−0.1	.035
Concentrations (mmol/L)				
Acetate	55.6	58.5	2.9	.633
Proprionate	10.3	7.5	−2.8	.257
Butyrate	10.8	8.0	−2.7	.365
Isobutyrate	1.4	0.9	−0.6	.179
Valerate	2.1	1.0	−1.1	.189
Isovalerate	1.8	1.0	−0.8	.159
Acetate/propionate	6.6	14.6	8.0	.144

been demonstrated that those antibiotics that lower serum cholesterol inhibit colonic bacterial activity, as judged by their inability to convert primary to secondary bile acids [2]. The lack of bile acid data is a deficiency in our paper. The original studies with neomycin indicated that the cholesterol lowering related to the reduction in fecal 7α-hydroxylase activity and the conversion of cholate to deoxycholate [1]. There have however been no reports of increased bile acid losses [1], and the suppressant effect of either increased primary bile acids or reduced secondary bile acids on hepatic cholesterol synthesis has not been clearly elucidated. For this reason we wondered whether the suppression of 7α-hydroxylase activity in feces was an indicator of overall reduced fermentative activity.

In the present study there were no associations between blood lipids and markers of colonic fermentation, including fecal fiber recovery, SCFA output, or breath hydrogen and methane levels during the day. It is also possible that an increase in the ratio of propionate to acetate or an absolute increase in propionate synthesis might reduce serum cholesterol levels [38] by an effect on HMG CoA-reductase activity [39]. However, no treatment difference was seen in propionate or in the propionate/acetate ratio in the present studies nor did these measurements relate to serum lipid treatment differences.

The increase in bifidobacteria and the reduction in the remaining anaerobes provide one of the few positive findings linking altered colonic microbial metabolism to changes in blood lipids. Increased bifidobacterial numbers because of feeding fructoligosaccharides or inulin have been reported to reduce serum cholesterol concentrations [6,7]. As in the present study, these studies also failed to provide a mechanism. No alteration was seen in total SCFA output and their molar ratios or in total bile acid excretion [7]. However, the increase in bifidobacteria was associated with increased secondary bile acid losses [7]. There are reports that bifidobacteria can sequester cholesterol [40,41]. Nevertheless, because this action is largely limited to the colon the effect is likely to be small.

The increase in bifidobacteria counts on metronidazole was an unexpected finding. However, previous reports indicated that metronidazole either had no measurable effect on fecal microflora or caused a significant increase in aerobic bacteria with no change in anaerobic microflora [42,43]. This has been suggested to be due to the rapid metabolism of the drug by the bowel microflora under the usual anaerobic conditions in the colon. The reason for the increase in bifidobacteria seen in the present study is unclear but may be due to the greater sensitivity to metronidazole of other anaerobes leaving increased amounts of fermentable material in the fecal stream for the growth of the less antibiotic-sensitive bifidobacteria. In this respect, studies also indicate that most strains of bifidobacteria are resistant to kanamycin, neomycin, paromomycin sulfate, and metronidazole [44]. It is of interest that kanamycin, neomycin, and paromomycin together with chlortetracycline and paraaminosalicylic acid were the same antibiotics, which Samuel et al originally demonstrated to lower serum cholesterol. Furthermore, agar containing neomycin sulfate has been used for isolation and enumeration of bifidobacteria [45] because bifidobacteria are resistant to neomycin [44]. The unexpected reduction of fecal anaerobes with ciprofloxacin has been observed by others [45] and is likely related to the high antibiotic fecal concentration despite poor in vitro anaerobic activity.

There are a number of weaknesses in the present study. The period of antibiotic use was relatively short and the maximum cholesterol-lowering effect might not have been reached. Nevertheless, a substantial fall in serum cholesterol was seen with metronidazole over 10 days and similar early major reductions in serum cholesterol have been reported with dietary interventions [46,47]. Furthermore, sustained reductions in serum cholesterol would be required for CHD risk reduction. Current trials are testing longer-term antibiotic administration. The value of the present data is therefore to focus attention on the potential importance of antibiotic use, possibly through altering colonic function, in reducing blood lipids. Finally, there was no placebo tablet in the ciprofloxacin study. Nevertheless, the cholesterol-lowering effect was greatest with metronidazole where the control period involved a placebo. The absence of a placebo was therefore not responsible for a false-positive result.

We conclude that physiologically significant reductions in serum lipid risk factors for cardiovascular disease can result from antibiotic use. The possible underlying microbiological explanation could relate to the increase in the numbers of bifidobacteria. These findings may be relevant to studies assessing the effect of antibiotic use in the prevention of CHD and in confounding routine blood cholesterol measurements. Possibly of greater relevance is the emphasis on the potential for colonic events to modify serum cholesterol and the need to explore nonantibiotic means to lower serum cholesterol by altering colonic bacterial numbers and functions.

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