Short-Chain Fatty Acids Suppress Cholesterol Synthesis in Rat Liver and Intestine

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ABSTRACT We previously showed that plasma cholesterol levels decreased following ingestion of a short-chain fatty acid (SCFA) mixture composed of sodium salts of acetic, propionic, and butyric acids simulating cecal fermentation products of sugar-beet fiber (SBF). In the present study, we investigated whether hepatic and small intestinal cholesterol synthesis is involved in the cholesterol-lowering effects of SCFA and SBF. In vitro (expt. 1) and in vivo (expt. 2) cholesterol synthesis rates and the diurnal pattern of SCFA concentrations in portal plasma (expt. 3) were studied in three separate experiments in rats fed diets containing the SCFA mixture, SBF (100 g/kg diet), or the fiber-free control diet. Cholesterol synthesis was measured using ^3H_2O as a tracer. The in vitro rate of cholesterol synthesis, measured using liver slices, was greater in the SBF group, but not in the SCFA group, than in the fiber-free control group. In contrast, the hepatic cholesterol synthesis rate in vivo was lower in the SCFA group, but not in the SBF group, than in the control group. The mucosal cholesterol synthesis rate for the whole small intestine was <50% of the hepatic rate. The rate in the proximal region was slightly but significantly lower in the SCFA group, and was significantly higher in the SBF group than in the fiber-free group. The rate in the distal small intestines was also significantly greater in the SBF group than in the fiber-free group. Plasma total cholesterol concentrations were lower in the SCFA and SBF groups than in the fiber-free group in both experiments 2 and 3. Diurnal changes in portal SCFA and cholesterol levels were studied in the experiment 3. SCFA concentrations increased rapidly after the start of feeding the SCFA diet, and changes in plasma cholesterol were the reciprocal of those observed in SCFA. These results show that a decrease in hepatic cholesterol synthesis rate mainly contributes to the lowering of plasma cholesterol in rats fed the SCFA mixture diet. Changes in portal SCFA and cholesterol concentrations support this conclusion. In SBF-fed rats, SCFA produced by cecal fermentation are possibly involved in lowering plasma cholesterol levels by negating the counteractive induction of hepatic cholesterol synthesis caused by an increase in bile acid excretion. J. Nutr. 129: 942–948, 1999.

KEY WORDS: • *cholesterol synthesis* • *short-chain fatty acids* • *liver* • *small intestine* • *rats*

An important physiological action of dietary fiber is the lowering of plasma cholesterol levels (Anderson 1985, Kritchevsky and Stony 1986). Inhibition of the absorption of bile acids and cholesterol, or suppression of cholesterol synthesis, may be involved in the effects of dietary fiber on lowering blood cholesterol. Carr et al. (1996) and Gallaher et al. (1993) showed that the viscosity of dietary fiber is related to its cholesterol lowering effect in hamsters fed cholesterol-supplemented diets; however, Younes et al. (1995) demonstrated that, in rats, a fermentable component, resistant starch, lowers plasma cholesterol levels more effectively than cholestyramine.

Ingestion of highly fermentable dietary fiber, sugar-beet fiber (SBF)[2], decreases plasma cholesterol concentrations in rats (Aritsuka et al. 1989, Johnson et al. 1990), and the effect requires the large intestine (Nishimura et al. 1993). We previously demonstrated that a short-chain fatty acid mixture simulating the cecal fermentation products of SBF lowered plasma cholesterol levels to a degree similar to that obtained by SBF feeding in rats (Hara et al. 1998). This finding suggests that the suppressive effect of SBF on plasma cholesterol levels is at least partly caused by the cecal fermentation products, short-chain fatty acid (SCFA). One of these fermentation products, propionate, is viewed as a potential candidate as an effective agent for lowering plasma cholesterol levels (Chen et al. 1984, Nishina and Freedland 1990); however, the results of studies examining the effects of propionate on cholesterol metabolism are controversial (Beaulieu and McBurney 1992, Berggren et al. 1996, Kishimoto et al. 1995). Also, we showed that acetate was an effective component of the SCFA mixture for lowering plasma cholesterol levels, whereas propionate was not (Hara et al. 1998).

The aims of the present study were to clarify the mechanism of the cholesterol-lowering effect of SCFA and SBF. Using ^3H_2O as a tracer, we examined the changes in cholesterol synthesis in the liver and the small intestine after feeding diets containing the SCFA mixture and SBF to rats. We also studied the diurnal changes in portal plasma SCFA concen-

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[2] Abbreviation used: DPS, digitonin-precipitable sterol; SBF, sugar-beet fiber; SCFA, short-chain fatty acid.

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TABLE 1

Composition of the fiber-free (basal) diet

	Fiber-free diet[1]
	g/kg diet
Casein[2]	250
Corn oil[3]	50
Mineral mixture[4]	40
Vitamin mixture[5]	10
Choline bitartrate	4.0
Sucrose	to make 1 kg

[1] Sugar-beet fiber (100 g/kg diet) and short-chain fatty acid mixture were added to the fiber-free diet at the expense of the whole diet. The mixture of short-chain fatty acids (SCFA diet, sodium salts of acetic, propionic and butyric acids [average purity of each of the three reagents was 96%, Wako Pure Chemical Industry, Osaka, Japan] were added in amounts up to 35 g (0.43 mol), 22 g (0.23 mol) and 9.0 g (0.082 mol)/kg diet, respectively).

[2] Casein (ALACID; New Zealand Dairy Board, Wellington, New Zealand)

[3] Retinyl palmitate (7.66 μmol/kg diet) and ergocalciferol (0.0504 μmol/kg diet) were added to corn oil.

[4] The mineral mixture was prepared based on report of the AIN-76 Workshop held in 1989 (Reeves 1989). It provids (mg/kg diet): Ca, 4491; P, 2997; K, 3746; Mg, 375; Fe, 100; I, 0.32; Mn, 10.0; Zn, 34.7; Cu, 6.00; Na, 4279; Cl, 6542; Se, 1.05; Mo, 1.00; Cr, 0.50; B, 0.50; V, 0.25; Sn, 2.00; As, 1.00; Si, 20.0; Ni, 1.00; F, 2.72; Co, 0.20.

[5] The vitamin mixture without vitamin E was prepared in accordance with the AIN-76 mixture (American Institute of Nutrition 1977), except that menadione and L-ascorbic acid were added to make 5.81 μmol/kg (American Institute of Nutrition 1980) and 284 μmol/kg (Harper 1959) diet, respectively.

trations to evaluate the absorptive behavior of orally administered SCFA.

MATERIALS AND METHODS

Animals and diets. Male Wistar-ST rats (Japan SLC, Hamamatsu, Japan), weighing ~100 g each, were fed a semi-purified, sucrose-based diet containing 250 g casein/kg diet (basal diet) **(Table 1)** for 10–14 d to acclimate the animals to an inverse lighting cycle (lights on: 21:00–09:00 h), with feeding during the dark phase. In all experiments, the diets were given at the beginning of the dark cycle. Three separate experiments were conducted to examine the effects of feeding diets containing the SCFA mixture and SBF on in vitro cholesterol synthesis activity in the liver, in vivo cholesterol synthesis rates in the liver and the small intestinal mucosa, and diurnal changes in portal plasma SCFA and cholesterol concentrations compared to those in rats fed a fiber-free diet. The SCFA mixture was composed of sodium salts of acetic, propionic, and butyric acids simulating fermentation products of SBF produced by cecal bacteria. Preparation and analysis of the fermentation products of SBF were described previously (Hara et al. 1998). In the SCFA mixture diet, sodium salts of acetic, propionic, and butyric acids (average purity of each of the three reagents was 96%) (Wako Pure Chemical Industry, Osaka, Japan) were added in amounts up to 35 g (0.43 mol), 22 g (0.23 mol), and 9.0 g (0.082 mol)/kg diet, respectively. The amounts of SCFA added to the diets were comparable to those produced by cecal fermentation from SBF added to diet in the present study (Hara et al. 1998). Rats were divided into three blocks based on plasma cholesterol concentration, and then in each of the three blocks three diet groups were formed based on body weight. The artificial SCFA mixture and SBF (100 g/kg diet, Nippon Sugar Beet Manufacturing, Obihiro, Japan) were added to the fiber-free diet at the expense of the whole diet. Throughout all experiments, the rats were housed in individual cages in a temperature-controlled room at 22°C. The study was approved by the Hokkaido University Animal Committee, and the animals were maintained in accordance with the guidelines for the care and use of laboratory animals of Hokkaido University.

In experiment 1, we examined the effects of feeding SCFA and SBF on in vitro cholesterol synthesis in liver slices. Rats from three groups were fed the fiber-free diet (basal diet) or diets containing SBF or the SCFA mixture for 14 d. At the mid-point of the dark phase (15:00 h) on the last day of feeding, the rats were killed after collection of aortic blood under anesthesia (Nembutal: sodium pentobarbital, 50 mg/kg body wt; Abbott Laboratories, North Chicago, IL). The liver was immediately removed, rinsed with saline, and weighed. Liver slices (~1 mm thick, 300 mg wet wt) were cut out and incubated in 2 mL of Krebs-Ringer bicarbonate buffer (pH 7.4, gassed with 95% O_2: 5% CO_2) containing 18.5 MBq of ^3H_2O (New England Nuclear, Boston, MA) and 130 mg of bovine serum albumin (fatty acid-free; Sigma Chemical, St. Louis, MO) for 1.5 h. Cholesterol synthesis in the slice was stopped by adding 2 mL of 15 mol KOH/L. Plasma and the remaining portions of liver were stored at -40°C.

In experiment 2, we examined the effects of feeding SCFA and SBF on the in vivo cholesterol synthesis rate in the liver and the small intestinal mucosa. Rats were fed one of the three test diets as in experiment 1 for 14 d. At the mid-point of the dark phase (15:00 h) on the last day of feeding, ^3H_2O in saline (740 MBq/kg body wt) was injected into the jugular vein of the rats under light anesthesia by diethyl ether inhalation. One hour after the injection, aortic blood was collected under pentobarbital anesthesia, the rats were killed, and the liver and the small intestine were immediately removed. The small intestine was divided into two segments of equal length, the proximal half and distal half. These tissues were rinsed with cold saline and stored at -40°C until subsequent analyses.

In experiment 3, we examined the effects of feeding SCFA and SBF on diurnal changes in portal plasma SCFA and cholesterol concentrations. Rats were fed the basal diet for 13 d to acclimate them to a meal feeding protocol of 5.5 h (9:00–15:30 h) to coordinate the postprandial condition. Rats from the three groups were fed one of the three test diets described for experiment 1 under a 5.5 h meal feeding for 14 d. On the last day, rats of each diet group were divided into five sub-groups, and 0, 5, 10, 15, and 20 h after the start of feeding, rats in each sub-group were anesthetized with pentobarbital and portal blood was withdrawn.

Analyses. Aortic and portal plasma total cholesterol concentrations were measured by an enzymatic procedure (T-CHO, Kainos Laboratories, Tokyo). Cholesterol and triglyceride concentrations in the liver were estimated by enzymatic procedures (T-CHO and TG-EN, respectively, Kainos Laboratories) after Folch's extraction (Folch et al. 1957) of saponified liver and hexane extraction of liver, respectively. Cholesterol concentration in the intestinal mucosa was measured by the same method as that used for the liver.

Cholesterol synthesis in vitro and in vivo in the liver and the small intestinal mucosa were evaluated by measuring ^3H_2O incorporation into digitonin-precipitable sterol (Dietschy and Spady 1984). Briefly, the liver (~500 mg) and the intestinal mucosa sampled from the frozen intestine (Kasai et al. 1993) were saponified by heating in 15 mol KOH/L, and cholesterol was extracted from the saponified solution with hexane. The solvent was replaced by acetone: ethanol (1:1), and cholesterol was precipitated by digitonin as digitonin-precipitable sterols (DPS). After washing with acetone followed by diethyl ether, the radioactivity of ^3H in DPS was measured by means of a liquid scintillation counting system (LSC-700, Aloka, Tokyo, Japan). To calculate in vivo cholesterol synthesis rates, specific radioactivity in precursor pools (blood and liver water) were measured in condensed water made by heating aortic plasma or a small piece of the frozen liver in a nitrogen-filled tube. We found that values in the water of plasma and tissue were the same, and the plasma water radioactivity in each rat [means ± SEM for 18 rats was 133 ± 0.50 Bq/μmol water] was used for subsequent calculation in the case of both liver and intestinal mucosa.

Short-chain fatty acid concentrations in the portal plasma were evaluated after extraction by the previously described method (Murase et al. 1995). Individual SCFA were measured by gas-liquid chromatography (Shimadzu GC-14A with a prepacked glass column [1600 mm x 3 mm, SP-1220 + H_3PO_4 (15% + 1%)] on 80–100 mesh Chromosorb W-AW DMCS, Shimadzu Corporation, Kyoto, Japan) after adding phosphoric acid (final concentration 0.67 mol/L).

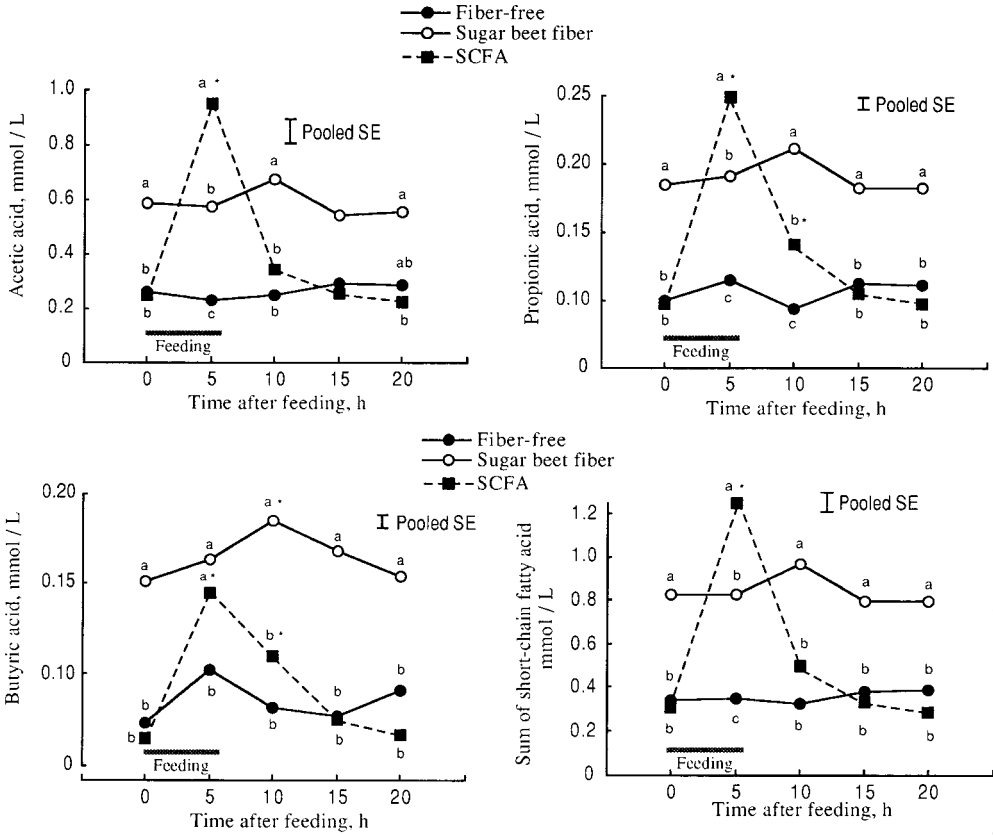

FIGURE 1 Diurnal changes in plasma concentrations of short-chain fatty acids (SCFA), i.e., acetic, propionic, and butyric acids, and the sum of these three acids (Sum of SCFA), on d 14 in rats fed fiber-free, sugar-beet fiber (100 g/kg diet), and three short-chain fatty acid mixture diets (expt. 3). Each value is the mean for 6 rats. The pooled SEM are shown. *P* values estimated by two-way ANOVA for acetic acid were <0.001 for Diet, 0.008 for Time, and <0.001 for Diet x Time; for propionic acid were <0.001 for Diet, <0.001 for Time, and <0.001 for Diet x Time; for butyric acid were <0.001 for Diet, <0.001 for Time, and 0.001 for Diet x Time; and for the sum of the three acids were <0.001 for Diet, <0.001 for Time, and <0.001 for Diet x Time. Mean values at a time not sharing a letter are significantly different($P < 0.05$).

Calculations. Cholesterol synthesis rates in vivo were calculated by following equations (Dietschy & Spady 1984):

Specific radioactivity of tissue cholesterol (Bq/μmol)

= Radioactivity in DPS (Bq)/Cholesterol in DPS (μmol) (1)

Fractional turnover rate (for 1 h)

= (1)/Specific radioactivity of plasma water (Bq/μmol) x 1.09 (2)

1.09 is the correction factor for the estimation of precursor pool by measuring plasma water specific activity 1 h after ^3H_2O injection

Cholesterol synthesis rate [μmol/(g tissue · h)]

= (2) x cholesterol concentration in tissue (μmol/g tissue)

To calculate in vitro cholesterol synthesis activity, specific activities of ^3H_2O in the incubation medium were used.

Data were analyzed by one-way or two-way (Diet and Time, **Figs. 1** and **2**) ANOVA, and significant differences among diet groups were determined by Duncan's multiple range test (Duncan 1955, $P < 0.05$). These statistical analyses were done by the GLM procedure of SAS (SAS version 6.07, SAS Institute, Cary, NC).

RESULTS

Body weight gain and energy intake during the test period were not different among the three diet groups in the three experiments. The overall mean values for body weight gain and energy intake in all rats were 7.43 g/d (pooled SE, 0.147, $P = 0.761$, $n = 18$) and 272 kJ/d (pooled SE, 5.14, $P = 0.261$, $n = 18$), respectively, in experiment 1; 5.68 g/d (pooled SE, 0.123, $P = 0.326$, $n = 18$), and 225 kJ/d (pooled SE, 1.28, $P = 0.205$, $n = 18$), respectively, in experiment 2; and 5.00 g/d (pooled SE, 0.082, $P = 0.392$, $n = 90$) and 177 kJ/d (pooled SE, 2.00, $P = 0.126$, $n = 90$), respectively, in experiment 3.

The plasma cholesterol concentrations in rats fed SBF and SCFA diets for 14 d were lower than those in rats fed a fiber-free diet in experiments 1 and 2 **(Table 2)**.

Relative liver weight was not significantly affected by diet

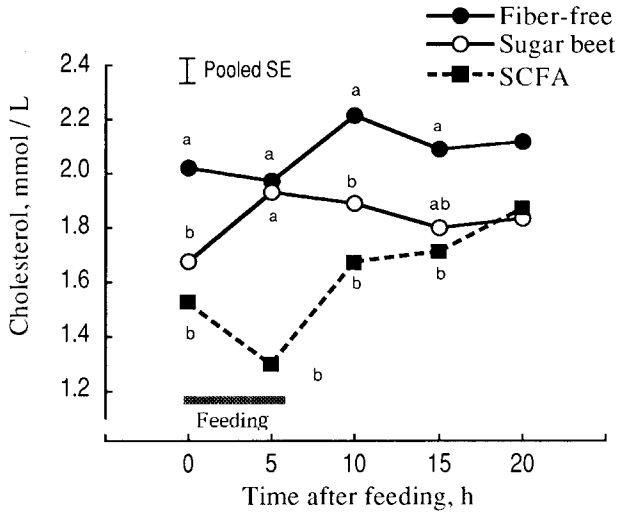

FIGURE 2 Diurnal changes in plasma cholesterol concentrations on d 14 in rats fed fiber-free, sugar-beet fiber (100 g/kg diet), and short-chain fatty acid mixture diets (expt. 3). Each value is the mean for 6 rats. The pooled SEM are shown. *P* values estimated by two-way ANOVA were <0.001 for Diet, 0.003 for Time, and 0.105 for Diet x Time. Mean values at a time not sharing a letter are significantly different ($P < 0.05$).

TABLE 2

Plasma total cholesterol concentration in rats fed fiber-free, sugar-beet fiber and short-chain fatty acid mixture diet for 14 d[1]

Diet group	Cholesterol concentration[2]
	μmol/L
Expt. 1	
Fiber-free	2.36[a]
Sugar-beet fiber[3]	2.00[b]
SCFA mixture[4]	1.90[b]
pooled SEM	0.121
ANOVA *P* value	0.040
Expt. 2	
Fiber-free	2.41[a]
Sugar-beet fiber	1.89[b]
SCFA mixture	1.74[b]
pooled SEM	0.110
ANOVA *P* value	0.002

[1] Values are means, $n = 6$. Values not sharing a superscript letter differ significantly, $P < 0.05$. Abbreviation used: SCFA, short-chain fatty acid.

[2] Initial concentrations of plasma cholesterol were 2.74 μmol/L (pooled SEM, 0.183, $P = 0.999$, $n = 18$) in experiment 1 and 2.86 μmol/L (pooled SEM, 0.083, $P = 0.996$, $n = 18$) in experiment 2.

[3] Sugar-beet fiber was added to the fiber-free diet (100 g/kg diet) at the expense of the whole diet.

[4] Short-chain fatty acid mixture diet contained sodium salts of acetic, propionic, and butyric acids.

in experiments 1 and 2. The overall data for wet weight for the three groups were 4.91 g/100g body wt (pooled SE, 0.183, $P = 0.938$) in experiment 1 and 4.43 g/100g body wt (pooled SE, 0.106, $P = 0.240$) in experiment 2. The liver cholesterol pool and concentration were also not different among the diet groups (data not shown); however, the triglyceride pool and concentration were markedly lower ($P < 0.01$) in the SBF and SCFA groups than in the fiber-free group in experiment 1 **(Table 3)**.

Mucosal wet weights of the proximal and distal halves of the small intestine, and the cholesterol pool and concentration of these tissues, 2 wk after feeding the three test diets were not significantly influenced by the diets (expt. 2). The overall relative mucosal wet weight for the three groups were 1.03 g/100g body wt (pooled SE, 0.066, $P = 0.250$) in the proximal half of the small intestine and 0.743 g/100g body wt (pooled SE, 0.054, $P = 0.224$) in the distal half of the small intestine.

TABLE 3

Liver triglyceride levels in rats fed fiber-free, sugar-beet fiber, and short-chain fatty acid mixture diets for 14 d[1]

Diet group	Liver triglyceride	
	μmol/g liver	*μmol/100 g body wt*
Expt. 1		
Fiber-free	27.1[a]	124[a]
Sugar-beet fiber[2]	14.7[b]	63.9[b]
SCFA mixture[3]	12.0[b]	53.2[b]
pooled SEM	2.74	12.5
ANOVA *P* value	0.003	0.003

[1] Values are means, $n = 6$. Values not sharing a superscript letter differ significantly, $P < 0.05$. Abbreviation used: SCFA, short-chain fatty acid.

[2] Sugar-beet fiber was added to the fiber-free diet (100 g/kg diet) at the expense of the whole diet.

[3] Short-chain fatty acid mixture diet contained sodium salts of acetic, propionic, and butyric acids.

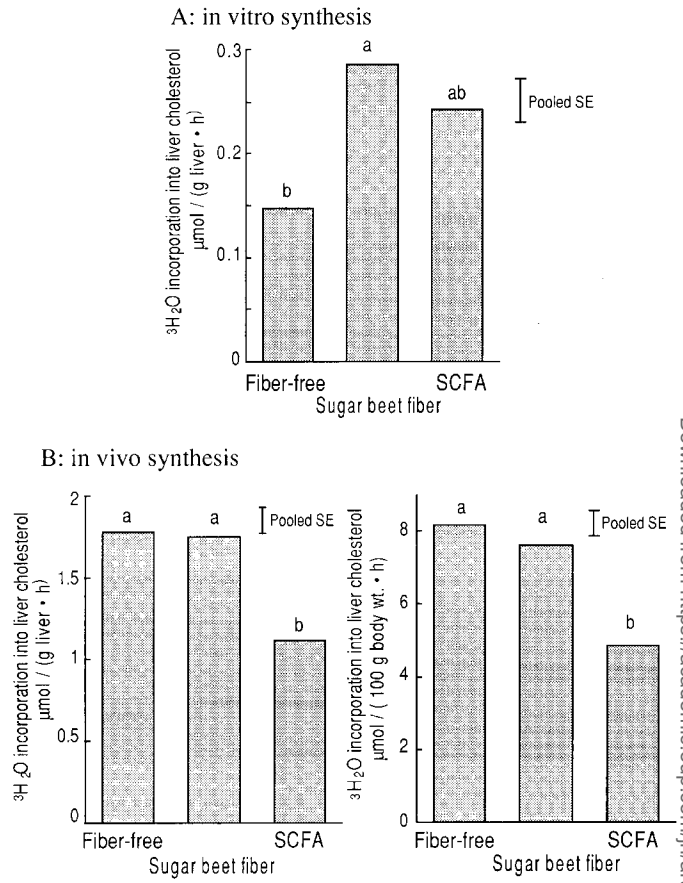

FIGURE 3 Liver cholesterol synthesis rate in vitro in liver slices (*A*, expt. 1) and in vivo (*B*, expt. 2) in rats fed fiber-free, sugar-beet fiber (100 g/kg diet), and short-chain fatty acid (SCFA) mixture diets for 14 d. The SCFA mixture diet contained sodium salts of acetic (35 g/kg diet), propionic (22 g/kg diet), and butyric (9.0 g/kg diet) acids. The synthesis rate was determined by measuring the incorporation of ^3H_2O into digitonin-precipitable sterol. Each value is the mean for 6 rats. The pooled SEM are shown. *P* values estimated by ANOVA were 0.050 for in vitro synthesis and 0.010 and 0.009 for in vivo synthesis per unit liver and per 100 g body wt, respectively. Mean values not sharing a letter are significantly different ($P < 0.05$).

In vitro cholesterol synthesis rates in liver slices from rats fed the SBF diet were higher than those from rats fed the fiber-free diet (expt. 1, **Fig. 3**A), with rates for the SCFA group intermediate. In vivo hepatic cholesterol synthesis rates per unit weight of liver and per 100 g body wt are shown in Figure 3B (expt. 2). Cholesterol synthetic rates in the SCFA group were lower than those of the fiber-free and SBF groups, which did not differ.

The cholesterol synthesis rate in the proximal and distal regions of the small intestinal mucosa are shown in **Figure 4**. The rates per unit weight of mucosa and per 100 g body wt were significantly higher in the SBF group in the proximal and distal small intestine compared to the fiber-free group, except for the rate per g mucosa in the distal site. The proximal small intestine mucosal cholesterol synthesis rate per 100 g body wt

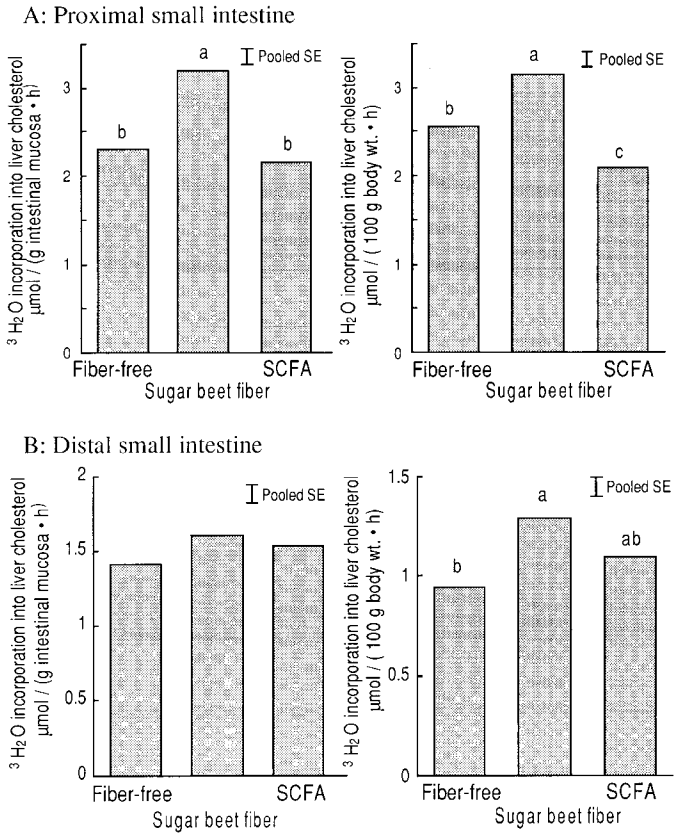

FIGURE 4 In vivo cholesterol synthesis rate in the mucosa of the proximal half (***A***) and distal half (***B***) of the small intestine in rats fed fiber-free, sugar-beet fiber (100 g/kg diet), and short-chain fatty acid mixture diets for 14 d (expt. 2). Other details are as shown in Fig. 3. Each value is the mean for 6 rats. The pooled SEM for the are shown. *P* values estimated by ANOVA were <0.001 and 0.002 for proximal small intestinal synthesis per unit liver and per 100 g body wt, and 0.532 and 0.034 for distal small intestinal synthesis per unit liver and per 100 g body wt, respectively. Mean values not sharing a letter are significantly different ($P < 0.05$).

in the SCFA group was lower than that in the fiber-free and SBF groups.

Diurnal changes in portal concentrations of acetic, propionic, and butyric acids and cholesterol in rats adapted to meal feeding the test diets on d 14 are shown in Figures 1 and 2. In SCFA-fed rats, portal concentrations of all SCFA were markedly increased 5 h after the start of feeding, and significantly higher than those in the fiber-free and SBF-diet groups. The values 15 h after feeding in the SCFA group had returned to the prefeeding value. In the fiber-free and SBF groups, there were no significant changes in the three SCFA concentrations in the 24-h period, except for butyrate levels in rats fed the SBF diet. The butyrate concentration in the portal blood was significantly higher 10 h after the start of feeding compared to the prefeeding value. Except for the acetic acid concentration at 15 h, the three SCFA concentrations in the SBF group were higher than those in the fiber-free group. Cholesterol concentrations in the SCFA group (Fig. 2) were decreased 5 h after feeding compared to the prefeeding values. The concentrations before and 10 h after feeding were lower in the SCFA and SBF groups than in the fiber-free group, and the value 5 h after feeding in the SCFA group was lower than that in the SBF and fiber-free groups. There was a significant negative correlation between plasma cholesterol and portal acetic acid ($r = -0.649$, $P < 0.01$) or total SCFA ($r = -0.658$, $P < 0.01$) 5 h after the start of feeding.

DISCUSSION

In a previous report, we demonstrated that ingestion of SCFA, a mixture of acetic, propionic, and butyric acids simulating cecal fermentation products of SBF, lowers plasma cholesterol concentration (Hara et al. 1998). The mechanism involved in the cholesterol-lowering effects of SCFA was examined in the present study. In vivo hepatic cholesterol synthesis rates were significantly decreased as a result of feeding the SCFA mixture, and also mucosal cholesterol synthesis was weak but significantly decreased in the proximal small intestine. Decreased synthesis rates (hydrogen atoms of water incorporated in cholesterol) obtained as a result of feeding SCFA were ~3 μmol/(100 g body wt • h) in the liver (Fig. 3) and 0.5 μmol/(100 g body wt • h) in the proximal small intestine (Fig. 4), which suggests that the lower hepatic cholesterol synthesis is mainly associated with the lowering of plasma cholesterol levels in rats fed the SCFA mixture.

In contrast, in vitro cholesterol synthesis using liver slices of rats fed the SCFA diet was not lower, but tended to be higher than that in the control group (Fig. 3). The in vitro synthetic rates were much lower than the in vivo rates. This shows that the slice system may not present actual synthetic rates; however, the differences in the in vitro rate reflect activity of whole process of hepatic cholesterol synthesis. The result of the in vitro study reveals that cholesterol synthetic capacity (activity of synthetic pathway) is not reduced by SCFA feeding, and suggest that the rate of synthesis in vivo is suppressed by higher levels of SCFA in the liver when SCFA absorbed from the intestine reaches that organ. The tendency for higher of in vitro cholesterol synthesis in liver slices from the SCFA group shows that the lower plasma cholesterol level induces hepatic cholesterol synthetic activity, for example hydroxmethylglutaryl CoA reductase activity.

In rats fed the SBF diet, in vitro cholesterol synthesis was greater than in the control; however, the in vivo synthesis rate did not differ in the liver (Fig. 3) and was increased in the small intestine (Fig. 4). Plasma cholesterol concentrations were lower in rats fed the SBF diet (Table 2). There was no correlation between the hepatic cholesterol synthesis rate and the lowering of plasma cholesterol levels in the SBF group. It has not been reported that water-soluble and fermentable fibers suppress hepatic cholesterol synthesis as measured with the use of ^3H_2O. Furthermore, pectin feeding markedly increases hepatic cholesterol synthesis in rats(Arjmandi et al. 1992, Stark and Madar 1993). They suggested that the increase in hepatic cholesterol synthesis compensates for the greater excretion of bile acids with pectin feeding, and that the cholesterol synthesis rate in the liver is not involved in lowering cholesterol levels in rats fed fermentable fiber. However, the present study shows that hepatic cholesterol synthesis is involved in the lowering of plasma cholesterol levels in rats fed the SCFA mixture diet, and the amount of SCFA added to the diet is that produced by cecal fermentation in rats fed the SBF diet described above. Feeding SBF increases fecal bile acid excretion (Hara et al. 1998, Overton et al. 1994), and this increase probably induced in vitro hepatic cholesterol synthetic activity in the SBF group (Fig. 3). We speculate that SCFA produced by SBF fermentation suppress cholesterol synthesis activity, which is increased by greater bile acid excretion with SBF feeding. This resulted in no change in the in vivo hepatic cholesterol synthesis rate as shown in Figure 3. The decrease in plasma cholesterol levels in the SBF group

may be resulted because the induction of cholesterol synthetic activity with the higher bile acid excretion is canceled by fermentation products. In turn, enhancement of bile acid excretion worked effectively to lower plasma cholesterol concentration in the SBF group. As described above, pectin feeding increases in vivo hepatic cholesterol synthesis. In rats fed pectin, suppression by SCFA is not sufficient to prevent the increase in cholesterol synthesis. Bile acid excretion is higher in rats fed viscous fiber than in those fed SBF (Overton et al. 1994).

We demonstrated the suppressive effect of SCFA on hepatic cholesterol synthesis. It was suggested that propionic acid is the agent present among the cecal and colonic fermentation products that lowers cholesterol levels (Chen et al. 1984). Demigné et al. (1995) using ^3H_2O as a tracer showed that a nearly physiological concentration of propionate (0.6 mmol/L) slightly but significantly suppressed in vitro cholesterol synthesis in hepatocytes; however, in other studies using ^3H_2O, a higher concentration (1 mmol/L) did not (Nishina & Freedland 1990, Wright et al. 1990). The inhibitory effect of propionate is controversial. The maximum concentration of portal propionic acid was about 0.25 mmol/L in rats fed the SCFA mixture (Fig. 1), and Illman & Topping (1985) reported a similar propionate concentration in the portal blood of rats fed oat bran. Cecal infusion and oral feeding of propionate have no effect on plasma cholesterol levels (Beaulieu & McBurney 1992, Levrat et al. 1994). These results suggest that the effective component of the SCFA mixture for suppression of cholesterol synthesis is not propionate. We demonstrate in the previous study (Hara et al. 1998) that among cecal fermentation products of SBF, acetate, not propionate, was effective in lowering plasma cholesterol levels.

In the present study, plasma cholesterol concentrations were lower in rats fed SCFA mixture and SBF; however, liver cholesterol was not. Mazur et al. (1992) reported a similar result for SBF-fed rats. In rats fed inulin or cyclodextrin, plasma, but not liver, cholesterol levels were decreased (Levrat et al. 1994). It is not known why suppression of hepatic cholesterol synthesis affects plasma cholesterol levels but does not affect liver cholesterol. In experiments using diets supplemented with cholesterol, both plasma and liver cholesterol levels were decreased as a result of feeding soluble dietary fiber to rats (Evans et al. 1992, Fernandez 1995). In rats fed a cholesterol-free diet, lowering liver cholesterol levels may not always have beneficial effects.

We examined the absorptive behavior of orally administered SCFA and of SCFA produced in the cecal fermentation in rats fed SBF (Fig. 1). The portal SCFA concentrations in rats fed the SCFA mixture reached peak values 5 h after the start of feeding and returned to prefeeding levels 10h after feeding, which shows that dietary SCFA were readily absorbed, consistent with the results obtained by Illman et al. (1988). Cholesterol concentrations changed reciprocally with those of SCFA (Fig. 2). This finding supports the view that absorbed SCFA suppresses hepatic cholesterol synthesis. Portal SCFA concentrations in the SBF group did not substantially change, but higher levels were maintained than those in the fiber-free group. The concentrations are comparable to the peak level observed in the case of SCFA-fed rats. This result shows that SCFA produced in cecal and colonic fermentation of SBF are capable of contributing to the suppression of hepatic cholesterol synthesis. In the SCFA group, plasma cholesterol levels were lower than those in the fiber-free group at 0 or 15 h (Fig. 2);at these times, portal SCFA concentrations returned to the same level as those in the fiber-free group. However, during these periods, the hepatic cholesterol synthesis rate is very low and extrahepatic synthesis predominantly contributes to whole body synthesis (Jeske and Dietschy 1980).

In conclusion, a dietary SCFA mixture decreased the cholesterol synthesis rate, which probably contributes to the lowering of plasma cholesterol levels as observed in rats fed the SCFA mixture. The suppressive effects of SCFA may be partly involved in lowering plasma cholesterol levels as observed in rats fed SBF.

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