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The effect of high-amylose cornstarch on lipid metabolism in OVX rats is affected by fructose feeding

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Abstract

We examined whether the effects of high-amylose cornstarch (HACS) on lipid metabolism in ovariectomized (OVX) rats were affected by high-fructose feeding. Sucrose (482 g/kg diet) was used as fructose source. OVX rats were fed one of the following four diets for 21 days: a sucrose-based or cornstarch-based cholesterol-free diet with or without HACS (150 g/kg diet). Body weight and food intake were increased by sucrose. Plasma total cholesterol and low-density lipoprotein cholesterol concentrations were increased by sucrose and decreased by HACS in cornstarch-fed rats, but not in sucrose-fed rats. Liver total lipids and concentrations of plasma and liver triacylglycerol (TAG) were increased by sucrose, whereas plasma TAG concentration was decreased by HACS, in sucrose-fed rats. However, liver cholesterol concentration was not affected by diet. The amount of cholesterol in small-intestinal contents was increased in sucrose-fed rats, but not in cornstarch-fed rats, but that of bile acids was not affected by diet. Fecal excretions of bile acids and neutral sterols were increased by HACS. The level of sterol-regulatory element-binding protein-1c mRNA was increased by sucrose and decreased by HACS in sucrose-fed rats, but not in cornstarch-fed rats. The level of farnesoid X receptor mRNA was decreased by sucrose and increased by HACS in cornstarch-fed rats, but not in sucrose-fed rats, as was the level of cholesterol 7α-hydroxylase mRNA. These results show that the effect of HACS on hyperlipidemia induced by ovarian hormone deficiency would be affected by the consumption of fructose-rich sweeteners such as sucrose and high-fructose syrup.

Keywords: Amylose content; Resistant starch; Ovariectomized rat; Plasma lipids; Bile acids

1. Introduction

It is well known that lipid metabolism is influenced by sex hormones in animals and humans [1]. Sex hormones such as estrogen have a major impact on atherosclerotic processes, and studies in animal models have shown that estrogen inhibits the development of atherosclerotic lesions [2]. Estrogen deficiency is associated with changes in blood lipid levels. It is now clear that estrogen deficiency plays a key pathogenetic role in the development of coronary heart disease (CHD) in women, as supported by several epidemiological findings [3]. The risk of CHD in women increases dramatically at the onset of menopause.

One of the factors that influences plasma lipids is the amount and type of dietary carbohydrates. It is well-known that lipid metabolism is influenced by high-fructose intake [4]. Studies based on high-fructose versus high-glucose diets have shown that high-fructose diets produce an increase in the concentrations of plasma triacylglycerol (TAG), total cholesterol, very-low-density lipoprotein (VLDL) cholesterol and low-density lipoprotein (LDL) cholesterol [5]. Fructose in the diet comes from fructose that occurs naturally in food and fructose that is added to food during food processing and preparation with the use of sweeteners (added fructose). The availability of fructose increased substantially

Abbreviations: ACAT-1, acyl-coenzyme A:cholesterol acyltransferase 1; ACAT-2, acyl-coenzyme A:cholesterol acyltransferase 2; ApoB, apolipoprotein B; CYP7A1, cholesterol 7α-hydroxylase; CYP8B1, cholesterol 12α-hydroxylase; DF, dietary fiber; FXR, farnesoid X receptor; HDL, high-density lipoprotein; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; LDL, low-density lipoprotein; LDL-R, low-density lipoprotein receptor; LXR, liver X receptor; MTP, microsomal triglyceride transfer protein; RS, resistant starch; SCFA, short-chain fatty acid; SREBP-1a, sterol-regulatory element-binding protein-1a; SREBP-1c, sterol-regulatory element-binding protein-1c; SREBP-2, sterol-regulatory element-binding protein-2; TAG, triacylglycerol; VLDL, very-low-density lipoprotein.

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when it became possible in the 1960s to produce high-fructose syrup economically from cornstarch and other starches [6]. The most recent available data suggest that fructose consumption is increasing worldwide [7].

Resistant starch (RS) is reported to have physiological effects that are similar to those of dietary fiber (DF). RS is divided into four types: RS type 1 to RS type 4. High-amylose cornstarch (HACS), which is RS type 2, reduces plasma cholesterol and TAG concentrations in rats [8,9]. However, there are limited data examining the influence of HACS on lipid metabolism in rats during estrogen deficiency, in conjunction with increased fructose intake.

Estrogen can affect cholesterol absorption and the endogenous metabolism of cholesterol. Ovariectomy can minimize the interference of endogenous estrogens and mimics postmenopausal conditions. The aim of this experiment was to consider the effects of HACS and fructose on endogenous cholesterol metabolism.

Therefore, we compared the effects of HACS on cholesterol metabolism in OVX rats that had been fed a cholesterol-free diet with or without high fructose. Sucrose was used as fructose source.

2. Materials and methods

2.1. Materials

Gelatinized normal cornstarch (G-CS; Nisshoku Alstar E) and HACS (Nisshoku High-Amylose Starch) were purchased from Nihon Shokuhin Kako (Tokyo, Japan). The amylose contents of G-CS and HACS were 26 and 68 g/100 g, respectively. The amount of RS in G-CS and HACS, as determined by the method of MaClearly and Monaghan [10], was 5.6 and 50.7 g/100 g, respectively. The DF content of G-CS and HACS, as determined by the method of the Association of Official Agricultural Chemists, was 0.1 and 19.3 g/100 g, respectively.

2.2. Animals and diets

This study was approved by the Laboratory Animal Care Committee of Ehime University. Rats were maintained in accordance with the Guidelines for the Care and Use of Laboratory Animals of Ehime University.

Six-month-old female Wistar rats were housed individually in screen-bottomed stainless-steel cages in a room maintained at 23±1°C with a 12-h light/dark cycle (lights on, 0700–1900 h). The rats were acclimated by feeding them a commercial solid diet (Roden Lab Diet EQ; PMI, USA) for 7 days. After acclimation, the rats were anesthetized by intraperitoneal injection of sodium pentobarbital (30 mg/kg body weight; Nembutal; Abbott Laboratories, Chicago, IL) and bilaterally ovariectomized (OVX). The rats were fed the commercial solid diet during the 7-day recovery period, after which they were randomly divided into four groups (n=6) and allowed free access to one of the experimental diets for 21 days. The compositions of each diet are shown in Table 1 [11]. Body weight and food intake were recorded daily for each rat in the morning before the food was replaced.

2.3. Sampling and analytical procedures

Before the rats were killed, feces were collected from each rat over the final 4 days of the experimental period. The feces were freeze-dried, weighed and milled. On the night of the last day of the experimental period, blood sample was collected from the neck of each rat into a blood collection tube (Vacutainer; Becton Dickinson, Franklin Lakes, NJ) that contained heparin as anticoagulant. Plasma was separated by centrifugation at 1400×g at 4°C for 15 min and stored at −50°C until analyzed. After blood collection, the liver was immediately perfused with cold saline (9 g/L NaCl), removed, washed with cold saline, blotted dry on filter paper, weighed and stored at −50°C until analyzed. After the liver had been removed, the small intestine and cecum were removed. The contents of the small intestine were transferred into a preweighed tube, freeze-dried and weighed. The cecum was weighed, then 0.4 g of the cecal contents was transferred into a tube, and 2 ml of 10 mmol/L sodium hydroxide was immediately added. The mixture was used for short-chain fatty acid (SCFA) analysis; an aqueous solution containing 0.5 g/L crotonic acid was used as internal standard. The pH of the cecal contents was measured immediately after removal with a compact pH meter using a sampling sheet (Model C-1, calibrated at 20°C; Horiba, Tokyo, Japan). The moisture level of the cecal contents was determined as the difference between the wet mass and the dry mass of the cecal contents after freeze drying. The cecal wall was flushed with ice-cold saline (9 g/L NaCl, 4°C), blotted onto filter paper and weighed.

Table 1
Composition of experimental diets

	Diet with sucrose (SU diet)		Diet without sucrose (ST diet)	
	G-CS (g/kg)	HACS (g/kg)	G-CS (g/kg)	HACS (g/kg)
Casein	200	200	200	200
Sucrose	482	482	–	–
G-CS[1]	150	–	632	482
Soybean oil	70	70	70	70
AIN-93 mineral mixture[2]	35	35	35	35
AIN-93 vitamin mixture[3]	10	10	10	10
L-Cystine	3	3	3	3
Cellulose[4]	50	50	50	50
HACS[5]	–	150	–	150
tert-Butylhydroquinone	0.014	0.014	0.014	0.014

[1] G-CS, which contained 26 g of amylose and 0.1 g of DF per 100 g.
[2] Based on AIN-93G [22].
[3] The AIN-93 vitamin mixture used in this study contained 20 g of choline bitartrate per 100 g.
[4] PC200 (Danisco Japan Ltd., Tokyo, Japan).
[5] HACS, which contained 68 g of amylose and 19.3 g of DF per 100 g.

2.4. Biochemical analysis

The concentrations of total cholesterol, TAG and phospholipids in the plasma were determined enzymatically using commercial diagnostic kits (Cholesterol E-Test Wako, Triglyceride E-Test Wako and Phospholipid C-Test Wako; Wako Pure Chemical Industries, Osaka, Japan).

Plasma lipoprotein fractions [VLDL, d<1.006; LDL, d=1.006–1.063; high-density lipoprotein (HDL), d=1.063–1.210] were separated by stepwise density gradient ultracentrifugation in a TL-100 ultracentrifuge (Beckmann Instruments, Inc., Palo Alto, CA). The cholesterol concentration in the lipoprotein fractions was determined enzymatically using a commercial kit (Cholesterol E Test Wako).

The level of liver total lipids was determined gravimetrically after extraction in accordance with the method of Folch et al. [12]. The liver triglyceride and cholesterol levels were determined enzymatically as previously described [13]. Steroids were extracted from the feces and digestive tract contents (small intestine and cecum) with a chloroform/methanol mixture (1:1, vol/vol) at 70°C for 60 h. The concentrations of bile acids in the cecal contents and feces were determined enzymatically by the 3α-dehydrogenase assay method of Sheltaway and Losowsky [14] using taurocholic acid as standard. The concentrations of cholesterol and coprostanol in the small-intestinal contents and feces were analyzed by capillary gas–liquid chromatography using a Hewlett Packard gas chromatograph (Model HP5890A; Hewlett Packard, Palo Alto, CA) that was equipped with a flame ionization detector and a capillary column (30 m×0.53 mm, inner diameter) coated with DB-1 (J&W Scientific, Folsom, CA). The oven temperature was 260°C, and the flow rate of helium carrier gas was 16.9 ml/min. 5α-Cholestane (Nacalai Tesque, Inc., Kyoto, Japan) was used as internal standard for neutral sterol analysis. The concentration and composition of bile acids in the small-intestinal contents were analyzed by capillary gas–liquid chromatography using a model HP5890A gas chromatograph (Hewlett Packard) equipped with a flame ionization detector and a capillary column (30 m×0.25 mm, inner diameter) coated with DB-210 (J&W Scientific). The oven temperature was programmed to increase from 60°C to 235°C at a rate of 10°C min, and the flow rate of helium carrier gas was 1.5 ml/min. Nordeoxycholic acid (Steraloids, Inc., Wilton, NH) was used as internal standard for bile acid analysis. Standard bile acids, cholic acid (CA), deoxycholic acid (DCA), 12-oxo-chenodeoxycholic acid (12-oxo-CDCA), 12-oxo-lithocholic acid (12-oxo-LCA), chenodeoxycholic acid (CDCA), α-muricholic acid (α-MCA), β-muricholic acid (β-MCA), ω-muricholic acid (ω-MCA), lithocholic acid (LCA), hyodeoxycholic acid (HDCA) and ursodeoxycholic acid (UDCA) were purchased from Steraloids, Inc. The levels of cecal organic acids were measured using high-performance liquid chromatography (LC-6A; Shimadzu, Kyoto, Japan) using the internal standard method [13].

2.5. RNA extraction from the liver and RT-PCR analysis of gene expression

Total RNA was extracted from frozen livers in accordance with the method described by Chomczynski and Sacchi [15]. RNA integrity was verified by agarose gel electrophoresis after purification of the mRNA using Oligotex-dT30 (Takara Bio, Shiga, Japan). One microgram of mRNA was used for cDNA synthesis with 10 U of AMV Reverse Transcriptase (Takara Bio) and 2 μl of oligo(dT) primer (Novagen, Inc., Madison, WI) in accordance with the manufacturer's instructions. Expression of the mRNAs for acyl-coenzyme A:cholesterol acyltransferase 1 (ACAT-1), acyl-coenzyme A:cholesterol acyltransferase 2 (ACAT-2), apolipoprotein B (ApoB), cholesterol 7α-hydroxylase (CYP7A1), cholesterol 27-hydroxylase (CYP27), cholesterol 12α-hydroxylase (CYP8B1), farnesoid X receptor (FXR), 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, low-density lipoprotein receptor (LDL-R), liver X receptor (LXR), microsomal triglyceride transfer protein (MTP), sterol-regulatory element-binding protein-1a (SREBP-1a),

Table 2
Primer sequence, product sizes and annealing temperatures

Gene	Primer sequence		Product size (bp)	Annealing temperature (°C)
	Sense	Antisense		
ACAT-1	ATGTGGGAAGTAAATGAAGC	AAATACTAGCCAGACCGAAT	193	53
ACAT-2	CGGTCATGCTGATCCTCTTT	GTGTCACCAGCTCCCAAAAT	204	55
ApoB	TTGACACACTGAAGTTCCTA	ACATCAAACCCTGGTATTAG	124	60
CYP8B1	ATGAAGGCTGTGCGAGAG	TCTCTTCCATCACGCTGTC	127	59
CYP27	CTCAAGAGACTGTCGGCAC	CTTCCTGGATCTCTGGGTT	114	57
FXR	CGTTCAGCGGAGATTTTCAA	ATTTTCAGCTCCCCGACACT	140	57
HMG-CoA reductase	GCTGGTGAGTTGTCCTTGAT	CTTCTTGGTGCATGTTCCCT	117	60
LDL-R	CTTGCCCTGATGGTATGCTA	CTTGCGCTTCAGTGACACA	137	60
LXR	TGCTAATGAAGCTGGTGA	AGAGGACACGGAGAAACAGT	144	57
MTP	AGCGACATCACAGTGGACTC	GGCTCTCTTTTCTTCTCCGA	223	62
SREBP-1a	CCGAGATGTGCGAACTGGAC	TGTCTCCGTTCTCACCCCCA	129	55
SREBP-1c	GGAGCCATGGATTGCACATT	AGGAAGGCTTCCAGAGAGGA	191	53
SREBP-2	CACAATATCATTGAAAAGCGCTACG	TTTTTCTGATTGGCCAGCTTCAGCA	200	55
β-Actin	CTATGAGCTGCCTGACGGTC	AGTTTCATGGATGCACAGG	115	53

sterol-regulatory element-binding protein-1c (SREBP-1c), sterol-regulatory element-binding protein-2 (SREBP-2) and β-actin (as a housekeeping gene for normalization) was determined by real-time monitoring of PCR using a Light Cycler instrument (Roche Diagnostics, Mannheim, Germany). Two microliters of cDNA was amplified in a total volume of 20 μl using the 2× QuantiTect SYBR Green RT-PCR Master Mix (Qiagen, Hilden, Germany) and specific primers each at 0.5 M. After initial denaturation and activation of the polymerase at 95°C for 15 min, cycling was performed for 50 cycles with annealing at the temperatures shown in Table 2 for 25 s, synthesis was performed at 72°C for 30 s and denaturation was performed at 94°C for 15 s. Fluorescence was measured at the end of the elongation step at 72°C. The sequences of the gene-specific primers (Carl Roth, Karlsruhe, Germany) used in this study are listed in Table 2. Relative gene expression was calculated by using the crossing point of each target gene, with the crossing point of the β-actin gene used as reference.

2.6. Statistical analyses

Data are expressed as mean±S.E.M. (n=6). Two-way analysis of variance (ANOVA; StatView Version 4.5; Abacus Concepts, Berkeley, CA) was used to test the significance of the effects of sucrose and HACS, and their interaction. When significant F ratios were found, individual comparisons were made by Tukey's multiple range test using the Super ANOVA statistical software package (Abacus Concepts). Student's t test was used to estimate the significance of the differences between the G-CS group and the HACS group in rats that had been fed the SU diet (with sucrose) or the ST diet (without sucrose). Differences were considered to be significant at P<.05.

3. Results

3.1. Body weight gain, food intake, plasma lipids and liver lipids

Body weight gain and food intake were increased by sucrose ingestion, but were not affected by HACS ingestion (Table 3). Body weight gain increased as food intake increased (r=.895, P<0001). RS daily intake was decreased by feeding on the SU diet, but increased by HACS ingestion. Plasma total cholesterol, LDL-cholesterol and HDL-cholesterol concentrations were increased by feeding on the SU diet, whereas the plasma VLDL-cholesterol concentration was not affected. The plasma total TAG concentration was

Table 3
Effects of dietary sucrose and HACS on body weight gain, food intake, plasma lipids, liver weight and liver lipids[1,2]

	Diet with sucrose (SU diet)		Diet without sucrose (ST diet)		Two-way ANOVA[3]
	G-CS	HACS	G-CS	HACS	
Body weight gain (g/21 days)	57±5	60±6	38±5	31±5	Sucrose
Food intake (g/days)					
Total	21.6±0.6	20.3±0.9	17,7±0.7	17.4±0.7	Sucrose
RS	0.18±0.01	1.55±0.07	0.63±0.03	1.79±0.07	Sucrose, HACS
Plasma lipids (mmol/L)					
Cholesterol					
Total	4.06±0.12	4.35±0.14	3.75±0.08	2.86±0.05**	Sucrose, HACS
VLDL	0.18±0.01	0.14±0.01	0.13±0.01	0.14±0.02	–
LDL	1.73±0.07	1.78±0.08	1.59±0.06	1.07±0.07**	Sucrose
HDL	1.52±0.03	1.62±0.03	1.48±0.02	1.19±0.03**	Sucrose
TAG					
Total	2.36±0.05[b]	1.82±0.07[a]	1.46±0.10[a]	1.31±0.07[a]	Sucrose, HACS, interaction
VLDL	2.02±0.08	1.51±0.09*	1.22±0.07	1.11±0.09	Sucrose, HACS
LDL	0.26±0.01	0.24±0.00	0.19±0.01	0.17±0.01	–
HDL	0.07±0.00	0.06±0.00	0.05±0.00	0.04±0.00	–
Liver weight (g)	12.07±0.67	11.84±0.52	9.15±0.21	8.72±0.40	Sucrose
Liver lipids					
Total lipids (mg/g liver)	107±5	109±14	64±3	67±2	Sucrose
Triglyceride (μmol/g liver)	73.6±4.0	78.3±7.6	38.3±3.4	32.1±3.2	Sucrose
Cholesterol (μmol/g liver)					
Total	10.8±0.3	10.5±0.6	10.7±0.5	10.5±0.2	–
Free	8.4±0.3	8.3±0.5	8.4±0.3	7.5±0.1	–
Esterified	2.4±0.2	2.3±0.2	2.3±0.2	3.0±0.2	–

(–) Not significant (P>.05).
Values in a row with no common superscript letters are significantly different (P<.05).
* P<.05, compared with the G-CS group in SU-diet-fed rats.
** P<.05, compared with the G-CS group in ST-diet-fed rats.
[1] OVX rats were fed a test diet for 21 days.
[2] Results are expressed as mean±S.E. (n=6).
[3] Significant effect of the interaction between dietary sucrose and HACS (Sucrose×HACS), and significant effect (P<05) of dietary sucrose (Sucrose) and HACS.

decreased by HACS ingestion in rats that had been fed the SU diet, and this decrease was proportionally greater in SU-diet-fed rats than in ST-diet-fed rats. The plasma VLDL-TAG concentration was increased by the SU diet, but was decreased by HACS ingestion. Plasma LDL-TAG and HDL-TAG concentrations were not affected by diet. Liver weight and the concentrations of total lipids and TAG in the liver were increased by the SU diet. The concentrations of total, free and esterified cholesterols in the liver were not affected by diet.

3.2. Intestinal contents

Analysis of the contents of the small intestine showed that the dry weight and the amounts of coprostanol and total neutral sterols were increased by HACS ingestion (Table 4). The amount of cholesterol in the small-intestinal contents was significantly decreased by HACS ingestion in SU-diet-fed rats, but not in ST-diet-fed rats. The amounts of bile acids were not affected by the changes in diet; however, the CA group/CDCA group ratio tended to decrease in SU-diet-fed rats compared with ST-diet-fed rats (P=.0764).

3.3. Amounts of SCFAs in cecal contents and in fecal excretions of bile acids and neutral sterols

Cecal tissue weight and the dry weight of the cecal contents were increased by HACS ingestion (Table 5). The wet weight of the cecal contents was also increased by HACS ingestion, and this increase was proportionally greater in SU-diet-fed rats than in ST-diet-fed rats. The moisture level of the cecal contents and the amount of bile acids in the cecal contents were not affected by the changes in diet. The pH value of the cecal contents was lowered by HACS ingestion. The amounts of acetic acid, propionic acid, *n*-butyric acid and total SCFAs in the cecal contents were increased by HACS ingestion, and these increases were proportionally greater in SU-diet-fed rats than in ST-diet-fed rats. Fecal dry weight and the fecal excretions of bile acids, cholesterol and coprostanol were increased by HACS ingestion.

3.4. Hepatic gene expression

The levels of ACAT-1, ACAT-2, CYP27, LDL-R, LXR, HMG-CoA reductase and SREBP-1a mRNA were not

Table 4
Effect of dietary sucrose and HACS on the dry weight of the small intestine, amounts of bile acids and neutral sterols, and composition of bile acids in small-intestinal contents[1,2]

	Diet with sucrose (SU diet)		Diet without sucrose (ST diet)		Two-way ANOVA[3]
	G-CS	HACS	G-CS	HACS	
Small-intestinal contents					
Dry weight (g)	0.45±0.02	0.64±0.05*	0.44±0.05	0.60±0.04**	HACS
Bile acids (μmol)	69.7±5.1	75.4±6.5	58.7±4.4	72.8±5.4	–
Neutral sterols (μmol)					
Cholesterol	0.15±0.01[a]	0.27±0.04[b]	0.20±0.03[ab]	0.21±0.02[ab]	HACS, interaction
Coprostanol	0.12±0.01	0.22±0.03*	0.16±0.02	0.20±0.02	HACS
Total[4]	0.26±0.02	0.48±0.06*	0.37±0.05	0.40±0.04	HACS
Composition of bile acid (%)					
CA group[5] (%)					
CA	12.46±1.67	16.84±2.60	10.79±1.57	12.46±1.89	–
DCA	1.00±0.12	1.07±0.17	1.12±0.15	1.24±0.19	–
12-oxo-CDCA	2.27±0.12	2.99±0.23	1.72±0.22	2.32±0.18	Sucrose, HACS
12-oxo-LCA	22.20±0.88	21.96±1.21	31.93±1.70	29.11±2.44	Sucrose
CDCA group[6] (%)					
CDCA	1.66±0.18	3.04±0.51	1.29±0.11	2.42±0.32	Sucrose
α-MCA	39.60±2.01	35.16±2.30	32.43±1.20	32.51±4.86	–
β-MCA	0.16±0.02	0.33±0.06*	0.18±0.02	0.22±0.04	HACS
ω-MCA	1.85±0.16	2.16±0.26	1.95±0.39	1.66±0.22	–
LCA	0.43±0.04	0.80±0.16*	0.66±0.05	0.72±0.12	HACS
HDCA+UDCA	18.36±1.67	15.64±1.59	17.94±1.76	17.35±2.23	–
CA group/CACD group ratio[7]	0.62±0.07	0.77±0.09	0.84±0.05	0.86±0.12	–

(–) Not significant (P>.05).
Values in a row with no common superscript letters are significantly different (P<.05).
* P<.05, compared with the G-CS group in SU-diet-fed rats.
** P<.05, compared with the G-CS group in ST-diet-fed rats.
[1] OVX rats were fed a test diet for 21 days.
[2] Results are expressed as mean±S.E. (n=6).
[3] Significant effect (P<.05) of dietary sucrose (Sucrose) and HACS, and significant effect of the interaction between dietary sucrose and HACS (Sucrose×HACS).
[4] Total=cholesterol+coprostanol.
[5] CA group=CA+DCA+12-oxo-CDCA+12-oxo-LCA.
[6] CDCA group=CDCA+α-MCA+β-MCA+ω-MCA+HDCA+UDCA.
[7] CA group/CDCA group ratio=(CA+DCA+12-oxo-CDCA+12-oxo-LCA)/(CDCA+α-MCA+β-MCA+ω-MCA+LCA+HCDA+UDCA).

Table 5
Effects of dietary sucrose and HACS on cecal tissue weight and dry weight, bile acids and SCFAs in the cecal contents and in fecal excretion in OVX rats[1,2]

	Diet with sucrose (SU diet)		Diet without sucrose (ST diet)		Two-way ANOVA[3]
	G-CS	HACS	G-CS	HACS	
Cecal tissue wet weight (g)	1.19±0.07	1.44±0.06	1.10±0.06	1.30±0.07	HACS
Cecal contents					
Wet weight (g)	2.59±0.15^{a}	5.13±0.31^{c}	3.47±0.35^{ab}	4.30±0.23^{bc}	HACS, interaction
Dry weight (g)	0.61±0.06	1.22±0.12*	0.80±0.09	0.95±0.16	HACS
Moisture (g/100 g)	75.0±2.6	77.3±0.5	75.0±2.6	77.3±0.8	—
Bile acids (μmol)	22.7±2.3	29.9±4.3	24.9±4.0	23.3±4.8	—
pH	7.53±0.17	6.88±0.07*	7.18±0.07	6.80±0.05**	HACS
SCFA (μmol)					
Acetic acid	198±13^{a}	578±23^{c}	248±16^{a}	424±32^{b}	Sucrose, HACS, interaction
Propionic acid	49±3^{a}	115±4^{c}	59±5^{ab}	78±6^{b}	Sucrose, HACS, interaction
n-Butyric acid	30±2^{a}	76±5^{b}	37±2^{a}	64±5^{b}	HACS, interaction
Total SCFA[4]	277±14^{a}	769±20^{c}	344±21^{a}	566±40^{b}	HACS, interaction
Fecal excretion[5]					
Dry weight (g/day)	1.17±0.07	1.74±0.14*	1.09±0.08	1.57±0.14**	HACS
Bile acids (μmol/day)	25.8±1.6	28.6±1.9	23.3±1.4	30.2±1.8**	HACS
Neutral sterols (μmol/day)					
Cholesterol	2.57±0.16	3.48±0.26*	2.53±0.30	3.30±0.20	HACS
Coprostanol	2.31±0.15	2.89±0.22	1.96±0.15	3.20±0.26**	HACS
Total neutral sterol[6]	4.89±0.30	6.37±0.41*	4.50±0.23	6.51±0.40**	HACS

(–) Not significant ($P>.05$).
Values in a row with no common superscript letters are significantly different ($P<.05$).
* $P<.05$, compared with the G-CS group in SU-diet-fed rats.
** $P<.05$, compared with the G-CS group in ST-diet-fed rats.
[1] OVX rats were fed a test diet for 21 days.
[2] Results are expressed as mean±S.E. ($n=6$).
[3] Significant effect ($P<.05$) of dietary sucrose (Sucrose) and HACS, and significant effect of the interaction between dietary sucrose and HACS (Sucrose×HACS).
[4] Total SCFA=acetic acid+propionic acid+*n*-butyric acid.
[5] Feces were collected on the last 3 days of the experimental period.
[6] Total neutral sterol=cholesterol+coprostanol.

affected by diet (data not shown). The level of ApoB mRNA was decreased by HACS ingestion in SU-diet-fed rats ($P=.0064$; Table 6). The levels of CYP7A1 and CYP8A1 mRNA were increased by HACS ingestion. The level of SREBP-1c mRNA was increased by feeding with the SU diet, but was decreased by HACS ingestion. The level of SREBP-2 mRNA was increased by feeding with the SU diet.

4. Discussion

Fermentation of HACS in the large intestine results in the production of SCFAs. Butyrate is the key SCFA: it increases the secretion of gut peptides such as peptide YY (PYY) and glucagon-like peptide-1 (GLP-1), which regulate food intake and increase satiety [16]. The amount of butyric acid in the

Table 6
Effects of dietary sucrose and HACS on the mRNA levels of genes upon cholesterol metabolism in OVX rats[1,2]

Gene	Diet with sucrose (SU diet)		Diet without sucrose (ST diet)		Two-way ANOVA[3]
	G-CS (arbitrary unit)	HACS (arbitrary unit)	G-CS (arbitrary unit)	HACS (arbitrary unit)	
ApoB	0.84±0.06	0.60±0.04*	0.76±0.06	0.74±0.05	HACS
CYP7A1	0.92±0.07^{ab}	1.14±0.08^{b}	0.83±0.05^{a}	1.45±0.10^{c}	HACS, interaction
CYP8B1	0.72±0.09	0.88±0.08	0.75±0.05	0.91±0.04**	HACS
FXR	0.64±0.06	0.83±0.05	0.75±0.06	0.92±0.06**	Sucrose, HACS
SREBP-1c	1.06±0.05	0.78±0.06*	0.83±0.02	0.71±0.04	Sucrose, HACS
SREBP-2	0.92±0.05	0.91±0.09	0.66±0.04	0.75±0.06	Sucrose

Values in a row with no common superscript letters are significantly different ($P<.05$).
* $P<.05$, compared with the G-CS group in SU-diet-fed rats.
** $P<.05$, compared with the G-CS group in ST-diet-fed rats.
[1] OVX rats were fed a test diet for 21 days.
[2] Results are expressed as mean±S.E. ($n=6$).
[3] Significant effect ($P<.05$) of dietary sucrose (Sucrose) and HACS, and significant effect of the interaction between dietary sucrose and HACS (Sucrose×HACS).

cecal contents was increased by HACS ingestion. HACS ingestion has been shown to increase plasma PYY and GLP-1 [17]. In this study, however, food intake and body weight gain were not affected by HACS ingestion. These findings agree with the report of de Roos et al. [18] stating that the consumption of HACS has little influence on appetite and food intake in healthy young men.

It is well known that a deficiency in total energy intake immediately leads to lowering of plasma cholesterol levels [19]. In the small intestine of rats, the digestibility of normal cornstarch, which includes 300 g/kg amylose, is 98%, whereas the digestibility of HACS, which includes 700 g/kg amylose, is only 60% [20]. HACS is almost completely fermented in the large intestine in rats [21]. The energy produced by the fermentation of indigestible cornstarch is estimated to be 7.1 kJ/g (1.7 kcal/g) [22]. Energy intake in rats fed the diet with or without sucrose was decreased by HACS ingestion by approximately 10% and 6%, respectively, but there was no significant difference between all the dietary groups. The concentration of plasma total cholesterol was decreased by HACS ingestion in ST-diet-fed rats, but not in SU-diet-fed rats. Therefore, the hypocholesterolemic effect of HACS on ST-diet-fed rats must depend on factors other than energy intake.

Because the experimental diets did not contain cholesterol, it is clear that this hypocholesterolemic effect must be due to changes in endogenous sterol metabolism.

Fecal excretion of bile acids and the level of CYP7A1 mRNA were increased by HACS ingestion in ST-diet-fed rats, but not in SU-diet-fed rats. The hypothesis that propionate generated by bacterial fermentation of DF could exert a rate-controlling effect on liver cholesterol synthesis has been the subject of in vivo and in vitro studies [23]. However, there are also findings that appear inconsistent with a physiological role for propionate in the control of cholesterol synthesis [24]. In this study, the amount of propionate in the cecal contents was not increased by HACS ingestion in ST-diet-fed rats. Hara et al. [25] showed that acetate, another component of the SCFA mixture, was effective at lowering the plasma cholesterol concentration, whereas propionate was not. The amount of acetate in the cecal contents was increased by HACS ingestion in ST-diet-fed rats, but also in SU-diet-fed rats.

Plasma total concentration and VLDL-TAG concentration were significantly decreased by HACS ingestion in SU-diet-fed rats, but not in ST-diet-fed rats. It is known that the expression of fatty acid synthase (FAS) is promoted by the activation of SREBP-1c, a transcription factor that activates fatty acid synthesis [26]. Fatty acids are the major components of TAG. In SU-diet-fed rats, HACS ingestion decreased the mRNA level of SREBP-1c (which might result in a decreased mRNA level of FAS) and decreased the mRNA level of ApoB (the primary apolipoprotein of LDL). Therefore, the decreased plasma VLDL-TAG concentration caused by HACS ingestion might depend on the decreased synthesis and secretion of TAG in the liver.

In this study, HACS decreased the plasma cholesterol concentration in SU-diet-fed rats, but not in ST-diet-fed rats, whereas HACS decreased the plasma TAG concentration in ST-diet-fed rats, but not in SU-diet-fed rats. Sucrose has been shown to increase both plasma cholesterol and TAG concentrations in guinea pigs [27], but a study in rats has demonstrated that substituting sucrose with starch results in a decrease in plasma cholesterol and TAG concentrations [28]. In addition, greater cholesterogenesis in hepatocytes has been observed in rats fed sucrose compared with those fed starch [29]. Sucrose is a dimer of fructose and glucose. Numerous studies report that dietary fructose induces hyperlipidemia in rats [30]. The activity of hepatic enzymes that regulate lipid metabolism is increased with diets that contain fructose in place of starch [31]. Therefore, sucrose and starch would appear to influence lipid metabolism to different extents and in different ways, which might determine the effect of HACS on plasma cholesterol and TAG concentrations. The reason would appear to be due largely to the differences in the metabolism of the two hexoses by the liver. However, the reasons why the cholesterol concentration in SU-diet-fed rats and the TAG concentration in ST-diet-fed rats were not decreased by HACS ingestion remained unclear in this study.

The amounts of bile acids and neutral sterols in the small-intestinal contents were not affected by diet. However, the mRNA level of CYP7A1, an enzyme catalyzing the rate-limiting step of the bile acid synthetic pathway, was increased by HACS ingestion in ST-diet-fed rats and tended to increase in SU-diet-fed rats. Bile acid secretion induces biliary lipid secretion [32]. On the other hand, undigested starch can bind bile acids [33]. Therefore, the increased amounts of bile acids and cholesterol in the small-intestinal contents might be due to increased biliary secretion and decreased reabsorption. FXR, a bile-acid-activated nuclear receptor that plays a major role in the regulation of bile acid metabolism, is activated by hydrophobic bile acids such as CDCA and DCA [34], and has been proposed to play a central role in the feedback repression of the CYP7A1 gene [35]. The level of FXR mRNA decreased as the level of CDCA in the small-intestinal contents increased (r=−.458, P=.0242). However, no negative correlation was found between the mRNA levels of CYP7A1 and FXR.

Sterol 12α-hydroxylase (CYP8B), which is required for CA synthesis, is a key enzyme in the regulation of the CA/CDCA ratio in bile acid synthesis [36], as CA production is dependent on the activity of CYP8B1. The level of CYP8B1 mRNA was increased by HACS ingestion in ST-diet-fed rats, but not in SU-diet-fed rats. Serum insulin suppresses the expression of CYP8B [37]. The secretion and biosynthesis of insulin are elevated in rats fed sucrose compared to rats fed starch [38]. In humans, serum insulin concentration rises more rapidly after a meal with a high-sucrose content than after one with a high-starch content [39]. We did not measure serum insulin concentrations in this study. However, the ratio of the CA group/CDCA group bile acids in

the small-intestinal contents was not affected by the changes in diet.

Cecal tissue weight and cecal contents were increased by HACS ingestion. It has been reported that fermentation primarily takes place in the cecum in rats and that HACS is almost completely fermented in the rat large intestine [21]. Fermentable carbohydrates increase the growth of cecal mucosal tissue in rats, very likely via the trophic effects of SCFAs [40].

The amounts of total SCFAs, acetic acid, propionic acid and *n*-butyric acid in the cecal contents were increased by HACS ingestion. The fermentation of HACS increases SCFA production, which reduces the pH value of the cecal contents. The pH value of the cecal contents decreased logarithmically as the amount of SCFAs increased (r=−.955, P=.0065). The amounts of acetic and propionic acids in the cecal contents were higher in SU-diet-fed rats than in ST-diet-fed rats, but we do not have an explanation for this.

In conclusion, the concentration of plasma cholesterol was decreased, and the fecal excretion of bile acids was increased by HACS in ST-diet-fed rats, but not in SU-diet-fed rats. The mRNA levels of CYP7A1, CYP8B1 and FXR, which are the genes related to bile acid metabolism, were increased by HACS in ST-diet-fed rats, but not in SU-diet-fed rats. These suggest that HACS cannot prevent ovarian-hormone-deficiency-induced hypercholesterolemia when fructose consumption is high. The hypercholesterolemic effects of ovarian hormone deficiency and fructose might be synergistic. HACS may possess the counter-mechanism that protects against ovarian-hormone-deficiency-induced hypercholesterolemia; however, that mechanism of HACS may not be linked to hypercholesterolemia induced by fructose.

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