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ORIGINAL CONTRIBUTION

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Comparison of enzymatically synthesized inulin, resistant maltodextrin and clofibrate effects on biomarkers of metabolic disease in rats fed a high-fat and high-sucrose (cafeteria) diet

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Abstract *Background* While naturally occurring inulin has anti-hyperlipidemic effects in animals and humans, health effects of synthetic inulin with different degrees of fructose polymerization remain poorly understood. *Aim of the study* Our study aimed at distinguishing health effects of synthetic inulin with different degrees of fructose polymerization (DP) from those of resistant maltodextrin and clofibrate. *Methods* We examined effects of synthetic inulin on serum and liver lipid profiles and blood biochemical parameters in rats fed a high-fat and high-sucrose (HF, cafeteria) diet when compared to resistant maltodextrin and clofibrate. *Results* Treatment with inulin (average DP = 6–8, 16–17 and 23) and resistant maltodextrin for 3 weeks reduced the elevation in liver levels of triacylglycerol and total cholesterol of rats fed the cafeteria diet but not the standard diet. In these groups, inulin (average DP = 16–17) significantly reduced the portal plasma glucose level. Moreover, the levels of portal plasma propionate and circulating serum adiponectin, which were decreased in cafeteria rats, recovered to nearly normal levels after administration of inulin (average DP = 16–17). In addition, the dietary inulin suppressed elevation in levels of portal plasma insulin and circulating serum leptin and induction of acetyl-CoA carboxylase and fatty acid synthase mRNAs in the liver of cafeteria rats, consistent with the reduction of liver lipids. The dietary inulin and clofibrate markedly reduced triacylglycerol levels in serum very low density lipoprotein (VLDL) and liver and epididymal adipose tissue weights of cafeteria rats; the extent of suppression by the dietary inulin was higher than that by clofibrate. No additive or synergistic effect of the dietary inulin and clofibrate was found in decrease in circulating serum VLDL and liver lipid levels. *Conclusion* These observations indicate that the dietary inulin may prevent the development of metabolic disease such as hyperlipidemia and hyperinsulinemia caused by intake of cafeteria diet, in association with suppression of liver lipogenesis.

Key words synthetic inulin – anti-metabolic disease – clofibrate – propionate – glucose

Abbreviations AST: Aspartate aminotransferase; ALT: Alanin aminotransferase; ChREBP:

Carbohydrate responsive element-binding protein; DP: Degree of polymerization; HMG-CoA reductase: 3–hydroxy-3-methylglutaryl CoA reductase; PPAR: Peroxisome proliferator- activated receptor; SREBP: Sterol regulatory element-binding protein; wat: White adipose tissue

Introduction

Inulin is a polysaccharide with β (2-1) linkages through D-fructoses that are polymerized with a terminal glucose molecule. Inulin is widely distributed throughout the plant kingdom and exists as a reserve substance in the tubers or tuberous roots of *Asteraceae* plants such as the dahlia and British inula, as well as chicory roots [20]. These naturally occurring molecules have degrees of fructose polymerization (DP) that vary from 2 to greater than 60 (the average DP ranges from 32 to 34). In previous studies [21, 22], we found a microorganism *Bacillus sp. 217C-1* expressing a highly efficient enzyme that converts sucrose into inulin molecules having similar chain-lengths and structures to plant-origin inulin, and we developed methods to industrially produce inulin from sucrose using one of these inulin-producing enzymes. Although our synthetic inulin had many similar properties to plant-derived inulin, there was a marked difference in the polydispersity of the inulin chain length. Since synthetic inulin has a narrow range of fructose polymerization, it shows better solubility in water than plant-derived inulin and a lower viscosity than Raftiline HP and Raftiline ST [22].

Naturally occurring inulin and oligofructans are soluble and fermentable dietary fibers, in contrast to starch. Inulin and oligofructans are resistant to hydrolysis by pancreatic amylase and saccharidases (sucrase, maltase, isomaltase or lactase) in the upper gastrointestinal tract, reach the large intestine unabsorbed, and are utilized as carbohydrate substrates for the growth of indigenous bifidobacteria [8, 10, 14, 15, 19]. Since synthetic inulin is not hydrolyzed by digestive enzymes (i.e. α-amylase and amyloglucosidase), it is also considered to reach the large intestine essentially intact in the same manner as plant-origin inulin and function as a dietary fiber. The effects of dietary inulin or inulin-type fructans on carbohydrate and lipid metabolism and disease risk are considered to depend on chemical-physical properties such as solubility in water, viscosity, and fermentability by microorganisms in the large intestine. Chemical-physical properties of synthetic inulin depend on the extent of fructose polymerization. Accordingly, in the present study, we investigated whether changes in the degree of fructose polymerization affect health effects on serum and liver parameters such as lipid profiles, and glucose, insulin, leptin, and adiponectin levels. We have reported that high-fat and high-sucrose (cafeteria) diet modulates expression of liver drug-metabolizing phase I and II enzymes in rats, leading to alteration in metabolism of drugs such as acetaminophen [13] and induction of liver failure by phenobarbital and dexamethasone, that is ameliorated through modulation of circulating serum and liver lipid profiles by synthetic inulin [17]. However, little is known about the mechanism of modulation of serum and liver lipids by dietary synthetic inulin. In addition, activation of peroxisome proliferators-activated receptor α (PPARα), which regulates the transcription of many genes involved in lipid catabolism, increases fatty acid β-oxidation and reduces the level of circulating serum lipids [4]. Therefore, in this study, we examined effects of synthetic inulin on circulating serum and liver profiles and hepatic expression of lipid metabolism-related enzyme and transcription factor genes, when compared to resistant maltodextrin and PPARα agonist clofibrate.

Materials and methods

Chemicals

Inulin (average DP = 16–17; DP range, 5–30) enzymatically synthesized from sucrose by an inulin-producing enzyme was prepared as previously reported [22]. Inulin (average DP = 6–8; DP range, 3–16) (supplemental Fig. 1) was prepared as follows: a 67 % sucrose solution was added to the inulin-producing enzyme and incubated in citrate buffer (pH 6.0–7.0) at 65°C for 2 days. The reaction was stopped by heat treatment at 85°C for 30 min. The reaction mixture was decolorized with activated charcoal, filtered through a reverse osmosis membrane to remove low molecular materials, desalted with ion-exchange resin, and spray-dried to obtain inulin powder. Inulin from chicory (average DP = 23, raftiline HP; DP range, 12–60) and clofibrate were purchased from Sigma-Aldrich (St. Louis, MO) and resistant maltodextrin (pinefiber C) obtained by heating and enzyme treatment of potato starch was from Matsutani Chemical Industries (Itami, Japan). Sweetness of average DP = 6–8 and 16–17 inulins was 0.3-fold and 0.5-fold lower than that

of sucrose, respectively. The water solubility of average DP = 6–8 and 16–17 inulins was 50 and 20% (w/w) at room temperature, respectively. The viscosity of average DP = 6–8 inulin was similar as that of fructooligosaccharide, and the viscosity of average DP = 16–17 inulin was lower than that of raftiline HP and raftiline ST. The thermal stability order at pH 3 was raftiline HP > average DP = 16–17 inulin > average DP = 6–8 inulin and raftiline ST > fructooligosaccharide. The growth order of *Bifidobacterium adolescens* was average DP = 6–8 inulin > average DP = 16–17 inulin and resistant maltodextrine > raftiline HP (average DP = 23 inulin). Average DP = 6–8 and 16–17 inulins suppressed more strongly the growth of harmful bacteria such as *Clostridium butyricum* and *Eubacterium aerofaciens* than fructooligosaccharide (data not shown).

Experimental animals

All studies followed protocols approved by the Institutional Animal Care and Life Committee, University of Shizuoka. Male Wistar rats were obtained from Japan Charles River (Tokyo, Japan) at 6 weeks of age. Animals were acclimatized for one week prior to the experiment, housed in stainless-steel hanging cages with free access to food and water, and maintained on a 12-h light-dark cycle. All animals were randomly assigned to the standard diet (SD), 5% inulin or resistant maltodextrin-supplemented standard diet (SD + I or P), high-fat and high-sucrose diet (HF, cafeteria), or 5% inulin or resistant maltodextrin-supplemented high-fat and high-sucrose diet (cafeteria + I or P) for 3 weeks. The cafeteria diet consisted of 19.7% casein, 1% soybean oil, 10% lard, 4% mineral mixture, 1% vitamin mixture, 0.15% choline chloride, 0.5% cholesterol, 0.25% sodium cholate, 3.4% cellulose and 60% sucrose (23.9% lipid, 56.8% carbohydrate and 19.3% protein [kJ]), and the SD diet consisted of 23.8% crude protein, 5.1% crude fat, 3.2% crude fiber, 6.1% ash, 54% nitrogen-free extract and 7.8% humidity (12.9% lipid, 60.4% carbohydrate and 26.7% protein [kJ]). All rats were weighed three times per week, and food intake in grams was monitored.

Administration of clofibrate (CFB): After one-week-consumption of each diet, each diet group was divided into three subgroups consuming each diet containing 0, 0.1, and 0.25% clofibrate, and then continued to consume the diets for another 2 weeks.

Biochemical analyses

Blood and tissue sampling was done between 11:00 and 12:00 AM as described previously [22]. Plasma glucose concentrations were determined by the hexokinase method using commercial reagents (R-Biopharm AG, Darmstadt, Germany). Plasma insulin was measured using a double antibody radioimmunoassay kit specific for rat insulin (Eiken Chemical, Tokyo, Japan). Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured using kits from Wako Pure Chemicals (Osaka, Japan). Serum adiponectin and leptin were measured using kits from Cyclex (Nagano, Japan) and Yanaihara Laboratory (Shizuoka, Japan), respectively. Frozen livers (about 0.5 g) were homogenized in 20 volumes (the SD group) or 100 volumes (the cafeteria group) of 0.9% NaCl containing 0.1% Triton X-100, and the concentrations of triacylglycerol, total cholesterol, and nonesterified fatty acid were estimated enzymatically with kits from Shino Test (Tokyo, Japan).

Plasma short chain fatty acids were analyzed by using an HPLC system comprised of a CO-8020 injector, DP-8020 pump, TSKgel OApak-P precolumn (6.0 mm × 40 mm), TSKgel OApak-A column (7.8 mm × 300 mm), and a PD-8020 spectrophotometer (TOSOH, Tokyo, Japan) with 0.75 mM H_2SO_4 as the mobile phase at 0.7 ml/min according to the manufacturer's protocol. Detection was performed by measuring absorption at 207 nm. Portal plasma was added crotonic acid (3 μg/ml) as internal standard. Propionic acid and butyric acid were measured against standards of these short chain fatty acids according to the manufacturer's protocol.

Triacylglycerol and total cholesterol levels in serum lipoproteins were determined by a dual detection HPLC system with two tandem connected TSKgel Lipopropak XL columns (300 mm × 7.8 mm; TOSOH) in Skylight Biotech Inc. (Akita, Japan) [18].

Determination of mRNA levels

Total RNA was prepared from the liver using TRIZOL reagent (Invitrogen Life Technologies, Carlsbad, CA). Samples were quantitated by spectrophotometry, and 1 μg of total RNA was used to generate cDNA by reverse transcription (RT) using a Prime Script RT reagent kit (Takara Bio. Inc., Otsu, Japan) according to the manufacturer's protocol. cDNA synthesized from 50 ng of total RNA was subjected to quantitative real-time polymerase chain reaction (PCR) as described previously [16] with an 7500 Real Time PCR System (Applied Biosystems, Foster City, CA) using Premix Ex Taq reagent (TaKaRa Bio Inc.) for the TaqMan probe method or SYBR Premix Ex Taq reagent (TaKaRa Bio Inc.) for the intercalation reaction with SYBR Green I according to the manufacturer's specifications. The TaqMan probes and primers for rat fatty acid synthase (FAS,

Table 1 Initial and final body weight, food intake, energy intake, liver lipids, and biochemical measurements of rats fed standard (SD), inulin (I)- or resistant maltodextrin (P)-supplemented standard (SD + I or P), high-fat and high-suxrose (HF, cafeteria), and inulin- or resistant maltodextrin-supplemented high-fat and high-sucrose (cafeteria + I or P) diets for 3 weeks

	SD	SD + I (6–8)	SD + I (16–17)	SD + I (23)	SD + P	Cafeteria	Cafeteria + I (6–8)	Cafeteria + I (16–17)	Cafeteria + I (23)	Cafeteria + P
n	10	5	10	5	5	10	5	10	5	5
Initial body weight (g)	220.1 ± 2.8	217.3 ± 4.3	215.2 ± 2.3	216.7 ± 3.2	227.7 ± 3.0	218.5 ± 2.7	213.5 ± 2.7	213.2 ± 2.7	215.9 ± 5.0	217.7 ± 2.6
Final body weight (g)	342.4 ± 6.8	348.2 ± 10.3	329.5 ± 7.6	333.3 ± 8.9	344.0 ± 6.1	341.8 ± 5.4	318.4 ± 8.9	325.9 ± 6.3	338.0 ± 17.3	339.6 ± 6.2
Food intake (g/d)	22.9 ± 0.5	23.4 ± 0.6	23.2 ± 0.5	23.5 ± 0.8	22.2 ± 0.4	20.7 ± 0.3	18.5 ± 0.6	20.1 ± 0.5	19.2 ± 0.8	20.0 ± 0.4
Energy intake (kJ/d)	337.2 ± 6.7	332.6 ± 8.0	330.0 ± 7.6	334.8 ± 11.3	316.1 ± 5.2	357.6 ± 5.1	313.4 ± 10.2$^{\#\#}$	339.7 ± 8.5	324.4 ± 13.9	339.5 ± 6.9
Food efficiency (g gain/g food)	0.26 ± 0.01	0.27 ± 0.01	0.23 ± 0.01	0.24 ± 0.01	0.24 ± 0.01	0.28 ± 0.01	0.27 ± 0.01	0.27 ± 0.01	0.30 ± 0.02	0.26 ± 0.01
Liver weight (g)	12.42 ± 0.49	12.71 ± 0.56	11.61 ± 0.51	12.35 ± 0.40	12.50 ± 0.44	18.25 ± 0.31^{+++}	15.23 ± 0.71$^{\#\#\#}$	16.28 ± 0.57	16.69 ± 0.99	17.42 ± 0.57
Liver weight (g)/body weight (g)	0.036 ± 0.001	0.036 ± 0.001	0.034 ± 0.001	0.037 ± 0.001	0.036 ± 0.001	0.053 ± 0.001^{+++}	0.048 ± 0.001$^{\#\#}$	0.050 ± 0.001	0.049 ± 0.001	0.052 ± 0.002
Liver triacylglycerol (mg/g)	13.04 ± 1.46	10.34 ± 1.36	10.87 ± 1.00	9.52 ± 1.70	11.45 ± 1.64	81.82 ± 4.83^{+++}	42.58 ± 7.26$^{\#\#\#}$	50.63 ± 4.05$^{\#\#\#}$	50.22 ± 7.35$^{\#\#}$	54.31 ± 7.39$^{\#\#\#}$
Liver total choresterol (mg/g)	5.42 ± 0.56	3.67 ± 0.22	3.66 ± 0.31	3.60 ± 0.40	3.88 ± 0.26	47.07 ± 1.72^{+++}	34.35 ± 0.69$^{\#\#\#}$	39.21 ± 2.97$^{\#}$	39.25 ± 2.52	34.15 ± 1.32$^{\#\#\#}$
Liver free fatty acid (mg/g)	7.98 ± 0.31	8.55 ± 0.46	8.93 ± 0.54	9.67 ± 0.71	9.76 ± 0.13	35.03 ± 1.34^{+++}	31.07 ± 1.36	36.37 ± 1.83	31.22 ± 0.83	33.79 ± 1.56
Serum AST (U/l)	15.0 ± 2.1	15.1 ± 1.8	7.3 ± 1.2**	7.3 ± 1.4	20.5 ± 2.9	4.1 ± 1.8^{+++}	3.0 ± 2.6	0.7 ± 0.7	1.0 ± 1.0	2.9 ± 1.9
Serum ALT (U/l)	1.20 ± 0.47	0.43 ± 0.29	1.50 ± 0.50	0.91 ± 0.37	3.98 ± 1.45	0.3 ± 0.3	6.56 ± 2.97$^{\#\#}$	0.26 ± 0.26	2.51 ± 2.00	0.00 ± 0.00

Values are the means ± S.E.; *n* number of animals. Numbers in parenthesis show average degree of fructose polymerization of synthetic inulin (6–8 and 16–17) and plant-origin inulin (23). Food intake represents the daily mean over the 3-weeks-protocol. Statistical significance between treatment groups was determined by ANOVA and Bonferroni's test
**$P < 0.01$ for inulin-supplemented SD animals versus SD animals; $^{+++}P < 0.001$ for HF animals versus SD animals; $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$ for inulin (or resistant maltodextrin)-supplemented cafeteria animals versus cafeteria animals

NM_017332), rat 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA reductase, NM_013134) and rat β-actin [6], rat carnitine palmitoyltransferase 1 [11] and rat sterol regulating element-binding protein 1 (SREBP1, AF286470) and rat SREBP2 (XM_216989) [9] were as reported previously, and those for rat PPARα (NM_013196) (assay identification number Rn00566193_ml), rat PPARγ (NM_013124) (assay identification number Rn00440945_ml) and rat β-actin (NM_031144) (assay identification number Rn00667869_ml) were assay-on-demand gene expression products (Applied Biosystems). 5′-CCCAACAGAATAAAGCTACTCTGG-3′ and 5′-TCCTTTTGTGCAACTAGGAACGT-3′ primers for rat acetyl-CoA carboxylase (NM_022193) and 5′-CGGGACATGTTTGATGACTATGTC-3′ and 5′-AAAGGTCGGATGAGGATGCTGAA-3′ primers for rat carbohydrate-responsive element-binding protein (ChREBP) (NM_133552) were used.

Statistics

Values are expressed as the mean ± standard error. The data were analyzed by ANOVA unless otherwise stated. Fisher's Protected Least Significant Difference test was used to determine the significance of differences among the groups. The level of statistical significance was set at $P < 0.05$.

Results

Effects of fructose polymerization of enzymatically synthesized inulin on biomarkers of metabolic disease in rats fed a cafeteria diet

We have reported that DP = 16–17 inulin consumption for 4 and 12 weeks in the cafeteria group reduced the rat body weights, while the consumption in the SD group did not affect them (3). In this study, while food efficiency in rats fed inulin (average DP = 6–8, 16–17, and 23)-supplemented diets did not vary significantly between groups, intake of inulin (average DP = 6–8 and 16–17) for 3 weeks showed a trend toward body weight reduction ($P < 0.10$), but not the weight of rats fed the SD diet (Table 1). In the liver of cafeteria rats in a 3-week feeding study, dietary inulin (average DP = 6–8, 16–17, and 23) and resistant maltodextrin suppressed the elevation of triacylglycerol and total cholesterol levels (Table 1). There was no significant difference in liver lipid profiles between inulin and maltodextrin. Dietary inulin with average DP = 16–17 significantly reduced portal plasma glucose levels in rats fed not only the cafeteria diet but also the SD

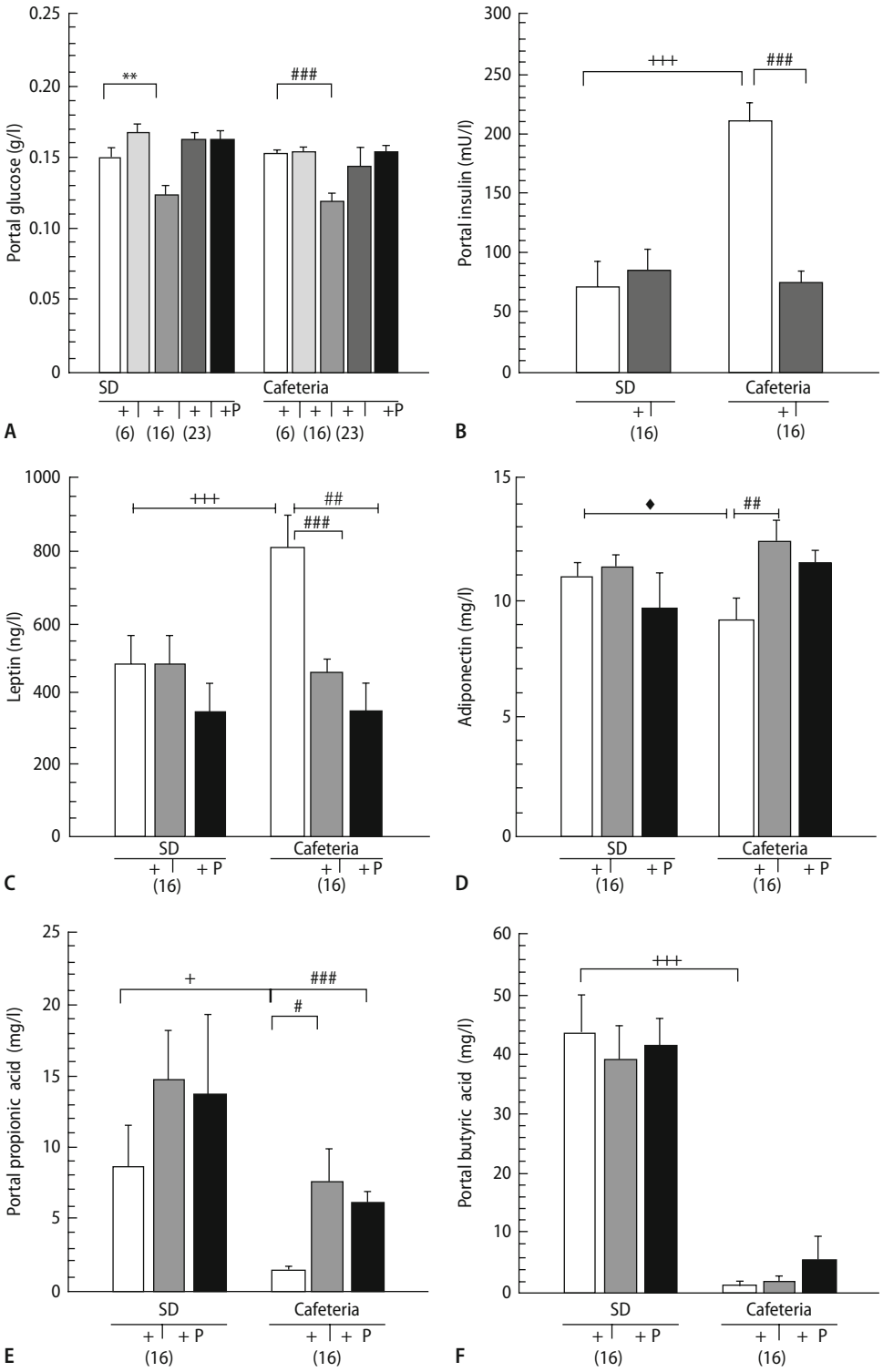

Fig. 1 Comparison of synthetic inulin with different degrees of fructose polymerization and resistant maltodextrin effects on blood biomarkers of metabolic disease in SD and cafeteria rats. Rats were fed SD-, inulin (I)- or resistant maltodextrin (P)-supplemented SD (SD + I or P)-, cafeteria and inulin- or resistant maltodextrin-supplemented cafeteria (cafeteria + I or P)-diets for 3 weeks, and then sacrificed. Values are the means ± SE of 5–9 determinations in each group. $^{**}P < 0.01$ for SD + I (16) rats versus SD rats; $^{\blacklozenge}P < 0.10$, $^{+}P < 0.5$, $^{+++}P < 0.001$ for cafeteria rats versus SD rats; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ for cafeteria + I (16) rats or cafeteria + P rats versus cafeteria rats. *Numbers* in parenthesis show the average degree of fructose polymerization (6; 6–8, 16; 16–17)

diet (Fig. 1a). Resistant maltodextrin did not reduce the portal plasma glucose level. Intake of the cafeteria diet elevated portal plasma insulin levels and circulating serum leptin levels in rats, and the dietary inulin with average DP = 16–17 induced recovery to near control levels in cafeteria rats

(Fig. 1b, c). In addition, intake of the cafeteria diet tended to reduce circulating serum adiponectin levels, and the dietary inulin (average DP = 16–17) recovered to near control levels (Fig. 1d).

Effects of dietary inulin and resistant maltodextrin on portal plasma short chain fatty acid levels

Inulin-type fructans in the large intestine are reported to be metabolized by digestive microflora to produce short-chain fatty acids [8, 10, 14, 15, 19]. We examined whether intake of synthetic inulin (average DP = 16–17) and resistant maltodextrin affected portal plasma short chain fatty acid levels in rats fed standard and cafeteria diet. Rats fed the cafeteria diet for 3 weeks showed markedly reduced portal plasma propionate and butyrate levels (Fig. 1 e, f). Dietary inulin (average DP = 16–17) and resistant maltodextrin tended to increase portal plasma propionate levels in SD rats and induce recovery to near control levels in cafeteria rats (Fig. 1e), although dietary inulin (average DP = 16–17) and resistant maltodextrin did not affect portal plasma butyrate levels in SD and cafeteria rats (Fig. 1f).

Effects of dietary inulin and clofibrate on biomarkers of metabolic disease in cafeteria rats

Since clofibrate at the concentration of 0.25% but not 0.1% elevated liver weights in SD and cafeteria rats, in this study, we compared health effects of dietary inulin (average DP = 16–17) and clofibrate (0.1%). Dietary inulin and clofibrate reduced the elevation of liver triacylglycerol levels but not total cholesterol levels in cafeteria rats (Fig. 2a, c, d). The extents of the reduction in epididymal adipose tissue weights and liver triacylglycerol levels by the dietary inulin were higher than those by 0.1% clofibrate (Fig. 2b, c). The majority of circulating serum lipoprotein triacylglycerol in SD and cafeteria rats was in very low density lipoprotein (VLDL) (85.2 ± 12.7 and 87.9 ± 10.2%, respectively), and VLDL total cholesterol content in cafeteria rats was high level (79.4 ± 9.5%) among serum lipoproteins (Fig. 2e, f). Dietary inulin and clofibrate markedly reduced serum VLDL triacylglycerol levels in SD and cafeteria rats (Fig. 2e). The extent of suppression by dietary inulin was higher than by 0.1% clofibrate in cafeteria rats, and the additive or synergistic suppression by both inulin and clofibrate was not found. In contrast, the elevated levels of serum VLDL total cholesterol in cafeteria rats was reduced by 0.1% clofibrate but not by dietary inulin (Fig. 2f).

Liver gene expression

Next, in order to investigate the mechanism of anti-hyperlipidemic effects by dietary inulin, we examined the effects of inulin (average DP = 16–17) on the hepatic expression of genes such as HMG-CoA reductase, acetyl-CoA carboxylase and fatty acid synthase, that are involved in syntheses of cholesterol and fatty acid, as well as genes involved in carbohydrate and lipid metabolism. Consistent with the increase in serum VLDL and liver triacylglycerol levels, rats fed the cafeteria diet for 3 weeks showed significant elevation of acetyl-CoA carboxylase and fatty acid synthase mRNA levels in the liver (Table 2). HMG-CoA reductase mRNA level was reduced in the liver of cafeteria rats (Table 2). Accumulation of cholesterol in the liver may inhibit the gene transcription (Table 2). Dietary inulin suppressed the upregulation of acetyl-CoA carboxylase and fatty acid synthase mRNAs (Table 2). The expression of PPARα target gene carnitine palmitoyltransferase 1a mRNA in SD and cafeteria rats was not affected by the dietary inulin (0.99 ± 0.15 and 0.45 ± 0.12 fold induction) when compared to rats fed fiber-free diets (1.00 ± 0.24 and 0.47 ± 0.16 fold induction, respectively), while the levels in rats fed SD, SD + I, cafeteria, and cafeteria + I diets tended to be increased by administration of clofibrate (1.57 ± 0.63, 1.29 ± 0.23, 0.74 ± 0.10, and 0.72 ± 0.05 fold induction, respectively). These results were consistent with facts that PPARα mRNA levels were not significantly changed by intake of the cafeteria diet and inulin in this model (Table 2). Moreover, dietary inulin did not affect the expression of PPARγ and SREBP2 mRNAs in cafeteria rats (Table 2). The messenger RNA levels of liver lipogenesis gene-regulating transcription factors such as SREBP1 and ChREBP were not significantly increased by intake of the cafeteria diet, but the mRNA levels of SREBP1 and ChREBP, that are induced by glucose, were reduced or tended to be reduced by the dietary inulin, consistent with the decrease in portal plasma glucose levels (Fig. 1 and Table 2).

Discussion

In a previous study [22], we reported that synthetic inulin and plant inulin had similar properties for in vitro fermentation, and that synthetic inulin suppressed the growth of harmful bacteria more strongly than fructooligosaccharide. However, it has not been determined whether the effects of synthetic inulin depend on its physico-chemical properties. In the present study, in order to investigate whether the anti-hyperlipidemic effects of inulin are dependent on

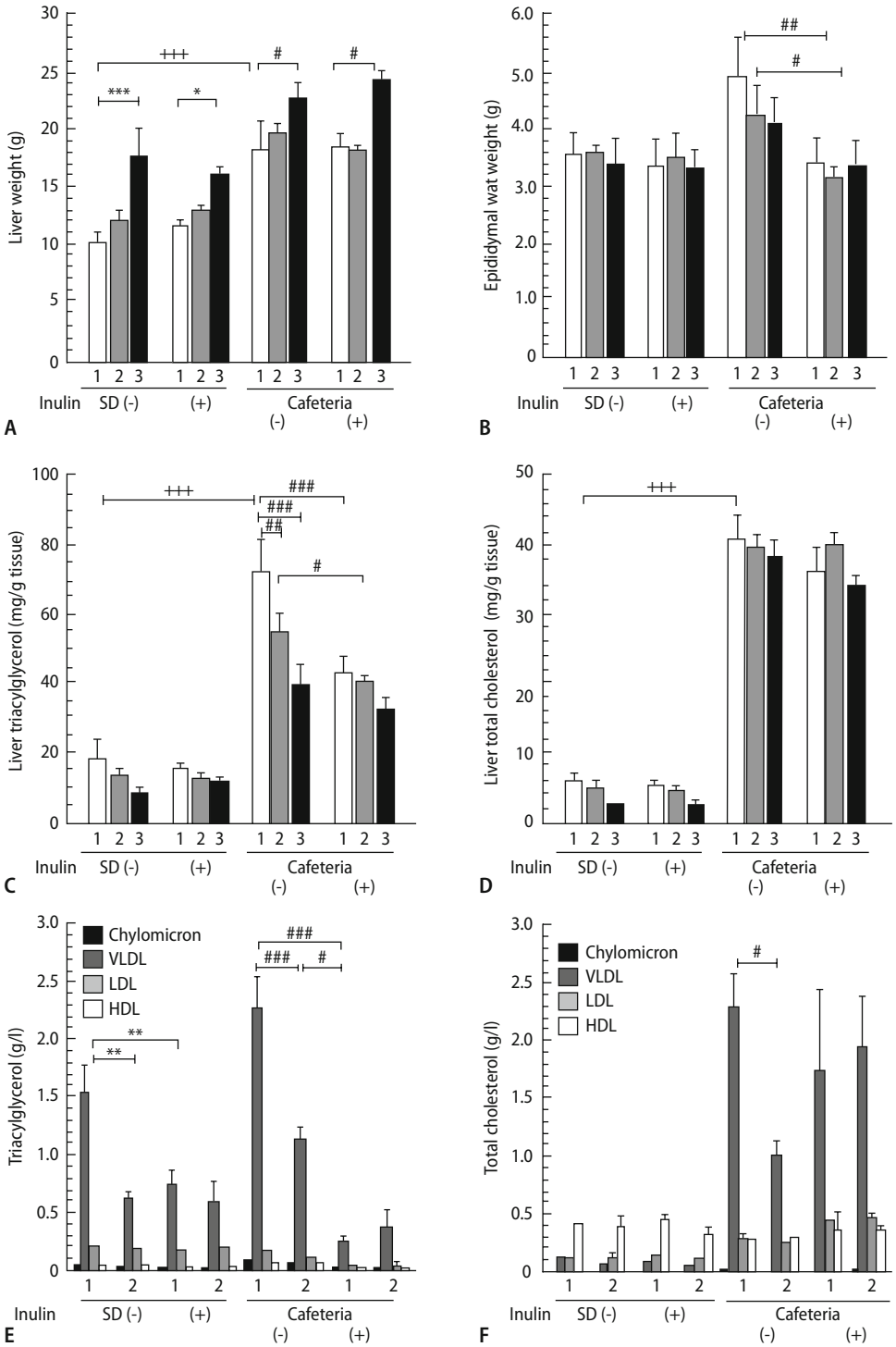

Fig. 2 Comparison of synthetic inulin (average DP = 16–17) and clofibrate effects on tissue weights and lipid profiles in the liver and circulating serum lipoproteins in SD and cafeteria rats. Rats (7 weeks of age) were fed SD-, inulin (I)-supplemented SD (SD + I)-, cafeteria -, and inulin-supplemented cafeteria (cafeteria + I) diets for 1 week, continued to consume each diet containing 0, 0.1, and 0.25% (w/w) clofibrate for another 2 weeks, and then sacrificed. Values are the means ± SE of 4 determinations in each group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ for SD + 0.25% clofibrate rats versus SD control rats, or SD + I + 0.25% clofibrate rats versus SD + I control rats; $^{+++}P < 0.001$ for cafeteria control rats versus SD control rats; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ for cafeteria + 0.1% clofibrate, cafeteria + 0.25% clofibrate, or cafeteria + I control rats versus cafeteria control rats, cafeteria + I + 0.25% clofibrate rats versus cafeteria + I control rats, cafeteria + I control or cafeteria + I + 0.1% clofibrate rats versus cafeteria 0.1% clofibrate rats

its physico-chemical properties based on its degree of fructose polymerization, we compared the health effects of synthetic inulin (average DP = 6–8 and 16–17 inulins) and chicory inulin (average DP = 23) with those of resistant maltodextrin. In rats fed the cafeteria diet, inulin (5%, average DP = 16–17) reduced the elevation of serum and liver triacylglycerol levels (Table 1). Although Levrat et al. [10] reported that addition of 20%, but not 5 or 10%, chicory inulin to a similar diet to the standard diet in the present study

Table 2 Liver gene expression in rats in response to 3-week standard (SD), inulin-supplemented standard (SD + I), high-fat and high-sucrose (HF, cafeteria), and inulin-supplemented high-fat and high-sucrose (cafeteria + I) diets

Liver gene	Fold induction			
	SD	SD + I (16–17)	Cafeteria	Cafeteria + I (16–17)
n	5	5	5	5
Acetyl-CoA carboxylase	1.00 ± 0.18	0.71 ± 0.18	1.89 ± 0.21^{++}	0.56 ± 0.15$^{\#\#\#}$
Fatty acid synthase	1.00 ± 0.12	0.76 ± 0.26	5.26 ± 1.30^{+++}	0.49 ± 0.15$^{\#\#\#}$
HMG CoA reductase	1.00 ± 0.16	0.98 ± 0.22	0.35 ± 0.06^{++}	0.08 ± 0.02
Carnitine	1.00 ± 0.24	0.99 ± 0.15	0.47 ± 0.16	0.45 ± 0.12
Palmitoyltransferase Ia				
PPARα	1.00 ± 0.14	1.59 ± 0.23	1.07 ± 0.09	0.77 ± 0.18
PPARγ	1.00 ± 0.10	0.67 ± 0.15	0.60 ± 0.04	0.62 ± 0.10
SREBP 1	1.00 ± 0.32	0.19 ± 0.08	1.27 ± 0.16	0.41 ± 0.13$^{\#\#}$
SREBP 2	1.00 ± 0.30	0.43 ± 0.06	0.38 ± 0.09	0.09 ± 0.02
ChREBP	1.00 ± 0.23	0.46 ± 0.12	0.70 ± 0.10	0.29 ± 0.09

Values are the means ± S.E.; *n* number of animals. Numbers in parenthesis show average degree of fructose polymerization of synthesized inulin. Fold induction is expressed by taking the control values obtained from SD-fed animals as 1.0. Statistical significance between treatment groups was determined by ANOVA and Bonferroni's test.
$^{++}P < 0.01$; $^{+++}P < 0.001$ for cafeteria-fed animals versus SD-fed animals; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$ for inulin-supplemented cafeteria-fed animals versus cafeteria-fed animals

decreased the plasma triacylglycerol level, 5% synthetic inulin in the cafeteria diet but not in the SD diet suppressed the triacylglycerol accumulation in blood and liver. While there were no differences in the suppression of liver triacylglycerol or total cholesterol accumulation among the three types of inulin and resistant maltodextrin, inulin (average DP = 16–17) only decreased portal plasma glucose levels in rats fed the SD and cafeteria diets (Table 1 and Fig. 1). Degree of fructose polymerization of synthetic inulins is less than 30, while there are plant-derived inulins with over than 30 of DP (supplemental Fig. 1). The difference in portal plasma glucose levels among these groups may result from differences in chemical and physical properties including solubility in water, viscosity, and fermentability. As reported previously [22], inulin (5%, average DP = 16–17) significantly suppressed the elevation of blood glucose in healthy subjects after dextrin loading. In addition, it has been reported that a high-fructose diet (60% fructose w/w) for 8 weeks can cause hyperinsulinaemia in rats with elevated plasma insulin and leptin concentrations [7]. As well as the high-fructose diet [7], the high-fat (10% lard) and high-sucrose (60% sucrose w/w) diet in this study resulted in increase in portal insulin and circulating serum leptin levels. In addition, whereas adiponectin increases insulin sensitivity by increasing fatty acid oxidation in skeletal muscle and suppresses hepatic glucose production [2, 5], the high-fat and high-sucrose diet showed a tendency to decrease circulating serum adiponectin levels. The levels of insulin, leptin, and adiponectin were recovered to nearly normal levels by intake of synthetic inulin (average DP = 16–17) (Fig. 1b–d). Accordingly, inulin with an average DP of 16–17 is likely the most effective compound in terms of its anti-hyperlipidemic and anti-hyperglycemic activities.

In this study, we found that portal plasma propionate levels were markedly reduced in rats fed the cafeteria diet (Fig. 1e). Interestingly, propionate is reported to inhibit fatty acid synthesis in rat hepatocytes [3, 12]. The reduction in propionate levels in portal blood may be associated with induction of liver fatty acid synthase. Inulin is fermented by colonic microflora, and short-chain fatty acids such as acetate, propionate and butyrate are produced and can be absorbed from the colon [8, 10, 19]. The fact that propionate absorption is higher than that of butyrate [10] is consistent with our observations that propionate levels recovered to nearly normal levels and butyrate levels were remained reduced after dietary inulin intake (Fig. 1e, f). Thus, the recovery of portal plasma propionate levels by dietary inulin could possibly result in suppression of fatty acid synthesis. Taken together, these data suggest that a link likely exists between the modulation of glucose and propionate levels in portal blood by dietary inulin and its anti-lipogenic effects. The different physical and biological properties of synthetic and plant-derived inulins with the different degrees of polymerization might be associated with the different nutritional effects.

The mechanism underlying the effect of inulin on serum and liver lipid accumulation has not been accurately determined. Since lipid levels of liver and serum VLDL were markedly reduced, we compared the hepatic expression of genes involved in carbohydrate and lipid metabolism between rats fed SD and cafeteria diets. As shown in Table 2, mRNA levels of acetyl-CoA carboxylase and fatty acid synthase, which regulate de novo lipogenesis, were elevated in the liver of rats fed the cafeteria diet. Dietary inulin (average DP = 16–17) reduced the mRNA levels of acetyl-CoA carboxylase and fatty acid synthase, leading to sup-

pression of liver fatty acid synthesis. Activation of PPARα with PPARα agonists has been shown to reduce circulating serum lipid levels and suppress fatty liver formation in humans [1]. Since dietary inulin did not affect PPARα mRNA levels, we examined health effects of coadministration with synthetic inulin and clofibrate in this study. Although clofibrate reduced circulating serum and liver lipid levels in cafeteria rats, no additive or synergistic effect of clofibrate on reduction in serum and liver lipid levels by dietary inulin was found (Fig. 2). The anti-lipogenic action of synthetic inulin is likely to be more effective than that of clofibrate (Fig. 2). Transcription factors SREBP1 and ChREBP, of which the expression is regulated by glucose, activate genes required for fatty acid synthesis. Portal glucose levels were decreased in SD and cafeteria rats by intake of synthetic inulin and then hepatic mRNA levels of SREBP1 and ChREBP tended to be reduced, consistent with suppression in elevated mRNA levels of acetyl-CoA carboxylase and fatty acid synthase. Thus SREBP1 and ChREBP may play a key role in fatty acid synthesis in rats fed the cafeteria diet, leading to lipid accumulation. Further studies are required to confirm whether synthetic inulin can decrease blood glucose and insulin levels and circulating and liver lipid levels in humans.

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