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Low molecular weight chitosan inhibits obesity induced by feeding a high-fat diet long-term in mice

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Abstract

Three low molecular weight chitosans (molecular weight: 21, 46 and 130 kDa) obtained by enzymatic hydrolysis of a high molecular weight chitosan (average molecular weight: 650 kDa) had low viscosity and were water-soluble. The effects of these water-soluble chitosans on pancreatic lipase (in-vitro) and the elevation of plasma triacylglycerol concentration after the oral lipid tolerance test were examined in mice. The water-soluble 46-kDa chitosan was the most effective at inhibiting pancreatic lipase activity (in-vitro) and plasma triacylglycerol elevation after the oral lipid tolerance test. Based on this result, the effects of the 46-kDa chitosan on increases in bodyweight, various white adipose tissue weights, and plasma and liver lipids were examined in mice fed a high-fat diet for 20 weeks. Water-soluble 46-kDa chitosan (300 mg kg^{-1}, twice daily) prevented increases in bodyweight, various white adipose tissue weights and liver lipids (cholesterol and triacylglycerol) in mice fed a high-fat diet, and further increased the faecal bile acid and fat. The results suggest that the lipid-lowering effects of the 46-kDa chitosan may be mediated by increases in faecal fat and/or bile acid excretion resulting from the binding of bile acids, and by a decrease in the absorption of dietary lipids (triacylglycerol and cholesterol) from the small intestine as a result of the inhibition of pancreatic lipase activity. Water-soluble 46-kDa chitosan (100 and 300 mg kg^{-1}, twice daily) did not cause liver damage with the elevation of glutamic oxaloacetic transaminase and glutamic pyruvic transaminase, or kidney damage with the elevation of blood nitrogen urea. It was concluded that water-soluble 46-kDa chitosan is a safe functional food.

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Introduction

Chitin and chitosan are polymers that have a molecular weight of 1000 kDa and contain more than 5000 acetylglucosamine and glucosamine units, respectively. Chitin is widely distributed in natural products such as the protective cuticles of crustaceans and insects, as well as in the cell walls of some fungi and microorganisms, and it is usually prepared from the shells of crabs and shrimp. Chitin is converted to chitosan by deacetylation with 45% NaOH at 100°C for 2 h. Chitosan is commercially produced on a large scale in various countries (Japan, North America, Russia, Norway, Korea, India and China) (Singla & Chawla 2001). Chitosan has the characteristic of dietary fibre, being a polysaccharide that is indigestible by mammalian enzymes. Several studies have shown that chitosan has antihypertension (Kato et al 1994), hypocholesterolaemic (Sugano et al 1988; Ormrod et al 1998; Yao & Chiang 2002), and anti-obesity (Han et al 1999) effects, and preventive effects against adverse reactions induced by cancer chemotherapeutic drugs (Kimura & Okuda 1999; Kimura et al 2000, 2001) in animal models. Although chitosan was shown to have clinical hypocholesterolaemic effects (Wuolijioki et al 1999; Tai et al 2000), it had no effect on obesity (Guercioline et al 2001; Ho et al 2001; Gades & Stern 2002). The molecular weights of the chitosans used in those studies were over 650 kDa, and the chitosans were insoluble in water. Since the greater viscosity of the intestinal content induced by dietary fibre is strongly associated with a reduction of plasma and liver cholesterol, it is possible that the hypocholesterolaemic action of chitosan is correlated with an increase in the viscosity of the intestinal contents (Gallaher et al 1993). Deuchi et al (1995) reported that an increase in the viscosity or the degree of deacetylation of chitosan resulted in a pronounced effect on the apparent digestibility of fats. However, the hypocholesterolaemic action of chitosan is independent of the viscosity of chitosan (Sugano et al 1988). Furthermore, we

reported that low molecular weight chitosans (oligochitosan, 21- and 46-kDa chitosans) with a lower viscosity might be useful in preventing tumour growth through the activation of intestinal immune functions (Maeda & Kimura 2004). Thus, it seems likely that the discrepancies regarding the physiological actions of water-insoluble chitosan may be due to differences in the viscosity and/or other characteristics of chitosans. The effects of water-soluble low molecular weight chitosan on lipid and carbohydrate metabolism are unclear. In a preliminary experiment, we examined the effects of various water-soluble low molecular weight chitosans on pancreatic lipase activity. Among the chitosans tested, the 46-kDa water-soluble chitosan inhibited pancreatic lipase activity most effectively. We therefore examined the effects of the water-soluble 46-kDa chitosan on obesity induced in mice fed a high-fat diet for 20 weeks.

Materials and Methods

Materials

Water-soluble chitosan with an average molecular weight of 46 kDa was supplied by Mind Ace Co. Ltd (Miyazaki, Japan). Triolein and pancreatic lipase were purchased from Sigma Co. (St Louis, MO, USA). Triglyceride E-Test, Total Cholesterol E-Test and Total Bile Acid-Test kits were purchased from Wako Pure Chemical (Osaka, Japan). Non-salt butter was purchased from Yotsuba Nyugyou Co. (Hokkaido, Japan). Cornstarch, casein, mineral mixture (AIN-76) and vitamin mixture (AIN-76) were purchased from Oriental Yeast (Tokyo, Japan). Other chemicals were of reagent grade.

Diet composition

The low-fat diet consisted of 3.0% (w/w) butter, 41.5% corn starch, 5.0% sucrose, 20% casein, 3.0% cellulose, 3.5% mineral mixture (AIN-76), 1.0% vitamin mixture (AIN-76), 0.4% choline chloride and 22.6% water (total kcal: 292.8). The high-fat diet consisted of 45.0% butter, 17.1% corn starch, 10.0% sucrose, 20.0% casein, 3.0% cellulose, 3.5% mineral mixture, 1.0% vitamin mixture and 0.4% choline chloride (total kcal: 535.2).

Animals

Male C57BL/6J strain mice (4 weeks old) were obtained from Japan SLC (Shizuoka, Japan). The mice were housed in a room with a 12-h light/dark cycle and controlled for temperature and humidity. The animals had free access to food and water and were studied after 1 week of adaptation to the lighting conditions. Mice were treated according to the ethical guidelines of the Animal Center, School of Medicine, Ehime University. The Animal Studies Committee of Ehime University approved the experimental protocol.

In-vitro pancreatic lipase activity

The assay of pancreatic lipase activity in porcine pancreas was performed as described previously (Tsujita & Okuda 1983). Enzyme activity (μmol oleic acid released L^{-1} reaction mixture h^{-1}) was expressed as a percentage of the value obtained with buffer alone (control).

Plasma triacylglycerol (TG) concentration after oral administration of butter emulsions to mice

Various chitosans with average molecular weights of 21, 46 and 130 kDa (500 mg) were dissolved with distilled water (10 mL). The mice were deprived of food overnight and then the chitosans (500 mg kg^{-1}) were administered orally 20 s before oral administration of 0.2 mL of the butter emulsion. Blood samples were taken from the tail vein at 0, 0.5, 1, 2, 3 and 4 h after administration of the butter emulsion using a heparinized capillary tube, and centrifuged at 5500 *g* for 5 min in a Model KH-120 M (Kubota, Japan) centrifuge to obtain the plasma. The plasma TG concentration was determined using the Triglyceride E-Test kit.

Fat excretion and bile acid in faeces of mice

The 46-kDa water-soluble chitosan (100 and 300 mg kg^{-1}) was administered orally twice daily (0700 and 1900 hours) to the mice fed the high-fat diet for 10 weeks; control mice were given the low-fat diet or the high-fat diet alone for 10 weeks. Samples of faeces were obtained from each group at 24-h intervals for 4 weeks and the TG and total cholesterol (TC) in the faeces was measured by the methods of Fletcher (1968) and Zak et al (1954), respectively. Bile acids in faeces were measured using the Total Bile Acid-Test kit.

Bodyweight, liver and white adipose tissue weights, and liver TG and TC concentrations

The bodyweight of each mouse was measured once a week and the total amount of food consumed was recorded weekly. The 46-kDa water-soluble chitosan (100 and 300 mg kg^{-1}) was administered orally twice daily (0700 and 1900 hours) to mice fed the high-fat diet for 20 weeks. Control mice fed the low-fat diet or the high-fat diets alone were given distilled water on the same schedule. On Week 20, for mice fed the low-fat and high-fat diets, blood was obtained by venous puncture under diethyl ether anaesthesia. The plasma was prepared by centrifugation and frozen at −80°C until analysis. The plasma TG and TC concentrations were determined using Triglyceride E-Test and Total Cholesterol E-Test kits. The liver and white adipose tissue were dissected and weighed. To measure the liver TG and TC concentrations, liver (1 g) was homogenized with distilled water (10 mL). The liver TG and TC concentrations were measured by the methods of Fletcher (1968) and Zak et al (1954), respectively.

Histological examination

White epididymal adipose tissues were fixed in buffered 10% formalin for at least 2 h, and then progressively dehydrated in solutions containing an increasing percentage of ethanol (70, 80, 95 and 100%). They were then cleared in Histoclear, embedded in paraffin under vacuum, sectioned at 5-μm thickness, deparaffinized, and stained with Harris hematoxylin and

eosin. Four different microscopic fields (magnification 40×) per plate were photographed and more than 100 adipose cells were selected and their cell diameters measured.

Statistical analysis

All values are expressed as means ± s.e.m. Data were analysed by one-way analysis of variance, and then differences among means were analysed using Fisher's protected LSD test. Differences were considered significant at $P < 0.05$.

Results

In-vitro pancreatic lipase activity

Figure 1 shows the dose–response curve of the effects of various chitosans on pancreatic lipase activity. Among the low molecular weight chitosans (average molecular weight: 21, 46 and 130 kDa), the 46-kDa chitosan inhibited the pancreatic lipase activity most effectively. Based on this finding, we examined the effects of 46-kDa chitosan on the elevation of plasma TG concentration after oral administration of a butter emulsion containing cholesterol to mice.

Plasma TG and TC in the oral lipid tolerance test

Among the three chitosans, the 46-kDa chitosan significantly reduced the elevation of the plasma TG concentration 0.5–3 h after oral administration of the butter emulsion. The 130-kDa chitosan also reduced the elevation of plasma TG concentration 0.5 and 1 h after the oral administration of butter emulsion, but, at 3 h, the 21-kDa chitosan and the 130-kDa chitosan increased the TG concentration compared with that in mice administered butter alone (control mice) (Figure 2). In the preliminary in-vitro and in-vivo experiments, it was found that the 46-kDa chitosan inhibited pancreatic lipase activity (in-vitro) and the elevation of the plasma TG concentration in the oral lipid tolerance test in mice (in-vivo) most effectively. We therefore examined the effects of water-soluble 46-kDa chitosan on lipid metabolism in mice fed a high-fat diet for 20 weeks.

Energy intake, bodyweight and tissue weight, plasma and hepatic lipids in mice fed a high-fat diet

The mean food consumption per day per mouse for 8 days was not different among mice fed the high-fat diet, the high-fat diet plus 46-kDa chitosan (100 or 300 mg kg^{-1}, twice daily) and the low-fat diet, being 48.27 ± 1.13 kJ (high-fat diet), 46.34 ± 1.40 kJ (high-fat diet plus 100 mg kg^{-1} 46-kDa chitosan), 44.21 ± 1.43 kJ (high-fat diet plus 300 mg kg^{-1} 46-kDa chitosan) and 51.42 ± 1.48 kJ (low-fat diet). Figure 3 shows the changes in bodyweight of the groups during the experiment. Mice fed the high-fat diet showed significant increases in bodyweight at 4–20 weeks compared with mice fed the low-fat diet. The 46-kDa chitosan (100 and 300 mg kg^{-1}, twice daily) had no effect on the increase of bodyweight induced by feeding a high-fat diet up until Week 17; the 46-kDa chitosan at a dose of 300 mg kg^{-1} twice daily

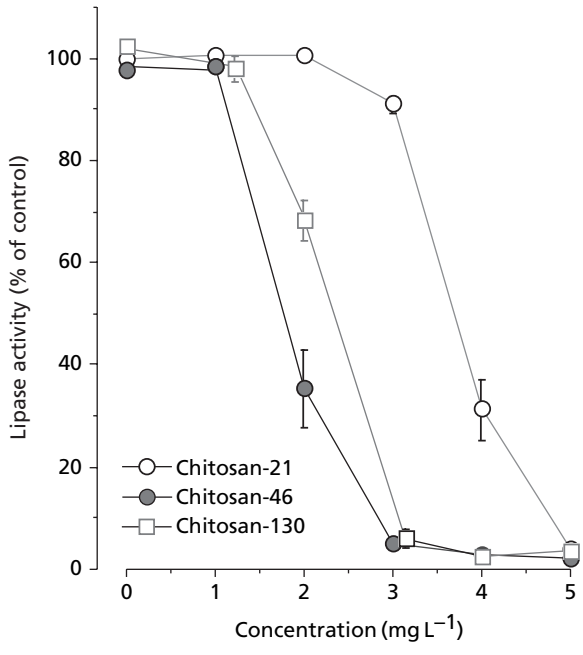

Figure 1 Effects of three water-soluble chitosans (molecular weight: 21, 46 and 130 kDa) on pancreatic lipase activity. ○, 21-kDa chitosan; ●, 46-kDa chitosan; □, 130-kDa chitosan. Values are means ± s.e.m., n = 4.

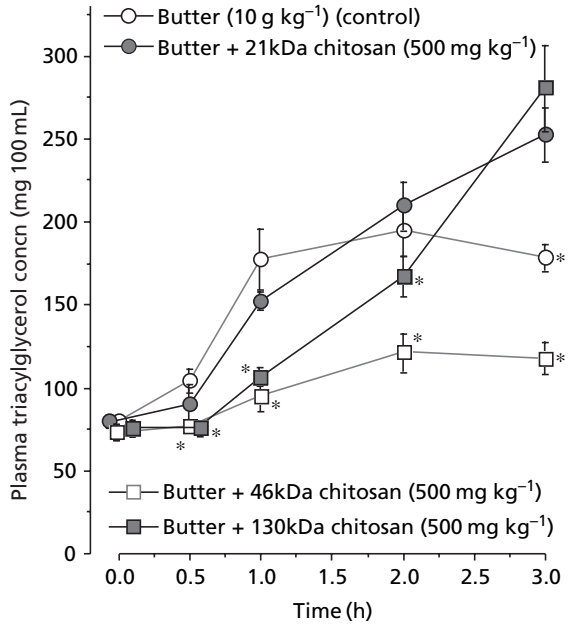

Figure 2 Effects of three water-soluble chitosans (molecular weight: 21, 46 and 130 kDa) on plasma triacylglycerol concentration after the oral lipid tolerance test in mice. ○, Butter (10 g kg^{-1}) (control); ●, butter + 21-kDa chitosan (500 mg kg^{-1}); □, butter + 46-kDa chitosan (500 mg kg^{-1}); ■, butter + 130-kDa chitosan (500 mg kg^{-1}). Values are means ± s.e.m., n = 6. *$P < 0.05$, significantly different compared with the control.

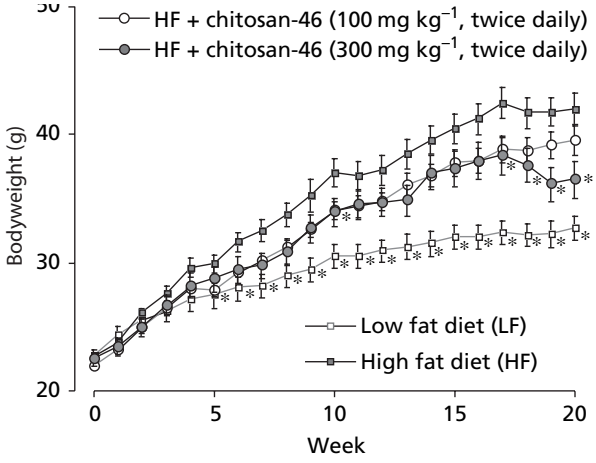

Figure 3 Effects of water-soluble low molecular weight chitosan (molecular weight: 46 kDa) on bodyweight in mice fed a high-fat diet for 20 weeks. ○, High-fat diet + 46-kDa chitosan (100 mg kg^{-1}, twice daily) (n = 7); ●, high-fat diet + 46-kDa chitosan (300 mg kg^{-1}, twice daily) (n = 7); □, low-fat diet (n = 7); ■, high-fat diet (n = 8). Values are means ± s.e.m., n = 7–8. *$P < 0.05$, significantly different compared with mice fed the high-fat diet.

inhibited the increase of bodyweight from Weeks 17 to 20 (Figure 3).

The plasma TG concentration was not significantly different among mice fed the low-fat diet (54.6 ± 3.80 mg 100 mL^{-1}), the high-fat diet (74.0 ± 8.12 mg 100 mL^{-1}), high-fat diet plus 46-kDa chitosan (100 mg kg^{-1}, twice daily) (89.7 ± 14.7 mg 100 mL^{-1}), and the high-fat diet plus 46-kDa chitosan (300 mg kg^{-1}, twice daily) (84.6 ± 13.4 mg 100 mL^{-1}).

The plasma TC concentration was significantly increased at Week 20 in mice fed a high-fat diet (245.2 ± 15.5 mg 100 mL^{-1}) compared with mice fed a low-fat diet (140.2 ± 24.1 mg 100 mL^{-1}). The 46-kDa chitosan (300 mg kg^{-1}, twice daily) inhibited the increase of the TC concentration compared with the level in mice fed the high-fat diet (198.8 ± 5.96 mg 100 mL^{-1} vs 245.2 ± 15.5 mg 100 mL^{-1} for mice fed the high-fat diet plus 300 mg kg^{-1} 46-kDa chitosan and the high-fat diet, respectively)

Liver weight and hepatic lipids (TG and TC) were not significantly different between mice fed the high-fat diet (liver weight: 1.87 ± 0.13 g; TG: 234.2 ± 24.8 mg g^{-1}; TC: 13.9 ± 1.05 mg g^{-1}) and mice fed the low-fat diet (liver weight: 1.87 ± 0.19 g; TG: 196.6 ± 19.9 mg g^{-1}; TC: 18.1 ± 1.48 mg g^{-1}). The 46-kDa chitosan (300 mg kg^{-1}, twice daily) significantly reduced the liver weight (1.37 ± 0.09 g), and hepatic TG (79.6 ± 15.8 mg g^{-1}) and TC (10.7 ± 0.96 mg g^{-1}) contents compared with those in mice fed the high-fat diet. The 46-kDa chitosan (100 and 300 mg kg^{-1}, twice daily) did not cause liver damage with the elevation of glutamic oxaloacetic transaminase and glutamic pyruvic transaminase, or kidney damage with the elevation of blood nitrogen urea in mice fed the high-fat diet for 20 weeks (data not shown). We did not examine the effects of 46-kDa chitosan on pancreatic function (for example, amylase, lipase and insulin secretion). The toxic side-effects of 46-kDa chitosan on pancreatic function need to be further clarified in mice fed the high-fat diet for 20 weeks.

The weights of subcutaneous, mesenteric and epididymal white adipose tissue were increased together with bodyweight by feeding the high-fat diet compared with feeding the low-fat diet (Table 1). The 46-kDa chitosan (300 mg kg^{-1}, twice daily) significantly reduced the weight of subcutaneous, mesenteric and epididymal white adipose tissues compared with those in mice fed the high-fat diet (Table 1). Furthermore, we examined the effects of 46-kDa chitosan on cell diameter in mice fed the high-fat diet for 20 weeks. The frequency of cells with a diameter of 120–160 μm in epididymal adipose tissue in mice fed the high-fat diet (30.71 ± 2.31%) was greater than that in mice fed the low-fat diet (0.17 ± 0.17%). The 46-kDa chitosan (300 mg kg^{-1}, twice daily) significantly reduced (to 20.79 ± 2.28%) the frequency of cells with a diameter of 120–160 μm in adipose tissue compared with that in mice fed the high-fat diet (30.71 ± 2.31%) (Figure 4). Thus, the 46-kDa chitosan prevented the increases of bodyweight, and subcutaneous, mesenteric and epididymal adipose tissue weights and cell diameter of white adipose tissue induced by feeding the high-fat diet for 20 weeks.

Fat excretion in faeces of mice fed a high-fat diet

The dry weight (0.750 ± 0.060 g per mouse per day) of faeces collected during 4 days at Week 10 in mice fed the high-fat diet were significantly ($P < 0.007$) reduced compared with the dry weight (1.065 ± 0.047 g per mouse per day) of faeces collected from mice fed the low-fat diet. The dry weight of faeces collected during 4 days at Week 10 did not differ between mice fed the high-fat diet alone and mice fed the high-fat diet plus 46-kDa chitosan (100 and 300 mg kg^{-1}, twice daily) (data not shown). The TG content

Table 1 Effects of low molecular weight chitosan (46 kDa) on the weight of subcutaneous, mesenteric and epididymal white adipose tissues in mice fed a high-fat diet for 20 weeks

Treatment	Subcutaneous adipose tissue (g)	Mesenteric adipose tissue (g)	Epididymal adipose tissue (g)
Low-fat diet (n = 7)	0.322 ± 0.057*	0.283 ± 0.044*	1.188 ± 0.098*
High-fat diet (n = 8)	1.162 ± 0.095	0.887 ± 0.068	2.493 ± 0.076
High-fat + 46-kDa chitosan (100 mg kg^{-1}, twice daily) (n = 7)	1.067 ± 0.098	0.741 ± 0.077†	2.553 ± 0.082†
High-fat + 46-kDa chitosan (300 mg kg^{-1}, twice daily) (n = 7)	0.698 ± 0.085*†	0.620 ± 0.088*†	2.031 ± 0.219*†

Results are means ± s.e.m, n = 7–8. *$P < 0.05$, significantly different compared with mice fed a high-fat diet. †$P < 0.05$, significantly different compared with mice fed a low-fat diet.

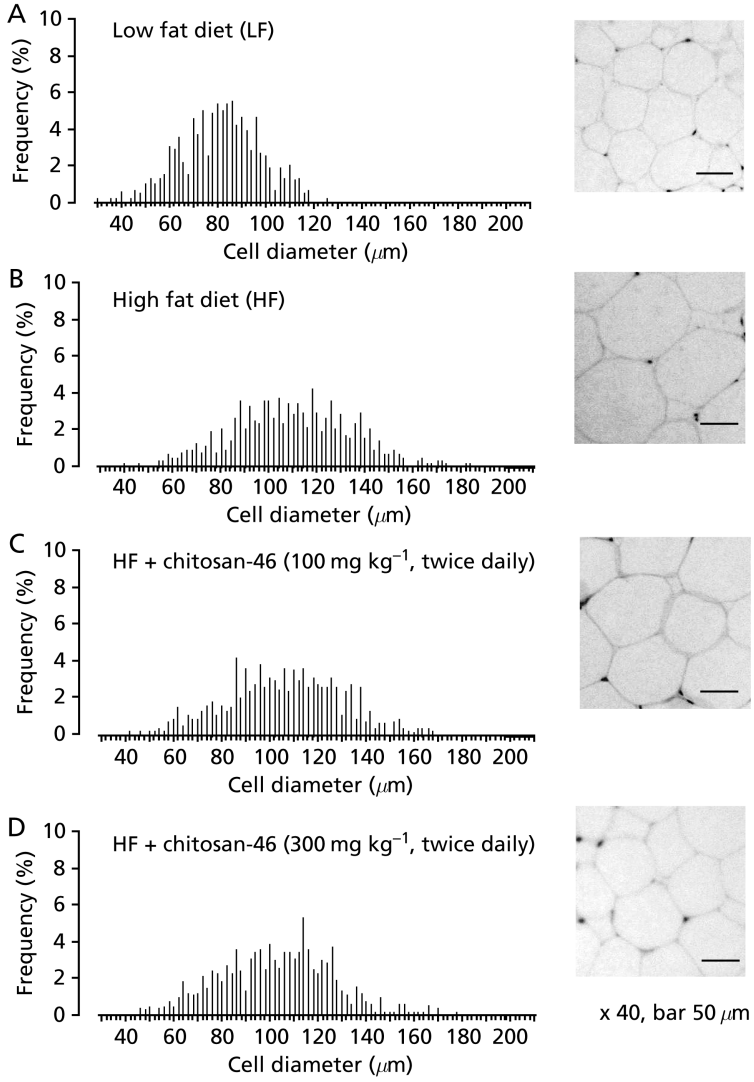

Figure 4 Effects of water-soluble low molecular weight chitosan (molecular weight: 46 kDa) on cell diameter in white adipose tissue in mice fed a high-fat diet for 20 weeks. A. Low-fat diet; B. high-fat diet; C. high-fat diet + 46-kDa chitosan (100 mg kg^{-1}, twice daily); D. high-fat diet + 46-kDa chitosan (300 mg kg^{-1}, twice daily). Values are expressed as % frequency of cells with the indicated cell diameter.

in faeces was not significantly different between the low-fat diet and high-fat diet groups. The TG content in faeces of mice fed the high-fat diet was increased by the administration of 46-kDa chitosan compared with that of mice fed the high-fat diet alone. The TC content in faeces during 4 days at Week 10 in mice fed the high-fat diet was significantly increased compared with that for mice fed the low-fat diet, but there was no significant difference between that in mice fed the high-fat diet and mice fed the high-fat diet plus the 46-kDa chitosan (100 and 300 mg kg^{-1}, twice daily) (Table 2). The total bile acid content of faeces during 4 days at Week 10 did not differ between mice fed the high-fat diet and mice fed the low-fat diet. The total bile acid content of faeces was significantly increased in mice fed the high-fat diet plus the 46-kDa chitosan (100 and 300 mg kg^{-1}, twice daily) compared with that in mice fed the high-fat diet alone (Table 2).

Discussion

We previously reported that water-insoluble, high molecular weight chitosan (650 kDa) at a level of 30 g kg^{-1} in a high-fat diet (corresponding to approx. 2–3 g chitosan kg^{-1} per day per mouse) prevented the increases in bodyweight and white adipose tissue weights, hyperlipidaemia and fatty liver induced by feeding the high-fat diet for 9 weeks, by inhibiting the intestinal absorption of dietary fat. The doses and viscosity of the high molecular weight chitosan used in the previous study were very high, and the chitosan was water-insoluble. In a clinical study, administration of chitosan (400 mg, twice daily for 8 weeks) did not significantly alter serum total cholesterol or bodyweight, but slightly increased serum triacylglycerols compared with placebo (Pitter et al 1999; Wuolijioki et al 1999). The doses of the various chitosans were between 0.1 and 15 g kg^{-1} per day for the evaluation of various pharmacological

Table 2 Effects of low molecular weight chitosan (46 kDa) on fat excretion into faeces during 4 days at Week 10 in mice fed a high-fat diet

Treatment	Triacylglycerol (mg/mouse/day)	Total cholesterol (mg/mouse/day)	Total bile acid μEq/mouse/day
Low-fat diet (n = 7)	0.347 ± 0.041	0.506 ± 0.018*	43.78 ± 3.84
High-fat diet (n = 8)	0.334 ± 0.024	0.843 ± 0.082	57.83 ± 2.93
High-fat + 46-kDa chitosan (100 mg kg^{-1}, twice daily) (n = 7)	0.301 ± 0.021	1.006 ± 0.116	71.30 ± 6.45*†
High-fat + 46-kDa chitosan (300 mg kg^{-1}, twice daily) (n = 7)	0.629 ± 0.183*†	1.051 ± 0.097	74.10 ± 5.31*†

Results are means ± s.e.m, n = 7–8. *$P < 0.05$, significantly different compared with mice fed a high-fat diet; †$P < 0.05$, significantly different compared with mice fed a low-fat diet.

actions in animal studies (Sugano et al 1988; LeHoux & Grondin 1993; Han et al 1999; Gallaher et al 2000; Kimura et al 2001; Hayashi & Ito 2002; Shon et al 2002; Maeda & Kimura 2004). In particular, doses of 2–5 g kg^{-1} were used for the evaluation of the hypcholesterolaemic effects of chitosan. Chitosan with an average molecular weight of 650 kDa or over was approved by the Ministry of Health, Labour and Welfare (Japan) in 1997 as a specific healthy functional food; the oral dose of chitosan approved was 0.5–3 g per day in humans. In this study, the doses of 46-kDa chitosan used were 100 and 300 mg kg^{-1} bodyweight twice daily; these doses are approximately 6- to 10-fold the doses used in humans. Sugano et al (1988) investigated the relationship between the hypocholesterolaemic action and the average molecular weight of chitosan in rats fed a cholesterol-enriched diet, and suggested that the hypocholesterolaemic action of chitosan was independent of its molecular weight. On the other hand, LeHoux & Grondin (1993) reported that 70-kDa chitosan at a level of 50 g kg^{-1} food lowered plasma and liver cholesterol levels by 54% and 64%, respectively, in rats fed a high cholesterol diet, but that a high molecular weight chitosan (>750 kDa) had less hypocholesterolaemic potential than a 70-kDa preparation. Thus, it is unclear if the hypocholesterolaemic effects depend on the molecular weight of chitosan. That the anti-obesity effects of chitosan were not observed in clinical studies may be due to the dependence of such action on the molecular weight of chitosan. In the present study, to investigate the preventive effects on the increases of bodyweight and fat storage in adipose tissue induced by feeding a high-fat diet long term together with lower doses of chitosans, we prepared three water-soluble chitosans with low molecular weight obtained from a high molecular weight chitosan (Hatanaka 1996). We examined the effects of the three water-soluble chitosans (molecular weight: 21, 46 and 130 kDa) on pancreatic lipase activity (in-vitro) and the oral lipid tolerance test (in-vivo). Among the three chitosans, the 46-kDa chitosan had potent activity in both in-vitro and in-vivo experiments. The 46-kDa chitosan was therefore selected for evaluation in mice fed a high-fat diet for 20 weeks. Water-soluble 46-kDa chitosan (300 mg kg^{-1}, twice daily) prevented the increases of bodyweight, subcutaneous, mesenteric and epididymal adipose tissue weights and cell diameter in adipose tissues induced by feeding a high-fat diet for 20 weeks. This suggests that 46-kDa chitosan may inhibit the absorption of dietary fat from the small intestine through the inhibition of pancreatic lipase. A number of clinical studies have demonstrated the contribution of visceral fat accumulation to the development of metabolic disorders, including glucose intolerance and hyperlipidaemia (Fujioka et al 1987). It seems likely that the 46-kDa chitosan may prevent high-fat diet induced insulin resistance through the preventive action on the increase of visceral adipose tissue, but further work is needed to clarify this. Roberts et al (2004) reported that mRNA expression of hepatic HMG-CoA reductase, a key enzyme of hepatic cholesterol biosynthesis, was increased, and the protein abundance and activity of this enzyme were unchanged in rats fed a high-fat sucrose diet for 20 months. On the other hand, Wang et al (2001) reported that hamsters became hypercholesterolaemic when fed a high-fat diet containing cholesterol, with a dramatic down-regulation of hepatic HMG-CoA reductase mRNA expression, suggesting inhibition of endogenous cholesterol synthesis by feeding a high-fat diet containing cholesterol. In the present study, plasma total cholesterol level and HMG-CoA reductase activity were elevated by feeding a high-fat diet for 20 weeks. Water-soluble 46-kDa chitosan prevented the elevation of plasma total cholesterol caused by feeding the high-fat diet for 20 weeks, and further reduced the liver total cholesterol content; however, 46-kDa chitosan had no effect on hepatic HMG-CoA reductase activity in mice fed the high-fat diet (data not shown). Therefore, the effect of 46-kDa chitosan on the plasma and liver total cholesterol in mice fed the high-fat diet could not be explained by effects on hepatic HMG-CoA reductase activity. The total cholesterol content was 2.2 g kg^{-1} food in the non-salted butter used in this study. Young & Hui (1999) reported that inhibition of pancreatic lipase resulted in a significant decrease in the absorption of dietary cholesterol through the gastrointestinal tract. Chitosan is a weak anion exchanger and consequently would be expected to be able to bind bile acids. Such ability has been demonstrated in several studies in-vitro; chitosan has approximately half or the same bile acid binding capacity as cholestyramine, a strong anion exchanger with a high capacity for binding bile acids (Sugano et al 1980; Lee et al 1999). It is well known that increased bile acid excretion reduces cholesterol levels because plasma or liver cholesterol is utilized to maintain the bile acid pool. The present study showed that the 46-kDa chitosan increased faecal fat and bile acid excretion in mice fed a high-fat diet. Therefore, the hypocholesterolaemic actions of the 46-kDa chitosan may be mediated by increases of faecal fat and/or bile acid excretion as a result of the binding of bile acids, and by a decrease in the absorption of dietary cholesterol

from the small intestine as a result of the inhibition of pancreatic lipase activity.

In conclusion, we found that among three water-soluble low molecular weight chitosans prepared from a high molecular weight 650-kDa chitosan, a novel 46-kDa chitosan prevented the increases of bodyweight and adipose tissue weight induced by long-term feeding of a high-fat diet in mice. Further clinical studies are needed to clarify the preventive effect on diet-induced obesity of the water-soluble 46-kDa chitosan. It was found that the water-soluble 46-kDa chitosan did not cause liver or kidney damage in mice fed a high-fat diet for 20 weeks. To date, toxic side-effects such as liver and kidney damage after oral administration of high molecular weight chitosan (0.5 to 3 g per day per human) have not been reported. Therefore, high or low molecular weight chitosan is a safe functional food.

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