British Journal of Nutrition (2013), **109**, 254–262
doi:10.1017/S0007114512000980

A novel soluble β-1,3-D-glucan Salecan reduces adiposity and improves glucose tolerance in high-fat diet-fed mice

Ying Zhang, Lin Xia, Wenqiang Pang, Tao Wang, Peng Chen, Bin Zhu and Jianfa Zhang*

Center for Molecular Metabolism, Nanjing University of Science and Technology, B508, No. 364 Building, 200 Xiaolinwei Street, Nanjing 210094, People's Republic of China

(Submitted 22 August 2011 – Final revision received 25 January 2012 – Accepted 15 February 2012 – First published online 13 April 2012)

Abstract

Salecan is a recently identified water-soluble viscous extracellular β-1,3-D-glucan polysaccharide from an *Agrobacterium* species. It is a high-molecular-mass polymer (about 2×10^6 Da) and composed of a linear chain of glucosyl residues linked through a repeat unit of seven β-(1,3) and two α-(1,3) glucosidic bonds. In the present study, we examined the effects of dietary Salecan fed at 2 and 5% in a high-fat diet (64% energy) in C57BL/6J mice. After 6 weeks, mice fed 2 and 5% Salecan had significantly lower body weight, fat mass and percentage of body fat mass compared with those fed a high-fat cellulose (control) diet. Both the Salecan groups significantly and dose-dependently improved glucose tolerance, with a 9 and 26% reduction of glucose AUC, respectively. Liver and adipose tissue weights were also significantly decreased by the Salecan treatment. Supplementation with 5% Salecan led to lower serum TAG, total cholesterol (TC) and HDL-cholesterol (52, 18 and 19%, respectively) and lower hepatic TAG by 56% and TC by 22% compared with the high-fat cellulose control group. Dietary Salecan intake caused an obvious elevation of fat in the faeces. Supplementation with Salecan disturbed bile acid-promoted emulsification and reduced the size of emulsion droplets *in vitro*. These results indicate that Salecan decreases fat absorption, improves glucose tolerance and has biologically important, dose-related effects on reducing high-fat diet-induced obesity.

Key words: Salecan: β-Glucans: High-fat diet: Obesity: Glucose tolerance

Obesity represents one of the most serious global health issues. Environmental and genetic factors play an important role in the increase in obesity that is affecting the whole of mankind on a large scale. Among these, diet-induced obesity has become one of the most critical medical problems in the world[1]. Obesity is defined medically as a state of increased body weight, more specifically adipose tissue, of sufficient magnitude to produce adverse health consequences[2,3]. The excessive fat accumulation in adipose tissue, liver and other organs strongly predisposes obese individuals to the development of metabolic changes that increase overall morbidity risk. Obesity is associated with insulin resistance[4], a state of low-grade chronic inflammation[5] and the metabolic syndrome. The metabolic syndrome is related to higher circulating levels of inflammatory markers, many of which enhance tumour growth[6]. Clearly, prevention and management of obesity are relevant to health promotion.

The predominant obesity-causing factor is energy imbalance. While pharmaceutical treatments for obesity have been extensively researched, only a few drugs have been approved for long-term use in significantly obese patients by the Food and Drug Administration. However, they have adverse effects including gastrointestinal discomfort, flatulence and diarrhoea[7]. In addition to prescription drugs, nutritional supplements for weight loss are popular in the over-the-counter market. Although such treatments are widely used, few have been proved to be safe and effective[8]. Hence, it is necessary to find more effective and safe treatments through the inhibition of digestion and absorption of dietary fat which is the target for obesity treatment.

A number of studies have documented that dietary fibres provide a variety of human health benefits. β-Glucans are non-digestible polysaccharides that are widely found in nature in sources such as cereal grains, including oats and barley, as well as in yeast, bacteria, algae and mushrooms[9–11]. Cereals are the most common source of dietary β-glucans and are linear glucose polymers of three to four glucose residues of (1 → 4)-β-D linkages and separated by β-(1 → 3) linkages, and exhibit only partial solubility in water[9]. Yeast β-glucan is a branched polysaccharide consisting of a backbone chain of (1 → 3)-β-D-glucopyranosyl units, along which are randomly dispersed side chains of D-glucopyranosyl units attached by β-(1 → 6) linkages, and is insoluble in water[10]. The cholesterol-lowering effect of cereal or yeast has been reported[12–14],

Abbreviations: HFD, high-fat diet; HF-2%S, high-fat diet with 2% Salecan; HF-5%S, high-fat diet with 5% Salecan; LFD, low-fat diet; TC, total cholesterol.

* **Corresponding author:** Professor J. Zhang, fax +86 25 84318533, email jfzhang@mail.njust.edu.cn

and the mechanisms of action of glucans in lowering plasma cholesterol have been wildly studied. Specifically, Marlett *et al.*[15] have shown that induction of bile acid excretion is one possible mechanism. The increased viscosity of gastrointestinal content by β-glucans is also a key factor for the decrease in cholesterol level. Viscosity depends on the solubility of β-glucan and its molecular weight[16]. Raw oat bran, because of the low solubility of β-glucans, has no significant effect on cholesterol level[17]. However, commercial oat bran has already been heated and forms viscous solutions and has a significant cholesterol-lowering effect[18]. The solubility of fibre leads to a change in viscosity, which has a powerful impact on plasma cholesterol[19].

Salecan is a novel water-soluble glucan produced by *Agrobacterium* sp. ZX09. It is an extracellular polysaccharide consisting of the following repeating unit: → 3)-β-D-Glc*p*-(1 → 3)-(β-D-Glc*p*-(1 → 3)-β-D-Glc*p*-(1 → 3))$_3$-α-D-Glc*p*-(1 → 3)-α-D-Glc*p*-(1 → . The production and chemical properties of Salecan have been reported in a previous study[20]. The average molecular weight of Salecan was estimated to be about 2×10^6 Da from a calibration curve of standard dextrans obtained by gel filtration on Sepharose CL-4B[20]. Salecan solutions are highly viscous even at low polymer concentrations. Acute and subchronic studies have demonstrated that administration of Salecan induced no toxicity[21] and Salecan has excellent rheological properties and could be utilised in the food industry[22]. The aim of the present study was to investigate the effects of Salecan on diet-induced obesity and fat accumulation in mice.

Materials and methods

Preparation of Salecan

The preparation of Salecan has been described previously[20]. Briefly, the strain ZX09 used in the present study was isolated from a soil sample from the ocean coast of Shandong, China. Cultures were maintained on Htm agar consisting of 1 g NaH_2PO_4, 3 g KNO_3, 0·07 g $CaCl_2$, 0·2 g $MgCl_2$, 0·0125 g $FeSO_4.7H_2O$, 0·003 g $MnSO_4$, 0·0075 g $ZnCl_2$, 20 g sucrose, 9 g agar and 1000 ml water, pH 7·2. A colony of the strain ZX09 was inoculated into a 250 ml flask containing 50 ml medium consisting of 2% sucrose and mineral salt solution. The inoculated preparation was incubated at 28°C on a rotary shaker at 220 rpm for 24 h. A 0·5 ml portion was transferred to a 250 ml flask containing 50 ml fermentation medium. Fermentation was performed on a rotary shaker at 220 rpm for 48 h. The culture broth was diluted more than three times with deionised water and centrifuged at 12 000 ***g*** for 30 min to separate cells from the supernatant. The supernatant was added to two volumes of 95% ethanol. Recovery of Salecan was expressed in terms of weight after ethanol precipitation collected by centrifugation at 6000 ***g*** for 15 min and dried under reduced pressure.

Animals and diets

Female C57BL/6 mice, 6 weeks old, were used in the present study. Animals were maintained under controlled environmental conditions of temperature (22 ± 2°C) and 12 h light–12 h dark cycles with lights on from 07.00 to 19.00 hours, and with free access to food and water. All animal care and use procedures were in accordance with the guidelines of the Institutional Animal Care and Use Committee at Nanjing University of Science & Technology. Mice were divided into four groups (n 8): low-fat diet (LFD), high-fat diet (HFD) only, HFD with 2% Salecan (HF-2%S) and HFD with 5% Salecan (HF-5%S) for 6 weeks. Preparation of the experimental diets was according to the method described previously with some modifications[23]. Salecan has the composition of 77% sugar, 6% protein and 13·8 kJ/g (3·3 kcal/g) energy[20]. By adding the different amounts of casein, maize starch, lard oil and cellulose to the last three different diets (HFD, HF-2%S and HF-5%S), the total energy and the final proportion of major macronutrients are the same across the experimental diets. The LFD provided a total energy of 16·3 kJ/g (3·9 kcal/g), i.e. 12% from fat, 67% from carbohydrate and 21% from protein, and the HFD, HF-2%S and HF-5%S provided a total energy of 23·4 kJ/g (5·6 kcal/g), i.e. 64% from fat, 21% from carbohydrate and 15% from protein. The compositions of each diet are shown in Table 1. The amounts of food and water in the feeding containers were measured every day at 09.00 hours. Food and water intake were expressed as g/d per mouse.

Body composition measurements

Body weights were measured for each animal in duplicate just before body composition measurements using a standard scale, and the mean body weight was used for analysis. The Bruker Minispec (Bruker Optics), which employs NMR technology to estimate the body composition of the animals, was used to assess body composition. The percentage of fat mass of each animal was calculated at each time point as fat mass divided by body weight.

Glucose tolerance tests

Intraperitoneal glucose tolerance tests were performed after 4 weeks of treatment. After 14 h of fasting, mice were

Table 1. Composition of the experimental diets

	Diets (g/kg)			
Ingredients	LFD	HFD	HF-2%S	HF-5%S
Casein	200	200	198	196
Maize starch	650	150	136	113
Sucrose	0	150	150	150
Maize oil	50	0	0	0
Lard oil	0	400	400	400
Cellulose	50	50	47	41
Mineral mixture*	35	35	35	35
Vitamin mixture†	10	10	10	10
DL-Met	3	3	3	3
Choline bitartrate	2	2	2	2
Salecan	0	0	20	50

AIN, American Institute of Nutrition.
LFD, low-fat diet; HFD, high-fat diet; HF-2%S, high-fat diet with 2% Salecan; HF-5%S, high-fat diet with 5% Salecan.
* AIN-93M mineral mixture (ICN).
† AIN-93VX vitamin mixture (ICN).

injected intraperitoneally with glucose (2 g/kg body weight). Oral glucose tolerance tests were performed after 6 weeks of treatment. Mice fasted for 18 h were given glucose orally by intragastric tube to provide 1 g glucose/kg body weight. Blood glucose was determined before and after treatment using a One Touch Blood Glucose Meter (AW063-436-01A; LifeScan Inc.) with 3 μl of the whole blood obtained by tail bleed. AUC values were calculated and normalised to baseline to measure glucose tolerance.

Sampling and biochemical assays

Faeces obtained during 24 h were collected and weighed, and lipids were extracted with chloroform–methanol (2:1, v/v) according to the method described previously[24]. After 6 weeks of dietary treatment, mice were anaesthetised at 09.00 hours (i.e. non-fasting) and blood was collected from a carotid artery. Liver and parametrial white adipose tissues were collected, weighed and immediately frozen in liquid N_2 for further analysis. Serum was then separated by centrifugation at 3000 ***g*** for 15 min and stored at −80°C until analysis. Extraction of lipids from the liver was according to the method described previously[25]. Briefly, hepatic tissue was homogenised (10 %, w/v) in isopropanol for 20 s. To extract TAG and cholesterol, the homogenate was kept at 4°C for 2 d, and then centrifuged at 1000 ***g*** for 10 min. Aliquots of the supernatants were analysed for TAG and cholesterol. Concentrations of serum TAG, total cholesterol (TC), HDL-cholesterol, hepatic TAG, TC and faecal TAG were determined using the Triglyceride Test Kit (Beijing BHKT Clinical Reagent) by an enzymatic method.

Histopathology

Liver and parametrial adipose samples were fixed in 10 % formalin–saline, embedded in paraffin, and then sectioned. Sections (5 μm) were stained with haematoxylin and eosin dye for histopathological examination by light microscopy.

Emulsion preparation

Preparation of emulsions was according to the method described previously with some modifications[26]. Briefly, Salecan was dissolved in buffer solution (50 mM-Tris–HCl, 150 mM-NaCl, pH 7·5) under stirring at room temperature. The emulsions were prepared by mixing 4 μl olive oil into 10 μl buffer solution (with 0·3, 0·6 % Salecan or without Salecan), and a fixed volume of fresh murine bile samples (obtained just after killing) was added to a final volume of 16 μl. The tubes were capped and shaken at 1000 strokes/min for 20 min at 37°C. The samples were examined and photographed immediately after preparing the emulsion under bright field illumination on a photomicroscope. The procedure was duplicated by taking a second sample from the same emulsion.

Statistical analysis

All data are expressed as means with their standard errors. Statistical analysis was performed using SPSS 13.0 software (SPSS Inc.), and differences between groups were analysed by one-way ANOVA followed by Tukey's *post hoc* test. Differences between means were considered statistically significant at $P < 0{\cdot}05$.

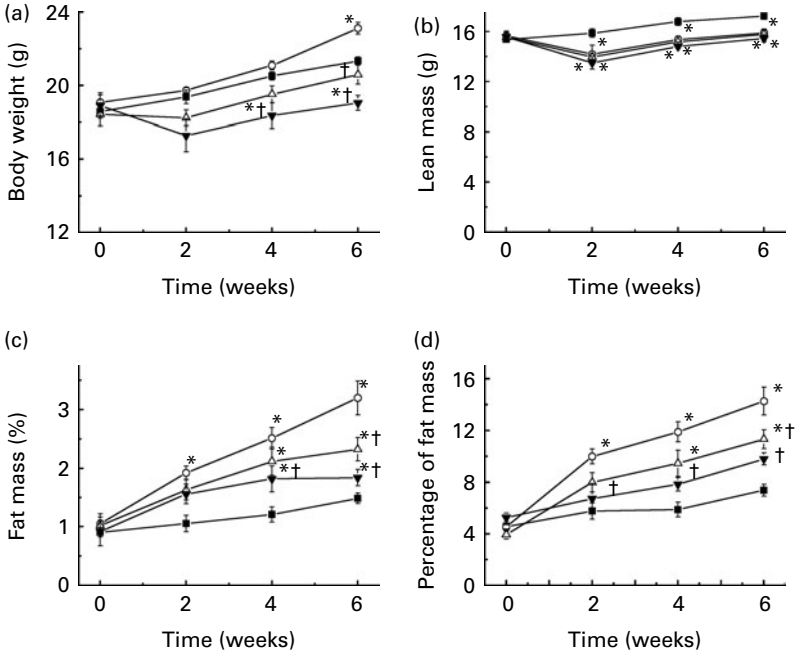

Fig. 1. Effects of Salecan on body weight and body composition in high-fat diet-fed mice. (a) Body weight, (b) lean mass and (C) fat mass were determined at weeks 0, 2, 4 and 6. (d) The percentage of fat mass of each animal was calculated at each time point as fat mass divided by body weight. Values are means of eight animals, with standard errors represented by vertical bars. *Mean values were significantly different from those of the low-fat diet (LFD, —■—) group ($P < 0{\cdot}05$). †Mean values were significantly different from those of the high-fat diet (HFD, —○—) group ($P < 0{\cdot}05$). –△–, High-fat diet with 2 % Salecan; —▼—, high-fat diet with 5 % Salecan.

Table 2. Effect of Salecan on liver and adipose tissue weight
(Mean values with their standard errors; *n* 8)

	Liver weight (g)		Liver (percentage of total body weight)		Parametrial white adipose tissue (g)		Parametrial white adipose tissue (percentage of total body weight)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
LFD	1·02	0·098	4·78	0·47	0·55	0·030	2·58	0·14
HFD	1·44*	0·021	6·23*	0·14	1·38*	0·048	5·98*	0·12
HF-2 %S	1·22	0·022	6·03	0·13	0·74†	0·11	3·55†	0·44
HF-5 %S	1·12†	0·037	5·87†	0·31	0·61†	0·11	2·82†	0·36

LFD, low-fat diet; HFD, high-fat diet; HF-2 %S, HFD with 2 % Salecan; HF-5 %S, HFD with 5 % Salecan.
* Mean values were significantly different from those of the LFD group ($P < 0·05$).
† Mean values were significantly different from those of the HFD group ($P < 0·05$).

Results

Decreased body weight and fat in mice fed with Salecan

Compared with the LFD group, mice fed on the HFD (HFD group) gained more body mass during the feeding periods, and reached a higher body weight at the end of the experiment ($P<0·05$). In the HF-2 %S and HF-5 %S groups, body weight was reduced by 11 and 18 % compared with the HFD group, respectively (Fig. 1(a)). No significant differences were found between the HFD, HF-2 %S and HF-5 %S groups for lean mass (Fig. 1(b)). Both HF-2 %S and HF-5 %S groups markedly decreased body fat mass (28 and 43 %, respectively) and the percentage of fat mass (21 and 31 %, respectively) compared with the HFD group (Fig. 1(c), (d)).

Feeding Salecan lowered lipid levels in the liver and adipose tissue

As shown in Table 2, the 5 % Salecan treatment (HF-5 %S group) decreased liver weight by 22 %, and decreased parametrial white adipose tissue weight by 56 %. Histological analysis of hepatocytes revealed that the HFD group had severe fat anomalies with a large area of hepatocytes taken over by fat vacuoles compared with the LFD group (Fig. 2(a), (b)). There were some lipid vacuoles in the HF-2 %S group, but the granularity and quantity of lipid vacuoles were much less compared with the HFD group (Fig. 2(c)). There were only a few small lipid vacuoles in the HF-5 %S group, and the appearance was very similar to that of the LFD group (Fig. 2(d)). The adipocyte diameter in parametrial adipose tissue was much larger in the HFD group than in the LFD group (Fig. 2(e), (f)). Haematoxylin–eosin staining showed an apparent reduction of adipocyte size in the Salecan treatment groups (Fig. 2(g), (h)). The lipid profiles of plasma and liver are shown in Table 3. Mice in the HFD group had higher TAG and cholesterol compared with the LFD group. The elevated lipid profiles in HFD-fed mice were alleviated by the Salecan treatment. There were differences between the HF-2 %S and HFD groups in serum and hepatic TAG levels ($P<0·05$). Compared with the HFD group, the HF-5 %S group showed lower serum TAG

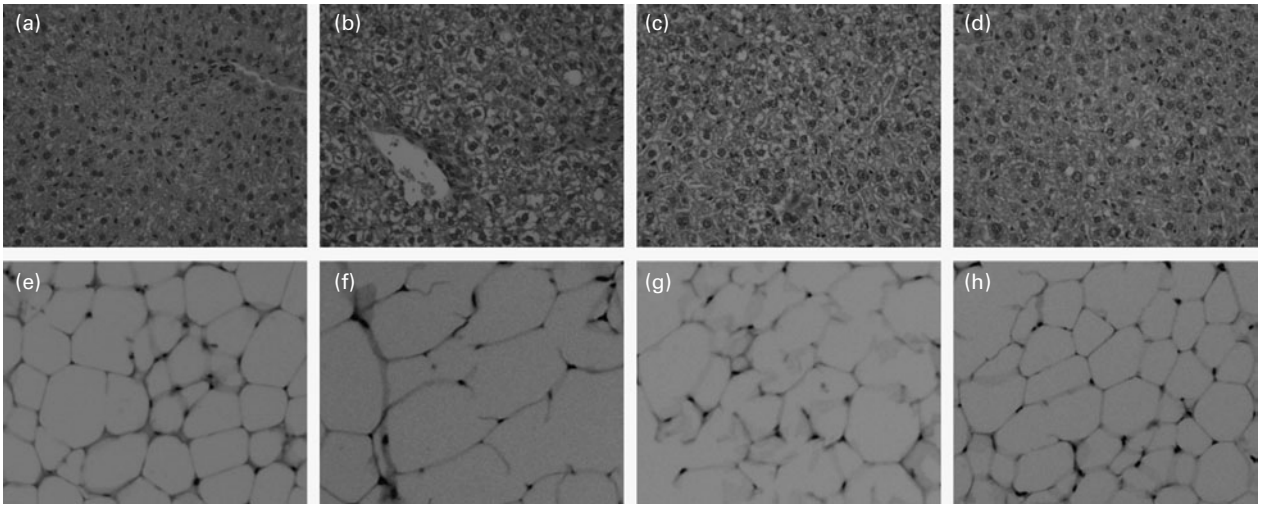

Fig. 2. Effects of Salecan on liver fat accumulation and adipocyte size. (a–d) Hepatic pathological variations of the experimental animals. (e–h) Adipocyte size variations of the experimental animals. Mice (6 weeks old) were provided a low-fat diet (LFD) or high-fat diets (HFD) containing 0, 2 or 5 % Salecan for 6 weeks, and liver and parametrial adipose were removed for histological examination. For each group, six mice were examined and sixty images were taken. The image shown here for each group was chosen randomly from the sixty images taken from this group. Original magnification, 400 ×. HF-2 %S, high-fat diet with 2 % Salecan; HF-5 %S, high-fat diet with 5 % Salecan.

British Journal of Nutrition

Table 3. Effect of Salecan on serum and hepatic lipid composition
(Mean values with their standard errors; *n* 8)

	Serum						Liver			
	TAG (mmol/l)		TC (mmol/l)		HDL-C (mmol/l)		TAG (mg/g liver)		TC (mg/g liver)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
LFD	0·47	0·061	1·51	0·10	1·48	0·098	18·03	7·39	1·78	0·17
HFD	0·95*	0·057	2·58*	0·077	2·23*	0·040	39·23*	8·14	2·66*	0·40
HF-2 %S	0·68*†	0·043	2·55*	0·11	2·21*	0·072	21·04*†	1·45	1·91†	0·098
HF-5 %S	0·46†‡	0·047	2·11*†	0·21	1·81*†‡	0·098	17·41†	4·86	2·07†	0·21

TC, total cholesterol; HDL-C, HDL-cholesterol; LFD, low-fat diet; HFD, high-fat diet; HF-2 %S, HFD with 2 % Salecan; HF-5 %S, HFD with 5 % Salecan.
* Mean values were significantly different from those of the LFD group ($P < 0·05$).
† Mean values were significantly different from those of the HFD group ($P < 0·05$).
‡ Mean values were significantly different from those of the HF-2 %S group ($P < 0·05$).

by 52 %, TC by 18 %, HDL-cholesterol by 19 %, and lower hepatic TAG and TC by 56 and 22 %, respectively. Serum and hepatic TAG levels in the HF-5 %S group were almost similar to those in the LFD group.

Diet and glucose tolerance

HFD-induced obesity was associated with alterations in liver and adipose lipid metabolism, displaying impaired glucose tolerance in HFD mice. Intraperitoneal glucose tolerance tests were performed on mice after 4 weeks of dietary treatment. The concentrations of blood glucose in LFD, HFD, HF-2 %S and HF-5 %S mice reached a maximum at 30 min after the intraperitoneal administration of glucose, and then declined to the basal value. The HFD group significantly impaired the glucose response during 2 h glucose tolerance tests compared with the LFD group (Fig. 3(a)). The rise and fall of blood glucose in the HF-5 %S group was similar to that in the LFD group (Fig. 3(a)), suggesting that Salecan could improve the glucose tolerance of mice fed the HFD. Compared with the HFD group, the AUC for glucose was lower in the HF-5 %S group ($P< 0·05$), and tended to be lower in the HF-2 %S group (Fig. 3(b)). Additionally, an oral glucose tolerance test was conducted after 6 weeks of food consumption. Glucose concentrations 30 min after the

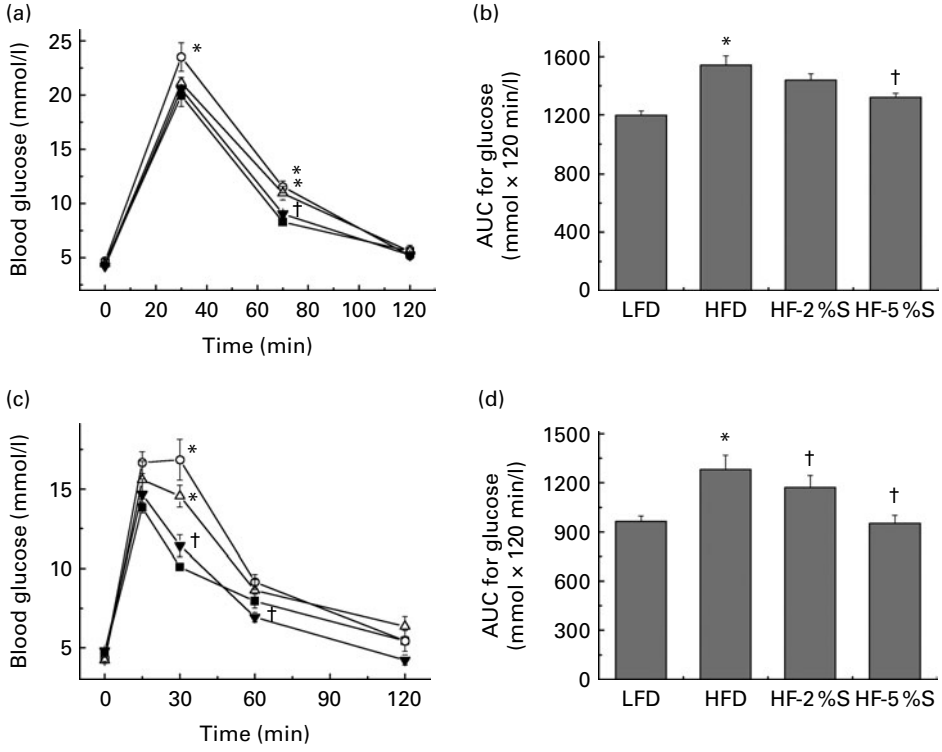

Fig. 3. Effects of Salecan on glucose tolerance in high-fat diet (HFD)-fed mice. (a) Intraperitoneal glucose tolerance tests were performed after 4 weeks of food consumption and (b) AUC values were calculated and normalised to baseline. Mice were intraperitoneally given 2 g glucose/kg body weight after 14 h of food deprivation. (c) Oral glucose tolerance tests were performed after 6 weeks of food consumption and (d) AUC values were calculated and normalised to baseline. Mice were intragastrically given 1 g glucose/kg body weight after 18 h of food deprivation. Values are means of five to six animals, with standard errors represented by vertical bars. * Mean values were significantly different from those of the low-fat diet (LFD, —■—) group ($P < 0·05$). † Mean values were significantly different from those of the HFD (—○—) group ($P < 0·05$). —△—, HFD with 2 % Salecan (HF-2 %S); —▼—, HFD with 5 % Salecan (HF-5 %S).

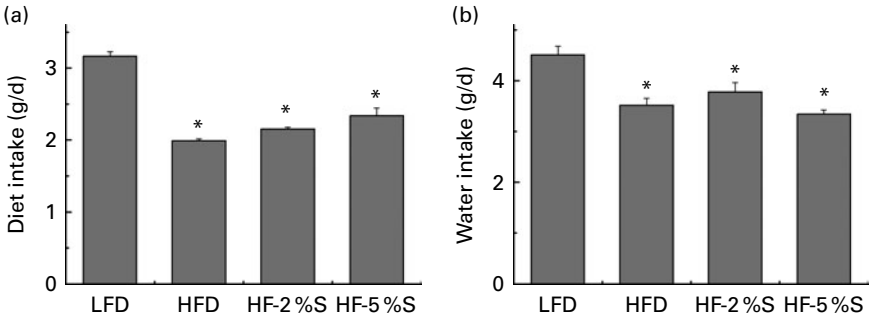

Fig. 4. Effect of Salecan on food and water intake. (a) Food intake and (b) water intake are expressed as g/d per mouse. Values are means of eight animals, with standard errors represented by vertical bars. * Mean values were significantly different from those of the low-fat diet (LFD) group ($P < 0·05$). † Mean values were significantly different from those of the high-fat diet (HFD) group ($P < 0·05$). HF-2 %S, high-fat diet with 2 % Salecan; HF-5 %S, HFD with 5 % Salecan.

oral administration of glucose in the HFD group were higher than those in the LFD group ($P<0·05$; Fig. 3(c)). Salecan markedly and dose-dependently reduced the blood glucose level (Fig. 3(c)), with a 9 and 26 % reduction of glucose AUC in the HF-2 %S and HF-5 %S groups compared with the HFD group (Fig. 3(d)).

Mice fed on Salecan increased fat excretion in the faeces

We further investigated whether Salecan could change food consumption. After having free access to the diets and water for 6 weeks, there were no obvious differences among the HFD, HF-2 %S and HF-5 %S groups in mean diet and water intake (Fig. 4(a), (b)). Next, we measured faeces weight and composition. As shown in Table 4, all groups fed on the HFD showed a lower faeces weight compared with the LFD group. Mice fed the HFD containing 2 and 5 % Salecan (HF-2 %S and HF-5 %S groups) increased faecal mass (23 and 51 %) and had a higher TAG level in the faeces compared with the HFD group (27 and 69 %), implying that Salecan could disturb fat digestion and absorption.

Salecan altered bile acid-promoted emulsification of fat in vitro

To clarify the effect of Salecan on fat digestion, an *in vitro* test of emulsification of fat by bile acids in the presence of Salecan was performed. Emulsion droplet size was determined by light microscopy. Without Salecan, the droplets were densely packed, small in mean diameter and distributed over a narrower size range (Fig. 5(a)). Fig. 5(b) shows large oil droplets produced by 0·3 % Salecan dispersed among small oil droplets. Fig. 5(c) shows the micrographs of 0·6 % Salecan, showing much larger emulsion droplet size compared with the 0 % Salecan group. The size of the emulsified droplets was increased by raising the concentration of Salecan. These data suggested that Salecan disturbed the bile acid-promoted emulsification of fat.

Table 4. Effect of Salecan on fat excretion in the faeces of mice fed a high-fat diet (HFD)

(Mean values with their standard errors; *n* 6–7)

	Faeces weight (g/d)		TAG in faeces (μmol/g faeces)	
	Mean	SEM	Mean	SEM
LFD	1·40	0·041	15·02	2·37
HFD	0·43*	0·023	51·39*	6·06
HF-2 %S	0·53*	0·026	65·35*	0·53
HF-5 %S	0·65*†‡	0·050	86·70*†‡	17·66

LFD, low-fat diet; HF-2 %S, HFD with 2 % Salecan; HF-5 %S, HFD with 5 % Salecan.
* Mean values were significantly different from those of the LFD group ($P < 0·05$).
† Mean values were significantly different from those of the HFD group ($P < 0·05$).
‡ Mean values were significantly different from those of the HF-2 %S group ($P < 0·05$).

Discussion

Bacterial extracellular polysaccharides (such as xanthan or gellan gum) can be easily produced and applied as thickeners and stabilisers in foods as food additives. Salecan, a novel soluble extracellular β-glucan, produced by a marine bacterium ZX09, contains a large amount of β-1,3-D-glucosidic linkages as the main backbone structure, together with a small proportion of α-1,3-D-glucosidic linkages[20]. The differences between the β-glucan linkages and the chemical structure are significant with regard to solubility and overall biological activity[27,28]. β-Glucans are not digestible due to a lack of hydrolase in both humans and mice, and are constituents of dietary fibre. NSP are major components of dietary fibre, which resist human small-intestinal digestion. Glucans, by their very nature, are components of fibre. The actual direct energy dilution by NSP is generally very small in human diets as only 20–30 g/d are consumed. Then, it is well recognised that dietary fibres provide less energy as a substitute for nutrients in diets, promote satiation and prolong satiety[29]. Soluble dietary fibre increases stool volume and stool water content[30,31], and can increase the viscosity of gut contents, carrying undigested food into the colon. A short-term feeding study of Salecan in mice showed no toxic effects[21]. Salecan, as a novel soluble dietary fibre, has a potential for promoting good heath and preventing obesity-related diseases.

Many studies have been carried out to investigate the effects of soluble fibres on body weight in mice and human subjects. Oat bran β-glucan at a concentration of 10 % lowers body weight[32]. However, a meta-analysis that combined the results of eleven randomised controlled trials found that guar gum supplements were not effective in reducing body weight[33].

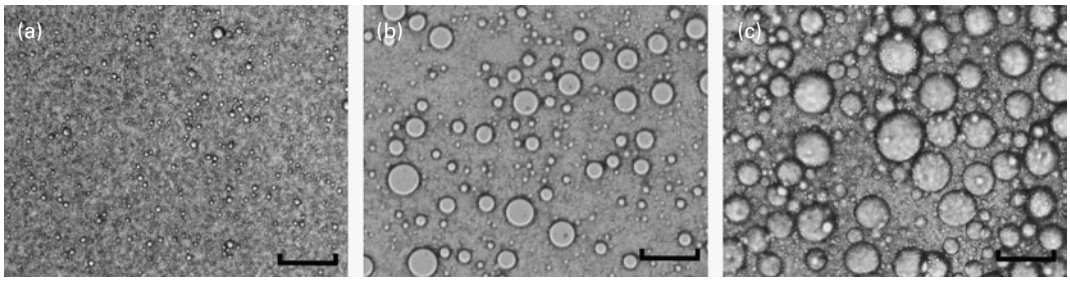

Fig. 5. Effects of Salecan on lipid emulsification. Photomicrographs of emulsion prepared with (a) 0 %, (b) 0·3 % or (c) 0·6 % Salecan under bright field illumination. Each experiment was performed at least three times. For each group, thirty images were taken. The image shown here for each group was chosen randomly from the thirty images taken from this group. Original magnification, 100 × . Scale bar = 100 μm.

In the present study, the HF-2 %S and HF-5 %S groups displayed a lower body weight than the HFD group. No significant differences were found between the HFD, HF-2 %S and HF-5 %S groups for lean mass. Fat mass and the percentage of fat mass were lower in the HF-2 %S and HF-5 %S groups compared with the HFD group. Previous studies showed that viscous soluble dietary fibres reduced lipid emulsification[34]. Similarly, we found that Salecan could disturb bile acid-promoted emulsification and increase the size of emulsified fat droplets. The size and composition of the emulsified droplets alter the binding and the hydrolytic activity of pancreatic lipase[35,36]. Then, TAG excretion was significantly increased in the faeces of mice by Salecan supplementation. It has been suggested that Salecan reduced the rate of fat digestion and absorption by reducing lipid emulsification.

Liver and adipose tissue weights and adipose size were increased in HFD-fed mice[37,38]. A previous study has reported that barley β-glucan significantly reduced liver weight, but not adipose tissue weight[39]. Another previous study has found that barley significantly reduced adipose tissue in mice fed a HFD[40]. According to the present results, Salecan treatment significantly reduced both liver and adipose tissue weights. Oats were first found to have a cholesterol-lowering effect and the active component was identified as β-glucan[41]. Oats reduced both serum TC and LDL-C compared with the control[42,43], and barley has also been shown to have a similar effect[44,45]. However, oat and barley β-glucans do not improve TAG[46]. Similarly to the previous studies, we also observed that the intake of Salecan might help mice maintain lower serum levels of TC and HDL-cholesterol compared with the HFD group; moreover, we also found that serum TAG levels decreased by the Salecan treatment. Consequently, HFD-induced impaired glucose tolerance was improved by feeding Salecan, indicating a potential application in treating the metabolic syndrome. Increasing the intake of fibre in one's diet may lead to socially unacceptable symptoms such as bloating, cramping and flatulence[47,48]. These side effects are the result of colonic fermentation of soluble fibre leading to the production of gases and volatile SCFA[49]. However, most studies have reported no complaints of gastrointestinal distress after a high intake of dietary fibre[50,51]. Further studies will be needed to see whether Salecan has any gastrointestinal side effects.

In summary, consumption of Salecan suppressed weight gain, decreased body fat content and markedly improved glucose tolerance in mice fed the HFD. Furthermore, feeding Salecan lowered liver and parametrial adipose tissue weights, and protected against liver and adipocyte fat accumulation. The present findings suggest that foods containing Salecan could be useful in treating diet-induced obesity.

Acknowledgements

This study was supported by the National Science Foundation of China (30730030). The authors' contributions are as follows: Y. Z. performed the experiments, analysed the data, prepared the images and drafted the manuscript; L. X. performed the experiments, analysed the data and edited the manuscript; W. P. performed the experiments and analysed the data; T. W. prepared the images; P. C. analysed the data; B. Z. analysed the data; J. Z. conceived and designed the research, edited and revised the manuscript, and approved the final version of the manuscript. The authors declare that they have no conflict of interest.

References

1. Ogden CL, Carroll MD, Curtin LR, *et al.* (2006) Prevalence of overweight and obesity in the United States, 1999–2004. *JAMA* **295**, 1549–1555.
2. Spiegelman BM & Flier JS (2001) Obesity and the regulation of energy balance. *Cell* **104**, 531–543.
3. Metges CC (2009) Early nutrition and later obesity: animal models provide insights into mechanisms. *Adv Exp Med Biol* **646**, 105–112.
4. Kahn BB & Flier JS (2000) Obesity and insulin resistance. *J Clin Invest* **106**, 473–481.
5. Stienstra R, Duval C, Müller M, *et al.* (2007) PPARs, Obesity, and Inflammation. *PPAR Res* **2007**, 95974.
6. Pischon T, Nöthlings U & Boeing H (2008) Obesity and cancer. *Proc Nutr Soc* **67**, 128–145.
7. Heck AM, Yanovski JA & Calis KA (2000) Orlistat, a new lipase inhibitor for the management of obesity. *Pharmacotherapy* **20**, 270–279.
8. Pittler MH & Ernst E (2004) Dietary supplements for body-weight reduction: a systematic review. *Am J Clin Nutr* **79**, 529–536.
9. Delaney B, Nicolosi RJ, Wilson TA, *et al.* (2003) Beta-glucan fractions from barley and oats are similarly antiatherogenic in hypercholesterolemic Syrian golden hamsters. *J Nutr* **133**, 468–475.
10. Babícek K, Cechová I, Simon RR, *et al.* (2007) Toxicological assessment of a particulate yeast (1,3/1,6)-beta-D-glucan in rats. *Food Chem Toxicol* **45**, 1719–1730.

11. McIntosh M, Stone BA & Stanisich VA (2005) Curdlan and other bacterial (1(3)-beta-D-glucans. *Appl Microbiol Biotechnol* **68**, 163–173.
12. Reyna-Villasmil N, Bermúdez-Pirela V, Mengual-Moreno E, *et al.* (2007) Oat-derived beta-glucan significantly improves HDLC and diminishes LDLC and non-HDL cholesterol in overweight individuals with mild hypercholesterolemia. *Am J Ther* **14**, 203–212.
13. AbuMweis SS, Jew S & Ames NP (2010) β-Glucan from barley and its lipid-lowering capacity: a meta-analysis of randomized, controlled trials. *Eur J Clin Nutr* **64**, 1472–1480.
14. Nicolosi R, Bell SJ, Bistrian BR, *et al.* (1999) Plasma lipid changes after supplementation with beta-glucan fiber from yeast. *Am J Clin Nutr* **70**, 208–212.
15. Marlett JA, Hosig KB, Vollendorf NW, *et al.* (1994) Mechanism of serum cholesterol reduction by oat bran. *Hepatology* **20**, 1450–1457.
16. Asp NG, Mattsson B & Onning G (1992) Variation in dietary fibre, beta-glucan, starch, protein, fat and hull content of oats grown in Sweden 1987–1989. *Eur J Clin Nutr* **46**, 31–37.
17. Würsch P & Pi-Sunyer FX (1997) The role of viscous soluble fiber in the metabolic control of diabetes. A review with special emphasis on cereals rich in beta-glucan. *Diabetes Care* **20**, 1774–1780.
18. de Groot A, Luyken R & Pikaar NA (1963) Cholesterol-lowering effect of rolled oats. *Lancet* **ii**, 303–304.
19. Carr TP, Gallaher DD, Yang CH, *et al.* (1996) Increased intestinal contents viscosity reduces cholesterol absorption efficiency in hamsters fed hydroxypropyl methylcellulose. *J Nutr* **126**, 1463–1469.
20. Xiu A, Kong Y, Zhou M, *et al.* (2010) The chemical and digestive properties of a soluble glucan from *Agrobacterium* sp. ZX09. *Carbohydr Polym* **82**, 623–628.
21. Xiu A, Zhan Y, Zhou M, *et al.* (2011) Results of a 90-day safety assessment study in mice fed a glucan produced by *Agrobacterium* sp. ZX09. *Food Chem Toxicol* **49**, 2377–2384.
22. Xiu A, Zhou M, Zhu B, *et al.* (2011) Rheological properties of Salecan as a new source of thickening agent. *Food Hydrocol* **25**, 1719–1725.
23. Bae IY, Lee S, Kim SM, *et al.* (2009) Effect of partially hydrolyzed oat β-glucan on the weight gain and lipid profile of mice. *Food Hydrocol* **23**, 2016–2021.
24. Folch J, Lees MS & Stanley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* **226**, 497–509.
25. Haug A & Høstmark AT (1987) Lipoprotein lipases, lipoproteins and tissue lipids in rats fed fish oil or coconut oil. *J Nutr* **117**, 1011–1017.
26. Pasquier B, Armand M, Guillon F, *et al.* (1996) Viscous soluble dietary fibers alter emulsification and lipolysis of triacylglycerols in duodenal medium *in vitro*. *J Nutr Biochem* **7**, 293–302.
27. Schmid F, Stone BA, McDougall BM, *et al.* (2001) Structure of epiglucan, a highly side-chain/branched (1 → 3;1 → 6)-beta-glucan from the micro fungus *Epicoccum nigrum* Ehrenb. ex Schlecht. *Carbohydr Res* **331**, 163–171.
28. Šandula J, Kogan G, Kačuráková M, *et al.* (1999) Microbial (1 → 3)-β-D-glucans, their preparation, physico-chemical characterization and immunomodulatory activity. *Carbohydr Polym* **38**, 247–253.
29. Lairon D (2007) Dietary fiber and control of body weight. *Nutr Metab Cardiovasc Dis* **17**, 1–5.
30. Burkitt DP (1971) Epidemiology of cancer of colon and rectum. *Cancer* **28**, 3–13.
31. Burkitt DP (1973) The role of refined carbohydrate in large bowel behaviour and disease. *Plant Food Man* **1**, 5–9.
32. Sanchez D, Muguerza B, Moulay L, *et al.* (2008) Highly methoxylated pectin improves insulin resistance and other cardiometabolic risk factors in Zucker fatty rats. *J Agric Food Chem* **56**, 3574–3581.
33. Pittler MH & Ernst E (2001) Guar gum for body weight reduction: meta-analysis of randomized trials. *Am J Med* **110**, 724–730.
34. Pasquier B, Armand M, Castelain C, *et al.* (1996) Emulsification and lipolysis of triacylglycerols are altered by viscous soluble dietary fibres in acidic gastric medium *in vitro*. *Biochem J* **314**, 269–275.
35. Borel P, Armand M, Ythier P, *et al.* (1994) Hydrolysis of emulsions with different triglycerides and droplet sizes by gastric lipase *in vitro*. Effect on pancreatic lipase activity. *J Nutr Biochem* **5**, 124–133.
36. Armand M, Borel P, Ythier P, *et al.* (1992) Effects of droplet size, triacylglycerol composition, and calcium on the hydrolysis of complex emulsions by pancreatic lipase: an *in vitro* study. *J Nutr Biochem* **3**, 333–341.
37. Nozaki Y, Fujita K, Yoneda M, *et al.* (2009) Long-term combination therapy of ezetimibe and acarbose for non-alcoholic fatty liver disease. *J Hepatol* **51**, 548–556.
38. Madsen L, Guerre-Millo M, Flindt EN, *et al.* (2002) Tetradecylthioacetic acid prevents high fat diet induced adiposity and insulin resistance. *J Lipid Res* **43**, 742–750.
39. Choi JS, Kim H, Jung MH, *et al.* (2010) Consumption of barley beta-glucan ameliorates fatty liver and insulin resistance in mice fed a high-fat diet. *Mol Nutr Food Res* **54**, 1004–1013.
40. Yokoyama WH & Shao Q (2006) Soluble fibers prevent insulin resistance in hamsters fed high saturated fat diets. *Cereal Foods World* **51**, 16–18.
41. Kerckhoffs DA, Brouns F, Hornstra G, *et al.* (2002) Effects on the human serum lipoprotein profile of beta-glucan, soy protein and isoflavones, plant sterols and stanols, garlic and tocotrienols. *J Nutr* **132**, 2494–2505.
42. Kerckhoffs DA, Hornstra G & Mensink RP (2003) Cholesterol-lowering effect of beta-glucan from oat bran in mildly hypercholesterolemic subjects may decrease when beta-glucan is incorporated into bread and cookies. *Am J Clin Nutr* **78**, 221–227.
43. Theuwissen E & Mensink RP (2007) Simultaneous intake of beta-glucan and plant stanol esters affects lipid metabolism in slightly hypercholesterolemic subjects. *J Nutr* **137**, 583–588.
44. Davy BM, Davy KP, Ho RC, *et al.* (2002) High-fiber oat cereal compared with wheat cereal consumption favorably alters LDL-cholesterol subclass and particle numbers in middle-aged and older men. *Am J Clin Nutr* **76**, 351–358.
45. Behall KM, Scholfield DJ & Hallfrisch J (2004) Diets containing barley significantly reduce lipids in mildly hypercholesterolemic men and women. *Am J Clin Nutr* **80**, 1185–1193.
46. Colhoun HM, Betteridge DJ, Durrington PN, *et al.* (2004) Primary prevention of cardiovascular disease with atorvastatin in type 2 diabetes in the Collaborative Atorvastatin Diabetes Study (CARDS): multicentre randomised placebo-controlled trial. *Lancet* **364**, 685–696.
47. Anderson JW & Gustafson NJ (1988) Hypocholesterolemic effects of oat and bean products. *Am J Clin Nutr* **48**, 749–753.
48. Eastwood MA & Passmore R (1983) Dietary fibre. *Lancet* **ii**, 202–206.

British Journal of Nutrition

49. Maki KC, Davidson MH, Torri S, *et al.* (2000) High-molecular-weight hydroxypropylmethylcellulose taken with or between meals is hypocholesterolemic in adult men. *J Nutr* **130**, 1705–1710.
50. Delaney B, Carlson T, Zheng GH, *et al.* (2003) Repeated dose oral toxicological evaluation of concentrated barley beta-glucan in CD-1 mice including a recovery phase. *Food Chem Toxicol* **41**, 1089–1102.
51. Story L, Anderson JW, Chen WJ, *et al.* (1985) Adherence to high-carbohydrate, high-fiber diets: long-term studies of non-obese diabetic men. *J Am Diet Assoc* **85**, 1105–1110.

British Journal of Nutrition