Diet Supplement Based on Radiation-Modified Chitosan and Radiation-Synthesized Polyvinylpyrrolidone Microgels: Influence on the Liver Weight in Rats Fed a Fat- and Cholesterol-Rich Diet

Renata Czechowska-Biskup,[1] Piotr Ulański,[1] Alicja K. Olejnik,[1] Grażyna Nowicka,[2] Bogumiła Panczenko-Kresowska,[2] Janusz M. Rosiak[1]

[1]*Institute of Applied Radiation Chemistry, Technical University of Łódź, 93-590 Łódź, Poland*
[2]*National Food and Nutrition Institute, 02-903 Warsaw, Poland*

Received 11 March 2006; accepted 23 June 2006
DOI 10.1002/app.26103
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: A polymer-based preparation (a chitosan–microgel preparation called CHM), intended for use as a diet supplement reducing fat and cholesterol absorption from the digestive tract, was obtained by the combination of chitosan of a reduced molecular weight with a polyvinylpyrrolidone microgel in a 1 : 1 (w/w) ratio. Both components were obtained with ionizing radiation (controlled degradation of chitosan in the solid state and crosslinking polymerization of neat vinylpyrrolidone). The chitosan component was shown in previous *in vitro* studies to exhibit a high fat-binding capacity, whereas the microgels, able to swell to about 40 times their original volume, were intended to serve as stomach-filling agents. The influence of the CHM preparation on the body mass, the weight of the liver and other principal organs, and the serum levels of triglycerides and cholesterol was examined in rats fed a fat- and cholesterol-rich diet (FChD). It was found that the preparation administered as a diet additive at 4% CHM (2% chitosan) in rats effectively prevented body mass gain, fat accumulation in the liver, and liver enlargement, which otherwise developed in the animals fed FChD only, whereas the weight of the other main organs (heart, liver, kidney, spleen, testicles, and lungs) remained unaltered. CHM was also capable of exerting this effect when administered to animals already in an atherosclerotic, overweight condition. No adverse effects were observed during the 10 weeks of feeding the animals the CHM-containing diet. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 105: 169–176, 2007

Key words: biological applications of polymers; chitosan; microgels; ionizing radiation

INTRODUCTION

Chitosan, a linear cationic polysaccharide obtained by the chemical deacetylation of chitin, is currently the main ingredient of numerous commercially available dietary supplements advertised to have a body-weight-reduction effect based on trapping dietary fat in the digestive tract in a way that prevents fat absorption. The chitosan contained in these formulations is also believed to reduce the level of total plasma cholesterol and triglycerides.

It is known that chitosan is able to bind fat *in vitro*, not only in the simplest tests based on mixing these two components[1,2] but also in somewhat more sophisticated biophysical models of the digestive tract,[3–5] in which the temperature, movement, residence time, and pH in subsequent parts of the tract are simulated. Upon a change in the pH from acidic to slightly basic, chitosan precipitates, encapsulating and trapping micelles and microdroplets of fat. Typically, 1 g of dry chitosan is reported to bind 5–20 g of fat, depending on the experimental procedure and kind of chitosan used (although in leaflets on commercial preparations, values >700 g/g are claimed[6]). There are also convincing, albeit limited, data resulting from studies on rats, in which the fat-trapping action of chitosan in the intestine and increase in fecal fat excretion have been clearly documented.[3]

The ability of chitosan to bind dietary fat and prevent its absorption is expected to reduce body weight or at least to partially prevent body weight increase caused by a fat-rich diet. Numerous studies on animals and humans have been focused on proving this idea. The results are not univocal. In several studies on rats, a pronounced reduction in body

This article is dedicated to the memory of Professor Marian Kryszewski.

Correspondence to: P. Ulanski (ulanskip@mitr.p.lodz.pl).

Contract grant sponsor: International Atomic Energy Agency (through a Technical Co-operation Project); contract grant number: POL/6/007.

Contract grant sponsor: Ministry of Science and Higher Education of Poland; contract grant numbers: 138/E-370/SPB/IAEA/KN/DWM77/2005-2007, 3 T08E 078 29, and 3 T08E 043 30.

weight gain has been in fact observed;[3,7] however, there are also reports on experiments in which no significant effect could be detected.[8–10] Similarly, in a large number of human studies, a significant reduction of body weight has been reported (e.g., refs. 11–14), whereas some other works indicate no effect or an effect that is statistically significant but too small to have real clinical meaning.[15–17] The differences in performance might be to some extent due to various testing procedures, including the chitosan dosage and time of the experiment. We believe, however, that a part of the problem lies in chitosan itself.

Chitosan is a copolymer of glucosamine and *N*-acetylglucosamine units. Its composition and chain length depend on the source and processing procedures. The physicochemical properties of chitosan largely depend on the weight-average molecular weight (M_w) and degree of deacetylation (i.e., the molar content of the glucosamine units), and these may vary significantly for samples of different origins. In our recent works,[4,5,18] we have shown that fat binding in an *in vitro* biopharmaceutical model of the digestive tract depends on M_w of chitosan, at least in the tested M_w range of 25–410 kDa. Within this range, the samples of the lowest M_w (25–50 kDa) exhibit over twice as high a fat-binding capacity as those of higher M_w, reaching about 20 g of fat/g of chitosan. Therefore, it seems that the application of low-molecular-weight chitosan (tens of kilodaltons) to diet additives should lead to relatively efficient fat binding.

Although unambiguous experimental confirmation of the correlation between the molecular weight of chitosan, *in vivo* fat binding, and body weight loss still requires systematic studies, in this communication, we focus not only on body mass changes but also on another effect expected to result from the oral administration of low-molecular-weight chitosan and binding of dietary fat, namely, reduced liver enlargement caused by fat accumulation in the liver of animals on a fat-rich diet. Such action, if proven on animals and on humans, could be an additional beneficial effect of chitosan-containing diet additives.

The aim of this study was to test, on rats, the influence of low-molecular-weight chitosan, administered for a long period at a low dietary level (2% pure chitosan) but with the average molecular weight in the range indicated by *in vitro* tests as the most effective in fat binding, on the body mass and on the weight of liver and other selected organs (heart, kidney, spleen, testicle, and lungs). The final levels of plasma triglycerides and cholesterol were tested as well.

Chitosan was administered in the form of a chitosan–microgel preparation (called CHM) being developed in our laboratory[19] and intended for use as a dietary food supplement of increased efficiency in reducing body weight and cholesterol levels. The formulation contains chitosan of a reduced molecular weight and a dry microgel (cf. ref. 20) made of a synthetic, hydrophilic polymer of proven nontoxicity and biocompatibility—polyvinylpyrrolidone (PVP; frequently used as an auxiliary substance in drug formulations and as the primary component in some biomaterials[21–23]). PVP microgels are designed to play a similar role to that of dietary fibers, that is, swelling in the stomach and thus inducing the feeling of the stomach being filled, which is one of the factors inducing the feeling of satiety. By using this component, we also expected to eliminate the effect observed in most previous studies,[3,9,10] in which the addition of chitosan was found to increase the food intake; this is an undesired phenomenon that could counterbalance the effects of chitosan on body weight reduction. The synthetic microgels used in our preparation, being covalently crosslinked polymer particles of high hydrophilicity, have a high equilibrium swelling ratio, may absorb hydrophilic food components, and move along the digestive tract without being destroyed, thus potentially providing also an additional antiabsorption effect. In future, such microgels may be, if necessary, also preloaded with a drug and/or a substance proven to enhance the fat-binding action of chitosan.

The influence of CHM on body mass and on the weight of the liver and other selected organs was investigated in rats fed an atherogenic, fat- and cholesterol-rich diet (FChD). The experiment plan included the formation of an animal group fed the high-fat diet (FChD), a group fed a diet supplemented with CHM for the whole experiment duration (10 weeks), and another group initially fed pure FChD for 4 weeks and subsequently fed FChD supplemented with CHM for the next 6 weeks. This setting was intended to check the efficiency of the CHM preparation under the metabolic conditions developing on a high-fat diet.

EXPERIMENTAL

Materials

Vinylpyrrolidone (VP; Fluka) was distilled under reduced pressure to eliminate the stabilizer. Chitosan (the starting material), a product of Primex Ingredients (Avaldsnes, Norway), had an M_w value of 395 kDa, as determined by multi-angle laser light scattering (see below). The degree of deacetylation (i.e., the molar fraction of glucosamine units estimated by potentiometric titration according to the method of Muzzarelli[24] adapted by Wojtasz-Pajak et al.[25]) was about 90% and did not change significantly during processing.

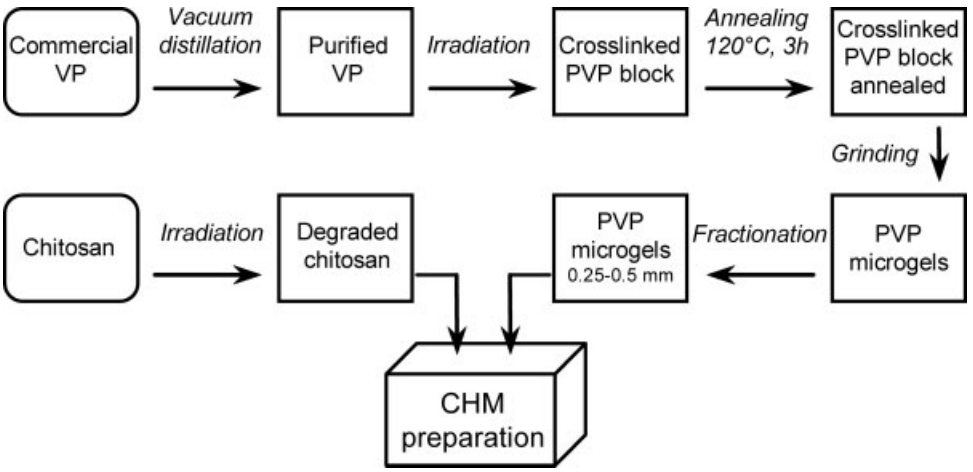

Figure 1 Production scheme for CHM.

All other chemicals were analytical-grade and were used as received.

Synthesis of the microgel component

Monomer samples (20 mL) were placed in glass ampules and saturated for 30 min with argon. Subsequently, the ampules were sealed and irradiated by ^{60}Co γ rays (average energy of the quantum = 1.25 MeV) with a dose of 25 kGy at a dose rate of 2.0 kGy/h. The monomer conversion was followed spectrophotometrically. The product, crosslinked PVP, was annealed for 3 h at 120°C to eliminate traces of the residual monomer and trapped free radicals. In the next step, the crosslinked PVP was ground; a 250–500-μm microgel fraction was separated and used in the CHM preparation.

Synthesis of chitosan of a defined average molecular weight

Chitosan in the form of a dry powder was placed in open glass ampules and irradiated by γ rays from a ^{60}Co source at a dose rate of 2.0 kGy/h. Changes in M_w were followed by multi-angle laser light scattering (a Brookhaven Instruments (Hottsville, NY) BI-SM 200 setup equipped with an Innova 90C argon-ion laser; λ = 514.5 nm) in a solution containing 8.5% formic acid and 0.5 mol/L sodium formate. The refractive-index increment of chitosan in this solvent was dn/dc = 0.174 mL/g.[26] Directly before the light scattering measurements, solutions were passed through filters of a 0.45-μm pore size (Sartorius, Göttingen, Germany). The intensity of scattered light was measured for five chitosan concentrations in the angular range of 30–120°. A Zimm plot algorithm[27] was applied for the evaluation of the results.

CHM preparation

The CHM dietary preparation was obtained through the mixing of dry modified chitosan with dry PVP microgel in a 1 : 1 weight ratio. This preparation was added to the diet of selected groups of animals (see below) at the 4% (by weight) level.

A scheme of the synthesis procedure is presented in Figure 1.

Animal study

Sixty male Wistar strain rats with an initial body weight average of 195.2 ± 1.5 g were used in the experiment. Animals were housed in individual cages and maintained in a 12-h/12-h light/dark cycle in a temperature- and humidity-controlled room. Rats were divided randomly into four treatment groups (for each group, n = 15). Rats in group A were fed laboratory pellet chow only, and rats in group B were fed FChD. Group BC rats were fed FChD for 4 weeks and for 6 weeks were fed FChD supplemented with CHM. Rats in group C were fed for 10 weeks FChD supplemented with CHM.

The experimental diet was prepared from basic laboratory pellet chow (powdered) with the addition of 10% lard and 0.5% cholesterol. Part of the experimental diet was supplemented with 4% CHM. The details are presented in Table I. The animals were given the diet and water *ad libitum*. In the fifth week of the experiment, the intake of the diet was evaluated: Rats were given a specified amount of the diet and after 24 h, the remaining diet was weighed.

At the end of the experiment, the rats were sacrificed by euthanasia. Selected organs (heart, liver, kidney, spleen, testicles, and lungs) were examined and weighted. The total serum cholesterol and triglyceride levels were measured by routine enzymatic tests.

TABLE I
Compositions of the Experimental Diets

	Animal group[a]				
			BC		
Component	A	B	0–4 weeks	5–10 weeks	C
Lard (wt %)	—	10	10	10	10
Cholesterol (wt %)	—	0.5	0.5	0.5	0.5
Cholic acid (wt %)	—	0.2	0.2	0.2	0.2
Methionine (wt %)	—	0.3	0.3	0.3	0.3
Cholic chloride (wt %)	—	0.2	0.2	0.2	0.2
CHM (wt %)	—	—	—	4	4
LSM (wt %)[b]	100	88.8	88.8	84.8	84.8

[a] A = standard animal chow only; B = cholesterol- and fat-rich diet; BC = cholesterol- and fat-rich diet followed by a cholesterol- and fat-rich diet supplemented by CHM; C = cholesterol- and fat-rich diet supplemented by CHM.
[b] Standard animal chow.

The animal studies were performed at the National Food and Nutrition Institute (Warsaw, Poland), and the study protocol was approved by the Local Ethical Commission on Animal Examination.

Each result is given as the mean plus or minus the standard error. The significance of the difference between the means of the control and test groups was determined by one-way analysis of variance followed by a Student test at $p < 0.05$.

RESULTS

Synthesis of the components by the radiation technique

The molecular weight of chitosan is often regulated by the controlled degradation of the raw biopolymer. Degradation can be preformed by a number of processes. A chemical treatment, such as acid hydrolysis, is often time- and energy-consuming, involves the use of chemicals, and generates waste. Moreover, being a multiparameter process, it is not always easy to control, although considerable progress in this technique has been achieved.[25,28–30] Also, enzymatic processing is a relatively complex procedure.[25,31,32]

Two alternative polysaccharide degradation methods are irradiation (i.e., treatment with ionizing radiation)[18,33–35] and sonication (treatment with ultrasound).[34,36] Irradiation can be applied to degrade a polysaccharide in any physical form (solid, suspension, paste, solution, etc.). The process is very efficient and can be easily controlled by the choice of a proper irradiation dose. No chemicals are needed, there is no additional processing, and there is no waste. Besides, under appropriate conditions, sterilization may be accomplished in parallel to the reduction in the molecular weight. Solid-state irradiation is probably the simplest chitosan degradation method in existence and thus has been employed in this work, although one should be aware of one drawback of this treatment, that is, the possibility of some radicals being trapped in crystalline regions of the polymer, resulting in further (albeit very slow) degradation after irradiation is stopped.[37,38]

The radiation–chemical yield of solid-state degradation for chitosan depends to some extent on the irradiation conditions and may also be influenced by the chemical composition and crystallinity of the starting material. Data in Figure 2 show the changes in M_w of the chitosan actually used for the synthesis of our preparation, along with more detailed data gained in a separate study performed at our laboratory on a chitosan sample of a similar starting molecular weight but a slightly lower degree of deacetylation. In the case of chitosan used in this work, our target M_w value of 60–70 kDa required γ irradiation of the starting material in a dry state with a dose of 60 kGy. This molecular weight is at the upper limit of the range in which fat binding is highly effective.[5,18] The purpose of targeting this upper limit is to allow for the very slow degradation processes (both natural hydrolysis in the presence of

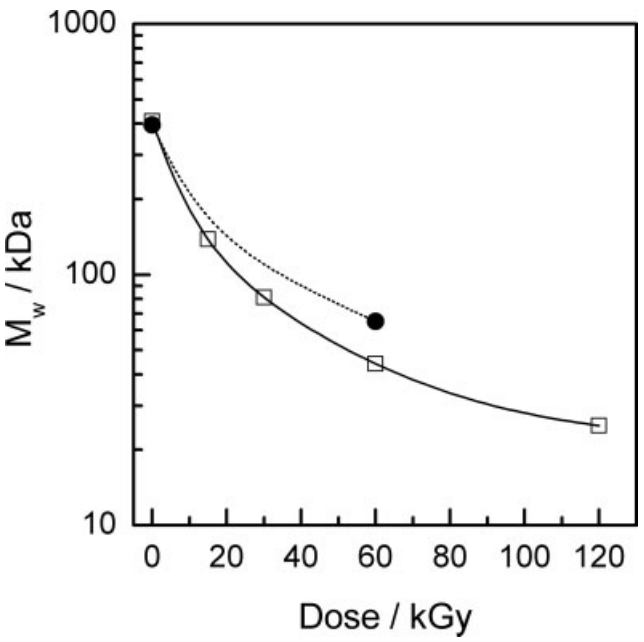

Figure 2 γ irradiation of solid chitosan in the presence of air: M_w as a function of the absorbed dose. The squares represent data for the model chitosan used for the detailed study of the action of ionizing radiation (Sea Fisheries Institute, Gdynia, Poland; M_{w0} = 408 kDa, degree of deacetylation = 88%); the circles represent data for the bulk chitosan used for the CHM preparation (Primex; M_{w0} = 395 kDa, degree of deacetylation = 90%).

moisture and the postirradiation degradation evidenced for chitosan irradiated in a solid state[33,38]) to occur within a reasonably long shelf-storage time without losing the high fat-binding capacity.

Irradiation may cause some side effects, including the formation of carbonyl and carboxyl groups, albeit at a very low yield (typically on the order of 10^{-7}–10^{-6} mol/J of absorbed energy,[39] which at our dose corresponds to the formation of a few millimoles of modified groups per kilogram of the substrate). On the basis of some previous observations, one could expect also some losses of amino groups; calculations based on the yields published by Ershov[39] would lead to an anticipated loss of less than 1% of these groups for the dose applied in the current work. Actually, within the accuracy limits of our detection method (3%), we did not observe any change in the amino group content upon irradiation. Because the extent of chemical changes accompanying our degradation procedure is very low, we assume that these effects do not significantly influence the physicochemical properties of chitosan other than the molecular weight.

Crosslinking polymerization is a standard method for synthesizing crosslinked polymeric structures, including products able to swell in water to equilibrium, that is, hydrogels. The use of this method often requires that the substrate mixture contains, besides the monomer, an initiator and a crosslinking agent. The application of ionizing radiation allows us to run the process without using the two latter additives, which is of some advantage in products for biomedical applications.[20,40]

The irradiation of bulk VP in the absence of oxygen with a dose of 25 kGy leads to the formation of solid, crosslinked blocks of PVP. In the first stage of irradiation, the dominating process is the radiation-induced polymerization of VP (Fig. 3). Under our experimental conditions, at a dose of about 2 kGy, most of the monomer is consumed, and crosslinking becomes the most prominent reaction. After the gelation dose is reached, determined previously for VP to be about 4 kGy,[41,42] an insoluble, crosslinked gel fraction is formed. Further crosslinking in this system proceeds with a relatively high radiation–chemical yield (5×10^{-8} mol/J[21]), and according to earlier studies on the crosslinking polymerization of VP, at 25 kGy (6.25 $\times$ the gelation dose), the gel fraction is of the order of 0.9.[21] Applying higher irradiation doses would not significantly increase the gel fraction and may reduce the swelling ability of our product because of a too high density of crosslinks (for typical swelling–dose dependences in PVP hydrogels, see refs. 42 and 43).

It is worth mentioning that a similar procedure, the radiation crosslinking of concentrated aqueous PVP solutions with a dose of 25 kGy to form swellable but mechanically resistant macroscopic hydrogels, is the basis of a fully commercialized technology used in the production of hydrogel wound dressings.[21,44–48]

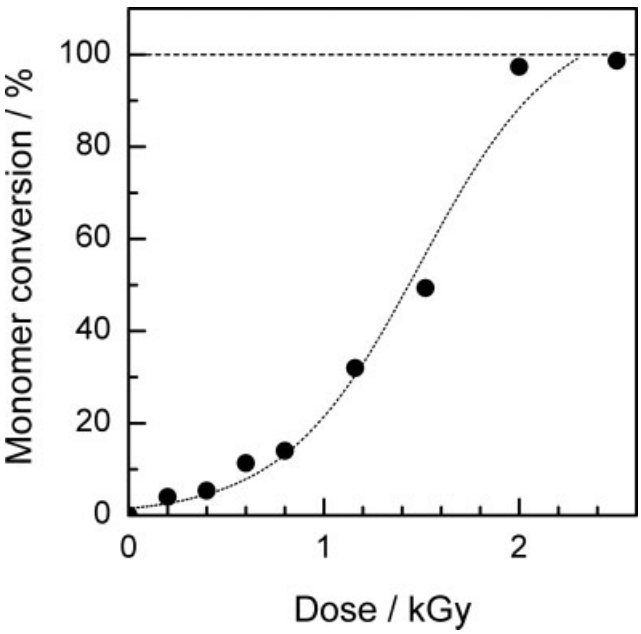

Figure 3 γ irradiation of Ar-saturated neat VP: the monomer conversion as a function of the absorbed dose.

The slightly brownish coloration of our water-free, solid product, being presumably due to both trapped radicals and coupled double bonds, is efficiently removed by annealing at 120°C. Grinding and subsequent separation on mechanical sieves allow us to obtain PVP microgel fractions of defined size ranges. As expected, the equilibrium degree of swelling (D_s), defined as $(m_s - m_d)/m_d$, where m_s is the mass of the swollen gel and m_d denotes the mass of the dry gel, is governed by the crosslink density, thus being independent of the microgel size, in the tested size ranges of 63–90 to 500–1000 μm. For our microgels, at 37°C in water, D_s is 45, and in 0.01 *M* HCl, D_s is 42. This can be considered the factor by which the dry gel volume is increased in contact with an aqueous medium in the digestive tract. This D_s corresponds to a network with an average molecular weight between adjacent crosslinks of the order of 150 kDa, as estimated on the basis of the modified Flory–Rehner theory.[49–53] When the soaking of microgels was performed in a 0.01 M HCl solution containing dissolved chitosan (1 : 1 w/w vs PVP), the observed D_s was similar to that in pure water, indicating that there was no negative effect of chitosan on the microgel swelling ability. For the microgel fraction selected for the CHM preparation (250–500 μm), a D_s of 50% was reached in less than 10 min after the dry gel had been dispersed in 0.01 *M* HCl.

TABLE II
Weight of Organs, Body Weight Gain, Levels of Serum Cholesterol and Triglycerides After 10 Weeks of Experimentation, and Daily Food Intake Measured During the Fifth Week of Experimentation

Group[a]		A	B	BC	C
Weight of organs (g/100 g of body weight)	Heart	0.31 ± 0.007	0.31 ± 0.009	0.31 ± 0.005	0.32 ± 0.008
	Liver	3.41 ± 0.100	4.06 ± 0.104[b]	3.63 ± 0.090[c]	3.67 ± 0.137[c]
	Kidney	0.81 ± 0.028	0.83 ± 0.017	0.81 ± 0.012	0.84 ± 0.017
	Spleen	0.16 ± 0.008	0.18 ± 0.006[b]	0.18 ± 0.007	0.16 ± 0.006[c]
	Testicles	0.80 ± 0.020	0.76 ± 0.017	0.82 ± 0.013[c]	0.83 ± 0.017[c]
	Lungs	0.48 ± 0.030	0.45 ± 0.013	0.45 ± 0.014	0.45 ± 0.020
Food intake (g/day)		25.32 ± 1.11	22.50 ± 0.71[b]	21.88 ± 0.89[b]	21.62 ± 0.86[b]
Body weight gain (g)		134.33 ± 6.43	149.07 ± 8.50	133.67 ± 6.45	136.67 ± 7.41
Serum cholesterol (mg/dL)		63.47 ± 2.37	97.13 ± 3.55[b]	89.40 ± 2.25[b]	93.00 ± 2.88[b]
Serum triglycerides (mg/dL)		91.07 ± 8.00	161.07 ± 12.82[b]	143.27 ± 12.62[b]	129.87 ± 12.11[b]

Data are expressed as the mean plus or minus the standard error for 15 rats.
[a] A = standard animal chow only; B = cholesterol- and fat-rich diet; BC = cholesterol- and fat-rich diet followed by a cholesterol- and fat-rich diet supplemented by CHM; C = cholesterol- and fat-rich diet supplemented by CHM.
[b] Significantly different from the control (group A), $p < 0.05$
[c] Significantly different from group B, $p < 0.05$ (in groups BC and C).

Animal studies on the action of the CHM preparation

The food intake in group B, fed the FChD diet, was 10% lower than that in group A, which was fed common fodder. In groups BC and C supplemented with CHM, the average food intake was somewhat lower than in group B, although the difference was not statistically significant.

In both animal groups treated with CHM (groups BC and C), the body mass was lower than that in the group on the fat-rich diet (group B) and similar to the body mass of animals from the control group (group A). A drop in serum cholesterol and triglycerides in animals supplemented with CHM (BC and C), compared with animals on a high-fat and -cholesterol diet (B), was also observed.

Feeding rats for 10 weeks with FChD (group B) caused a statistically significant increase in the liver weight in comparison with the animals fed the standard diet (group A). A comparison of the liver weight data for all groups clearly indicates that supplementing the high-fat diet with the CHM preparation efficiently prevents lipid infiltration and an increase in the weight of the liver. This effect was observed both in group C fed for 10 weeks FChD supplemented with CHM and in group BC, for which the supplementation of FChD with CHM started after 4 weeks of the experiment. No meaningful differences were observed between the four animal groups in the weights of other organs: the heart, kidneys, and lungs. Although feeding rats FChD seems to cause a statistically significant increase in the weight of the spleen, this effect is less pronounced in group BC and absent in group C. The observed lower weight of testicles in relation to the body weight in group B when compared with the other groups results from the higher average body weight of the animals in this group (Table II).

DISCUSSION

Feeding the animals FChD leads to hyperlipidemia and fatty degeneration of the liver. The accumulation of triglycerides in hepatic lobules causes an increase in the weight of this organ. It has been demonstrated before that the addition of chitosan to the diet exerts some effects on the liver function in rats, including changes in the rate of cholesterol synthesis and enzymatic activity.[10,54] It is not yet fully clear, however, whether and to what extent chitosan reduces lipid accumulation in the liver. Our data indicate that the CHM preparation used in our study [low-molecular-weight chitosan and microgel (1 : 1 w/w) at a 4% concentration in the diet (i.e., 2% chitosan)] significantly reduced the increase in the liver weight caused by the FChD diet.

It should be stressed that both components are easily obtained by simple solvent-, additive-, and waste-free radiation processing from relatively low-cost substrates, the product does not contain any complex biochemicals, and yet it is capable of exerting a desired physiological effect at a relatively low dietary content.

The observed effect may be compared with the results of Sugano et al.,[7] Fukada et al.,[9] and LeHoux and Grondin,[10] who fed rats a diet supplemented with pure chitosan. Such a comparison is not easy because of the different conditions of these tests. Fukada et al. did not observe any significant difference in the liver weight between the control and chitosan-fed groups, even at a 5% chitosan content.[9] The apparent discrepancy between these results and

our data may be attributed mainly to the higher molecular weight of the applied chitosan (250 kDa; which has been shown in *in vitro* tests to be less effective in fat binding), but there might be also some influence of the shorter duration of the experiment (3 weeks) and lower fat content in the diet in comparison with our group B. LeHoux and Grondin used chitosan of a molecular weight similar to that in our study and demonstrated that a 7.5% chitosan content in the diet prevented the liver weight gain induced by a sterol-rich diet (fat content not stated).[10] The same effect, albeit less pronounced, has been observed with 5 and 2.5% chitosan. The data of Sugano et al. indicate some reduction in the weight increase in the liver in rats fed a diet similar to our FChD already at a chitosan level of 2%, with no apparent difference between the efficiency of two chitosans of different but unstated molecular weights.[7] The extent of the preventive effect against the fat-rich-diet-induced increase in the liver weight observed by Sugano et al. is, however, difficult to quantitatively assess because no control group fed a standard diet was run. Nevertheless, our observations are, at least qualitatively, in line with the results of Sugano et al., indicating that chitosan can efficiently prevent lipid accumulation in liver caused by a fat-rich diet already when administered at the relatively low level of 2%, whereas a comparison of our data with the negative results of Fukada et al.[9] might indicate that low-molecular-weight chitosan seems to perform better than its high-molecular-weight analogue.

It has to be noticed that in humans, obesity and hypertriglyceridemia are related to a significant increase in the lipid accumulation in the liver and degeneration of liver cells.[55,56]

Although the PVP microgel, being physiologically inert, insoluble, and not absorbable in the digestive tract, is not expected to exert any direct action on lipid accumulation in liver, its presence may indirectly influence the liver weight increase caused by a fat-rich diet. In many cases when chitosan was used alone as the agent intended to reduce fat absorption, an undesirable side effect was observed, namely, an increase in the amount of consumed food (by 3–20%).[3,9,10] In our study, we observed a lower consumption of the diet in group B compared with control group A (the diet in group B was much more caloric in comparison with that in group A, so rats in group B tended to eat less), whereas the food intake in the groups fed the CHM-containing diet (C and BC) was slightly lower than that in group B. This may indicate that the presence of a microgel in the diet has some hunger-reducing influence, which, at our low microgel content (2%), was just enough to compensate for the chitosan-induced-hunger effect evidenced in previous works. This may have contributed to the lowering of the food intake and, as a result, of the liver weight gain in comparison with a situation (not studied in our work) in which chitosan would be used alone, without the microgel.

Further *in vivo* studies on the CHM preparation will include more detailed tests on its influence on the body weight gain, serum cholesterol, and triglycerides in rats.

CONCLUSIONS

Chitosan of a reduced average molecular weight (ca. 65 kDa), shown in previous *in vitro* studies to exhibit a high fat-binding capacity, combined at a 1 : 1 (w/w) ratio with a PVP microgel (CHM) and administered as a diet supplement at 4% CHM (2% chitosan) to rats fed FChD, effectively reduces the liver weight gain caused by FChD (by >60% under our experimental conditions), whereas the weight of the other main organs remains unaltered. The CHM preparation has been proved effective in exerting this effect also when administered to animals already in an atherosclerotic, overweight condition. In parallel, the CHM preparation has been shown to reduce the body weight gain and the levels of plasma cholesterol and triglycerides in rats fed FChD. No adverse effects were observed during 10 weeks of feeding the animals the CHM-containing diet.

The CHM preparation, combining biocompatible synthetic and natural polymers, is easily obtained from relatively low-cost substrates by simple radiation processing, involving neither solvents nor additives and generating no waste.

A treatment with ionizing radiation has been proved to be an efficient tool for synthesizing the two polymeric components of the CHM preparation. Irradiation in the solid state leads to controlled degradation of chitosan, for which the target molecular weight may be reached by the selection of the appropriate dose. The irradiation of neat, deaerated VP with a dose of 25 kGy, followed by thermal annealing, grinding, and fractionation, is a convenient way to produce microgels of a uniform size and desired swelling ability.

The authors thank Jadwiga Ignaczak and Julian Sikorski (Institute of Applied Radiation Chemistry, Technical University of Łódź) for their help with the microgel synthesis and characterization.

References

1. Knorr, D. J Food Sci 1982, 47, 593.
2. No, H. K.; Lee, K. S.; Meyers, S. P. J Food Sci 2000, 65, 1134.
3. Kanauchi, O.; Deuchi, K.; Imasato, Y.; Shizukuishi, M.; Kobayashi, E. Biosci Biotechnol Biochem 1995, 59, 786.
4. Meler, J.; Pluta, J.; Ulanski, P.; Krotkiewski, M. In Progress in Chemistry and Application of Chitin and Its Derivatives; Struszczyk, H., Ed.; Polish Chitin Society: Lodz, Poland, 2003; Vol. IX.

5. Czechowska-Biskup, R.; Ulanski, P.; Rosiak, J. M.; Kumor, A.; Kozak-Michałowska, I.; Lorenc, J.; Meler, J. In Progress in Chemistry and Application of Chitin and Its Derivatives; Struszczyk, H., Ed.; Polish Chitin Society: Lodz, Poland, 2004, Vol. X.
6. AbsorbitolPlus Brochure; Pharmanutrients: Lake Bluff: IL, 2003.
7. Sugano, M.; Watanabe, S.; Kishi, A.; Izume, M.; Ohtakara, A. Lipids 1988, 23, 187.
8. Chiang, M.-T.; Yao, H.-T.; Chen, H.-C. Biosci Biotechnol Biochem 2000, 64, 965.
9. Fukada, Y.; Kimura, K.; Ayaki, Y. Lipids 1991, 26, 395.
10. LeHoux, J.-G.; Grondin, F. Endocrinology 1993, 132, 1078.
11. Sciutto, A. M.; Colombo, P. Acta Toxicol Ther 1995, 16, 215.
12. Colombo, P.; Sciutto, A. M. Acta Toxicol Ther 1996, 17, 287.
13. Giustina, A.; Ventura, P. Acta Toxicol Ther 1995, 16, 199.
14. Zahorska-Markiewicz, B.; Krotkiewski, M.; Olszanecka-Gliniarowicz, M.; Zurakowski, A. Pol Merkuriusz Lek 2002, 13, 129.
15. Gades, M. D.; Stern, J. S. Int J Obes 2002, 26, 119.
16. Gades, M. D.; Stern, J. S. J Am Diet Assoc 2005, 105, 72.
17. Pittler, M. H.; Abbot, N. C.; Harkness, E. F.; Ernst, E. Eur J Clin Nutr 1999, 53, 379.
18. Czechowska-Biskup, R.; Rokita, B.; Ulanski, P.; Rosiak, J. M. Nucl Instrum Methods Phys Res Sect B 2005, 236, 383.
19. Rosiak, J. M.; Ulanski, P.; Czechowska-Biskup, R. Pol. Pat. Appl. P-378313 (2005).
20. Ulanski, P.; Rosiak, J. M. In Encyclopedia of Nanoscience and Nanotechnology; Nalwa, H. S., Ed.; American Scientific Publishers: Stevenson Ranch, CA, 2004.
21. Rosiak, J. M. In Radiation Effects on Polymers; Clough, R. C.; Shalaby, S. W., Eds.; ACS Symposium Series 475; American Chemical Society: Washington, 1991.
22. Buehler, V. Kollidon: Polyvinylpyrrolidone for the Pharmaceutical Industry; BASF: Ludwigshafen, Germany, 1993.
23. Robinson, B. V.; Sullivan, F. M.; Borzelleca, J. F.; Schwartz, S. L. PVP: A Critical Review of the Kinetics and Toxicology of Polyvinylpyrrolidone (Povidone); Lewis: Chelsea, MI, 1990.
24. Muzzarelli, R. A. A. Chitin; Pergamon: Oxford, 1977.
25. Wojtasz-Pajak, A.; Kolodziejska, I.; Debogorska, A.; Malesa-Ciecwierz, M. Bull Sea Fish Inst (Gdynia, Poland) 1998, 143, 29.
26. Muzzarelli, R. A. A.; Ferrero, A.; Pizzoli, M. Talanta 1972, 19, 1222.
27. Kratochvil, P. Classical Light Scattering from Polymer Solutions; Elsevier: Amsterdam, 1987.
28. Hasegawa, M.; Isogai, A.; Onabe, F. Carbohydr Polym 1993, 20, 279.
29. Allan, G. G.; Peyron, M. Carbohydr Res 1995, 277, 257.
30. Allan, G. G.; Peyron, M. Carbohydr Res 1995, 277, 273.
31. Nordtveit, R. J.; Varum, K. M.; Smidsrod, O. Carbohydr Polym 1994, 23, 253.
32. Varum, K. M.; Holme, H. K.; Izume, M.; Stokke, B. T.; Smidsrod, O. Biochim Biophys Acta 1996, 1291, 5.
33. Ulanski, P.; Rosiak, J. M. In Chitin World; Karnicki, Z. S.; Brzeski, M. M.; Bykowski, P. J.; Wojtasz-Pajak, A., Eds.; Wirtschaftsverlag: Bremerhaven, Germany, 1994.
34. Ulanski, P.; Wojtasz-Pajak, A.; Rosiak, J. M.; von Sonntag, C. In Advances in Chitin Science; Peter, M. G.; Muzzarelli, R. A. A.; Domard, A., Eds.; University of Potsdam: Potsdam, Germany, 2000; Vol. 4.
35. Ulanski, P.; von Sonntag, C. J Chem Soc Perkin Trans 2 2000, 2022.
36. Lorimer, J. P.; Mason, T. J.; Cuthbert, T. C.; Brookfield, E. A. Ultrason Sonochem 1995, 2, 55.
37. Ulanski, P.; Rosiak, J. Radiat Phys Chem 1992, 39, 53.
38. Rosiak, J. M.; Ulanski, P.; Kucharska, M.; Dutkiewicz, J.; Judkiewicz, L. J Radioanal Nucl Chem 1992, 159, 87.
39. Ershov, B. G.; Isakova, O. V.; Rogozhin, S. V.; Gamzazade, A. I.; Leonova, E. U. Dokl Akad Nauk 1987, 295, 1152.
40. Ivanov, V. S. Radiation Chemistry of Polymers; VSP: Utrecht, the Netherlands, 1992.
41. Rosiak, J.; Burczak, K.; Olejniczak, J.; Pekala, W. Polym Med 1987, 17, 99.
42. Darwis, D.; Hilmy, N.; Hardiningsih, L.; Erlinda, T. Radiat Phys Chem 1993, 42, 907.
43. Rosiak, J.; Olejniczak, J.; Burczak, K. Polym Med 1989, 19, 69.
44. Rosiak, J. M.; Rucinska-Rybus, A.; Pekala, W. U.S. Pat. 4,871,490 (1989).
45. Rosiak, J. Polym Prepr (Am Chem Soc Div Polym Chem) 1990, 31, 361.
46. Rosiak, J. M.; Ulanski, P.; Pajewski, L. A.; Yoshii, F.; Makuuchi, K. Radiat Phys Chem 1995, 46, 161.
47. Rosiak, J. M.; Ulanski, P. Radiat Phys Chem 1999, 55, 139.
48. Ajji, Z.; Othman, I.; Rosiak, J. M. Nucl Instrum Methods Phys Res Sect B 2005, 229, 375.
49. Flory, P. J.; Rehner, J. J Chem Phys 1943, 11, 521.
50. Rosiak, J.; Olejniczak, J.; Charlesby, A. Radiat Phys Chem 1988, 32, 691.
51. Sperling, L. H. Introduction to Physical Polymer Science; Wiley-Interscience: New York, 2001.
52. Sen, M.; Yakar, A.; Güven, O. Polymer 1999, 40, 2969.
53. Bray, J. C.; Merrill, E. W. J Appl Polym Sci 1973, 17, 3779.
54. Ikeda, I.; Sugano, M.; Yoshida, K.; Sasaki, E.; Iwamoto, Y.; Hatano, K. J Agric Food Chem 1993, 41, 431.
55. Tacikowski, T.; Dzieniszewski, J.; Nowicka, G.; Ciok, J. Med Sci Monit 2002, 8, CR697.
56. Tacikowski, T.; Godziemba-Maliszewska, E.; Dzieniszewski, J.; Nowicka, G. Pol Arch Med Wewn 2004, 50, 37.