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Fungal Chitin−Glucan from *Aspergillus niger* Efficiently Reduces Aortic Fatty Streak Accumulation in the High-Fat Fed Hamster, an Animal Model of Nutritionally Induced Atherosclerosis

ARLET BERECOCHEA-LOPEZ,[‡,†] KELLY DECORDÉ,[‡,†] EMILIE VENTURA,[†] MARLÈNE GODARD,[†] AURÉLIE BORNET,[‡] PIERRE-LOUIS TEISSÈDRE,[‡] JEAN-PAUL CRISTOL,[†] AND JEAN-MAX ROUANET*[,†]

Equipe d'Accueil 4188 Nutrition Humaine, Biodisponibilité et Athérogénèse, Université Montpellier 2, Place Eugène Bataillon, 34095 Montpellier, France, and Université Victor Ségalen Bordeaux 2, Faculté d'Œnologie-UMR 1219-ISVV-Laboratoire de Chimie Appliquée, 33405 Talence, France

The effects of chitin−glucan (CG) on early atherosclerosis, cardiac production of superoxide anion, and hepatic antioxidant enzymes were studied in an animal model of atherosclerosis. Three groups of 12 hamsters were fed an atherogenic diet for 12 weeks. They received by gavage either water (control group) or CG in water at a dose of 21.4 mg/kg BW·d^{-1} of chitin−glucan (CG ld) or 42.8 mg/kg BW·d^{-1} (GG hd). CG did not affect plasma cholesterol but lowered triglycerides. It also strongly reduced the area of aortic fatty streak deposition by 87−97%, cardiac production of superoxide anion by 25% and liver MDA by 77−85%, and enhanced liver superoxide dismutase activity by 7−45% and glutathionne peroxidase activity by 38−120%. These findings support the view that chronic consumption of chitin−glucan has potential beneficial effects with respect to the development of atherosclerosis. The underlying mechanism is related mainly to improving the antioxidant status.

KEYWORDS: Atherosclerosis; hamsters; fungal polysaccharides; antioxidant compounds; chitin−glucan

INTRODUCTION

Chitosan (poly-β-1,4-linked glucosamine) is a cationic polysaccharide produced by the alkaline N-deacetylation of chitin, an aminopolysaccharide found in some fungi (*1*). It can be used for its capacity to chelate transition metal (*2*) as edible coating for fruits and vegetables (*3*) in the form of packing films (*4*). Recently, Xie et al. (*5*) studied the antioxidant activity of the water-soluble chitosan derivatives, which are regarded as hydroxyl radical scavengers. In salmon, the antioxidant activity of different MW chitosans may be attributed to their ability to link metal ions. Several sources of iron bound to proteins exist in the tissues of fish, such as myoglobin, hemoglobin, ferritin, and transferrin. The iron associated with these proteins can be released during storage thereby activating oxygen and initiating the oxidation of lipids (*6*). Xue et al. (*7*) and very recently Kim and Thomas (*8*) reported that chitosans can bind metals or combine with negatively charged molecules as lipids, leading to a significant antioxidant effect, and suggested their possible application as an additive in food systems with high lipid levels. Peng et al. (*9*) and Winterowd and Sandford (*10*) have shown that chitosans retard the oxidation of lipids by chelating ferrous ions present in the medium, thereby eliminating their pro-oxidant activity or their conversion into ferric ion. In addition, amino groups in the chitosans may participate in the chelation of metal ions. Chitosans also behave as dietary fibers, being polysaccharides not hydrolyzed by mammalian digestive enzymes. Several studies have shown that they reduce cholesterol in animal models (*11, 12*). How chitosans reduce cholesterol remains uncertain. The anionic exchange properties of chitosans are the most likely explanation for their hypocholesterolemic properties.

While atherosclerosis was considered only a few years ago as an inevitable degenerative disease of the arteries, mainly due to age, it is now better recognized as an evolutionary pathology (*13*). Atherosclerosis may be characterized according to three theories (oxidative, inflammatory, and hyperlipemic), and since chitosans exhibited both antioxidant activity and hypolipidemic properties, it can be hypothesized that they might prevent this pathology. Indeed, it is now well recognized that an increased formation of oxygen radicals and other oxygen derivatives frequently accompanies tissue damage. Today, there is an explosive interest in the use of antioxidant nutritional supple-

* To whom correspondence should be addressed. University Montpellier 2, Equipe d'Accueil 4188 Nutrition Humaine, Biodisponibilité et Athérogénèse, Place Eugène Bataillon, Université Montpellier 2, 34095 Montpellier cedex 5, France. Tel/Fax: 33 (0)4 67 14 35 21. E-mail: jm.rouanet@univ-montp2.fr.

‡ These authors made an equal contribution to this work.

† Université Montpellier 2.

‡ Université Victor Ségalen Bordeaux 2.

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ments. Epidemiological evidence suggests that intake of some vitamins, minerals, fibers and other food constituents may help to protect against heart disease, cancer, and the aging process and that antioxidants may have a protective effect, either in preventing these diseases or in lessening the severity of the diseases upon their onset. Many of their activities are mediated by reactive oxygen species (ROS), which are generated during the oxidative burst (*14*, *15*). To our knowledge, only one study showed that chitosan from shells of crustacean inhibited atherogenesis in the apolipoprotein E-deficient mouse (*16*). A human clinical study also suggested a reduction of the atherosclerotic index by chitosan, although a direct effect on the pathology was not easily measurable (*17*). Chitosan comes from chitin, and in the fungal cell wall, chitosan and chitin occur in two forms, as free aminoglucoside and associated to β-D-glucan (*18*). In 1990, Wessels et al. proposed that initially chitin and β-glucan chains accumulate individually in the fungal cell wall and thereafter form the interpolymer linkage (*19*). In fungi, chitin is involved in cell walls in the form of a chitin−glucan complex. Similar to cellulose in plants and chitin in invertebrates, chitin−glucan is a component of the cell structure. The chemical structure of the chitin−glucan complex is still vague (linear polymer of *N*-acetyl-(D)-glucosamine linked through a 1,4-β osidic bond), even though it has been reported many years ago that at least some chitin chains are linked to β-glucans of some fungi (*20*). Hence, it is expected here that chronic diseases, particularly dietary induced atherosclerosis, may be prevented by chitin−glucan. There are few reports on chitin−glucan metabolic effects, and the presence of the two biologically active polysaccharides being able to enhance the effect of the complex led us to study its effect. To do so, an aortic wall response to a high-fat/high-cholesterol/low antioxidant diet was triggered in Syrian hamsters to induce fatty streak formation and atherosclerosis emergence; we then evaluated the possible preventive effect of the administration of chitin−glucan from fungal origin.

MATERIALS AND METHODS

Preparation and Characterization of Chitin−Glucan. Chitin−glucan was obtained from Kitozyme S.A. Herstal, Belgium. The experimental material derived from the cell walls of the mycelium of *Aspergillus niger* (*A. niger*) combined two types of polysaccharide chains, i.e., chitin (poly *N*-acetyl-D-glucosamine) and β (1,3)-D-glucan. The chitin−glucan ratio (w/w) was 35/65 as determined by ^{13}C-solid state NMR.

Animals. Weanling male Syrian golden hamsters (Elevage Janvier, Le Genest-St-Isle, France) weighing 60−80 g were randomly separated into three groups of 12 animals each. They were maintained in plastic cages in a temperature-controlled environment (23 ± 1 °C) subjected to a 12-h light/dark cycle and allowed free access to both food and water. Hamsters were handled according to the guidelines of the Committee on Animal Care at the University of Montpellier and NIH guidelines (*21*).

Diets and Feeding Procedures. Hamsters were fed for 12 weeks a semipurified hyperlipidic diet (**Table 1**) in which the cholesterol content had been set at 0.5% and which was supplemented with 15% lard at the expense of starch and sucrose; no selenium, vitamin C, or vitamin E was added to this diet. Uneaten food was weighed daily. The hamsters of each group additionally received daily by gavage either tap water (control) or a solution of chitin−glucan (CG) in water. The volumes for solutions force-fed were adjusted daily to the weight of hamsters. Hamsters received either 21.4 mg/kg BW·d^{-1} of chitin glucan (CG ld) or 42.8 mg/kg BW·d^{-1} of chitin−glucan (CG hd), i.e., 1.5 and 3.0 g/d equivalent doses for a 70 kg human. Chitin glucan was supplied by gavage at the same time that food was distributed (at 07 h p.m.).

Analytical Procedures. At the end of the 12-week experimental period, hamsters were deprived of food overnight, and blood samples were collected under anesthesia (Pentobarbital) by cardiac puncture. Plasma was prepared by centrifugation at 2,000*g* for 10 min at 4 °C, then stored at −80 °C until analysis. Plasma total cholesterol (TC) and HDL cholesterol (HDL-C) were determined by commercially available enzymatic methods (respectively nos. CH 200 and CH 203, Randox Laboratories LTD, Crumlin, UK). Plasma very low- and low-density lipoprotein cholesterol was precipitated with phosphotungstate reagent (*22*) and HDL-C was measured in the supernatant. Plasma triglycerides (TG) were also mesured using an automat Pentra 400 and a Randox enzymatic kit (no. TR 1697).

Table 1. Composition of the Diet

ingredients	g/kg
casein	200
DL-methionine	3
cornstarch	393
sucrose	154
cellulose	50
mineral mix[a]	35
vitamin mix[b]	10
lard	150
cholesterol	5

[a] The mineral mixture contained (mg/kg of diet) $CaHPO_4$, 17200; KCl, 4000; NaCl, 4000; MgO, 420; $MgSO_4$, 2000; Fe_2O_3, 120; $FeSO_4 \cdot 7H_2O$, 200; trace elements, 400 ($MnSO_4 \cdot H_2O$, 98; $CuSO_4 \cdot 5H_2O$, 20; $ZnSO_4 \cdot 7H_2O$, 80; $CoSO_4 \cdot 7H_2O$, 0.16; KI, 0.32; sufficient starch to bring to 40 g (per kg of diet). [b] The vitamin mixture contained (mg/kg of diet) retinol, 12; cholecalciferol, 0.125; thiamin, 40; riboflavin, 30; pantothenic acid, 140; pyridoxine, 20; inositol, 300; cyanocobalamin, 0.1; menadione, 80; nicotinic acid, 200; choline, 2720; folic acid, 10; *p*-aminobenzoic acid, 100; biotin, 0.6; sufficient sarch to bring to 20 g (per kg of diet).

The liver was perfused with 0.15 mol/L KCl to remove residual blood, rapidly excised, rinsed in ice cold saline, blotted dry, weighed, sectioned for analyses, and stored in liquid nitrogen. Concommitantly, samples were removed for histological analysis. Liver was homogenized in 4 volumes of ice cold 0.1 mol/L potassium phosphate buffer (pH 7.4), and the homogenate was spun at 13,000*g* for 15 min at 4 °C. The supernatant was then stored at −80 °C for subsequent assay of glutathione peroxidase (GSHPx) and superoxide dismutase (SOD) activity on an automat Pentra 400 (HORIBA ABX, Montpellier, France). GSHPx activity was measured by the method of Randox (Randox Laboratories LTD, Crumlin, UK) using a commercial kit (Ransel, no.RS505). Superoxide dismutase (SOD) activity was determined using a Randox kit (Ransod, no. SD 125). The cytosolic protein content was determined by using a commercial protein assay (Sigma, Saint Quentin Fallavier, France) according to the method of Smith et al. (*23*) and using bovine serum albumin as standard.

Lipid peroxidation was determined on the homogenate by measuring the formation of malondialdehyde (MDA), a thiobarbituric acid reactive substance (TBARS), which served as the index of lipid peroxidation. Homogenate (1 mL) was mixed with 1 mL of 17.5% trichloroacetic acid and 1 mL of 0.6% 2-thiobarbituric acid. This mixture was incubated for 1 h at 80 °C, and then 70% trichloraoacetic acid (1 mL) was added. After centrifugation at 1000*g* for 15 min, the absorbance of the supernatant was measured at 535 nm. TBARS concentration was expressed as ng/mg protein. Thiobarbituric acid 1,1,3,3-tetraethoxypropane was used as the standard of TBARS (*24*). Protein content was determined according to Smith et al. (*23*).

Determination of Superoxide Anion Production. Superoxide anion production was evaluated in tissues as previously described (*25*) by chemiluminescence using lucigenin bis-*N*-methyl acridinium. Briefly, the left ventricle (150 mg) was placed in Krebs buffer containing 250 μM lucigenin; the intensity of luminescence was measured on a luminometer (Perkin-Elmer Wallac, Victor, Turku, Finlande). Results were expressed as cps/mg protein.

Aortic Cholesterol Measurement. The thoracic aorta was removed and stored at 4 °C in PBS for subsequent analysis. After cleaning and weighing, samples of about 20 to 40 mg were placed in vials containing 4 mL of methanol and 10 mL of chloroform and treated according to Rudel et al. (*26*). The sample was mixed vigorously and left at room

Table 2. Body weight and Food Intake in Hamsters Fed an High Fat Diet (Control) or a High Fat Diet Plus Chitin−Glucan at a Low Dose (CG ld, 21.4 mg/kg BW·d^{-1}), or at a High Dose (CG hd, 42.8 mg/kg BW·d^{-1}) for 12 Weeks[a]

	food intake (g/day)	initial body wt (g)	final body wt (g)	weight gain (g/day)	feed gain ratio[b]
control	4.44 ± 0.40 a	116.3 ± 1.1 a	125.8 ± 2.4 ab	9.50 ± 1.60 a	2.14 ± 0.34 a
CG ld	4.83 ± 0.61 a	115.5 ± 1.1 a	127.6 ± 1.8 a	10.60 ± 1.00 a	2.21 ± 0.22 a
CG hd	4.68 ± 0.60 a	116.0 ± 1.0 a	122.6 ± 1.5 b	6.60 ± 0.50 b	1.40 ± 0.14 b

[a] Values are the means ± SEM; $n = 12$. Data were analyzed by one-way ANOVA followed by the least significant difference test. For each dietary treatment, means in a column with different letters differ, $P < 0.05$. [b] g weight gain per day/g feed per day.

temperature for 48 h before extraction. The solution was then placed at 37 °C under nitrogen. When one-half of the solution was evaporated, 1 mL of chloroform with 1% Triton-100 was added, mixed, and evaporated to dryness. Distilled water (250 μL) was added to the samples, vortexed, and placed at 37 °C for 20 min to solubilize the lipids. After incubation, total cholesterol concentration was determined enzymatically in triplicate with 25 μL of sample (no. CH 200, Randox Laboratories LTD, Crumlin, UK).

Aortic Tissue Processing. Following blood collection and liver removal, the intact aorta was first perfused with phosphate-buffered saline containing 1 mmol/L $CaCl_2$, 2.5% paraformaldehyde, and 1.5% glutaraldehyde for the fixation of the vasculature. The aorta was carefully dissected between sigmoid valves and 3−4 cm after the aortic arch, and thoroughly cleaned of loose adventitial tissue; the aortic arch was cut free, opened longitudinally along the outside of the arch, pin cork, immersed in fresh fixative solution, and stored at 4 °C until staining. The aortic arches were then first rinsed for 48 h in 0.1 mol/L sodium cacodylate buffer (pH 7.4) containing 30 mmol/L $CaCl_2$ and 250 mmol/L sucrose. The arches were then rinsed in distilled water, stained for 40 s in Harris hematoxylin, and then rinsed in distilled water, and quickly in 70% isopropyl alcohol; finally, they were stained in Oil Red O for 30 min according to the method of Nunnari et al. (*27*), rinsed in 70% isopropyl alcohol, and back in distilled water. Each aortic arch was then directly displayed on a glass slide, endothelium side up, covered with Aquamount mounting medium and coverslips, and observed en face by light microscopy. All segments were photographed using a video digitizer. The area covered by foam cells (aortic fatty streak lesion) was analyzed quantitatively using a computer-assisted morphometry system and expressed as a percentage of the total area surveyed.

Statistical Analyses. Data are shown as the means ± SEM. Data were subjected to logarithmic transformation when necessary to achieve homogeneity of variances. Statistical analyses of the data were carried out using Stat View IV software (Abacus Concepts, Berkeley, CA) by one-way ANOVA followed by Fisher's protected least significant difference test. Differences were considered to be significant at $P < 0.05$.

RESULTS

Compared to the control group under hyperlipidic diet alone, the food intake did not differ in hamsters under high fat diet with CG (**Table 2**). Feed efficiency (expressed as feed gain ratio) and weight gain was reduced only in the hamsters CG hd group (35% and 30%, respectively). In contrast, weight gain did not differ in hamsters fed CG ld as compared to the control group. There were no demonstrable undesirable or toxic effects or evidence of other clinical signs to the administration of the chitin−glucan at either level of supplementation. This indicates that at the low doses used here, chitin−glucan is a safe nutraceutical supplement.

The plasma lipid profile is shown in **Table 3**. TC and HDL-C concentration was not modified by the dietary treatments. However, the triglyceride concentration was significantly lower when CG was added to the hyperlipidic diet (20% for the CG ld group and 39% for the CG hd).

After 12 weeks, the $O_2^{\cdot -}$ production in the heart was not decreased in the CG ld group unlike that in the CG hd group.

Table 3. Plasma lipid Content of Hamsters Fed a High Fat Diet (Control) or a High Fat Diet Plus Chitin−Glucan at a Low Dose (CG ld, 21.4 mg/kg BW·d^{-1}) or at a High Dose (CG hd, 42.8 mg/kg BW·d^{-1}) on Blood Lipids for 12 Weeks[a]

	control	CG ld	CG hd
TC (g/L)	8.13 ± 0.15 a	7.86 ± 0.22 a	8.10 ± 0.29 a
TG (g/L)	1.76 ± 0.12 a	1.40 ± 0.15 b	1.08 ± 0.12 b
HDLC (g/L)	5.18 ± 0.07 a	4.65 ± 0.14 a	4.90 ± 0.24 a

[a] Values are the means ± SEM; $n = 12$. Data were analysed by one-way ANOVA followed by the least significant difference test. For each dietary treatment, means in a column with the different letters differ, $P < 0.05$. TC, total cholesterol; TG, triglycerides; HDLC, high-density lipoprotein cholesterol.

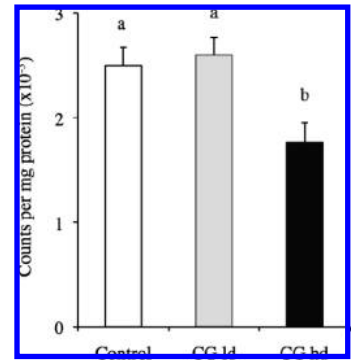

Figure 1. Cardiac superoxide anion production in hamsters fed an hyperlipidic diet without (Control) or with chitin−glucan at low dose (CG ld, 21.4 mg/kg BW·d^{-1}) and high dose (CG hd, 42.8 mg/kg BW·d^{-1}). Values are expressed as mean ± SEM of triplicate wells ($n = 6$). For each dietary treatment, bars with different index letters differ ($P < 0.05$).

Indeed, this ion production was significantly lower (25%) in the CG hd group (**Figure 1**).

Aortic cholesterol was significantly lowered by feeding either dose of chitin−glucan (79%) compared to the control (**Figure 2**). Average aortic fatty streak accumulation (AFSA), measured as the percentage of Oil Red O staining relative to the total area surveyed (**Figure 2**), was significantly decreased in hamsters receiving chitin−glucan at the high dose (CG hd) (97%) or at low dose (CG ld) (87%) compared to controls.

The daily consumption of the chitin−glucan at low or high dose significantly increased liver GSHPx activity (38% and 120%, respectively) and SOD activity (7% and 45%, respectively) (**Table 4**). The liver thiobarbituric acid reactive substance (TBARS) concentration is also shown in **Table 4** and was markedly lowered by the consumption of CG ld (77%) and CG hd (85%) according to the same pattern observed for AFSA.

DISCUSSION

In this study, we have demonstrated for the first time the cardiovascular and oxidative stress protective effect of chitin−glucan supplementation in high fat fed golden Syrian hamsters. It is well established that elevated blood lipids levels constitute

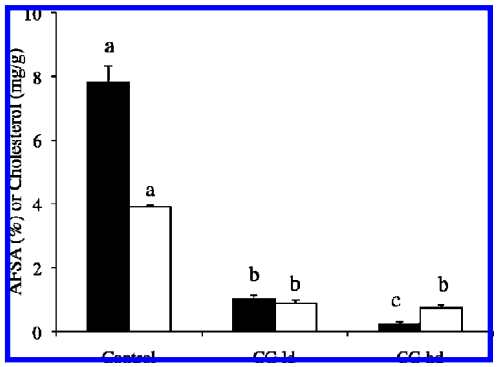

Figure 2. Aortic fatty streak area (AFSA, black bars) and aortic cholesterol level (white bars) in hamsters fed a high fat diet (Control) or an high fat diet supplemeted with chitin−glucan at low dose (CG ld, 21.4 mg/kg BW·d^{-1}) or at high dose (CG hd, 42.8 mg/kg BW·d^{-1}) for 12 weeks. Values are expressed as the mean ± SEM ($n = 6$). For each item, bars with different index letters differ ($P < 0.05$).

Table 4. Hepatic Antioxidant Enzyme Activities and Thiobarbituric Acid Reactive Substances (TBARS) in Hamsters Fed a Hyperlipidic Diet Plus a Daily Gavage of Water (Controls) or Chitin−Glucan at a Low Dose (CG ld) or Chitin−Glucan at a High Dose (CG hd)[a]

group	controls	GC ld	CG hd
	units·mg protein^{-1}		
SOD[b]	42 ± 2 a	45 ± 2 a	61 ± 4 b
GSHPx[c] (x 10^{-2})	1.55 ± 0.32 a	2.14 ± 0.11 b	3.42 ± 0.42 c
	ng·mg protein^{-1}		
TBARS	27.05 ± 3.27 a	6.11 ± 0.39 c	4.07 ± 0.33 d

[a] Values are the means ± SEM; $n = 12$. For each dietary treatment, means in a column with different superscripts differ, $P < 0.05$. [b] SOD: superoxide dismutase. [c] GSHPx: glutathione peroxidase.

the major risk factor for atheroclerosis (*28*). Many studies have been carried out to evaluate the effects of chitosan to lower blood cholesterol in animals and humans. To our knowledge, no study has been performed on chitin−glucan. Dietary supplementation with chitosan has been shown to limit the increase in blood cholesterol fed rabbits (*29*) and rats (*30*) but did not reduce blood cholesterol levels in rabbits with pre-existing hypercholesterolaemia (*29*). A study on Golden Syrian hamsters fed chitosan demonstrated a reduction of plasma total cholesterol from 10% to 55% and a decrease of triglycerides from 22% to 60% (*31*). However, in this study, the experimental conditions are different, and the dose of dietary chitosan compared to that of CG here is more important (five times as higher), and the diet composition is also different (only 5% lipids plus 0.4% cholesterol).

Our results showed that after 12 weeks plasma lipids profile indicated a decreased triglyceride level with treatment by chitin−glucan and that cholesterol was not modified. Previous studies have shown that elevated levels of serum triglycerides (TG) are an independent risk factor for coronary heart disease (*32*). The atherogenicity associated with high levels of TG is thought to be due to the atherogenic lipoprotein subclasses commonly associated with hypertriglyceridemia (*33*). High levels of TG lead to the production of large very low density lipoprotein (VLDL) particles. Degradation of these large TG-rich VLDL particles by lipoprotein lipase results in small VLDL and intermediate density lipoprotein (IDL) remnants, which are particularly atherogenic. Reducing levels of these TG-rich remnant lipoproteins is clinically important because of their atherogenic potential. Small VLDL and IDL are remnants derived from the metabolism of large, TG-rich VLDL particles, and in clinical studies, both of these remnant particles have been shown to be associated with atherosclerotic progression and increased cardiovascular risk, independent of total levels of fasting TG (*34*, *35*). Moreover, the same decrease of TG is observed after supplementation with fructooligosaccharides (FOS) in the rat (*36*, *37*) fed a high fat diet. One hypothesis to explain a possible effect of chitin−glucan on the modulation of TG metabolism is an indirect effect mediated by the production of short-chain fatty acids (SCFA) in the large intestine of CG-fed hamsters leading to an increase of acetate and propionate, as suggested for chitosan in rats (*38*).

The oxidative hypothesis of atherosclerosis allows to explain the initiation mechanism of the pathology according to an accumulation of oxidized LDL in monocyte-derived macrophages that leads to the development of foam cells (*39*). Oxidative stress results from an imbalance between ROS generation and antioxidant defense mechanisms, increasing superoxide production particularly when using a cholesterol-rich antioxidant-deficient diet that modifies the hamster's antioxidant status. Elsewhere, the development of atherosclerosis is associated with an increased cardiac ROS production (*40*), i.e., here a superoxide anion production induced by the atherogenic diet, which is closely dependent on the overexpression of NAD(P)H oxidase (*41*, *42*). As suggested by previous studies in the rat model (*25*) and in humans (*43*), we suspected that the source of superoxide anion generation may be NADPH oxidase. Moreover, high cardiac levels of superoxide play an important role in the pathogenesis of atherosclerosis and linked coronary arteries disease. In our model, the origin of cardiovascular alterations is accompanied by an increase of cardiac superoxide production. We have recently reported such a cardiovascular complication in the cholesterol-fed hamster and the implication of oxidative stress in the process of aortic fat deposition (*41*, *42*).

Determination of the activity of the hepatic antioxidant defense system as a marker of the putative protective role of dietary chitin−glucan exhibited major differences in our experimental conditions. Thus, we observed a significant increase of the superoxide dismutase and glutathione peroxidase activities in liver homogenates from CG ld- and particularly CG hd-fed hamsters after 84 days in comparison with that of the control animals. An increased activity of antioxidant enzymes may be a consequence of the antioxidant effect of chitin−glucan, reducing the requirement for enzymatic antioxidants, which catalyzes the detoxification of their substrates allowing the nonenzymatic antioxidants to be less consumed by reacting with ROS. Elsewhere, spontaneous hepatic lipid peroxidation has been shown to decrease with an increasing level of dietary antioxidants (*44*), and supplementation with chitin−glucan effectively decreases oxidative stress by decreasing TBARS formation in the liver, although they are not the better biomarkers of oxidative stress.

We have shown that chitin−glucan prevents the early development of atherosclerosis, characterized by the lipid deposition on the aorta and formation of foam cells. Tannin-Spitz et al. (*45*) reported the in vitro ability of cell-wall algal polysaccharide to inhibit autoxidation of linoleic acid and oxidative damage to 3T3 cells. In these assays, the polysaccharide inhibited oxidative damage in a dose-dependent manner. Very recently, Chen et al. (*46*) have shown that fungal polysaccharides from the genus *Ganoderma* exhibited strong DPPH free radical and superoxide anion radical scavenging activities and suggested that these purified polysaccharides could potentially be used as natural antioxidants. Polysaccharides from other origins such as tea (*47*), wolfberry (*48*), or ginseng (*49*)

have been shown to exert antioxidant activity. The antioxidant mechanism may be due to the supply of hydrogen by the polysaccharide, which combines with radicals and forms a stable radical to terminate the radical chain reaction. The other possibility is that the polysaccharides can combine with the radical ions, which are necessary for radical chain reaction; then the reaction is terminated. However, the exact explanation of the mechanism underlying the free radical scavenging activity exerted by polysaccharides is still not fully understood. Here, the decrease of aortic fatty streak area by chitin−glucan suggests that ROS-scavenging effect is a likely mechanism since the above studies have established that polysaccharides not only scavenge ROS but also act as potent antioxidants and inhibit the lipid peroxidation mediated by ROS. Moreover, improvement of hepatic SOD and GSHPx activities, lowered hepatic TBARS levels, and decreased cardiac superoxide anion production were observed, and aortic cholesterol deposit and fatty streak area development were prevented. These results suggest that chitin−glucan acted through an improvement in the antioxidant status indicating that even low amounts of ingested polysaccharides might decrease lipid oxidation. It is unlikely that the antioxidant effects of chitin−glucan polysaccharides are attributable to their systemic activity because they are not absorbed. The type of antioxidant responsible for the observed effects and the mechanism by which it acts remain major problems that are unresolved. At this stage, we can only posit a hypothesis. The most plausible is that these polysaccharides act only within the intestinal lumen by protecting the body against the absorption of radical species and/or by protecting nutrients against a possible oxidation before their absorption. Also, the antioxidant effect of some metabolites issuing from chitin−glucan fermentation by gut flora cannot be ruled out. Additionally, a hypolipemic effect of chitin−glucan should not be rejected. To our knowledge, there are no studies reported on the preventive effets of chitin−glucan on atherosclerosis. A comparison could be made with other close nonstarch polysaccharides such as chitosan and dietary fiber components such as β-glucan. Kamil et al. (*50*) demonstrated that among the different viscosity chitosans, 14 cP chitosan was more effective than the higher viscosity chitosans (57 cP and 360 cP) in preventing lipid oxidation in the herring flesh model system. Soluble and viscous dietary fibers, such as β-glucans are associated with two major health promoting effects, i.e., the attenuation of postprandial plasma glucose and insulin levels, and the control of cholesterol, that are important in atherosclerosis development. Increased viscosity in the intestine delays absorption of glucose and suppresses the absorption of cholesterol and the reabsorption of bile acids (*51*). Viscosity of β-glucan in foods and in food digestion depends on solubility, concentration, and MW. Since β-glucan can appear in the digestive tract after feeding hamsters with chitin−glucan, this could be another explanation for the decreased atherosclerotic plaques in our model. Unfortunately, our experimental conditions do not allow one to know whether the β-glucan is released from chitin−glucan in intestinal lumen or to know the MW of the issuing units. Although viscosity is a physicochemical property associated with dietary fibers, particularly soluble dietary fibers (*52*), chitin−glucan being a water-insoluble polysaccharide cannot acts directly due to such a property. Elsewhere, MW of the polysaccharides is another important parameter that could intervene in decreasing the atherosclerosis disease symptoms. The antioxidant activity of chitosans of different MWs (30, 90, and 120 kDa) has been investigated in salmon (*53*). In general, all chitosans exhibited antioxidative activities in salmon. The 90 kDa chitosan showed an increased free radical-scavenging activity. Another study (*54*) showed that antioxidative effects of chitosan were increased with larger MW and revealed that the scavenging activities also depended on the MWs of chitosans. However, while the radical-scavenging activity of low MW chitosans appears to play a role in their antioxidant activities, this is not the case for the high MW chitosan. In the latter case, its antioxidant activity must be explained by other mechanisms such as metal chelation or lipid binding. This could happen with chitin−glucan and fractions issuing from them.

These promising results obtained by dietary supplementation with chitin−glucan from *Aspergillus niger* in a diet-induced atherosclerosis animal model give rise to its use as a potential dietary therapy. Further investigation is warranted to define the mechanisms by which chitin−glucan provides protection.

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