PHYSIOLOGICAL EFFECTS OF DIFFERENT TYPES OF β-GLUCAN

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Background: Numerous types of glucans have been isolated from almost every species of yeast, grain, and fungi. These products have been extensively studied for their immunological and pharmacological effects.

Aim: In this paper we evaluated the possibility whether individual glucans will be similarly active against each of the tested biological properties or if each glucan will affect different reactions.

Methods: Immunological effects of glucans were measured by evaluation of phagocytosis of HEMA particles by peripheral blood leukocytes and production of IL-2 by mouse splenocytes. Next we measured the effects of long-term treatment with glucan on levels of blood glucose and blood cholesterol. Four different glucans differing in origin (yeast, grain and mushroom) were used.

Results: Our results showed that the same glucan, yeast-derived insoluble #300 glucan, stimulated phagocytosis of peripheral blood leukocytes, production of IL-2 by mouse splenocytes, lowered the cholesterol levels in mice with experimentally-induced cholesterolemia and lowered the level of blood sugar after induced hyperglycaemie. The remainder of tested glucans were only marginally active.

Conclusion: Taken together, our study showed that with respect to natural glucans, there is a yes-or-no effect suggesting that highly purified and highly active glucans will have pleiotropic impact, whereas poorly isolated and/or less active glucans will have only mediocre biological properties.

INTRODUCTION

Natural products, useful in treating or preventing various diseases, have been sought throughout the history of mankind. Most of these natural products are plagued with a common problem, i.e., the fact that they often represent a complex mixture of individual ingredients each of which can contribute to their biological activity. Natural (1,3)-beta-D-glucans from yeast, grain and mushrooms are well-established biological response modifiers[1,2], representing highly conserved structural components of cell walls in yeast, fungi, seaweed or grain seeds. 1,3-Glucan's role as a biologically active immunomodulator has been well documented for over 40 years. Interest in the immunomodulatory properties of polysaccharides was initially raised after experiments showing that a crude yeast cell preparation stimulated macrophages via activation of the complement system[3].

Numerous types of glucans have been isolated from almost every species of yeast, grain and fungi. (1,3)-beta-D-glucans have been extensively studied for their immunological and pharmacological effects. More than 2,000 papers describing the mostly immunological activities of glucans exist in the literature (for review see[4]).

However, multiple reports established additional effects of glucans. Nearly 50 years ago the possible effects of dietary fiber were first suggested by Keys[5] and these effects were later found to be associated with glucans[6]. The cholesterol-lowering effects of fibers are routinely associated with beta-glucans. Due to the high consumption of oats or oat bran, most of the attention has been focused on the relationship of oat-derived glucan to cholesterol levels. These glucans were shown to reduce serum cholesterol in both hypercholesterolemic animals[7-9] and humans[10], however there are some studies that found no such effects[11].

In addition, fiber-containing glucans, particularly the ones in human food, gained attention for their role in the metabolic control of diabetes[12-15]. However, most of these studies were accomplished using only partly purified glucans, making evaluation of the glucan role rather difficult.

Despite extensive investigations, no consensus on the source, size and other biochemical or physicochemical properties of beta-1,3-glucan has been achieved. In addition, numerous concentrations and routes of administration have been tested – including oral, intraperitoneal, subcutaneous and intravenous applications. Besides, there are probably more than a hundred of different samples on the U.S. market alone, which leads to a confusion about the quality, biological effects and overall efficiency of glucan. Therefore, we decided to compare the basic biological and immunological activities of a group of glucans.

The collection of techniques including phagocytosis and stimulation of IL-2 synthesis effects on cholesterol and blood sugar levels represents a general approach to the biological characteristics of tested glucans thus offering insight as to their biological activities.

Table 1.

	Control	#300	Krestin	Immunofibre	WGP	Now Yeast
Total weight	23.33 ± 1.06	24.34 ± 1.27	24.11 ± 1.48	24.99 ± 4.11	25.87 ± 3.38	26.99 ± 2.77
Liver	1.77 ± 0.23	1.49 ± 0.31	1.55 ± 0.19	1.54 ± 0.38	1.66 ± 0.35	1.47 ± 0.35
Spleen	0.16 ± 0.09	0.17 ± 0.06	0.14 ± 0.05	0.17 ± 0.07	0.15 ± 0.04	0.16 ± 0.03
Thymus	0.05 ± 0.02	0.06 ± 0.01	0.06 ± 0.01	0.05± 0.01	0.05 ± 0.05	0.08 ± 0.02
Heart	0.16 ± 0.06	0.17 ± 0.10	0.18 ± 0.08	0.16 ± 0.03	0.16 ± 0.05	0.17 ± 0.03
Kidneys	0.41 ± 0.10	0.38 ± 0.07	0.40 ± 0.12	0.40 ± 0.15	0.36 ± 0.08	0.42 ± 0.05
Lung	0.16 ± 0.02	0.17 ± 0.02	0.15 ± 0.02	0.18 ± 0.03	0.16 ± 0.04	0.18 ± 0.05

MATERIAL AND METHODS

Animals. Female, 8 weeks old BALB/c mice were purchased from the Jackson Laboratory (Bar Harbor, ME). All animal work was done according to the University of Louisville IACUC protocol. Animals were sacrificed by CO_2 asphyxiation.

Materials. RPMI 1640 medium, sodium citrate, Wright stain, Limulus lysate test E-TOXATE, Concanavalin A, cholesterol, streptozocin and were obtained from Sigma Chemical Co. (St. Louis, MO). Fetal calf serum (FCS) was from Hyclone Laboratories (Logan, UT).

Diet. All diets (Laboratory Rodent Diet 5001 enhanced with glucan and/or cholesterol) were formulated and prepared by Purina (Richmond, IN). Diet ingredients for all groups were identical except for the proportion of glucan and/or cholesterol. Glucan-enhanced diet contained various doses of individual glucan, cholesterol-enhanced diet contained cholesterol. The beta-glucans were quantified by using a Megazyme kit.

Beta-1,3 glucans. Yeast-derived insoluble glucan #300 were purchased from Transfer Point (Columbia, SC), mushroom-derived Krestin from (Kureha Chemical Industries, Tokyo, Japan), yeast-derived Now BETA glucan from Now Foods (Bloomingdale, IL), and grain-derived ImmunoFiber was purchased from (Whole Control, Arvada, CO).

Phagocytosis. The technique employing phagocytosis of synthetic polymeric microspheres was described earlier[15,16]. Briefly: peripheral blood cells were incubated with 0.05 ml of 2-hydroxyethyl methacrylate particles (HEMA; 5×10^8/ml). The test tubes were incubated at 37 °C for 60 min., with intermittent shaking. Smears were stained with Wright stain. The cells with three or more HEMA particles were considered positive. The same smears were also used for evaluation of cell types.

Evaluation of IL-2 production. Purified spleen cells (2×10^6/ml in RPMI 1640 medium with 5% FCS) were added into wells of a 24-well tissue culture plate. After addition of 1 μg of Concanavalin A into positive-control wells, cells were incubated for 72 hrs in a humidified incubator (37 °C, 5 % CO_2). At the endpoint of incubation, supernatants were collected, filtered through 0.45 μm filters and tested for the presence of IL-2. Levels of the IL-2 were measured using a Quantikine mouse IL-2 kit (R&D Systems, Minneapolis, MN).

Glucose evaluation. The mice were given drinking water freely and were not fed 24 hr prior to measurement of blood glucose level. In some experiments, blood glucose was determined in hyperglycaemic mice which were pretreated with ctreptozotocin (250 mg/kg ip.) 12 days before the start of feeding with glucan[17].

Biochemical Analysis. Mice were deprived of food for 24 hr and sacrificed. Serum was collected via the retro-orbital sinus and stored at – 80 °C for less than a week. Biochemical analyses were performed by Antech Diagnostics (Indianapolis, IN).

Statistics. Student's t-test was used to statistically analyze the data.

RESULTS

Phagocytosis is one of the biological activities traditionally connected with effects of natural immunomodulators, including glucans. Therefore, we initiated our study by comparison of the effects of intraperitoneally applied glucans used as a single dose (Fig. 1), From day 1, glucan #300 showed much stronger activation of blood neutrophils than other glucans. Two other glucans, Krestin and NOW glucan, also showed significant effects lasting up to 48 hrs. When we repeated the glucan administration for three consecutive days, we found not only higher phagocytic activity but also that it lasted significantly longer – in the case of #300 and Krestin up to 7 days (data not shown).

Production of IL-2 belongs to the valuable indicators of the immune activities. Therefore, we compared the effects of tested glucans on the secretion of IL-2 by spleen cells isolated from glucan-treated mice. The IL-2 production was measured after a 72 hr *in vitro* incubation of cells. The results summarized in Fig. 2 showed that even when all tested glucan stimulated IL-2 production, there were huge differences between individual glucans (i.e., #300 stimulated IL-2 secretion 3.5 times more than ImmunoFiber). The activity of all tested glucans slowly decreased with time but was still measurable 14 days after injection.

For cholesterol and blood sugar experiments we used the Laboratory Rodent Diet 5001 consisting of 23.9 % protein, 4.6 % fat, 5.5 % fiber, with 75.5 % total digestible nutrients. This diet was supplemented with either glucan or cholesterol corresponding to the final daily doses of
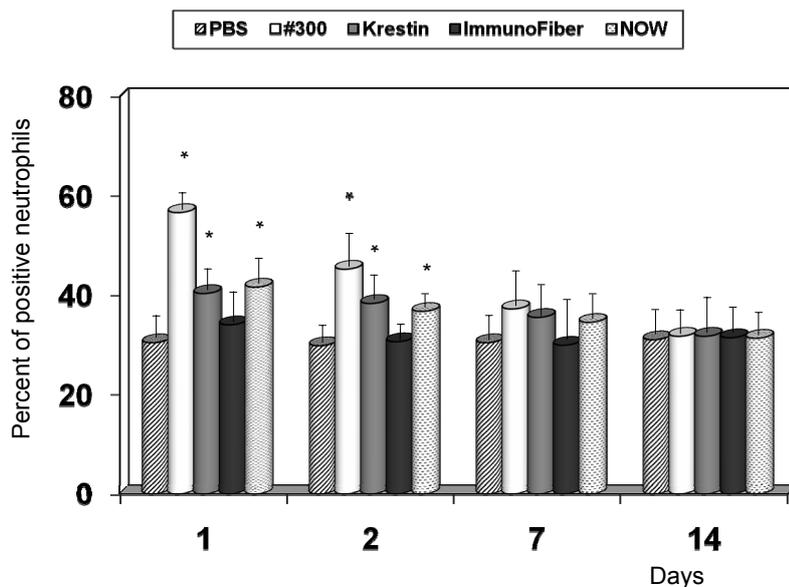
Fig. 1. Effect of long-term feeding with individual glucans (see Materials and Methods) on phagocytosis of HEMA particles by peripheral blood neutrophils. Each value represents the mean of three independent experiments ± SD.
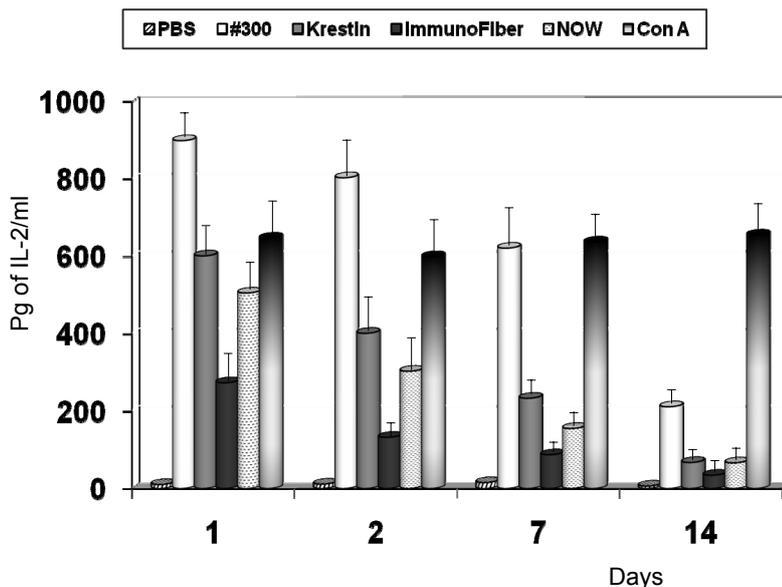*Represents significant differences between control (PBS) and glucan samples at P ≤ 0.05 level.

Fig. 2. Effects of glucan feeding on Con A-stimulated secretion of IL-2 by spleen cells.
*Represents significant differences between control (PBS) and samples at P ≤ 0.05 level.

100 μg of glucan or 16 μg of cholesterol, respectively. The mice adapted well to all diets and all survived the dietary treatment. Body weights and individual organ weights did not differ among the groups at each time point (Table 1).

First, we studied the effect of long-term feeding with glucan-added diet. Our data showed strong time-dependent effects of #300 and Krestin glucans on lowering cholesterol. Effects of other glucans were less pronounced and in the case of ImmunoFiber we found almost no effects (except after 50 days of feeding) – (Fig. 3)

The mice were then given a diet with added cholesterol. The blood cholesterol levels obtained after two weeks of cholesterol feeding were used as positive control. The cholesterol-rich diet was followed by 40 days of feeding with glucan-rich diet. Individual groups of mice were sacrificed in 10-day-intervals and cholesterol levels were evaluated. Results summarized in Fig. 4 showed that during short time intervals all glucans lowered the cholesterol levels in hypercholesterolemic animals, but in the long term only #300 glucan retained this activity.
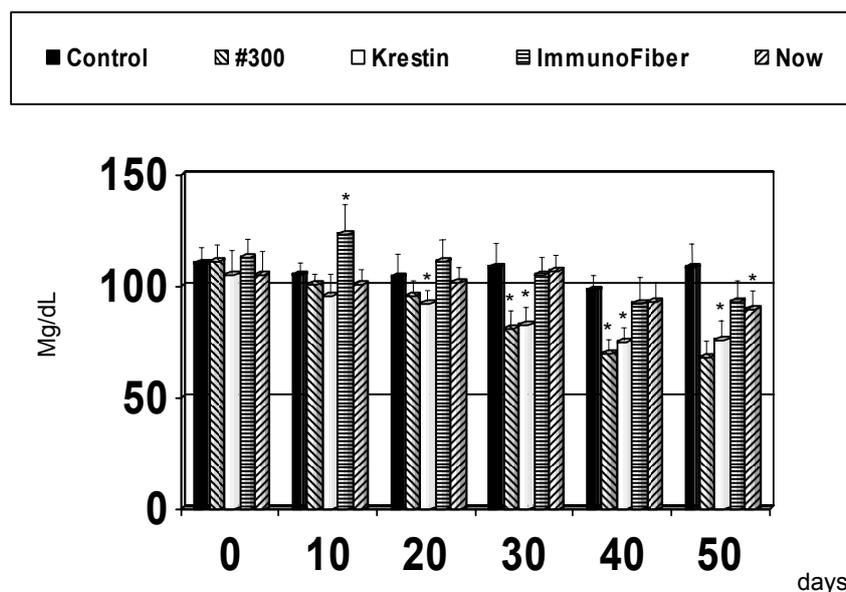
Fig. 3. Effect of long-term feeding with individual glucans (see MATERIALS AND METHODS) on blood cholesterol levels. Each value represents the mean of three independent experiments ± SD.
*Represents significant differences between control (PBS) and glucan samples at P ≤ 0.05 level.
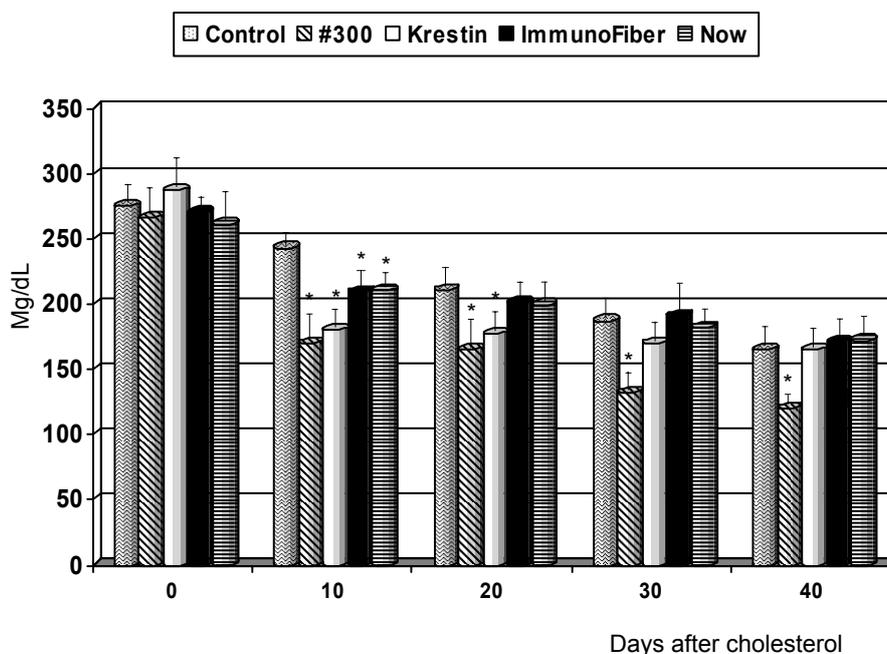

Fig. 4. Effect of long-term feeding with individual glucans on blood cholesterol levels in experimentally-induced hypercholesterolemia. The feeding with glucan started after two weeks of cholesterol-high diet (see Materials and Methods). Control group (Control) was fed with standard diet. Each value represents the mean of three independent experiments ± SD.
*Represents significant differences between control (PBS) and glucan samples at P ≤ 0.05 level.

The final part of our project was focused on effects of glucan administration on levels of blood sugar. Feeding with glucan did not significantly affect the sugar levels (Fig. 5). However, a different situation was found when we used mice with experimentally induced hyperglycaemie. After two weeks of feeding, #300 glucan significantly lowered the sugar levels to almost a normal (compare Figures 5 and 6). A longer application of glucan resulted in additional significant activity of ImmunoFibre glucan.

DISCUSSION

β-Glucans show notable physiological effects. This is the main reason they are of such great interest. They belong to a group of physiologically active compounds, collectively termed biological response modifiers. Thus far, among many known and tested immunomodulators of the first order, polysaccharides isolated from different microorganisms and plants hold a formidable place.
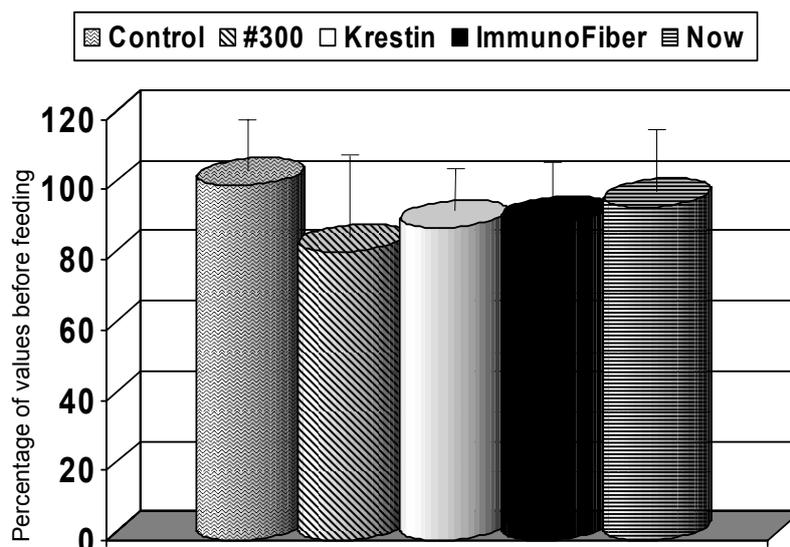
⊠ Control ⊠ #300 ☐ Krestin ■ ImmunoFiber ⊟ Now

Fig. 5. Effect of long-term feeding with individual glucans (see MATERIALS AND METHODS) on blood glucose
levels. The results are given as percentage of control values. Each value represents the mean of three independ-
ent experiments ± SD.
*Represents significant differences between control (PBS) and glucan samples at P ≤ 0.05 level.

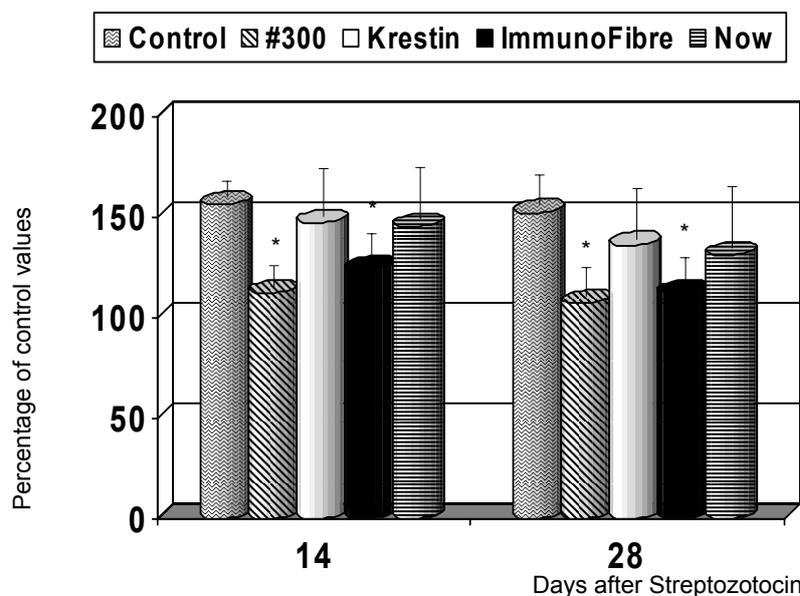**⊠ Control ⊠ #300 ☐ Krestin ■ ImmunoFibre ⊟ Now**

Fig. 6. Effect of long-term feeding with individual glucans (see MATERIALS AND METHODS) on blood glucose
levels in mice with experimentally-induced hyperglycaemie. The results are given as percentage of control
values. Each value represents the mean of three independent experiments ± SD.
*Represents significant differences between control (PBS) and glucan samples at P ≤ 0.05 level.

A large number of such polysaccharides, that act only as
immunopotentiators, are well known[18].

Binding of β-glucan to specific receptors (either
CR3 or Dectin-1) activates macrophages. The activation
consists of several interconnected processes including
increased chemokinesis, chemotaxis, migration of macro-
phages, degranulation leading to increased expression of
adhesive molecules, and adhesion to the endothelium. In
addition, β-glucan binding triggers intracellular processes,
characterized by the respiratory burst after phagocytosis
of invading cells (formation of reactive oxygen species

and free radicals), the increase of content and activity of
hydrolytic enzymes, and signaling processes leading to
activation of other cells and secretion of cytokines. For
an excellent review regarding interaction of glucans with
macrophages see[19].

The rationale for the choice of glucans parallels what
was explained in our previous paper[20]. We chose four glu-
cans widely sold and available in the US, Europe and
the Far East, representing grain-, mushroom- and yeast-
derived glucans in soluble and insoluble form. Briefly,
#300 is insoluble yeast-derived glucan; Krestin is soluble

mushroom-derived glucan; ImmunoFiber represents soluble grain-derived glucan; and NOW is a mixture of both insoluble glucans from yeast and soluble glucans from mushrooms.

There are very few comprehensive reviews focused on biological properties of glucans from various sources that exist. The comparative reviews focus mainly toward the reflection of chemical characteristics of glucans on their biological and immunological properties[21, 22].

In this paper, we continued the comparison of several commercially important glucans. Glucans are well known for their ability to stimulate the innate immunity and the cellular branch of immune reaction[18]. Therefore, our initial focus was phagocytic activity with the use of peripheral blood neutrophils and synthetic microspheres as a model. Our results confirmed our previous studies showing that glucan #300 was one of the most active glucans available.

It is assumed that glucan application results in signaling processes leading to activation of macrophages and other cells and subsequent secretion of cytokines and other substances initiating inflammation reactions (*e.g.*, interleukins IL-1, IL-2, IL-6, TNF-α)[23-25]. We found that all tested glucans stimulated splenocytes to produce IL-2, with #300 and Krestin showing the strongest and longest effects.

Most of experimental studies dealing with cholesterol-lowering effects of glucans used oats- or barley-derived glucans[8, 9], without finding significant differences between these glucans[7]. The cholesterol-lowering effects of glucans are well-established on numerous models, including humans[8, 26]. Without any direct proof, these effects are usually described as the result of fiber intake and subsequent decreased absorption of bile acids[27]. However, most of these studies suffer from the fact that they did not evaluate the effects of isolated glucans. They only used crude extracts[28] without any knowledge if these glucans are even digested.

The current study is not only the first to directly compare the cholesterol-lowering activity of two different yeast-derived β-glucans, but also the first to compare normal animals and mice with experimentally induced cholesterolemia.

The effects if glucans on blood sugar levels are less known. Some studies showed hypoglycemic activity of natural glucans[13, 29], additional studies demonstrated strong hypoglycemic activity of synthetic polysaccharides[17, 30]. The mechanisms remain, however, unknown. Some groups suggested similar mechanisms as in lowering cholesterol, i.e., changes in the increase of viscosity of the alimentary bolus and changes in the gastric emptiyng[31]. Our data found no correlation between these effects and source of the glucan or its solubility.

The present paper represents yet additional proof of the vast differences among commercially available glucans. Based on the presented data, it is clear that the individual glucans are either active in all tested areas or their activity is only mediocre. It is most likely that this yes-or-no effect suggests there are no glucans that can be tailor-made for stimulation of one particular reaction.

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