Nutrition

β-Glucan attenuates chronic fatigue syndrome in murine model

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The purpose of this study was to evaluate the beneficial effects of yeast-derived β-glucan on some immunological parameters in a mouse model of experimentally-induced chronic fatigue syndrome (CFS). This syndrome was induced by *Brucella* antigen injection. Our data show that the CFS manifested by a significant decrease of immune reactions including NK cell activity, cytokine production and lymphocyte proliferation. In addition, serious disruptions in activities of enzymes such as SOD, GSH and PGx both in blood and brain were observed. A two week supplementation of mice with glucan returned most of these values to normal. In addition, glucan supplementation improved the mean immobility time induced by long-term swimming. We conclude that glucan might be considered a potential natural treatment for chronic fatigue syndrome. *Journal of Nature and Science, 1(6):e112, 2015*

Glucan | fatigue | immunity | NK cells | cytokines | GSH

Despite decades of research, chronic fatigue syndrome (CFS) remains a serious disorder of unknown etiology, characterized by the major manifestation of profound fatigue. The most common diagnosis of CFS is a reduction in daily activity greater than 50% for at least 6 months [1]. However, fatigue is a prominent symptom in a number of infections and autoimmune diseases. Additional somatic and neuropsychiatric symptoms are common in possible cases of CFS, making both diagnosis and evidence-based treatment problematic. Since approximately 60% of CFS patients demonstrate immunological disturbances [2], possible etiology includes immunologically-mediated abnormalities associated with the central nervous system [3]. Recent studies have implicated oxidative stress in the pathogenesis of this disease [4,5].

It seems that the positive feedback loop represents PAMP activation leading to peripheral NF-κβ production, thence pro-inflammatory cytokine release in a positive feedback loop, leading to neuro-inflammatory conditions; as well as peripheral NF-κβ production leading to O and NS stress contributing to formation of DAMPs, which engage TLRs and trigger chronic inflammation. Neurological and biochemical effects are evidenced by changes in serum levels of anti-oxidant enzymes superoxide dismutases (SOD), oxidative stress marker malondialdehyde (MDA), and prostaglandins (PGx) in serum and by decreases in the levels of SOD and anti-oxidant glutathione (GSH) in the brain.

The abovementioned inflammatory cytokines include IL-2, which is a cytokine contributing to Th-1 dominance. With glucan, Th-1 dominance is typically induced by maitake D fraction, favoring tumor suppression [6]. It is interesting that we found IL-4, which contributes to Th-2 dominance, not particularly elevated, as this would be consistent with a hypothesis that Th-1 dominance can reduce CFS.

In recent years, interest has been renewed in therapeutic applications of natural molecules to CFS treatment, and their mechanisms of action. Although the data demonstrating effects of nutritional supplements on CFS are still minimal, some studies have already emerged, for example, on antioxidants as treatment [2]. In addition, dietary supplementation with different natural products (such as vitamin C or E) offered some palliative effects [7]. More recently, research has increasingly focused the immune system effects of natural supplements such as olive extract or green tea extract [8,9].

One of the natural immunomodulators shown to attenuate CFS is curcumin, possibly acting via oxidative stress reduction [10]. Additional studies found similar effects with polysaccharides from *Panax gingseng* [11] and Japanese herbal medicine Hochu-ekki-to [12]. A study showing strong effects of yeast extract [13] led us to the hypothesis that a molecule responsible for these effects might be glucan.

Glucans are non-cellulosic polymers of β-D-glucose, with glycosidic bonds in position β-(1,3) and various degrees of β-(1,6) branching. They represent highly conserved structural components of cell walls in yeast and fungi, and have been used as highly biologically active immunomodulators for over 50 years. Numerous studies (currently more than 10,000 research papers) have shown that β-glucans, either particulate or soluble, exhibit immunostimulating properties, including antibacterial and anti-tumor activities (for review see [14,15]). Besides effects on the immune system and other direct biological effects, glucans were found to improve the negative effects of stress by reducing the level of corticosterone [16,17], and to have positive effects following exercise stress [18].

The best known of the extensive immunological effects of glucans consists of the direct stimulation of phagocytosis of professional phagocytes – granulocytes, monocytes, macrophages and dendritic cells – and direct activation of natural killer cells (for a recent comprehensive review, see [14]). More and more studies suggest a strong relation between immune problems and CFS – indeed, the connection has been demonstrated in a murine model of CFS [13]. These data led us to the investigation of the possible immunologically mediated effects of highly purified β-glucan on CFS manifestation.

Several animal models of CFS have been established. One of these models induces CFS by administration of *Brucella abortus* [19]. Additional models use long-term swimming consistent with the contribution of peripheral tissues to CFS [20], or injection with *Corynebacterium parvum* antigen [21]. We used two different models – one induced CFS by injection of *Brucella* antigen; the second model used long-term swimming as stressor.

Material and Methods

Animals. Female, 8 week 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. Nine mice/group were used.

Material. Yeast-derived insoluble Glucan #300 was purchased from Transfer Point (Columbia, SC). The purity is over 85%. RPMI 1640 medium, glutamin, antibiotics and concanavalin A (Con A) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Fetal calf serum (FCS) was procured from Hyclone Laboratories (Logan, UT, USA). Biotrak cell proliferation ELISA system, version 2 was obtained from Amersham Pharmacia Biotech (Piscataway, NJ, USA).

Cells. Murine tumor cell line YAC-1 was provided by Dr. Julie Djeu of the Moffitt Cancer Research Center, Tampa, FL. Cells were maintained in RPMI 1640 medium supplemented with 10% FCS, 2 mM glutamine, and antibiotics.

Organ weights. All the mice were sacrificed at the end of experiments and the weight of individual organs were evaluated.

Experimental groups. A Control group of nine mice was administered PBS. The glucan-supplemented group received 100 μg/mouse of glucan orally for 14 days prior to the experiment.

Induction of CFS by Brucella abortus antigen. Fixed whole *Brucella abortus* ring test antigen was obtained from Central Research Institute, Kasauali, India. CFS was induced by a six time repeated injection of original antigen solution (0.2 ml per mouse) via tail vein every two weeks [22].

Water-immersion stress. Mice were forced to swim individually in a glass jar (30 cm x 12 cm x 30 cm) containing 18 cm-deep water at room temperature. After an initial period of vigorous activity each animal assumed a typical immobile posture. The mouse was considered to be immobile when it ceased to struggle and made minimal movements to keep its head above the water. This period of observation was 10 minutes.

Table 1. Effect of glucan on body weight and organ weight

	Body	Spleen	Thymus	Heart	Lungs
PBS	21.4 + 2.2	636 + 81	46 + 10	112 + 15	147 + 19
CFS	19.3 + 2.1	339 + 54	49 + 11	111 + 12	136 + 16
Glucan	22.7 + 1.9	519 + 45*	47 + 10	114 + 14	141 + 17

Weight of body is given in g, weight of organs in mg. Significant difference between CFS group and treated (Glucan) group at P < 0.05 level.

Table 2. Effect of glucan on serum levels of MDA, SOD and PGx

	MDA (nmol/ml)	SOD (U/ml)	PGx (U)
PBS	18.1 + 1.2	90.4 + 4.4	766.9 + 74.2
CFS	44.2 + 4.1*	54.3 + 3.9	554.6 + 64.2
Glucan	23.4 + 2.2*	92.8 + 6.2*	778.5 + 63.9*

Significant difference between CFS group and treated (Glucan) mice at P < 0.05 level.

Cell proliferation. A hundred µl of purified spleen cells (1×10^{6}/ml in RPMI 1640 medium with 5% FCS) from both groups of mice were added into each well of a 96-well plate in triplicates. After 0 to 72 hr. incubation in RPMI 1640 medium with 10% FCS, proliferation was evaluated using Biotrak cell proliferation ELISA system according to instructions given by the manufacturer.

Cytokines. After being cultured in a humidified incubator (37° C, 5% CO_2) for 72 hrs, levels of cytokines IL-2 and IL-4 secreted by splenocytes in the presence of 5 µg Con A were determined by ELISA: Supernatants were collected, filtered through 0.45 µm filters, and Il-2 and IL-4 measured using Quantikine mouse kits (R&D Systems, Minneapolis, MN).

Biochemical parameters. On the endpoint, animals were sacrificed. The brain was removed, rinsed in isotonic saline solution and weighed. A 10% w/v tissue homogenate was prepared in 0.1 M phosphate buffer pH 7.4. The homogenate was centrifuged at 12 000 g for 60 minutes at 4°C. GSH levels were estimated as described earlier [23]. Briefly, 0.75 ml of homogenate was precipitated with the same volume of 4% sulfosalicylic acid, centrifuged and mixed with 0.01 M 5,5-dithiobis-(2-nitrobenzoic) acid at ratio 1:9. The color was immediately measured using at 412 nm. Results were expressed as nanomoles of GSH/mg protein. Superoxide SOD activity was tested as described [24]. In summary, the reaction was initiated by the addition of hydroxylamine HCl to the mixture containing nitroblue tetrazolium and the homogenate. The reduction was measured at 560 nm. The results were expressed as units/mg of protein. Serum levels of MDA, SOD and GPx were measured as described [25].

NK cell activity. Spleen cells were isolated from the spleen of mice by standard methods. The viability was determined by trypan blue exclusion. Only cells with viability better than 95% were used in subsequent experiments. Splenocytes (10^{6}/ml; 0.1 ml/well) were added into V-shaped 96-well microplates and 50 µl of target cell line YAC-1 (three different concentrations of target cells were used so the final effector-target ratio was 50:1. After spinning the plates at 250x g for 5 min, the plates were incubated for 4 hrs. at 37°C. The cytotoxic activity of cells was determined by the use of CytoTox 96 Non-Radioactive Cytotoxicity Assay from Promega (Promega, Madison, WI, USA) according to the manufacturer's instructions. Briefly, 10 µl of lysis solution was added into the appropriate control wells 45 min before the end of incubation. Subsequently, the plates were spun at 250x g for 5 min, followed by transfer of 50 µl of supernatant into flat-bottomed, 96-well microplates. After 50 µl of reconstituted substrate was added into each well, plates were covered and incubated for 30 min at room temperature at dark. The optical density was determined by using a STL ELISA reader (Tecan U.S., Research Triangle Park, NC) at 492 nm. Specific cell-mediated cytotoxicity was calculated using the formula:

Percent-specific killing (% cytotoxicity) = 100 x [(OD_{492} experimental - OD_{492} spontaneous) divided (OD_{492} maximum - OD_{492} spontaneous)] as described in manufacturer's instructions, where spontaneous release was target cells incubated with medium alone and maximum release was that obtained from target cells lysed with the solution provided in the kit.

Statistics. Student's t-test was used to statistically analyze the data. Data at p<0.05 were considered significantly different.

Results

Induction of CFS did not result in significant changes in total body weight nor in weight of thymus, heart or lungs. However, the weight of the spleen was significantly decreased. This reduction was significantly improved by glucan treatment (Table 1). Chronic forced swimming for 6 minutes per session for 15 days increased the

immobility period in control animals, resembling CFS. Glucan treatment, administered daily, significantly reduced the immobility periods (Figure 1).
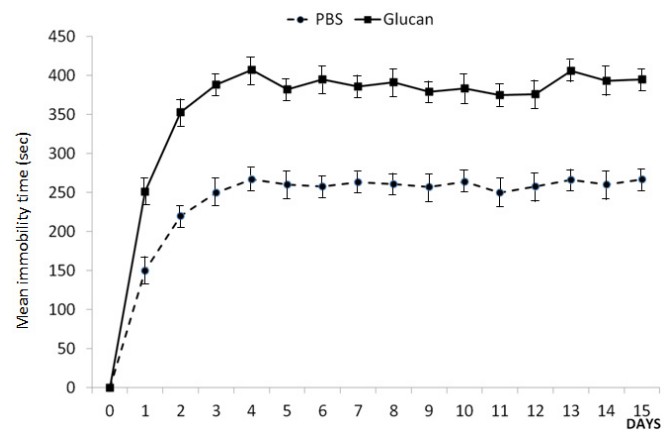

Figure 1. Effect of glucan on mean immobility time on day 1 through 15 in mice subjected to chronic swimming.

The same treatment significantly reduced levels of GSH and SOD in the brain. In both cases, glucan treatment reversed the decrease but the levels still did not fully reach levels in naïve mice (Figure 2). Testing of levels of enzymes such as MDA, SOD and PGx in serum, indicated a significant decrease in the CFS group, which was significantly reversed by the glucan treatment (Table 2).
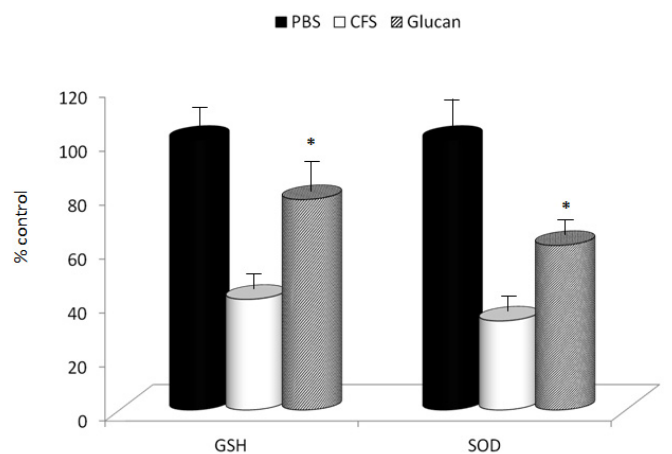

Figure 2. Effects of glucan on depletion of GSH and SOD in mice with CFS. Results represent mean from three experiments ± SD. *Represents significant differences between the CFS and glucan-treated mice at P≤ 0.05 level.

In order to establish the possible effects of CFS on the immune system, the proliferation of B lymphocytes was measured. Data summarized in Figure 3 showed that CFS caused long-term inhibition of splenic cell proliferation, which can be reversed by glucan supplementation. A similar trend was observed in experiments evaluating NK cell activity (Figure 4). Again, CFS resulted in depressed NK cell-mediated killing, which was reversed by glucan supplementation.
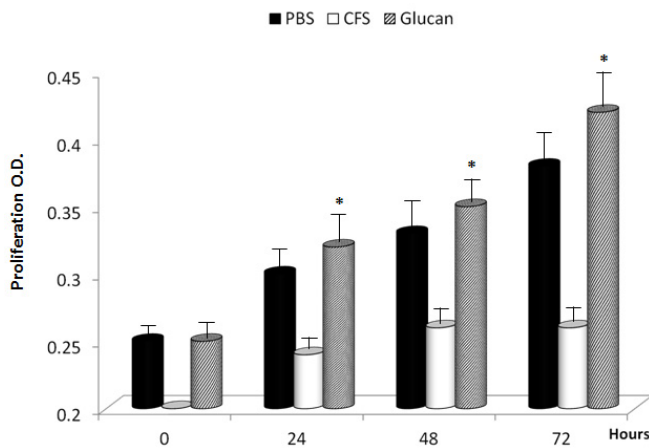
Figure 3. Effects of glucan on proliferation of cultured splenocytes. Results represent mean from three experiments ± SD. *Represents significant differences between the CFS and glucan-treated mice at P≤ 0.05 level.
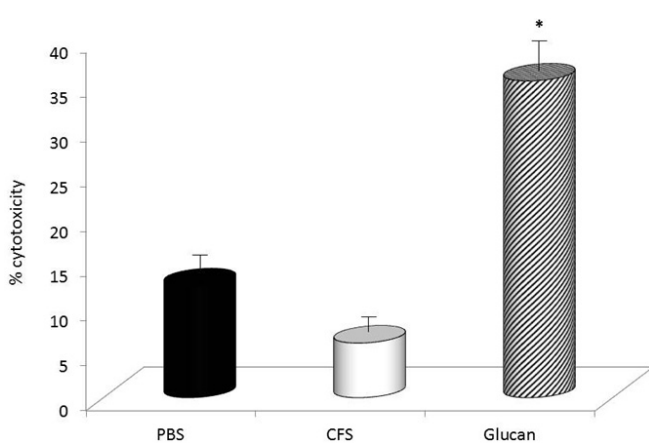

Figure 4. Effects of glucan on ability of splenic NK cells to kill YAC-1 targets. Results represent mean from three experiments ± SD. *Represents significant differences between the CFS and glucan-treated mice at P≤ 0.05 level.

In addition to the effects on cellular immunity, the effects on the humoral branch of immune reactions included strong depression of IL-2 formation, which was reversed by glucan treatment. The effects on IL-4 production were less pronounced, mostly because the production of IL-4 was generally rather low (Figure 5).

Discussion

In this study, we first demonstrated that the oral supplementation of food with highly purified 85% yeast-derived β-glucan could significantly improve the conditions of CSF. This treatment increased the activity of splenic NK cells, proliferation of splenic lymphocytes, production of important cytokines, and restored the activities of four enzymes in the blood and brain. In a separate model of chronic fatigue, glucan feeding improved the conditions induced by a swimming stress.
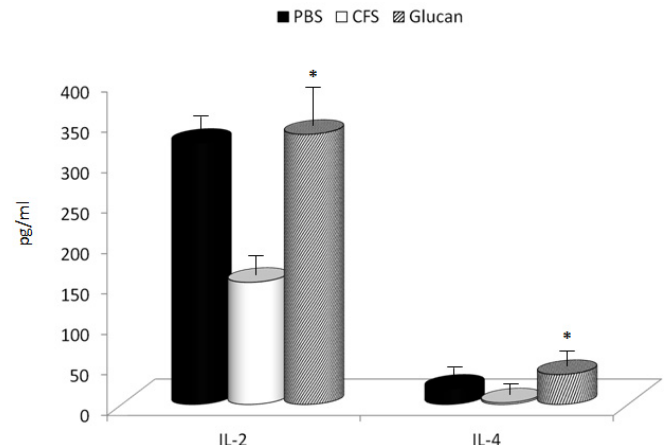

Figure 5. Effects of glucan on secretion of IL-2 and IL-4 by spleen cells. Results represent mean from three experiments ± SD. *Represents significant differences between the CFS and glucan-treated mice at P≤ 0.05 level.

Consistent with previous studies, we found that CFS induction resulted in suppression of cytokine formation [13]. The deregulated balance of Th1/Th2 related cytokines was previously found to play highly important role in the CFS immunopathogenesis [26]. In addition, we found significantly lower proliferation of splenic lymphocytes and activity of NK cells. Biochemical observations revealed significant changes in levels of MDA, SOD, and PGx in serum as well as changes in levels of SOD and GSH in the brain. These results confirmed a considerable increase in the lipid peroxidation and decrease in the glutathione levels, which was supported by the enzymatic evaluations of SOD. These data indicate increased oxidative stress in the brain and suggest the possible involvement of oxidative stress in the pathogenesis of CFS. Pretreatment of mice with glucan resulted in return to normal values, similar to the effects of antioxidants such as carvedilol and melatonin described earlier [27]. Another possibility is that glucan affects the endurance via its anti-fatigue properties [28] and subsequently improves the manifestation of the CFS. Effective mechanisms of polysaccharides on oxidative stress in CFS were also demonstrated by Wang et al. [11].

The current study indicates that treatment with yeast-derived b-glucan might be an effective treatment for the biological alterations associated with chronic fatigue syndrome and that these palliative effects might be mediated by the ability of glucan to suppress oxidative damage and to stimulate immune reactions. Based on our data we propose that β-glucan might be considered a potential treatment for CFS.

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