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Wellmune WGP® Clinical Data Supports Enhanced Immune System Benefits in Children

September 2013

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September 10, 2013

Objectives:

Wellmune WGP improves the functioning of the innate immune system by making white blood cells of the innate immune system (specifically leukocytes) better able to find and kill potential pathogens. This has been demonstrated in laboratory studies (in vitro), animal models and in human clinical trials that have measure both physical health benefits and changes in immune biomarkers. This paper will examine how this robust and varied research portfolio supports the benefit of Wellmune in young children ages 1 and older.

Description of the development of the immune system of children and adult

The adult immune system is considered to consist of the innate and adaptive components. The adult immune system has experienced significant encounters with pathogens, inflammation and other health challenges that provided "experience" to the adaptive immune system with a fully developed T-cell dependent antibody response capability. The innate immune system of the adult and the young child are comparable by age one. The immune system of the child consists of the same components (innate and adaptive). The innate immune system is developed and functioning in young children. The adaptive immune system of the child is present, but it is "inexperienced" due to minimal encounters with the health challenges encountered by the adult. In a child the innate immune system plays a major role in protecting the health of the child as evidenced by the higher total leukocyte population early in life vs. the adult; macrophages and neutrophils are considered fully developed at birth (1,2). The ability of the infant and young child to produce competent WBC's is normal from birth onward (1,2). Thus, the essential components for active processing and application of Wellmune WGP are present and functional in the young child at one year of age or less. Macrophages are capable of digesting Wellmune WGP into the active fragment, neutrophils are capable of receiving the active fragment onto the CR3 receptor and complement is present for opsonizing target cell such as pathogens.

Wellmune WGP Mechanism of Action (MOA)

The innate immune system is the primary line of defense for young children against common childhood diseases; the specific or adaptive immune system has not yet fully developed (1-3). The mechanism of action of Wellmune is complex, but can be delineated into a few simple steps (4).

1. The human gastrointestinal tract contains immune tissue with specific cells that actively collect and transport certain materials into the immune system; Wellmune is one of the materials that is actively collected by these gut immune cells.
2. Processing of Wellmune by specific immune cells (macrophages) produces a biologically active fragment of Wellmune. The Wellmune fragment binds to and enhances white blood cells called neutrophils.
3. The active fragment of Wellmune has some specific effects on neutrophils:
    a. When Wellmune fragment binds to neutrophils it increases the ability of these cells to move towards (chemotaxis) potential pathogens. (5, 6, 7, 8)
    b. The pathogen cells are marked and labeled by soluble blood proteins called complement in a process called complement activation.
    c. When neutrophils loaded with Wellmune encounter complement-activated cells (pathogens) it activates a specific biological mechanism that kills the pathogen. (9, 10, 11, 12, 13, 14)

Wellmune improves immune response leading to reduced impact of illness

We have provided data supporting that Wellmune WGP enhances the innate immune system leading to more effective immune response and killing of potential pathogens *in vivo*. Although it is important to study the biological immune biomarkers associated with Wellmune intake, it is equally important to see "real-life" clinical evidence that human subjects consuming Wellmune remain healthier than subjects not taking Wellmune.

Typical colds and flu (URTI) are caused by rhinovirus and influenza viruses. In multiple studies (summarized in Table 1), Wellmune WGP has reduced URTI symptoms and improved overall physical health.

Table 1. Clinical Studies with Wellmune WGP

Study Type	Subjects	Design & Dose	Results	Published/ Presented	Date
Physical Stress & Health Effects	54 wildland fire-fighters	10 day cross over @ 250 mg / day	Lower URTI symptoms ($p=0.06$), better overall perception of physical health (<0.006)	Am Soc of Sports Med (Presented)	May 2008
Cold/Flu	40 adults	30 day double blind placebo controlled @ 250 mg / day	Reduction in missed days of work or school, Reduced fever, better physical health component (SF36 v2 survey)	Feldman et al J. of Applied Research	July 2009
Physical Stress & Health Effects	75 adult marathoners	30 day double blind placebo controlled @ 250 mg / day	Reduced number of URTI symptoms ($p<0.05$), reduced fatigue, tension and mental confusion, increased vigor	Talbott and Talbott. Journal of Sports Science & Medicine	Dec 2009
Lifestyle Stress & Health Effects	150 stressed adults	30 day double blind placebo controlled @ 250 mg / day	Reduced number of URTI symptoms ($p<0.05$), reduced fatigue, tension and mental confusion, increased vigor	Talbott and Talbott. Agro Foods Industry Hi Tech	Feb 2010
Lifestyle Stress & Health Effects	122 stressed adults	90 day double blind placebo controlled @ 250 mg / day	Reduced number of URTI symptoms ($p<0.05$), improved global mood state and increased vigor	Talbott and Talbott. J Am Col. Nutr.	2013
Physical / Lifestyle Stress & Health Effects	100 4^{th} year med students	90 day double blind placebo controlled @ 250 mg / day	Reduced number of URTI symptoms ($p<0.06$), no changes in immune cell number or profile, no changes in cytokines from baseline	Fuller et al, J of Nutrition	June 2012
Physical Stress & Health Effects	182 adult marathoners	30 day double blind placebo controlled @ 250 mg / day	Reduced number of URTI symptoms ($p<0.05$) for both dispersible and soluble Wellmune	McFarlin et al J Dietary Suppl.	Aug 2013

As shown in Table 1, seven studies (15, 16, 17, 18, 19, 20, 21) have assessed the impact of Wellmune WGP on the physical and psychological health of individuals experiencing lifestyle and physical stress that often directly lead to illness. The studies featured members of the general population including firefighters, marathoners, students and individuals with moderate to high lifestyle stress. These studies were conducted by 5 independent research groups and have consistently found that Wellmune WGP improves physical health (measured as a reduction in URTI symptoms)

Studies with Wellmune WGP and young animals

As a surrogate model, studies with young animals consuming Wellmune WGP have demonstrated superior health and growth performance (22-24). In general, these studies were designed to look at animal growth performance and not immune biomarkers. However, the pattern of the studies is consistent in demonstrating better animal growth, weight gain, reduced mortality and reductions in common juvenile diseases such as diarrhea. In studies conducted by Land O'Lakes (largest feed company in North America), calves (functioning with a monogastric digestion system similar to pigs) fed a milk replacer diet with Wellmune WGP showed significant reductions in diarrhea, improved milk replacer intake and reductions in the use of electrolytes and antibiotic (22). In a study with 1200 piglets, Newport Laboratories observed that nursery piglets experiencing a growth challenge (underweight piglets) gained weight significantly better while consuming Wellmune WGP (23). In a second piglet study on a production farm, 1000 treatment piglets were fed a diet that included Wellmune WGP (32); 1000 control piglets received no additive to the diet. The study was conducted on a production facility with a history of viral infection (PRRS and CRCO viral infections). The Wellmune WGP treated group had reduced mortality and increased weight gain (24).

Clinical data for Wellmune WGP and children

In addition to animal models, a study recently presented at the 2013 Experimental Biology meeting compared incidence and duration of acute respiratory tract infections between children fed a follow-on formula (containing yeast beta glucan (Wellmune), DHA and a prebiotic blend) to cows milk (abstract attached). This study is the first direct evidence that the same benefits

documented in adults taking Wellmune (15, 16, 17, 18, 19, 20, 21) can also be measured in children.

Extrapolation of existing adult clinical data to children

A report by the National Research Council on the daily-recommended intake of the eight water-soluble B complex vitamins (thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline) cited "a nearly complete lack of usable data on the nutrient needs of infants, children and adolescents (25)." The RDA for numerous vitamins has been extrapolated from adult data (25). According to the authors, it is common to use the reference bodyweight method to extrapolate dose from adult data that of the child (25). Similar to the use of the extrapolation method for setting DRI's and RDA's of vitamins for children, mineral DRI's and RDA's have been extrapolated 26, 27). In a study reviewing the DRI's of calcium, phosphorus, magnesium, vitamin D, and fluoride it was concluded that extrapolation of data for mineral intake is a commonly used practice (26). Naturally, it is desirable to have data on the target population group; however, experts around the world have recognized that it is important to provide key nutrients to all populations while ongoing research optimizes dose and response. Another study provided clearly developed extrapolation procedures for using adult data for calcium, zinc, phosphorous and iron DRI's (27).

Extrapolation of adult intakes to child intakes is a common and accepted method

In general, data extrapolated from adult to child uses an accepted process. The use of Wellmune WGP as a dietary component intended to provide immune support fits with the extrapolation process. Wellmune WGP has a strong safety profile and can be safely used as part of the diet of a child.

The benefits of Wellmune WGP in children

The immune system of the young child has a developed innate immune system component, but an under-developed specific (adaptive) immune system. Major childhood diseases such as upper respiratory tract infections (URTI), otitis media and diarrhea are all caused by pathogenic bacteria or viruses. The innate immune system is the primary line of defense for young children against these diseases. Wellmune strengthens the innate immune system and enabling innate

immune cells to more easily detect, move towards and neutralize the pathogens commonly encountered by children. When one considers that the innate immune system of the one-year old child is similar to the composition and activity of the adult, there are sources of multiple data (MOA, adult human clinical studies, young animal model studies and a very recent clinical trial with young children) to support the benefits of Wellmune WGP in the child.

References

1. Janossy G, Bofill M, Poulter LW, et al. Separate ontogeny of two macrophage-like accessory cell populations in the human fetus. J Immunol. 1986;136:4354-4361.
2. Slayton WB, Li Y, Calhoun DA, et al. The first-appearance of neutrophils in the human fetal bone marrow cavity. Early Hum Dev. 1998;53:129-144.
3. Holsapple, M. P., D. J. Paustenbach, G. Charnley, L. J. West, M.I. Luster, R. R. Dietert and L. A. Burna-Naas. 2004. Symposium summary: Children's health risk-What's so special about the developing immune system? Toxicol. & Appl. Pharmacol, 199:61-70.
4. Hong, F., Yun, J. Baran J.T., Allendorf, D.J., Hansen, R. D. Ostroff, G.R., Xing, P.X., Cheung, N. V. and Ross, G. D. 2004. Mechanism by which orally administered β-1,3 gluco polysaccharides enhance the tumoricidal activity of antitumor monoclonal antibodies in murine tumor models. J. Immunol. 173:797-806.
5. Li, B., Allendorf, D. J., Hansen, R. D., Marroquin, J., Ding, C., Cramer, D. E., and Yan, J. 2006. Yeast ß-Glucan Amplifies Phagocyte Killing of iC3b-Opsonized Tumor Cells via Complement Receptor 3-Syk- Phosphatidylinositol 3-Kinase Pathway. J. Immunol. 177: 1661-1669.
6. Tsikitis, V.L., Albina, J.E. , and Reichner, J.S. , ß-glucan affects leukocyte navigation in a complex chemotactic gradient. 2004, Surgery 136:384-9
7. Harler, M. B., E. Wakshull, E. J. Filardo, J. E. Albina, and J. S. Reichner. 1999. Promotion of Neutrophil Chemotaxis Through Differential Regulation of $ß_1$ and $ß_2$ Integrins. J. Immunol. 162:6792 – 6799.
8. Leblanc, B. W., J. E. Albina and J. S. Reichner. 2006. The effect of PGG-ß-glucan on neutrophil chemotaxis in vivo. J. of Leukocyte Biology. 79:667-675.

9. Wakshull, E., D. Brunke-Reese, J. Lindermuth, L. Fisette, R. S. Nathans, J. J. Crowley, J. C. Tufts, J. Zimmerman, W. Mackin, and D. S. Adams. 1999. PGG-Glucan, a soluble beta1,3/-glucan, enhances the oxidative burst response, microbicidal activity, and activates an NF-kB-like factor in human PMN: Evidence for a glycosphingolipid beta-1,3/-glucan receptor Immunopharmacology 41:89–107.
10. Study 2004-01 Effect of Wellmune WGP given per os (PO) on the immune system of healthy human volunteers, an open-label study. Biothera, Inc.
11. Liang, J., D. Melican, L. Cafro, G. Palace, L. Fisette, R. Armstrong and M. L. Patchen. 1998. Enhanced clearance of a multiple antibiotic resistant Staphylococcus aureus in rats treated with PGG-glucan is associated with increased leukocyte counts and increased neutrophil oxidative burst activity. Intl. J. of Immunopharmacol. 20:595-614.
12. Niederman, R., H. Kelderman, S. Socransky, G. Ostroff, C. Genco, R. Kent, Jr., and P. Stashenko. 2002. Enhanced neutrophil emigration and *Porphyromonas gingivalis* reduction following PGG-glucan treatment of mice. Archives of Oral Biology 47:613-618.
13. Kernodle, D. S., H. Gates, and A. B. Kaiser. 1998. Prophylactic Anti-Infective Activity of Poly-[1-6]-b-D-Glucopyranosyl- [1-3]-b-D-Glucopyranose Glucan in a Guinea Pig Model of Staphylococcal Wound Infection. ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, 42:545-549.
14. Kaiser, A. B. and D. S. Kernodle. 1998. Synergism between Poly-(1-6)-b-D Glucopyranosyl-(1-3)-b-D-Glucopyranose Glucan and Cefazolin in Prophylaxis of Staphylococcal Wound Infection in a Guinea Pig Model. ANTIMICROBIAL AGENTS AND CHEMOTHERAPY. 42:2449-2451.
15. Feldman S, Schwartz HI, Kalman DS, Mayers A, Kohrman HM, Clemens R, et al. Randomized phase II clinical trials of Wellmune WGP® for immune support during cold and flu season. . J Appl Res Clin Expt Ther 2009;9:30-42.
16. Talbott S, Talbott J. Beta 1,3/1,6 Glucan Decreases Upper Respiratory Tract Infection Symptoms and Improves Psychological Well-Being in Moderate to Highly-Stressed Subjects. Agro Food Industry Hi-Tech. 2010;21:21-4.

17. Talbott S, Talbott J. Effect of BETA 1, 3/1, 6 GLUCAN on Upper Respiratory Tract Infection Symptoms and Mood State in Marathon Athletes. J Sports Sci Med. 2009;8:509-15.

18. Domitrovich SG, Domitrovich JW, Ruby BC. Effects of an immunomodulating supplement on upper respiratory tract infection symptoms in wildland firefighters. abstract published in Medicine & Science in Sports & Exercise. 2008;40(5):S353. (Study report available on request)

19. Talbott, S., Talbott, J. 2012. Beta-glucan supplement reduces upper respiratory tract infections and improves mood state in healthy stressed subjects. J Am. Col. Nutr. 31(4)

20. Fuller R, Yam T, Butt H, Calder P, Kenyon J, Noakes P (2012). A Randomised Controlled Trial to Assess the Ability of Yeast-Derived 1,3/1,6 Glucopolysaccharide to Reduce Upper Respiratory Tract Infection Symptoms. Nutrition 28: 665–669.

21. McFarlin B., Carpenter K. , Davidson T., McFarlin M. (2013) Baker's Yeast Beta Glucan Supplementation increases salivary IgA and decreases cold/flu symptomatic days after intense exercise. 2013 Journal of Dietary Supplements, Early Online:1–13, 2013 DOI: 10.3109/19390211.2013.820248

22. Land O'Lakes. 2003-2006. Proprietary research findings. Data available for review.

23. Newport Laboratory. 2004 Proprietary research findings. Report available.

24. Biothera piglet study. 2007. Report available.

25. Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride. 1997. Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine. National Academy Press.

26. Review of dietary reference intakes for selected nutrients challenges and implications for federal food and nutrition policy. 2007. U.S. Economic Research Service. USDA.

27. Suitor, C. W. and L. D. Meyers. 2006. Dietary Reference Intakes Research Synthesis: Workshop Summary from: Dietary Reference Intakes Research Synthesis: Workshop Summary http://www.nap.edu/catalog/11767.html

Reduced incidence and duration of acute respiratory infections (ARI) in children fed a follow-up formula containing docosahexaenoic acid (DHA), a prebiotic blend of polydextrose (PDX) and galactooligosaccharides (GOS), and yeast beta glucan

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Intakes of DHA, prebiotics, yeast-β-glucan, and other micronutrients have been associated with reduced incidence of respiratory infections.

To determine if a follow-up formula containing a combination of DHA, a prebiotic blend of PDX and GOS, and yeast β-glucan reduced the incidence of ARI in children.

In this double-blind, randomized, controlled trial, healthy children (3-4yrs) were fed 3 servings per day of a follow-up formula (FF; n=156) containing DHA (25mg/serving), PDX and GOS (1.2g/serving), and yeast β-glucan (12.8mg/serving), or a control powdered cow's milk (C; n=154) for 28 weeks. The incidence and duration of ARI were obtained from medical records. Incidence of ARI was analyzed with the CMH test. Duration of ARI was analyzed with ANOVA.

The FF group had fewer episodes and shorter average duration of ARI compared to the control group (Table).

When compared to cow's milk, daily intake of a follow-up formula containing DHA, a prebiotic blend of PDX and GOS, and yeast β-glucan over a 28 week period resulted in fewer episodes and shorter duration of acute respiratory infections.

Formula	**Number of ARI Episodes**				**p-value**
	0	**1**	**2**	**3**	
C; n (%)	73 (47)	68 (44)	11 (7)	2 (1)	**0.041**
FF; n (%)	90 (58)	58 (37)	8 (5)	0	
Duration (Days) of ARI (mean ± S.E.)					
C	4.3 ± 0.22				**0.007**
FF	3.5 ± 0.24				

Citation: Scalabrin, D., Jin, X., Zhuang, W., Strong, P., Liu, B.; *Reduced incidence and duration of acute respiratory infections (ARI) in children fed a follow-up formula containing docosahexaenoic acid (DHA), a prebiotic blend of polydextrose (PDX) and galactooligosaccharides (GOS), and yeast beta glucan;* FASEB J. April 2013; 27:lb328.