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Does larch arabinogalactan enhance immune function? A review of mechanistic and clinical trials

Carine Dion, Eric Chappuis* and Christophe Ripoll

Abstract

The common cold is a viral infection with important economic burdens in Western countries. The research and development of nutritional solutions to reduce the incidence and severity of colds today is a major focus of interest, and larch arabinogalactan seems to be a promising supportive agent. Arabinogalactan has been consumed by humans for thousands of years and is found in a variety of common vegetables as well as in medicinal herbs. The major commercial sources of this long, densely branched, high-molecular-weight polysaccharide are North American larch trees. The aim of this article is to review the immunomodulatory effects of larch arabinogalactan derived from *Larix laricina* and *Larix occidentalis* (North American *Larix* species) and more specifically its role in the resistance to common cold infections. In cell and animal models, larch arabinogalactan is capable of enhancing natural killer cells and macrophages as well as the secretion of pro-inflammatory cytokines. In humans a clinical study demonstrated that larch arabinogalactan increased the body's potential to defend against common cold infection. Larch arabinogalactan decreased the incidence of cold episodes by 23 %. Improvements of serum antigen-specific IgG and IgE response to *Streptococcus pneumoniae* and tetanus vaccination suggesting a B cell dependent mechanism have been reported in vaccination studies with larch arabinogalactan, while the absence of response following influenza vaccination suggests the involvement of a T cell dependent mechanism. These observations suggest a role for larch arabinogalactan in the improvement of cold infections, although the mode of action remains to be further explored. Different hypotheses can be envisaged as larch arabinogalactan can possibly act indirectly through microbiota-dependent mechanisms and/or have a direct effect on the immune system via the gut-associated lymphoid tissue (GALT).

Keywords: Larch arabinogalactan, Common cold infections, Immune system, Vaccine, SCFA, Polysaccharides, Dietary fibers, ResistAid®, *Larix*

Background

The common cold is an extremely common infection of the upper respiratory tract. This viral illness represents an enormous economic burden on Western society due to loss of productivity and high medical costs [1]. On average in the US, children have 6-8 and adults have 2-4 cold episodes per year [1]. Some authors estimated the economic cost of lost productivity due to the common cold as $25 billion each year ($16.6 billion due to on-the-job productivity loss, $8 billion due to absenteeism, and $230 million due to caregiver absenteeism) in the US [2]. On average each US American spends $8.30 per common cold episode on over-the-counter drugs. It is accepted that viruses, not bacteria, cause common cold infections [3] and more than 200 different types of viruses have been identified, with the rhinoviruses being the most common [4]. However, colds occasionally predispose individuals to bacterial complications. Nutrition is known to affect the immune system and can modulate resistance to infection [5]. The development of new nutritional solutions that can enhance the immune system's response to environmental pathogens has been of major interest in recent years. Amongst these solutions, larch arabinogalactan presents the advantage of enhancing the immune function [6], and thus is speculated to protect against common colds. So far, only a few reviews have been published on arabinogalactan

* Correspondence: eric.chappuis1@yahoo.fr
Naturalpha SAS, Parc Eurasanté, 885 avenue Eugène Avinée, 59120 Loos, France

[6, 7], while recent studies give more insights into their effect on the immune system along with proposed mechanisms of action. The purpose of this review is to provide a comprehensive overview of the immunomodulating properties of larch arabinogalactan derived from North American *Larix* species (Eastern and Western larch) and its related mechanisms of action.

Review

What is arabinogalactan?

Arabinogalactans (synonyms: Galactoarabinan, Arabogalactan, Galactoarabinin) belong to a major group of carbohydrates known as hemicelluloses, which are non-starch polysaccharides that occur abundantly in the primary and secondary cell walls of plant cells and are widely spread throughout the plant kingdom.

In most plants, arabinogalactans occur in covalent association with protein, either as proteoglycans or as glycoproteins [7]. The protein moiety of arabinogalactan associated proteins is typically rich in hydroxyproline, serine, alanine, threonine, and glycine and is resistant to proteolysis in its native state, a property that is presumably conferred by extensive glycosylation [8, 9]. Arabinogalactan extracted from *Larix spp.* heartwood is an exception, as it is not bound to protein, which is evidenced by the complete absence of nitrogen during elementary analysis of *Larix laricina* [10, 11].

Arabinogalactans have been part of the human diet for thousands of years. They have been detected in seeds, leaves, roots, fruit and xylem sap of representatives of all higher plant families. Dietary sources of arabinogalactans include leek seed, carrot, radish, pear, maize, wheat and tomato [7]. Sources also include medicinal herbs such as *Echinacea* species, *Baptisia tinctoria*, *Curcuma longa*, and *Angelica acutiloba* [12] which are cultivated all over the world.

In trees, arabinogalactans are widely present as minor, water-soluble components of softwoods such as hemlock, black spruce, parana pine, mugo pine, Douglas fir, incense cedar, and juniper [13].

The major commercial sources of arabinogalactan are the North American larch trees, which are genetically different from Eurasian larch tree species [14]. The genus *Larix* (Larches) is common throughout the world. Table 1 provides an overview of the different *Larix* species that grow in specific regions [Table 1].

Both the concentration and distribution of arabinogalactan varies between *Larix* species as well as within a single species, but may constitute up to 35 % by weight of dry heart wood of a larch tree [13, 15, 16]. Unique properties of larch arabinogalactan include its complete solubility and stability over a wide range of concentrations, pHs and temperatures [17].

Table 1 Overview of different species of the genus *Larix* growing throughout the world

Central Europe	European larch	*Larix decidua*
Japan	Japanese larch	*Larix leptolepis*/ *Larix kaempferi*
North America	Eastern larch, tamarack tree	*Larix laricina*
North America	Western larch	*Larix occidentalis*
Siberia	Dahurian larch/Mongolian larch	*Larix dahurica*/ *Larix gmelinii*
Siberia	Siberian larch	*Larix sibirica*

Arabinogalactan is composed of two monomers, D-galactose and L-arabinose (in a 6:1 and 7.5:1 ratio in Western larch and Siberian larch respectively), with traces of uronic acid [7, 18]. Western larch arabinogalactan consists of a $(1 \rightarrow 3)$-β-D-galactopyranan main chain with side $(1 \rightarrow 6)$-linked groups of varying length to every galactosyl unit; organised as a triple helical structure with varying morphologies. These features explain why arabinogalactan forms a hydrocolloid in solution [19, 20]. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) included arabinogalactan into section "Jellifying Agents, Thickening Agents, Stabilizers of Botanical Origin" and registered it under number E-409. Larch arabinogalactan was approved by the Food and Drug Administration in 1965 for direct addition to food and gained Generally Recognized As Safe (GRAS) notification in 2000. There is a Food Chemical Codex Monograph for arabinogalactan available and the larch arabinogalactan referred to here (ResistAid® brand) is produced in line with this monograph and the acceptance criteria listed therein. It is classified as a dietary fiber because it resists digestion by enzymes contained both in saliva and the small intestine, hence entering the large bowel intact, where it is fermented by the resident microflora. Larch arabinogalactan has a strong safety profile, according to a variety of toxicity studies carried out since the 1960s [10, 21].

North American larch arabinogalactan displays molecular masses ranging between 16,000 and 100,000 Daltons and presents a high molecular weight fraction (20 %), while Eurasian larch species (such as *Larix dahurica*, Mongolian larch) show neither of these characteristics [20]. In addition to composition variation existing across different species, the monosaccharide composition and molecular mass of arabinogalactan macromolecules observed can also differ within a single species depending on the specific isolation and extraction procedures employed [22]. This variability may account for the wide range of biological properties and activities documented, such as the protection of gastrointestinal mucosa and large bowel function [23], the support of digestive health by improving intestinal flora [6, 24, 25], the improvement of stress-

induced gastrointestinal dysfunction [26], the effect on vascular permeability [7], the effect in metastatic disease [7] and the enhancement of immune function [7].

Larch arabinogalactan and common cold infections: human trials

Larch arabinogalactan's effects on the immune system have been investigated through multiple human studies with different objectives [Table 2].

Three clinical trials performed in free-living healthy adults were retrieved from the literature. Two of these studies explored the effect of larch arabinogalactan on TNF-α in serum following four weeks' supplementation at 1.5 g/d. Results from both studies displayed different results as one reported an increase on this parameter while the other did not. Furthermore, other immune parameters explored (NK cells, immunoglobulins, immune cells counts) were not affected by the supplementation in either trial [12, 27]. The third study performed with a higher dose of larch arabinogalactan (4 g/d) in 51 young healthy adults did not evaluate the previous parameters but rather demonstrated that 4 g/d of larch arabinogalactan provided for 6 weeks in orange juice significantly increased the percentage of blood $CD8^+$ T-suppressor cells compared to a placebo ($p = 0.005$) and increased the proportion of monocytes in the lymphocyte fraction ($p = 0.05$), independent of time. Lymphocyte proliferation was significantly increased at 6 weeks compared to baseline in the arabinogalactan group, which was not the case in the control group. Other parameters including serum IgG levels, respiratory burst activity of neutrophils, NK cell number and B cell number remained unchanged [28]. These three studies performed in healthy adults suggest that larch arabinogalactan might influence TNF-α secretion and modulate the proportion of immune cells proportions while other parameters such as immunoglobulin levels, NK cells levels and activity or neutrophils activity seemed unaffected by the supplementation, though the pattern of effects exerted was different between studies. In these clinical trials however, the relevance of the model (healthy subjects and absence of immune challenge) and markers could be questioned, as improvement of immune response can be observed mainly in immune-challenged conditions. As discussed in an expert's review, the markers providing the most useful indication to assess the immune-modulating properties of nutraceuticals are those that involve either a standard assessment of relevant symptoms (symptoms of allergies or common infections) or those involving in vivo responses to a defined challenge with allergens or antigens (allergen provocation, vaccine response) [29].

Larch arabinogalactan has been tested in several of these immune-challenge models. Riede et al. evaluated the effect of larch arabinoglalactan on common cold infections in healthy adults. This placebo-controlled, double-blind and randomised trial was performed during the cold season of 2010/2011 with 199 healthy volunteers who had reported at least 3 upper respiratory tract infections in the last 6 months. After daily administration of either 4.5 g of an arabinogalactan preparation or placebo over a period of 12 weeks, it appeared that larch arabinogalactan (ResistAid® brand) increased the body's potential to defend against infections [30]. The incidence of common cold infections in the group supplemented with arabinogalactan was significantly decreased compared to the placebo group in both analysis sets: full analysis set (FAS, $p = 0.038$) and Per Protocol (PP, $p = 0.033$). The number of cold episodes strongly tended to decrease in the arabinogalactan group in the FAS ($p = 0.055$), while in the PP analysis this decrease of 23 % was statistically significant ($p = 0.04$) [Table 3] [30]. A trend for a reduction in the duration of cold episodes was observed in supplemented subjects ($p = 0.061$). Interestingly, self-reported severity of cold symptoms was higher on the first day of cold episodes in subjects supplemented with arabinogalactan while this difference was not observed on the fifth day of cold episodes [30]. It has been suggested that the highly variable subjective perception of a disease could be responsible for the difference noticed. However, these results could also be explained by a quicker and stronger immune response favoured by the supplementation with arabinogalactan. Therefore, the common symptoms of a cold such as redness, heat, swelling, and pain, experienced more intensely by participants on the first day of the trial could be attributed to such an immune response.

More specific information on the enhancement of an immune response following a challenge has been obtained using the vaccine challenge method. The impact of a 10-week supplementation period with 4.5 g/d of a proprietary arabinogalactan preparation from larch tree (ResistAid® brand) was studied in a vaccine model [31]. The researchers demonstrated that the preparation selectively enhanced the antibody response to vaccination against *Streptococcus pneumoniae* and observed an increase in pneumococcal IgG antibodies of various pneumococcal antigens [31].

A similar study performed by the same research group compared the effectiveness of the ResistAid® ingredient at a daily dose of 1.5 g to a placebo, and demonstrated a significant increase in IgG antibody response to tetanus vaccine, while no improvement was observed following influenza vaccine [32].

These results taken together suggest that larch arabinogalactan can improve immunity by decreasing infections and improving immunoglobulin response following a standardized immune challenge. Doses used in these trials suggest that larch arabinogalactan may improve immune response at a dose as low as 1.5 g/d taken for several

Table 2 Summary of clinical studies on the effect of larch arabinogalactan on the immune system

Article	Extract	Challenge (Vaccine)	Subjects	Day for the measures	Parameters measured	Results: effect of the extract on parameters
Udani et al. 2013 [32]	ResistAid™ Proprietary larch arabinogalactan 1.5 or 4.5 g/day For 60 days	Tetanus & influenza vaccines Vaccination at day 30	75 healthy adults	Day 0, 45 & 60	➢ Tetanus IgG	Group 1.5 g/day, day 60: significant rise in IgG levels compared to placebo ($p = 0.008$) Group 4.5 g/day group, day 45 & 60: significant rise in IgG levels compared to baseline ($p < 0.01$) but not compared to placebo
					➢ Influenza A & B IgG & IgM	No effect
Riede et al. 2013 [30]	ResistAid™ Proprietary larch arabinogalactan 4.5 g/day For 84 days	None	199 healthy adults		Common cold episode	Reduce the incidence of common cold infection ($p = 0.055$) Reduce the number of subject affected ($p = 0.038$) Increase of the severity of symptoms ($p = 0.028$) No effect on the duration of the common cold episodes
Udani et al. 2010 [31]	ResistAid™ Proprietary larch arabinogalactan 4.5 g/day For 72 days	*Streptococcus pneumonia* Vaccination at day 30	45 healthy adults	Day 0, 51 & 72	➢ Pneumococcal IgG (subtypes 4, 6B, 9 V, 14, 18C, 19 F & 23 F)	Significant rise in IgG levels compared to placebo in 2 antibodies subtypes (18C & 23 F) at day 51 ($p = 0.006$ and $p = 0.002$) and day 72 ($p = 0.008$ and $p = 0.041$)
					➢ Pneumococcal salivary IgA	No effect
				Day 0, 30, 31, 51 & 72	➢ WBC[a] count	No significant difference compared to placebo At day 72, rise in WBC compared to baseline ($p = 0.045$) No differences (clinically significant) in lymphocytes, neutrophils, monocytes or basophiles count Significant rise of eosinophil count at day 30 ($p = 0.006$) and day 51 ($p = 0.014$)
					➢ Inflammatory cytokines[a]: ENA-78, eotaxin, GM-CSF, IFNγ, IL10, IL12P40, IL1RA, IL2, IL4, IL5, IL6, IL8, MCP-1, MCP-3, PDGF-BB & TNF-α	Significant rise in IL6 between day 30 and 31 compared to placebo ($p = 0.046$)
					➢ Complement C3 & C4	No effect
Nantz et al. 2001 [28]	Arabinogalactan 4 g/day For 42 days	None	51 healthy adults	Day 1, 21 & 42	➢ Haematology: WBC[a], RBC[a], haemoglobin, hematocrit, neutrophils, lymphocytes, monocytes, platelets ➢ Count of CD19 B lymphocytes, CD4 T helper lymphocyte, CD8 T cytotoxic lymphocytes & NK1.1 natural killer cells ➢ PBMC[a] (lymphocytes) proliferation after exposure to PMA ➢ PMN[a] for oxidative burst activity ➢ Natural killer cells activity ➢ Ig[a]G	Significant increase of % CD8+ cells at 6 weeks after arabinogalactan compared to control group ($p = 0.005$) Significant increase in lymphocyte proliferation at 6 weeks compared to baseline in the arabinogalactan group ($p < 0.05$) only Significant effect of group detected on proportion of monocytes in the lymphocytes fraction ($p = 0.0497$), though no significant group[a]time effect detected ($p = 0.602$). No significant change in IgG levels, respiratory burst activity of neutrophils, NK cell number and B cell number.

Table 2 Summary of clinical studies on the effect of larch arabinogalactan on the immune system *(Continued)*

Kim et al. 2002 [27]	Larch arabinogalactan (90 %) 1.5 g/day For 28 days	None	48 healthy female adults	Day 0 & 28	➢ Vital signs: blood pressure, radial pulse, respiration rate, temperature	No effect
					➢ Complete blood count: WBC, neutrophils, lymphocytes & monocytes	No effect
					➢ NK cells quantitative	No effect
					➢ Complement properdin	No effect
					➢ TNF-α	Significant decrease ($p = 0.044$)
					➢ EBV VCA IgG Ab ➢ CMV IgG Ab	No effect No effect
					➢ *Lactobacillus acidophilus* stool culture ➢ Stool fungus culture for yeast	No effect No effect
					➢ Health related quality of life (SF-36)	Increase of the bowel movement (75 % of the subjects affected)
Kim et al. 2002 [12]	Larch arabinogalactan (90 %) – different concentration grades 1.5 & 4.5 g/day For 28 days	None	21 healthy adults	Day 0 & 28	➢ Haematology: WBC[a], RBC[a], haemoglobin, hematocrit, monocytes	No effect
					➢ TNF-α ➢ IFN-γ ➢ IL6	No effect No effect No effect
					➢ Stool culture	No effect
					➢ Health related quality of life (SF-36)	No effect

[a]*ENA* epithelial neutrophil activating peptide-78, *GM-CSF* granulocyte monocytes colony stimulating factor, *IFNγ* interferon gamma, *IL* interleukin, *MCP* monocyte chemotactic protein-1, MCP-3, *PDGF* platelet-derived growth factor-BB or *TNF* tumour necrosis factor-alpha, *PBMC* peripheral blood mononuclear cells, *PMN* polymorphonuclear neutrophils, *PMA* phorbol 12-myristate 13-acetate, *WBC* White blood cells, *RBC* red blood cells, *Ig* immunoglobulin, *EBV VCA IgG Ab* Epstein-Barr Virus viral capsid antigen IgG antibody, *CMV IgG Ab* Cytomegalovirus IgG antibody, *Ab* antibody

Table 3 Summary of Riede et al.'s results on the effect of larch arabinogalactan on common cold

Population analysed	FAS[a]		PP[a] set	
Groups	Placebo	AG[a]	Placebo	AG[a]
Number of common cold episodes	1.06 ± 0.85	0.83 ± 0.82	1.10 ± 0.85	0.85 ± 0.82 *
Number of subjects affected by a cold episode	72.4 %	58.4 % *	74.4 %	59.8 % *
Duration of common cold episodes	8.3 ± 2.9	8.5 ± 2.8	-	-
Intensity of symptoms after 5 days, documented in CRF[a]	8.5 ± 6.6	8.4 ± 6.8	-	-
Intensity of symptoms after 5 days, from subject diary	5.85 ± 8.35	4.73 ± 8.08	-	-
Intensity of symptoms at start, documented in CRF[a]	11.6 ± 6.3	13.3 ± 6.6	-	-
Intensity of symptoms at start, from subject diary	11.5 ± 6.5	13.7 ± 6.9 *	-	-

[a]*AG* Arabinogalactan, *CRF* Case Report Form, *FAS* Full analysis set, *PP* Per protocol
Mean values (± SD) significantly different from the placebo: * $p < 0.05$

weeks; however, more consistent results have been obtained at a dose level of 4.5 g/d over several weeks. This was seen both on vaccine models and on infection-prevention models. Further clinical studies would be required in order to confirm and clarify these findings, such as the lack of response following influenza vaccine.

Effect of larch arabinogalactan on immune parameters: preclinical studies

The immunostimulatory activity of larch arabinogalactan has been investigated in various in vitro and in vivo studies. These works have demonstrated activation of different components of the immune system. An effect on natural killer cells (NK cells), components of the nonspecific immediate immune response to antigens, has been observed. Hauer and Anderer's ex vivo study, using human peripheral blood mononuclear cells (PBMC), demonstrated larch arabinogalactan's ability to enhance NK cells'activity/cytotoxicity (i.e. ability to mediate spontaneous cytotoxicity against tumour cells and virus-infected cells without prior sensitisation by antigen) through a possible increase in interferon-gamma (IFN-γ) [33]. The investigators also highlighted larch arabinogalactan's ability to induce the production and/or release of pro-inflammatory cytokines such as tumour necrosis factors-alpha (TNF-α), Interleukin-1 beta (IL-1β) and Interleukin-6 (IL-6) [33]. It has been shown that tumoricidal and phagocytic activities of macrophages are enhanced by treatment with larch arabinogalactan, and these activated cells exhibit increased production of nitric oxide (NO), H_2O_2, TNF-α and IL-6 [34]. Furthermore, some but not all arabinogalactan-containing polysaccharides from other sources have been shown to have complement-fixing activity contributing to their immune-modulating effects [35].

Studies done in vivo report that the number of mouse spleen NK cells more than double compared to control after 14 days exposure to intra-peritoneally injected larch arabinogalactan [36]. The role played by larch arabinogalactan on the innate immune system is further substantiated by Grieshop et al.'s in vivo study on dogs, demonstrating that oral administration of larch arabinogalactan (at doses of 0.55 g/day or 1.65 g/day for 10 days) increases the number of circulating white blood cell counts, namely neutrophils and eosinophils [17]. The effect of larch arabinogalactan on the adaptive immune system has also been studied. Grieshop et al.'s study on dogs showed that the number of lymphocytes ($CD4^+$T helper, $CD8^+$ cytotoxic T cells or B $CD19^+$) was not affected by larch arabinogalactan administration. Serum IgG, IgM and IgA were also unaffected [17]. However, Choi's group affirms that the treatment of mice splenic lymphocytes with arabinogalactan increased their cytotoxic activity against tumour cells [34].

Pharmacokinetics of larch arabinogalactan

A number of studies aimed to investigate whether and how arabinogalactan reaches the systemic circulation in order to exert its effects on immunity. Larch arabinogalactan is resistant to digestion by enzymes in the upper gastrointestinal tract. It reaches the colon where it is slowly fermented by the gastrointestinal microflora and thus, promotes the growth of indigenous intestinal microflora such as *Bifidobacterium* and *Lactobacillus acidophilus* [12, 17, 25, 37] similarly to other oligosaccharides [38]. The fermentation of acacia gum arabinogalactan occurs both in proximal and distal parts of the colon while other oligosaccharides such as fructooligosaccharides may be fermented mostly in the proximal part of the colon as shown with an in vitro model of the human intestinal microbial ecosystem [39].

Carbohydrates of plant fibers are known to be digested to varying degrees by the large bowel flora [40] and *Isphagula* husk (an arabinoxylan of similar structure to arabinogalactan) metabolization by the gut flora reaches 85-100 % in humans [24, 41]. Moreover, Vince et al. have used an in vitro faecal incubation system and suggest complete fermentation of acacia gum arabinogalactan after 48 h [24]. The fermentation by the resident colonic microflora of larch arabinogalactan results in the production of the short chain fatty acids (SCFA) [Fig. 1], butyrate, acetate and propionate [12, 17, 24], with the

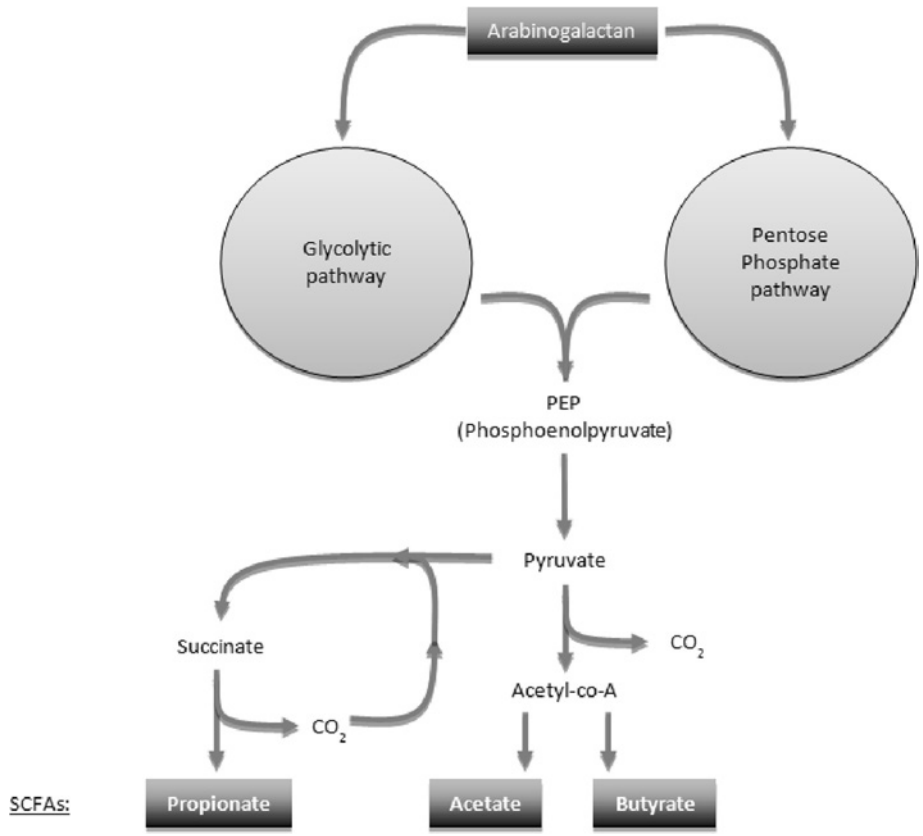

Fig. 1 Proposed mechanisms of action of larch arabinogalactan on immune system

latter two being predominantly produced [42]. Apart from this pathway, the existence of a transfer of the whole molecule of arabinogalactan to the systemic immune system via the M-cells of the GALT [34] is supported by the study of Yamashita et al. [43] on antitumor peptidomannan KS-2, providing evidence that orally administrated polysaccharides could be absorbed via portal vein and intestinal lymphatics into the general circulation with an intact molecular size.

According to these elements of evidence, arabinogalactan may potentially exert its effects indirectly, relying on SCFAs actions, or directly as a circulating agent.

Potential modes of action involved

Studies of the specific modes of action of larch arabinogalactan support in part the two pathways developed above. Indeed, arabinogalactan (similarly to other gut-fermented polysaccharides) can possibly act indirectly through microbiota-dependent mechanisms (i.e. rebalancing microbiota composition in the gut, production of SCFAs) and/or have a direct effect on the immune system after passage from the gut lumen through the GALT [Fig. 2].

The gut fermentation pathway generates SCFAs at high concentrations through the breakdown of carbohydrates [44]. These compounds, rapidly absorbed from the colonic lumen, enter the portal and peripheral circulation [45], regulate the metabolism, proliferation and differentiation of colonic epithelial cells [46] as well as intestinal immunity [38]. Their interactions with G-protein-coupled receptors 41 and 43 (GPR41 and 43), expressed on a range of immune cells [47, 48] may affect inflammatory responses [48]. SCFAs regulate the leukocyte production of cytokines, such as TNF-α, IFN-γ, IL-2, IL-6 and IL-10, as well as eicosanoids and chemokines (e.g., MCP-1 and CINC-2) [49, 50] and butyrate also affects leukocyte chemotaxis, limiting the migration and, thus, the microbial pathogens' destruction [49]. However, their exact and individual role in these effects remains unclear. This particularly applies to propionate and acetate, which are the two SCFAs predominantly generated by arabinogalactan fermentation [45]. In addition, Choi et al. suggested that mono- and disaccharides generated from complex carbohydrates during digestion could also exert an immunostimulating role, despite little evidence supporting the influence of simple carbohydrates on immune parameters [34].

There is also a possibility that larch arabinogalactan expresses its clinical effects as intact macromolecules rather than as fragments resulting from digestion [34], though the mode of action involved is still unclear.

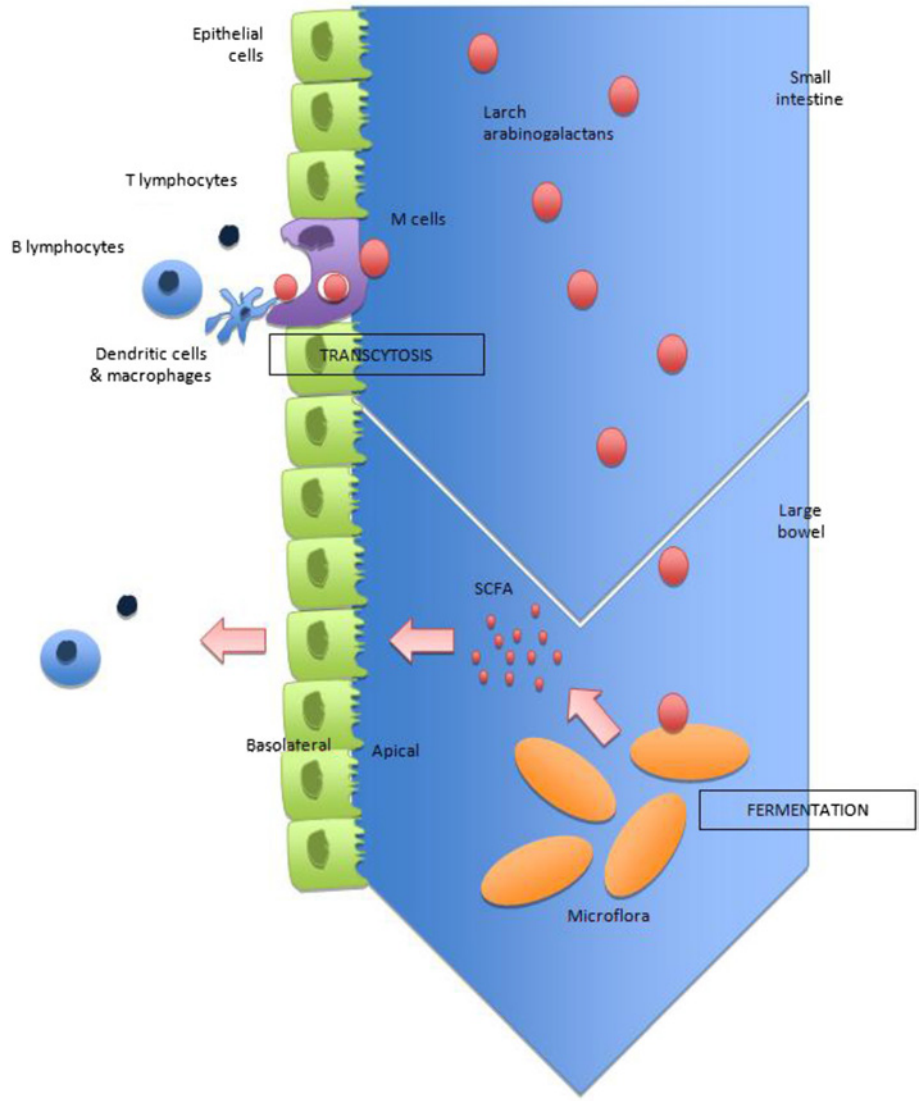

Fig. 2 Larch arabinogalactan metabolism: simplified diagram of polysaccharide breakdown and the main routes of carbohydrate fermentation in the large intestine

According to this second possible mode of action, complex carbohydrates could exert an effect on gut-associated immunity in the small intestine. This part of the gut contains the GALT, consisting of immunoreactive cells and organized lymphoid tissues, found in close contact with the mucosal lining of the gut, and thus the lumen. M-cells are specialised epithelial cells found in the follicle-associated epithelium (FAE) and continuously sample the lumen of the small intestine [51]. Soluble proteins, particles and live microorganisms traverse the M-cells by transcytosis and are delivered into a "pocket" on the basolateral side of the cell that is packed with T and B lymphocytes, macrophages and dendritic cells [51, 52]. Antigens seem to be unaltered by this translocation [52] and once across the M-cell, it is processed by antigen-presenting cells (APC) and presented to T lymphocytes that proliferate in place and stimulate local B lymphocytes [52]. These then migrate to distant sites [52], thus playing an important immunomodulatory role.

Despite these proposed mechanisms of action, several findings from clinical studies remain to be explained. As evidenced by Udani's research group, arabinogalactan supplementation failed to enhance immune response following influenza vaccine, suggesting that this polysaccharide confers a benefit in preparing the immune system to manage infection with bacterial antigens, but perhaps not with viral antigens [32]. Udani hypothesizes that arabinogalactan is capable of stimulating the Peyer's patches in the gut as it traverses the intestines. The larch polysaccharides may have a similar structure to these

potentially pathogenic bacteria, and therefore, provide a low level of stimulation, which keeps an array of antibodies ready in case the actual antigen appears [32]. This hypothesis is consistent for the *Streptococcus pneumoniae* vaccine, as the vaccine is made of bacterial sugars from 23 pneumococcal types. Vaccines produced from bacterial polysaccharides are generally known to trigger T-independent responses, i.e., directly inducing a B cell response in the absence of T cell help. Other features of this response include absence of immune memory and induction of low-affinity antibodies [53]. However, the effect of larch arabinogalactan on tetanus vaccine response seems to be due to other mechanisms that need to be identified. The tetanus vaccine is composed of toxoids, a modified and harmless form of the tetanus toxin protein (also named tetanospasmin and produced by *Clostridium tetani*). The protection is often mediated by B lymphocytes and IgG, as observed for *Streptococcus pneumoniae* and tetanus vaccination [53]. However, T cells could also be an important or the main effector of the immune response, as it is the case for tuberculosis vaccine ($CD4^+$ T cells) or live attenuated influenza intranasal vaccine ($CD8^+$ T cells) [53]. Thus, it is possible that arabinogalactan acts differently on these various immune cell types, influencing the efficiency of the vaccination through many different mechanisms. The latter assertion is consistent with the effects exerted by other plant polysaccharides that present the capacity to positively modulate the influenza vaccine response. A series of studies performed by Vos et al. shows that a mixture of oligosaccharides, consisting of short-chain galactooligosaccharides (scGOS) and long-chain fructooligosaccharides (lcFOS), influenced immune response to an influenza vaccine in mice by increasing vaccine-specific delayed-type hypersensitivity (DTH) response and modulating the lymphocyte T helper Th1/Th2 balance through enhancement of Th1-related and suppression of Th2-related parameters [54–57]. Regarding influenza vaccination, the hypothesis that the main immune cell type involved is T cells is supported by the results obtained in Bunout et al.'s clinical study, showing no influence of fructooligosaccharide consumption by healthy elderly on immunoglobulin levels (IgA, IgM, IgG and salivary secretory IgA) after influenza vaccine [58], which is consistent with Udani's results on arabinogalactan [32]. To date, a beneficial immunological effect of larch arabinogalactan was shown following challenges with *Streptococcus pneumoniae* and tetanus vaccination only, through increased concentration of antigen-specific IgG and IgE antibodies in serum. In future investigations, the study of different antibody isotypes could provide additional information on the type of immune response elicited (IgG1 and IgG3 indicating Th1-driven responses and IgG4 and IgE indicating Th2-driven responses) [5]. While measuring serum immune markers reflects in vivo response [5], measuring antibody production would allow to investigate the effect of larch arabinogalactan at the functional level. Regarding influenza, it is not obvious to identify a role for arabinogalactan in the improvement of the vaccine effect using serum immunoglobulins as biomarkers. However, the study of markers such as lymphocyte activation (i.e. surface expression of activation markers on $CD8^+$ lymphocytes) or lymphocyte-derived mediators (i.e. production of cytokines) could be more appropriate according to the mode of action involved.

Conclusion

Common cold infections are both a health problem and economic problem in Western countries, hence, it is important to develop supportive solutions. Recent human studies have demonstrated that dietary intervention with arabinogalactan from North American *Larix* species could increase resistance to infections. Larch arabinogalactan seems to positively influence NK cells, macrophage activities and pro-inflammatory cytokine production. A clinical study demonstrated that larch arabinogalactan supplementation reduced the incidence of common cold infections. In two vaccine models (*Streptococcus pneumoniae* and tetanus), larch arabinogalactan had an immunostimulatory effect. Therefore, these results suggest a role for larch arabinogalactan in the improvement of immune system and defence against pathogens in humans. It is interesting to note that both models (infection and vaccine) are considered relevant by the European Food Safety Authority (EFSA) to substantiate health claims on immune system in the frame of European regulation (EC) 1924/2006 on nutrition and health claims [59, 60].

To explain the mode of action, it has been suggested that it can interact with the immune system either indirectly through the production of SCFAs that affect inflammatory responses via leukocytes function and cytokine production, or directly through the capacity of M-cells to transfer intact arabinogalactan through the intestinal barrier, delivering it to immune cells (APC). However, the exact mode of action is not yet completely understood and further studies are required to better understand it and define more precisely the benefits of larch arabinogalactan to the immune system.

Abbreviations

APC: antigen-presenting cells; CD: cluster of differentiation; CINC-2: cytokine induce neutrophil chemoattractant-2; DTH: delayed-type hypersensitivity; EFSA: European food safety authority; FAE: follicle-associated epithelium; FAS: full analysis set; GALT: gut-associated lymphoid tissue; GPR: G-protein-coupled receptors; GRAS: generally recognized as safe; i.p.: intraperitoneal; IFN-γ: interferon-gamma; Ig: immunoglobulin; IL: interleukin; lcFOS: long-chain fructooligosaccharides; MCP-1: macrophage chemoattractant protein; NK cells: natural killer cells; NO: nitric oxide; pAOS: pectin derived acidic oligosaccharides; PBMC: peripheral blood mononuclear cells; PP: per protocol; SCFA: short-chain fatty acids; scGOS: short chain galactooligosaccharides; Th: T helper; TNF-α: tumour necrosis factors-alpha; Tregs: regulatory T-cells..

Competing interests
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Authors' contributions
The three authors, CD, EC and CR, have made similar contributions to the review. All authors have read and approved the final manuscript.

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Immunomodulatory Effects of ResistAid™: A Randomized, Double-Blind, Placebo-Controlled, Multidose Study

Jay K. Udani MD, FACN [a]
[a] Medicus Research LLC; Medical Director, Northridge Hospital Integrative Medicine Program , Northridge , California
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Original Research

Immunomodulatory Effects of ResistAid™: A Randomized, Double-Blind, Placebo-Controlled, Multidose Study

Jay K. Udani, MD, FACN

Medicus Research LLC; Medical Director, Northridge Hospital Integrative Medicine Program, Northridge, California

Key words: Clinical trial, immune system, adaptive immunity, tetanus, influenza, vaccination, Larix laricina, larch tree

Objective: To evaluate the ability of a proprietary arabinogalactan extract from the larch tree (ResistAid, Lonza Ltd., Basel, Switzerland) to change the immune response in healthy adults to a standardized antigenic challenge (tetanus and influenza vaccines) in a dose-dependent manner compared to placebo.

Methods: This randomized, double-blind, placebo-controlled trial included 75 healthy adults (18–61 years old). Subjects were randomized to receive either 1.5 or 4.5 g/day of ResistAid or placebo for 60 days. At day 30, subjects were administered both tetanus and influenza vaccines. Serum antigenic response (tetanus immunoglobulin G [IgG], influenza A and B IgG and immunoglobulin M [IgM]) was measured at days 45 (15 days after vaccination) and 60 (30 days after vaccination) of the study and compared to baseline antibody levels. Frequency and intensity of adverse events were monitored throughout the study.

Results: As expected, all 3 groups demonstrated an expected rise in tetanus IgG levels 15 and 30 days following the vaccine. There was a strongly significant difference in the rise in IgG levels at day 60 in the 1.5 g/day group compared to placebo ($p = 0.008$). In the 4.5 g/day group, there was significant rise in tetanus IgG at days 45 and 60 compared to baseline ($p < 0.01$) but these values were not significant compared to placebo. Neither group demonstrated any significant elevations in IgM or IgG antibodies compared to placebo following the influenza vaccine. There were no clinically or statistically significant or serious adverse events.

Conclusions: ResistAid at a dose of 1.5 g/day significantly increased the IgG antibody response to tetanus vaccine compared to placebo. In conjunction with earlier studies, this validates the effect of ResistAid on the augmentation of the response to bacterial antigens (in the form of vaccine).

INTRODUCTION

The adaptive immune system (also called the acquired immune system) is composed of specialized cells and actions that are involved in the elimination or prevention of pathogenic challenges. The adaptive immune response provides the immune system with the ability to recognize and remember specific pathogens and to mount a stronger response each time a pathogen is encountered. Adaptive immunity is triggered in humans when a pathogen invades the innate immune system and generates a threshold level of antigen. The adaptive immune response has been exploited by modern medicine through the use of vaccines [1]. By using live (attenuated) or inactivated pathogens or part of pathogens, vaccines trigger an immune response and development of vaccine-specific antibodies. The measurement of this response is frequently used as a way to measure the immunomodulatory effect of certain drug and dietary interventions [2]. It is a validated model to assess the *in vivo* functional capacity of the human immune system [3]. Vaccines used in clinical trials to measure antibody response have included tetanus and influenza vaccines.

Tetanus is an acute, often fatal, disease that causes painful tightening of the muscles, produced by an exotoxin (protein) secreted by the bacterium *Clostridium tetani*. *C. tetani* produces 2 exotoxins: tetanolysin and tetanospasmin. The latter is a neurotoxin and produces the clinical manifestations of the disease. Tetanus toxoid consists of formaldehyde-treated toxin (protein), which is a single antigen. Tetanus toxoid is a highly effective antigen, and a completed primary series generally induces protective levels of serum antitoxin that persists for 10 or more

Address correspondence to: Jay K. Udani, 28720 Roadside Drive #310, Agoura Hills, CA 91301. E-mail: jay.udani@medicusresearch.com

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years [4]. In a trial of 26 adults given a booster dose of tetanus toxoid, 81% of the subjects demonstrated a 2-fold or greater rise in serum antitoxin antibody levels [5]. The antigenic response to tetanus toxin is approximately 80% immunoglobulin G (IgG) [6]. A 4-fold increase in IgG levels is expected when comparing postvaccination to prevaccination results for previously unvaccinated individuals. For previously vaccinated individuals receiving a booster inoculation, the rise in IgG levels may be less than 4-fold.

Influenza is a respiratory tract infection caused by 3 types of RNA viruses: types A, B, and C. Each consists of 8 negative single-strand RNA segments encoding 11 proteins. The major surface glycoproteins of the virus are hemagglutinin (HA) and, to a lesser extent, neuraminidase. The antigenic drift of the HA protein results in the development of novel viral strains and a requirement for annual vaccination to keep up with the changes. The influenza vaccine contains 3 inactivated influenza viruses: one A (H3N2) virus, one regular seasonal A (H1N1) virus (in 2010 when this study took place this was replaced by the 2009 pandemic H1N1 virus), and one B virus. The vaccine produces antibody responses to both HA and neuraminidase. There is a rapid and robust influenza-specific response by antibody-secreting plasma cells that begins as early as 2 to 6 days after vaccination, peaks after 2 weeks, and then wanes over the next 6 months [7]. Influenza-specific antibodies are predominately IgG and immunoglobulin M (IgM) in serum and IgA in oral fluid [8].

Arabinogalactans are high-molecular-weight, highly branched, water-soluble polysaccharides that contain units of D-galactose and L-arabinose [9]. Dietary intake of arabinoglactans comes from plant food sources such as carrots, radishes, tomatoes, pears, and wheat. Gum arabic, a commonly used food additive, is composed of highly branched arabinogalactan [10]. The mean estimated intake of arabinogalactan from the diet is approximately 10.474 g [11]. The most common commercial source of arabinogalactans is from the wood of the larch tree (*Larix* spp.). Larch arabinogalactan consists of galactose and arabinose in a 6:1 ratio. It is a long, densely branched nonstarch polysaccharide with a galactan backbone with side chains of galactose and arabinose.

An *ex vivo* study with human peripheral blood mononuclear cells found that larch arabinogalactan stimulated natural killer cell activity through a possible increase in interferon-gamma [12]. A study with dogs demonstrated increased numbers of circulating white blood cell counts (primarily neutrophils and eosinophils) following oral administration of larch arabinoglactan [13].

A randomized, double-blind, placebo-controlled study evaluated the immunomodulating effects of 4 different preparations of echinacea, a proprietary larch arabinogalactan (1.5 g/day), and a combination of larch arabinogalactan and one of the echinacea preparations [14]. The study included 48 adult women (22–51 years old) who were divided into 6 groups of 8 women. After 4 weeks of treatment, there was a statistically significant increase in complement properdin in 2 of the echinacea groups and the group taking the larch arabinoglactan and echinacea combination. There was no significant increase in the group taking the larch arabinogalacton alone.

The proprietary arabinogalactan extract ResistAid (Lonza Ltd., Basel, Switzerland) was previously tested in a randomized, double-blind, placebo-controlled, parallel-group pilot study to determine immunomodulatory activity following vaccination against *Streptococcus pneumonia* [15]. This 72-day study included 45 healthy adult subjects who had not previously received the vaccine. The primary end points were 7 different pneumococcal IgG antibodies (4, 6B, 9V, 14, 18C, 19F, and 23F). The secondary objective was to determine whether the ResistAid product (4.5 g/day) would stimulate other arms of the immune system to which there was no direct antigenic stimulus. Secondary endpoints included salivary immunoglobulin A (IgA), white blood cell counts, complement (C3 and C4), and inflammatory cytokine levels. Subjects were randomized using a block design. In response to the vaccine, pneumococcal IgG plasma levels increased. The arabinogalactan group demonstrated a greater IgG antibody (Ab) response than the placebo group in two Ab subtypes (18C and 23F) at both day 51 ($p = 0.006$ and $p = 0.002$, respectively) and day 72 ($p = 0.008$ and $p = 0.041$). Ab subtypes 18C and 23F also demonstrated change scores from baseline in favor of the arabinogalactan group at day 51 ($p = 0.033$ and 0.001) and day 72 ($p = 0.012$ and $p = 0.003$). Change scores from baseline and mean values were greater in the arabinogalactan group than placebo for most time points in Ab subtypes 4, 6B, 9V, and 19F, but this was not significant. There was no effect from the vaccine or arabinogalactan on salivary IgA, white blood cell count, inflammatory cytokines, or complement. The proprietary larch arabinogalactan used in this study may have a selective immunomodulatory effect on acquired or adaptive immunity shown as an increase in antibodies without clinically significant changes to total white blood cells, cytokines, or complement. It is possible that rather than acting as a general immunostimulant, arabinogalactan can act in a specific manner.

It is hypothesized that the mechanism of this specific immunomodulation includes associated activation of the gut-associated lymphoid tissue as the long-chain-specific arabinogalactan passes through the gastrointestinal (GI) tract [16]. Presentation of polysaccharides to immune effector cells may resemble the capsular antigens of some potentially pathogenic encapsulated bacteria and the chronic low level stimulation of the gut-associated lymphoid tissue may prepare the body for similar presence of comparable pathogens [17]. Chronic low-level exposure to arabinogalactan in this manner may induce an immunomodulatory and immunostimlatory priming effect, allowing for faster response time of the immune system when a pathogenic antigen presents.

The current human clinical study was designed to test the hypothesis that the ingestion of ResistAid, a proprietary

arabinogalactan extracted from larch (*Larix laricina*), would selectively enhance the antibody response to the tetanus and influenza vaccines in a dose-related manner compared to placebo. The selected doses were 1.5 and 4.5 g, both of which had demonstrated effects in previous clinical studies.

METHODS

Study Sample

This study was conducted in accordance with Good Clinical Practice Guidelines and the ethical principles of the Declaration of Helsinki [18]. The study protocol and material were approved by an institutional review board (Copernicus Group IRB, Cary, NC) and all subjects gave written informed consent prior to participation.

This was a 60-day, 3-arm, randomized, double-blind, placebo-controlled, parallel-groups trial in healthy adults, conducted at one study center in Northridge, California (Staywell Research) and was designed and managed by the Medicus Research Contract Research Organization, also in Northridge, California. Subjects were recruited using existing databases and local advertising. Subjects were screened by phone prior to scheduling a screening visit. Inclusion criteria included assessment of being in good health, a body mass index between 18 and 30 kg/m^2, and 18–60 years of age (Table 1). Subjects included in the study must not have had an influenza vaccine in the past year or a tetanus vaccine in the past 5 years. They were asked to maintain their normal diet and exercise routine during the study and females were required to use an approved birth control method during the study. Potential participants were excluded from the study if they had any major systemic, inflammatory, or chronic disease; any active infection or infection in the past month requiring antibiotics or antiviral medication; used immunosuppressive drugs in the prior 5 years; were known to have alcohol or drug abuse; were pregnant or lactating; or had any medical condition that in the opinion of the investigator might interfere with the subject's participation in the trial. They were excluded if they had an allergy to eggs. Subjects using dietary supplements designed to boost the immune system and/or multivitamins were required to discontinue these products for at least 2 weeks before entering the study.

Table 1. Inclusion criteria, exclusion criteria, and study controls

Inclusion Criteria
Assessment of being in good health
BMI between 18 and 30 kg/m^2
Age between 18 and 60 years
Exclusion Criteria
Any major systemic, inflammatory, or chronic disease
Any active infection or infection in the past month requiring antibiotics or anti-viral medication
Used immunosuppressive drugs in the prior 5 years
Were known to have alcohol or drug abuse
Were pregnant or lactating
Allergy to eggs
Had any medical condition which in the opinion of the investigator might interfere with the subject's participation in the trial
Study Controls
Subjects asked to maintain their normal diet and exercise routine during study
Females were required to use an approved birth control method during the study
Subjects using dietary supplements designed to boost the immune system and/or multi-vitamins were required to discontinue these products for at least 2 weeks before entering the study.

Study Products

The intervention product tested was a proprietary arabinogalacton extract (ResistAid). The product is extracted from the wood of the larch tree (*Larix laricina*) using a water extraction patented process [19] (U.S. 5756098; EP 86608) in accordance with Hazard Analysis and Critical Control Points standards and in compliance with the monograph in the Food Chemicals Codex [20]. ResistAid is a fine, dry, light brown powder with a neutral taste that dissolves quickly in water or juice. The larch arabinogalactan used in the ResistAid product has been designated Generally Recognized as Safe (GRAS) by the U.S. Food and Drug Administration [21,22]. The placebo was maltodextrin (Maltrin M100, Grain Processing Corp., Muscatine, IA, USA).

The tetanus vaccine used in the study was the Massachusetts Biologic Labs Tetanus Diphth Tox AD NR SDV 0.5 mL 10/Pk.

The inactivated influenza vaccine used in the study was Fluzone (Sanofi Pasteur, Swiftwater, PA, USA) for the 2009–2010 influenza season (multidose vial, 5 mL). The vaccine formulation for the 2009–2010 season contains 3 strains of the influenza virus: the A/Brisbane/59/2007 (H1N1)-like virus, the A/Brisbane/10/2007 (H3N2)-like virus, and the B/Brisbane/60/2008-like virus. The 3 strains for the new influenza vaccine formulation were confirmed by the Food and Drug Administration's Vaccines and Related Biological Products Advisory Committee in February 2009 and correspond with recommendations made by the World Health Organization, also in February [23]. Influenza vaccine is formulated each year to match the strains predicted to circulate during the upcoming season. This formulation for the 2009–2010 influenza season introduced a new B strain. The two A strains were unchanged from the 2008–2009 season formulation.

Randomization and Dosing

Subjects were randomly assigned to one of 3 treatment groups in blocks of 5 with 10 subjects randomized per block. The atmospheric noise method was used to generate the randomization schema [24]. The treatment groups were as follows: (1) 1.5 g/day of ResistAid, (2) 4.5 g/day of ResistAid, or (3) placebo. In order to protect blinding, the study product was

produced in identical opaque sachets that contained either 4.5 g of ResistAid, 1.5 g of ResistAid with 3.0 g placebo, or 4.5 g placebo. Subjects were instructed to mix sachets in an 8 oz. cold beverage to be taken once a day in the morning with breakfast.

Each box was labeled with perforated labels provided by the Medicus Research Contract Research Organization with subject-specific information including a unique randomization number. Subjects, the medical director, and research staff were blinded to the treatment assignment for the duration of the trial.

Study Procedure

Subjects were required to be present for 5 clinic visits during the 60-day study. At screening (visit 1), eligibility was determined based on the inclusion and exclusion criteria. For eligible subjects, blood was drawn to measure baseline influenza A and B IgM and IgG levels as well as tetanus IgG levels. Subjects were counseled not to change their diet or exercise level during the study and they received the first dose of the assigned study product during the visit. Product was dispensed and subjects received a study-dosing diary. On day 15, subjects were called to check on compliance and as a reminder of their next visit. On day 30 (visit 2), subjects were administered the tetanus and influenza vaccines via intramuscular injection.

All subjects returned the next day (visit 3) to observe the vaccination site. On day 45 (visit 4), subjects had blood drawn to measure influenza A and B IgM and IgG levels and tetanus IgG levels. The blood draw and antigen measures were repeated on day 60 (visit 5).

During study visits, subjects were questioned about changes in health status (including concomitant therapies) and vital signs were taken. Adverse event monitoring was completed at each visit beginning with visit 2. During visits 2, 4, and 5, dosing diaries were collected and study compliance assessed (interview, diaries, and product wrappers were returned). Study product was dispensed and new dosing diaries were provided. A urine pregnancy test was completed for all female subjects at visits 1, 2, and 4.

Outcome Measures

The primary end points were the changes in the markers of immune response to the tetanus and influenza vaccines. These end points were measured in plasma samples and included tetanus IgG (measured by enzyme immunoassay) and influenza A IgM, influenza A IgG, influenza B IgM, and influenza B IgG (all measured by antibody enzyme-linked immunosorbent assay). The antibodies were measured using plasma samples.

Safety assessment included vital signs (temperature, blood pressure, pulse, and respiratory rate) as well as detailed adverse event (AE) monitoring to assess the frequency and intensity of AEs. Safety monitoring also included assessment of the vaccination site during visit 3.

Statistical Analysis

Paired sample *t* tests were used for within-subject means comparisons and independent sample *t* tests for between group comparisons (placebo vs each of the active groups individually).

Excel 2003 was used for data entry, validation, restructuring, calculating changes in variables over time, reorganizing and reformatting results, and preparing graphs. Statistical analyses were performed using SPSS Base System version 18 (IBM, Chicago, IL).

RESULTS

Characteristics of the Study Population

A total of 80 subjects were randomized for the study (see Fig. 1). Seventy-five subjects completed the 60-day study: 1.5 g/day ($n = 27$), 4.5 g/day ($n = 25$), and placebo ($n = 23$).

Five subjects (2 in the 1.5 g/day group, 1 in the 4.5 g/day group, and 2 in the placebo group) were lost to follow-up after visit 1 and never received the vaccines. They were not included in the analysis. The baseline characteristics of the subjects were not significantly different for gender, age, ethnicity, or marital status. The study began in May 2010 (first subject randomized) and ended in December 2010 (last subject out).

Tetanus IgG

All 3 groups demonstrated an increase in IgG levels at day 45. The increase appeared to peak at day 45 for the placebo group, while the 1.5 and 4.5 g/day groups continued to show a small increase at day 60. There was a strongly significant difference between the 1.5 g/day group and the placebo group in IgG levels at day 60 ($p = 0.008$). There were no other significant differences between groups at any time point (see Fig. 2).

Within-group changes in IgG levels from baseline were significant for the placebo group at day 60 ($p \leq 0.01$) and for the 4.5 g/day group at both days 45 and 60 ($p \leq 0.01$). There were no significant within-group changes in the 1.5 g/day group.

Influenza IgM and IgG Antibodies

All 3 groups demonstrated an expected physiological increase and peak in influenza A IgM by day 45 with a slight reduction at day 60 (see Fig. 3). Both the 1.5 and 4.5 g/day groups were not statistically different than placebo at baseline or day 60. The 1.5 and 4.5 g/day groups were not statistically different than each other at any time point. The within-group changes from baseline to day 45 and day 60 were not significant for any group at any time point with the exception of a significant increase from baseline to day 60 in the 1.5 g/day group ($p = 0.002$).

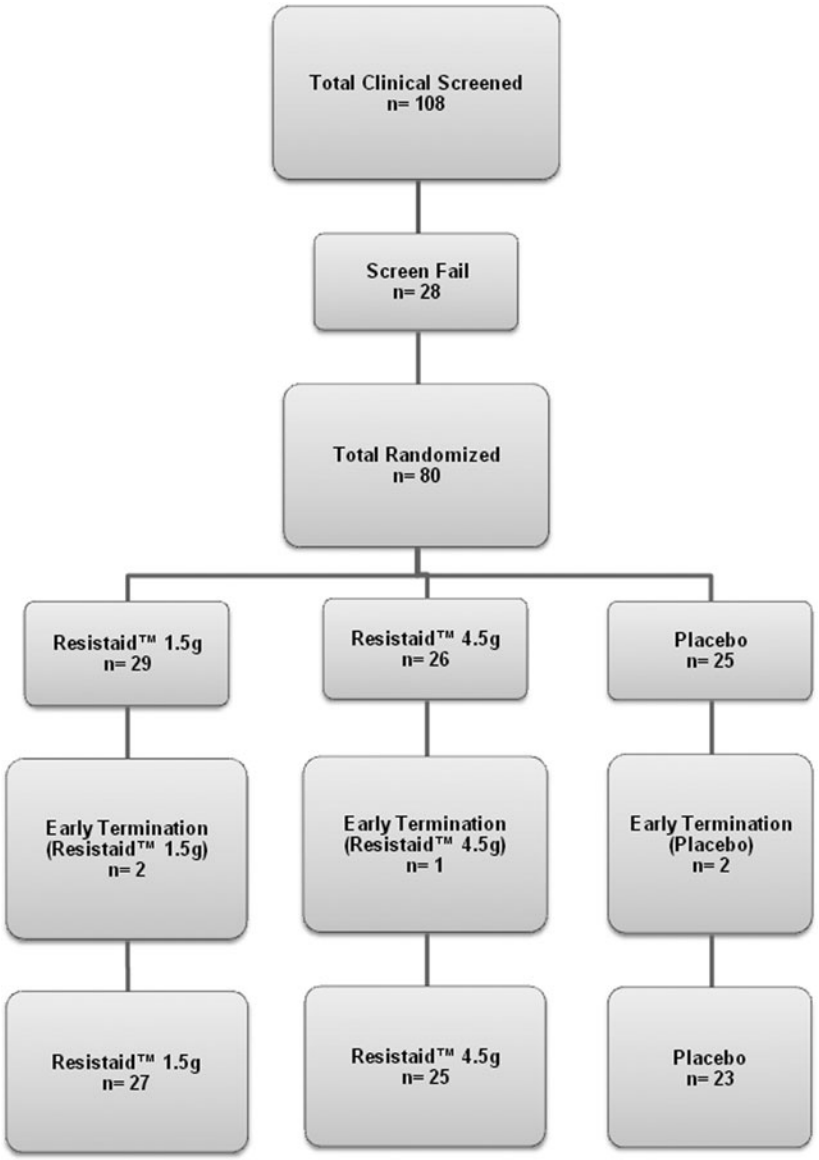

Fig. 1. Study Attrition Chart. 108 study participants were screened and 75 completed the study.

All 3 groups demonstrated an expected increase in influenza B IgM after vaccination (see Fig. 4). The 3 groups were not statistically different at any time point; however, there were statistically significant within-group changes from baseline.

All 3 groups demonstrated an expected rise in influenza A IgG following the vaccine, which peaked at day 45 for the 4.5 g/day and placebo groups and at day 60 for the 1.5 g/day group (see Fig. 3). The placebo group was significantly lower than the 4.5 g/day at baseline ($p = 0.029$); however, there were no significant differences between IgG levels in any of the 3 groups at day 45 or 60. The following within-group changes were statistically significant: (1) placebo group at day 45 ($p = 0.002$) and day 60 ($p = 0.0001$); (2) 1.5 g/day group at day 45 ($p = 0.006$); and (3) 4.5 g/day group at day 45 ($p = 0.001$) and day 60 ($p = 0.007$).

All 3 groups demonstrated an expected rise in influenza B IgG after the vaccine with a peak at day 45 for the 4.5 g/day group and day 60 for the 1.5 g/day group and placebo group (see Table 5). There were no significant differences between the values in any of the 3 groups at any time point. The within-group changes were statistically significant for all 3 groups at day 45 and day 60.

Adverse Events

There were no clinically significant or serious adverse events during the study. A total of 13 adverse events were reported

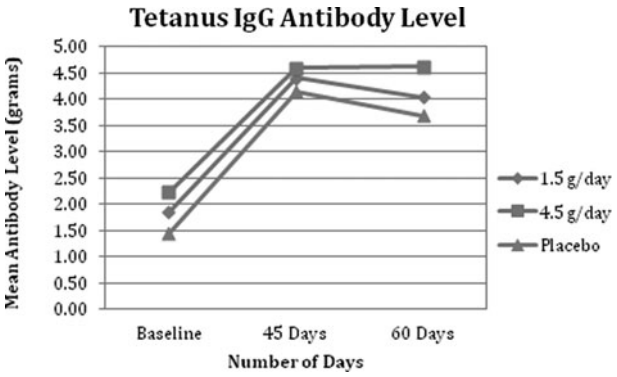

Fig. 2. Tetanus IgG Antibody Level. All 3 groups demonstrated an increase in IgG levels at day 45. There was a strongly significant difference between the 1.5 g/day group and the placebo group in IgG levels at day 60 ($p = 0.008$).

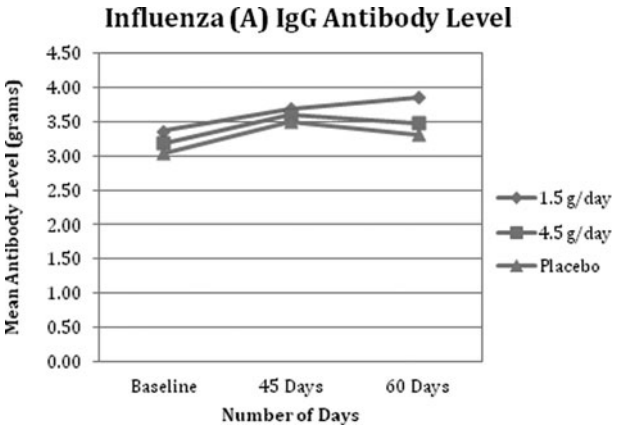

Fig. 3. Influenza (A) IgG Antibody Level. All 3 groups demonstrated an expected physiological increase and peak in influenza A IgM by day 45 with a slight reduction at day 60. All 3 groups demonstrated an expected rise in influenza A IgG following the vaccine, which peaked at day 45 for the 4.5 g/day and placebo groups and at day 60 for the 1.5 g/day group.

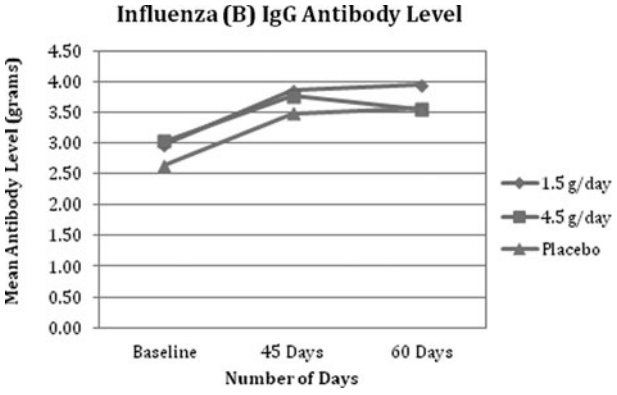

Fig. 4. Influenza (B) IgG Antibody Level. All 3 groups demonstrated an expected increase in influenza B IgM after vaccination.

during the study. In the placebo group, there were 5 AEs reported: upper respiratory tract infection (URI; 2 reports), sinus headache, hypertension, and lower abdominal pain. In the 1.5 g/day group there were 7 reported AEs: URI (3 reports), food poisoning, gastroenteritis, nausea, and headache. In the 4.5 g/day group there was one report of dizziness and no URIs reported. None of the adverse events in any group were attributed to the study product.

DISCUSSION

The present study employed a model antigenic stimulation using a vaccine-specific serum antibody production to evaluate the immunomodulatory effects of proprietary larch arabinogalactan product (ResistAid) in a healthy adult population. The IgM antibodies are the acute antibodies that provide short-term response to the antigen (in this case the vaccine). It is expected that they will rise and fall in a relatively short period of time (1 to 4 weeks). It is the IgG antibodies that provide the long-term protection and are a more significant immune marker. These tend to rise more slowly than the IgM antibodies but continue to rise for a longer period of time.

The study employed 2 different doses of arabinogalactan, 1.5 and 4.5 g/day, with the hypothesis that there would be a dose–response effect. We had previously observed an increase in IgG in response to the pneumococcal vaccine with the dose of 4.5 g/day [15]. A previous clinical study that measured levels of complement properdin reported that a dose of 1.5 g possibly augmented an effect due to echinacea species [14]. In the present study, the 1.5 g/day dose was found to significantly increase tetanus IgG antibody response at day 60 compared to placebo ($p = 0.008$). This is a confirmation that the ResistAid product confers a benefit in increasing the antibody response to a standard antigenic challenge. The ResistAid 4.5 g/day group showed statistically significant increases from baseline for this same vaccine and continued to show elevations in IgG levels at day 60 even when both the placebo and 1.5 g/day groups had already peaked, but this group did not show a statistically significant difference compared to the placebo group.

There were no significant differences between either ResistAid dose and placebo in the influenza antibodies. Both IgM and IgG were tested for influenza A and influenza B. Based on these results and on the prior results of the pneumococcal vaccine study [15], it appears that the ResistAid product confers a benefit in preparing the body to deal with bacterial antigens but perhaps not with viral antigens. As one considers other purported mechanisms of action in the GI tract for the product, the above may become clearer. The product may stimulate the Peyer's patches in the gut as it traverses the length of the intestines. The polysaccharide may have a structure similar to that of these potentially pathogenic bacteria and therefore provide

a low level of stimulation, which keeps an array of antibodies ready in case the actual antigen appears. If the structure of the polysaccharide is similar to that of bacteria, then it may not be similar to the structure of viruses and therefore may not confer the same benefit in that case. Another plausible explanation may be the noted prebiotic activity for larch arabinogalactan [25].

Prebiotics are noted to have immunomodulating activity, in part by increasing lactic acid bacteria and increasing production of short-chain fatty acids in the GI tract [26]. A combination of short-chain galactooligosaccharides and long-chain fructooligosaccharides was shown to influence immune response to an influenza vaccine in mice [27]. Supplementation with the prebiotic mix increased vaccine-specific delayed-type hypersensitivity (DTH) response when given prior to the primary vaccination. Supplementation after day 8 did not affect the DTH response. The study found a positive correlation between percentages of cecal lactobacilli at day 9 and DTH responses. A placebo-controlled study also found an effect on regulatory T-cells following influenza vaccine in mice supplemented with a prebiotic combination consisting of short-chain galactooligosaccharides, long-chain fructooligosaccharides, and pectin hydrolysate-derived acidic oligosaccharides [28]. The study found that the prebiotic mixture depleted $CD25^+$ regulatory T-cells, which resulted in enhanced Th1 vaccine responsiveness.

However, the results in animal studies have not been duplicated thus far in human studies examining the immunomodulating effect of prebiotics following vaccination. In a randomized, placebo-controlled trial, healthy elderly adults ($\geq$70 years old) were randomized to receive 6 g/day of a prebiotic fructooligosaccharide mixture 70% raftilose and 30% raftiline or placebo for 28 days [29]. At week 2 of the study, all subjects were vaccinated with influenza and pneumococcal vaccine. Though a slight increase in influenza A antibodies (saliva secretory IgA) was observed, there was no effect on serum influenza A and B and pneumococcal IgG or IgM levels in the prebiotic group compared to placebo.

Variables that affect the immune response to vaccines include age, gender, race, body mass index, and genetic characteristics [2,30]. One of the goals of this study was to determine the effect of the intervention on a relatively broad population—healthy adults from age 18 to 60 years old. The between-subject variability in response to vaccination is normally quite high and using a larger study population in future studies may clarify the clinical indications we have observed so far. In addition, because gender and age differences may affect immunity, these potentially confounding variables could be examined in future studies.

CONCLUSION

Daily ResistAid supplementation at a dose of 1.5 g/day for 30 days before the administration of the tetanus vaccine significantly increased the tetanus IgG antibody response compared to placebo. The 4.5 g/day dose of ResistAid also increased the IgG antibody response to the tetanus vaccine and this increase continued to rise by day 60; however, these values did not reach statistical significance. Neither group demonstrated any significant elevations in IgM or IgG antibody response to the influenza vaccine. The results suggest that ResistAid induces an elevated response to bacterial antigens (in the form of vaccine), but not viral antigens.

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Original article

Larch arabinogalactan effects on reducing incidence of upper respiratory infections

L. Riede
Analyze & realize AG, Berlin, Germany

B. Grube
General Practitioner, Berlin, Germany

J. Gruenwald
Analyze & realize AG, Berlin, Germany

Address for correspondence:
Linde Riede, analyze & realize AG, Waldseeweg 6, 13467 Berlin, Germany.
Tel.: +49 (0)30 4000 8501;
Fax: +49 (0)30 4000 8501;
aa@a-r.com

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ResistAid(TM) – Common cold – Immune system – Larch arabinogalactan – Randomized placebo-controlled study

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Abstract

Objective:
Larch arabinogalactan (ResistAid*) may prevent cold infections due to its immune-stimulatory properties. In a placebo-controlled, double-blind, randomized clinical trial, the effect of a proprietary larch arabinogalactan preparation on the incidences of common colds and its effect on cold symptoms, as a well established model for immune function, was compared to placebo.

Research design and methods:
A total of 199 healthy participants who had a self reported cold infection rate of three in 6 months were randomly assigned to receive a total of either 4.5 g of an arabinogalactan preparation ($n = 101$) or placebo ($n = 98$) over a period of 12 weeks.

Main outcome measures:
The participants documented each common cold episode in a diary, and rated 10 predefined infection symptoms on a 4 point rating scale during an infection period, resulting in an infection score. The common cold episodes were confirmed by medical doctors.

Clinical trial registration:
ISRCTN41183655.

Results:
In the full analysis set (FAS), arabinogalactan tended to decrease the incidence of common cold ($p = 0.055$). The number of participants affected by a cold was significantly reduced by arabinogalactan supplementation ($p = 0.038$). Concerning the per protocol (PP) collective, the incidences of common cold ($p = 0.040$) and the number of participants affected by the infection ($p = 0.033$) were significantly fewer after arabinogalactan compared to placebo consumption. The severity of symptoms at episode start as experienced by the participants was significantly higher after arabinogalactan supplementation ($p = 0.028$). The treatment was well tolerated with no significant differences between the study groups.

Conclusion:
The present study demonstrated that larch arabinogalactan increased the body's potential to defend against common cold infection. While the immunomodulatory effect of arabinogalactan can be assumed, its mechanism of action remains to be elucidated.

*ResistAid is a registered trade name of Lonza Ltd, Basel, Switzerland.

Introduction

Growing evidence obtained from *in vitro*, animal and human studies strongly suggest the immunomodulatory effect of arabinogalactan[1]. Arabinogalactan is a long, densely branched, polysaccharide with molecular weight ranging from 10,000 to 120,000 Daltons. It is mostly present in glycoprotein form, bound to a protein spine of threonine, proline or serine (arabinogalactan protein)[2]. In nature, arabinogalactans are found in microbial systems and plants. Among the many plants containing arabinogalactan are several immune-enhancing species such as Echinacea purpurea and Curucuma longa[3,4]. The major commercial source of arabinogalactan, however, is the larch tree. Arabinogalactans extracted from Larix spp. bark are water soluble, highly branched molecules composed of galactose and arabinose units in a 6:1 ratio, with trace amounts of glucuronic acid. Larch arabinogalactan has a galactan backbone that features β(1,3) linkages and galactose β(1,6) and arabinose β(1,6 and 1,3) sugar side chains[1].

Although generally harmless in symptomology, common cold infections count among the most frequent diseases in humans and, in fact, each person of the general population will catch a cold occasionally[5]. The common cold is, in most cases, a viral infectious disease of the upper respiratory system[5]. A well functioning immune system, including the innate and adaptive immune responses, is crucial for the defense against viral infections such as common colds.

According to the European Food Safety Authority (EFSA)[6], defense against pathogens at specific sites of the body, for example the upper respiratory tract, is a particular aspect of immune function. In this respect, an appropriate outcome measure is the number of episodes including severity or duration of the infection. This corresponds to the current scientific view on appropriate markers of the immune system[7,8].

The innate immune system comprises cells like neutrophils, monocytes, macrophages, complement factors and natural killer (NK) cells which rapidly recognize and respond to pathogens. This immune response depends largely on the recognition of pathogen associated molecular patterns (PAMP) by so-called pattern recognition receptors (PRR). The adaptive immune response, composed of highly specialized, systemic cells and processes, follows a few days later.

Arabinogalactan from larch was demonstrated to stimulate the innate immunity by increasing the NK cell cytotoxicity and the phagocytic capacity of macrophages and monocytes mediated by pro-inflammatory cytokines[9–12]. Udani *et al.*[13] suggested that arabinogalactan acts in a more specific manner on the adaptive immunity, as shown in the increase in antibody response to the pneumonia vaccine.

The aim of the present interventional study was to reveal the potential superiority of a proprietary arabinogalactan extract from the larch tree (ResistAid*) as compared to placebo on naturally acquired common cold episodes and the severity of the symptoms in otherwise healthy participants. The primary endpoint was the reduction of number of cold episodes over a period of 12 weeks in a comparison between arabinogalactan and placebo study arms.

The susceptibility to common colds is often related to a weak immune status or a lack of strong immune defense[14,15]. Thus, the common cold was used as a model system to determine the effect of an arabinogalactan preparation on the human system against invading pathogens.

Patients and methods

Trial design

The study was conducted as a prospective multi-center, randomized, double-blind, placebo-controlled study in healthy outpatient participants, with recurring upper respiratory tract infections (URTI). The study was approved by the local Ethics Advisory committee, Ethikkommission Charité-Universitätsmedizin, and carried out in accordance with the Declaration of Helsinki (Hong Kong 1989, Somerset 1996) as well as the ICH-GCP guidelines and EU recommendations (CPMP/ICH/135/95; Topic E6 [R1]). The study was registered in the International Standard Randomized Controlled Trial Number Register (http://www.isrctn.org/) as ISRCTN41183655.

Participants

Between October 2010 and February 2011, a total of 204 otherwise healthy participants who had a self reported cold infection rate of three in 6 months were enrolled at six study sites in Germany. Inclusion criteria were: age 18–70 years, written consent to participate, and at least three infections of upper airways within the last 6 months. The exclusion criteria were as follows: acute or chronic upper airways disease, suspected influenza or swine flu, vaccination against influenza or swine flu within 21 days before the study start, BMI > 30, clinically significant abnormal laboratory parameters, known sensibility to one of the ingredients of the study product, immune deficiency diseases, severe organ or systemic disorders, body temperature ≥38°C, pregnancy or nursing, intake of preparations that can influence the study outcome, incidence of alcohol, medication or drug abuse, use of pre- and probiotics, participation in a clinical study within the previous 30 days.

*ResistAid is a registered trade name of Lonza Ltd, Basel, Switzerland.

Participants were instructed not to change their eating habits. Participants gave written informed consent prior to the study.

Interventions

During the study period of 12 weeks, participants received a total of either 4.5 g of a proprietary water-based extract from larch tree (ResistAid†) or placebo. The active ingredients are the soluble fiber arabinogalactan and bioactive flavonoids. ResistAid† is a fine brown powder with a neutral taste and a fine pine-like aroma that dissolves quickly in water or juice. The placebo product was maltodextrin (Maltrin M100*). Verum and placebo were provided in sachets manufactured by Proderma, Switzerland. Participants were instructed to dissolve the content of a sachet in approximately 100–150 mL of liquid and take the prepared drink daily at breakfast. During the 12 week study period, a total of three basic visits were performed: at baseline, after 6 weeks and at the end after 12 weeks. Additionally, an episode visit was performed at the start and on the fifth day of each common cold episode. During an episode, the participants were instructed to record and assess their cold symptoms for a period of 14 days. For each occurring infection, the same procedure was performed. Thus, the total number of visits per participant varies depending on the number of infection episodes. Eating habits were recorded in a diet diary at start and end of the study. Compliance was checked by counting returned capsules. The accepted compliance rate was defined as 75–125% of capsules consumed. Biochemical parameters were assessed at baseline and at the end of the intervention.

Outcomes

The primary objective of the present study, the frequency of common cold episodes, was defined as the number of common cold infections during the study period. All the common cold episodes had to be documented in the subject diary and confirmed by an investigator during the 12 week intervention period. As secondary outcome parameters, the duration of cold episodes (based on subject diary), the episode intensity (as change of the total sum score after 5 days compared to start of episode at first episode visit, based on case report file [CRF] and subject diary), and the episode intensity at start of episode (sum score on day 1 based on subject diary) were assessed. For assessment of episode intensity, the participants had to rate ten predefined cold symptoms during the infection episode, on a rating scale (0 = complaint free, 1 = weak symptoms, 2 = moderate symptoms, 3 = strong symptoms) and had to document them in a diary. Symptoms were as follows: headache, joint pain, sore throat, difficulty swallowing, hoarseness, coughing, a watery nasal discharge, nasal congestion, cold related sleeping difficulties, body temperature >38°C. By summation of the scores of the individual symptoms, a sum of scores (=total score) was calculated at episode start and after 5 days. The duration of an episode was defined as the number of days since episode start until the first symptom-free day. Eating habits based on a 3 day record were assessed.

As a concurrent variable, the efficacy of the investigational product was evaluated by the participants and the investigator at the end of each common cold episode as 'very good', 'good', 'moderate', or 'poor'. The safety and tolerability of the product was evaluated by the documentation of adverse events, the assessment of laboratory parameters and by the global evaluation of the tolerability by the investigator and the subject at the end of the study. At each visit, the investigator asked the subject if any adverse events (AE) had occurred and recorded the respective AE. For any AE that occurred, the investigator recorded the seriousness, intensity, time of occurrence and duration of the observed AE. Further, the investigator recorded his/her judgment as to whether the observed AE has a causal relation to the intake of the investigational product as well as any actions taken due to an AE. The number of AEs was the basis for comparison between the study arms.

Sample size estimation

The sample size calculation, based on two sample *t*-tests, was determined by the effect size (group comparison), as well as the previously determined requirements of the significance level (5.0%, two-sided) and power (80%). The assumption of the effect size was based on the results of an unpublished superiority clinical study with a comparable design with an effect size of 0.49 for FAS and 0.42 for the PP population. No interim analysis was planned.

Randomization and blinding

The random allocation sequence was computer generated with a block size of four. The ratio of randomization between the verum and the placebo group was 1:1. Based upon the random list which was generated by an independent statistician, each container with sachets received a randomization number during packaging by a contract packaging company. Randomization was concealed from the study sites. The randomization sequence was stored under lock and key by Lonza Ltd. Investigators at the study sites enrolled participants and assigned them to random numbers in a sequential order. Verum and placebo were identical in appearance and taste. Both study

†ResistAid is a registered trade name of Lonza Ltd, Basel, Switzerland.
*Maltrin M100 is a registered trade name of the Grain Processing Corporation, Muscatine, Iowa, USA.

participants and investigators assessing outcomes were blinded to treatment assignment. Unblinding occurred only after closure of the database. No blinding envelope was opened.

Statistical analysis

All the variables contained in the data collection were presented descriptively using their statistical key data or their frequency distribution and statistically analyzed in view of the group specific differences ($p_{\chi 2}$-value). The Mann–Whitney *U*-test was employed to test for between-groups comparison (p_U). All statistical analyses were carried out on the FAS, including all randomized participants that received at least one intervention treatment and had data apart from baseline data, and on the PP set, including all randomized participants without major protocol deviations. Statistical analyses were performed with SPSS (SPSS for Windows, Release 19, LEAD Technologies Inc.). Values of $p < 0.05$ were considered significant. Means are given with standard deviation (SD) and confidence intervals (CI) of 95%.

Results

Subject recruitment

Out of 210 men and women assessed for eligibility, 204 were randomized (Figure 1). As three participants showed abnormal baseline laboratory values, 201 participants received treatment. Two participants were lost to follow up. The remaining 199 participants represent the FAS, thereof 101 in the verum and 98 in the placebo arm. Of these participants, twelve were excluded from the PP analysis due to major protocol deviations resulting in 187 participants (97 in the verum and 90 in the placebo arm). Participants were recruited between October 2010 and

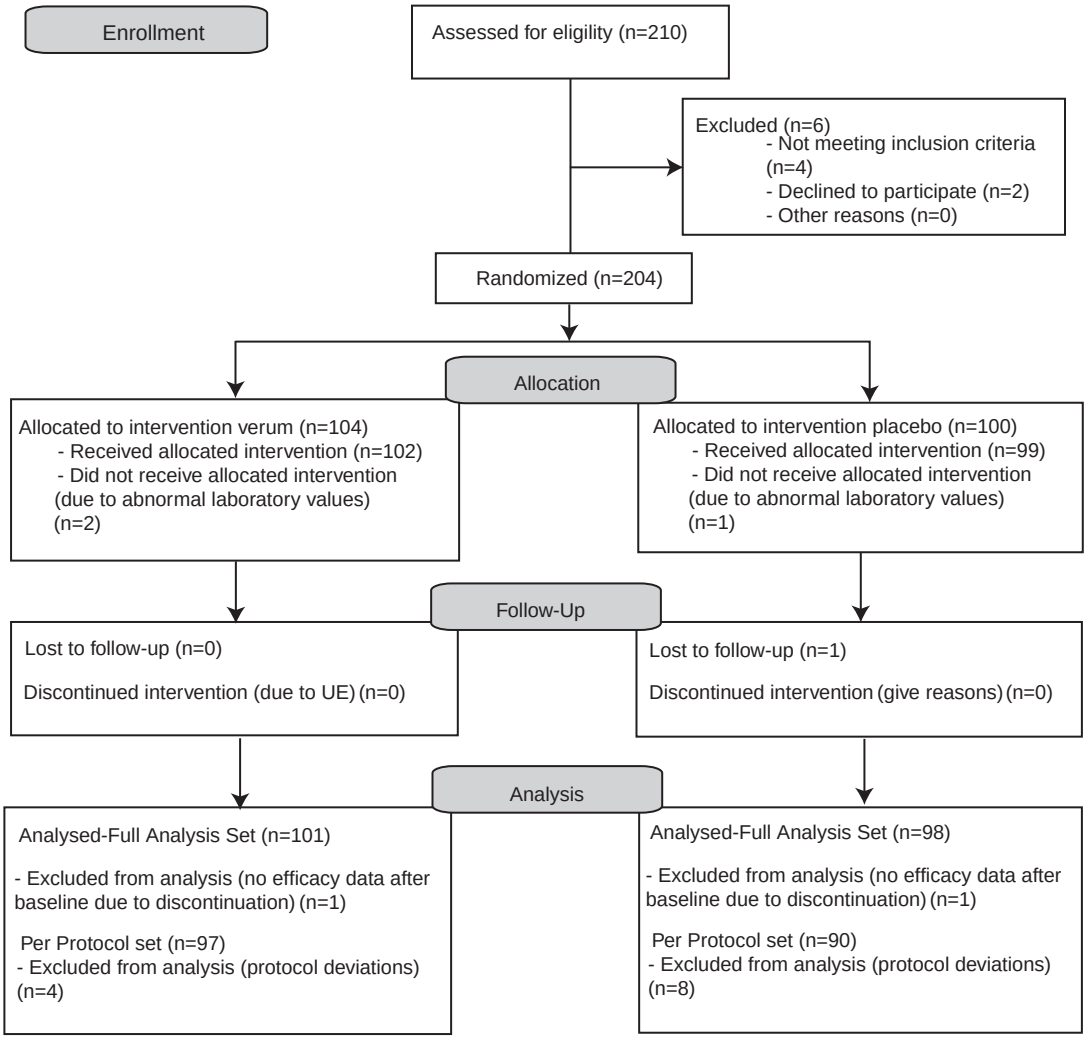

Figure 1. Subject flow.

Table 1. Baseline characteristics of the arabinogalactan and the placebo groups (FAS).

	All ($n=199$) mean ± SD	Arabinogalactan ($n=101$)[y] mean ± SD	Placebo ($n=98$)[z] mean ± SD	p
Sex (M/F)	65/134	37/64	28/70	0.225
Age (years)	42.2 ± 15.4	42.0 ± 14.9	42.4 ± 15.8	0.911
Height (cm)	171.1 ± 8.3	170.7 ± 9.0	171.6 ± 7.7	0.417
Weight (kg)	70.2 ± 11.8	69.5 ± 12.5	70.9 ± 11.2	0.240
BMI (kg/m^2)	23.9 ± 2.9	23.7 ± 3.0	24.0 ± 2.7	0.487
Systolic blood pressure (mmHg)	123.5 ± 15.1	123.9 ± 15.8	123.2 ± 14.6	0.742
Diastolic blood pressure (mmHg)	78.5 ± 7.1	78.8 ± 7.3	78.2 ± 6.8	0.636
Heart rate (1/min)	71.3 ± 6.9	71.4 ± 7.0	71.2 ± 6.9	0.795
Body temperature (°C)	36.6 ± 0.3	36.6 ± 0.3	36.5 ± 0.3	0.188

SD = standard deviation.
[y]Excluding three subjects' data (withdrawn due to abnormal laboratory values [$n=2$], no efficacy data after baseline [$n=1$]).
[z]Excluding two subjects data (withdrawn due to abnormal laboratory values [$n=1$], no efficacy data after baseline [$n=1$]).

February 2011. The last subject completed the study in May 2011.

Baseline data

Of the 199 participants, 65 (33%) were men and 134 (67%) were women. All participants reported that they had experienced at least three cold episodes in the 6 months prior to beginning the study. The baseline characteristics (Table 1) and the eating habits at baseline and at the end of the study period did not differ between interventional groups.

Incidence of common cold infection

Arabinogalactan treatment as compared to placebo tended to decrease the mean number of common cold episodes in the FAS population – verum group (VG): 0.83 (SD 0.82; CI 0.67–0.99) vs. placebo group (PG): 1.06 (SD 0.85; CI 0.89–1.23; $p_U=0.055$) (Figure 2). The number of participants affected by a cold episode significantly differed between the study arms ($n_{active}=59$ [58%]; $n_{placebo}=71$ [72%]; $p_{\chi 2}=0.038$). In the PP population, the mean number of episodes was 0.85 (SD 0.82; CI 0.68–1.10) in the verum and 1.1 (SD 0.85; CI 0.92–1.28) in the placebo group ($p_U=0.040$) (Figure 2). Significantly fewer participants suffered from a cold episode in the active group ($n=58$; 60%) as opposed to the placebo group ($n=67$; 74%) ($p_{\chi 2}=0.033$).

Intensitiy and duration of common cold episodes

The intensity of symptoms did not differ between the active group (8.4; SD 6.8; CI 6.6–10.3) and the placebo group (8.5; SD 6.6; CI 6.8–10.2) regarding the change in the sum of scores from day 1 to day 5 of a cold episode ($p_U=0.10$). This applies also for the change in the total sum of score documented in the subject diary with a mean

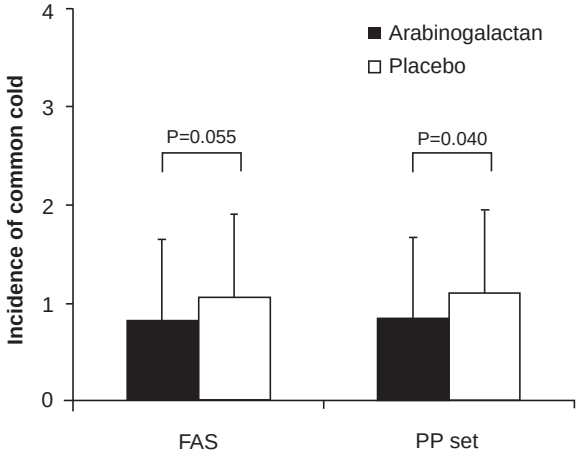

Figure 2. Incidence of common cold infections following 12 week arabinogalactan or placebo supplementation according to the FAS and the PP population. Values are mean ± SD.

of 5.85 (SD 8.35; CI 3.9–7.8) in the verum group and 4.73 (SD 8.08; CI 3.0–6.4) in the placebo group ($p_U=0.59$).

Regarding the sum of the cold symptom scores assessed at the first episode visit, there was a non-significant difference between the verum (13.3; SD 6.6; CI 11.8–14.7) and placebo group (11.6; SD 6.3; CI 10.4–12.9; $p_U=0.06$), while the sum of score documented in the subject diary at episode start differed between study arms – VG: 13.7 (SD 6.9; CI 12.2–15.2) vs. PG: 11.5 (SD 6.5; CI 10.2–12.8; $p_U=0.028$).

The duration of the common cold episodes did not differ between the study arms – VG: 8.5 (SD 2.8; CI 7.8–9.2) vs. PG: 8.3 (SD 2.9; CI 7.6–9.0; $p_U=0.61$).

Increase in symptom-free days

Analysis of symptom-free days was performed for a study duration of 10 weeks, which was chosen for comparison reasons as it had been achieved by the whole PP

population. The percentage of days that participants from the PP collective did not suffer from cold symptoms was significantly higher in the arabinogalactan group (91.2%) compared to the placebo group (88.5%) ($p_{chi} < 0.001$).

Global evaluation of the efficacy (FAS)

At the end of the study, the global assessment of efficacy for arabinogalactan treatment was rated as 'very good' or as 'good' by 83.7% of participants and by the physicians for 87.8% of the participants. For placebo, the efficacy was rated as 'very good' or 'good' by the investigators for 75.2% of the participants and by 73.7% participants in self-assessment. Both physicians and participants rated the efficacy of the arabinogalactan preparation better than the placebo ($p_{\chi 2} = 0.023$ and $p_{\chi 2} = 0.090$, respectively).

Safety evaluation

All measured clinical parameters, body weight, temperature, heart rate and blood pressure remained nearly constant during the study, with no significant differences between the two study populations.

A total of 16 adverse events occurred during the study period. Eight of them occurred in the active group (gastrointestinal infection [$n = 2$], cramp-like chest pain, purulent bronchitis, cervical syndrome, urinary infection, pneumonia right lower lobe, abnormal laboratory values at final visit). Another eight events occurred in the placebo group (gastroenteritis [$n = 2$], hypoglycemia with cold sweat, urinary infection, abnormal laboratory values at final visit, soft stool 30 minutes after intake of the investigational product, hay fever, lumbago). One adverse event (pneumonia right lower lobe) in the active group was classified as serious; this was, however, not related to the intervention. This serious adverse event led to study termination by the subject. One adverse event in the placebo group was judged as possibly related to the intake of the investigational product. The two study groups did not differ in the proportion of participants with adverse events ($p_{\chi 2} = 0.94$).

Discussion

The present study provided, in a placebo-controlled, randomized double blind intervention, clinical evidence for a link between consuming a proprietary larch arabinogalactan preparation and a reduction in the number of seasonal common cold episodes. The primary endpoint was reached with a statistical significance in the PP population. In the FAS collective, significance was just missed; however, a strong positive trend was shown.

The average number of episodes observed in the present study was smaller than expected, which possibly had an effect on the obtained results. Given a higher total number of episodes, the primary endpoint would most likely have been reached in the FAS population as well.

Regarding intensity and duration of cold symptoms, no significant differences between study arms could be observed, except for the endpoint intensity of symptoms at episode start, based on records in the subject diary. On the first day of the episode, there was a statistically significant slightly higher total symptom score in the arabinogalactan study arm. However, no significant differences were observed for the same secondary endpoint based on records from the first episode visit in the CRF. Further, since the two study arms do not significantly differ with respect to the change in symptom intensity after 5 days, the effect observed on day 1 does not prevail.

The large spread in the data might be the reason that no significant differences in the duration of cold episodes could be obtained. At the respective time point of starting the documentation in the subject diary, great differences in symptom strength were present, i.e. the participants presumably started documenting at different stages of the cold episodes. The reason could be the highly variable individual subjective perception of the disease process by the participants.

The ability of a person to defend against the common cold is influenced by their individual immunocompetencies and susceptibility to a cold infection depends in part on environmental factors including psychological stress, lack of vitamins or exposure to wet conditions and low temperatures. Therefore, every subject is at risk for getting a cold, at least occasionally[14,15]. As such, the participants included in the present clinical trial were of both sexes and aged 18 to 70 years and, hence, represent the general population.

The supplementation with an arabinogalactan preparation reduced the number of common cold episodes by 23%, which suggests an immunomodulatory effect of arabinogalactan. Indeed, our data are in line with *in vitro* and animal studies that showed various immunomodulating effects of arabinogalactan on markers of the innate as well as adaptive immunity. Hence, in response to arabinogalactan, human peripheral blood mononuclear cells (PBMC) and their subpopulations have been shown to release pro-inflammatory cytokines which stimulated NK cytotoxicity[11]. Moreover, larch arabinogalactan activated lymphocytes and macrophages *in vitro* which provokes a variety of cellular response including enhanced phagocytosis, oxidative burst and the modulation of cytokine production[9]. A study in dogs demonstrated increases in neutrophils and eosinophils in blood without effects on serum immunoglobulin (Ig) G, IgM or IgA following oral administration of arabinogalactan from larch[10]. The same proprietary larch arabinogalactan has recently been

demonstrated a selective immune-stimulatory effect on the adaptive immune system, as shown by the increase of the antibody response of healthy volunteers to the pneumonia vaccine[13]. Markers of the innate immune system, like total white blood cells, cytokines or complement, however, were not changed.

Although the receptor specificity of arabinogalactans is not well characterized, there is strong evidence that arabinogalactans have access to immune cells and are thereby involved in the elimination of invading pathogens. Thus, arabinogalactan may enhance the on-going immune response in order to react as quickly as possible to an infection by pathogens. Moreover, the symptoms of the common cold are primarily related to the immune response, which might explain why participants in the active group experienced the cold symptoms more severely. This in turn might suggest the effectiveness of arabinogalactan in activating the immune response.

As a unique dietary fiber, arabinogalactan impacts the digestive physiology. Grieshop *et al.*[10] revealed that larch arabinogalactan significantly enhanced gut microflora, especially increasing the beneficial fecal bacteria populations bifidobacteria and lactobacilli. The positive effect of these potentially protective bacteria on inhibition of invading pathogens has been demonstrated *in vitro* and *in vivo*[16,17]. This might support the proposed immunomodulating effects of arabinogalactan and is worthy of further investigation in human clinical studies. Indeed, further studies about the underlying mechanism and receptor specificity of arabinogalactan remain to be conducted.

The present study demonstrated the safety and tolerability of a larch arabinogalactan preparation. This is consistent with results of controlled animal studies demonstrating an absence of adverse effects, mortality and signs of toxicity after oral application of larch arabinogalactan[18].

Conclusion

Our study in healthy participants, representing the general population, confirms the hypothesis of a prophylactic effect of larch arabinogalactan supplementation on the incidence of common cold. The data showed for the first time the effectiveness of an arabinogalactan preparation in protecting against infections caused by pathogens and suggest a general increase in days free of cold symptoms, which might be of economic benefit.

Transparency

Declaration of funding

Lonza Ltd providing financial support for all aspects of this clinical study. The funders were involved in study design, manuscript writing, and made the decision to submit the paper for publication. Lonza Ltd had no role in data collection, analysis, and interpretation.

J.G. designed research; B.G. conducted research; L.R. and J.G. analyzed data; L.R. wrote the paper. L.R. had primary responsibility for final content. All authors read and approved the final manuscript.

Declaration of financial/other relationships

L.R., B.G. and J.G. are employed by a contract research organization that received funding from Lonza Ltd.

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Proprietary arabinogalactan extract increases antibody response to the pneumonia vaccine: a randomized, double-blind, placebo-controlled, pilot study in healthy volunteers

Jay K Udani[1,2*], Betsy B Singh[1], Marilyn L Barrett[3], Vijay J Singh[1]

Abstract

Background: Arabinogalactan from Larch tree (*Larix* spp.) bark has previously demonstrated immunostimulatory activity. The purpose of this study was to test the hypothesis that ingestion of a proprietary arabinogalactan extract, ResistAid™, would selectively enhance the antibody response to the pneumococcal (pneumonia) vaccine in healthy adults.

Methods: This randomized, double-blind, placebo-controlled, parallel group pilot study included 45 healthy adults who had not previously been vaccinated against *Streptococcus pneumoniae*. The volunteers began taking the study product or placebo (daily dosage 4.5 g) at the screening visit (V1-Day 0) and continued over the entire 72 day study period. After 30 days the subjects received the 23-valent pneumococcal vaccine (V2). They were monitored the following day (V3-Day 31), as well as 21 days (V4-Day 51) and 42 days (V5-Day 72) after vaccination. Responses by the adaptive immune system (antigen specific) were measured via pneumococcal IgG antibodies (subtypes 4, 6B, 9V, 14, 18C, 19F, and 23F) and salivary IgA levels. Responses by the innate immune system (non-specific) were measured via white blood cell counts, inflammatory cytokines and the complement system.

Results: Vaccination significantly increased pneumococcal IgG levels as expected. The arabinogalactan group demonstrated a statistically significant greater IgG antibody response than the placebo group in two antibodies subtypes (18C and 23F) at both Day 51 ($p = 0.006$ and $p = 0.002$) and at Day 72 ($p = 0.008$ and $p = 0.041$). These same subtypes (18C and 23F) also demonstrated change scores from baseline which were significant, in favor of the arabinogalactan group, at Day 51 ($p = 0.033$ and 0.001) and at Day 72 ($p = 0.012$ and $p = 0.003$). Change scores from baseline and mean values were greater in the arabinogalactan group than placebo for most time points in antibody subtypes 4, 6B, 9V, and 19F, but these differences did not reach statistical significance. There was no effect from the vaccine or arabinogalactan on salivary IgA, white blood cell count, inflammatory cytokines or complement.

Conclusions: The proprietary arabinogalactan extract (ResistAid™), tested in this randomized, double-blind, placebo-controlled, parallel-group pilot study, increased the antibody response of healthy volunteers to the 23-valent pneumococcal vaccine compared to placebo.

Trial Registration: ISRCTN98817459

* Correspondence: jay.udani@medicusresearch.com
[1]Medicus Research LLC, Northridge, CA 91325, USA
Full list of author information is available at the end of the article

Background

The immune system is a highly complex orchestration of cells, organs, tissues and active molecules which interact in an elaborate and dynamic network to protect the body from infection. The immune system can be divided into two categories: the innate immune system and the adaptive immune system. Innate immunity is an immediate but non-specific response. Adaptive or acquired immunity involves a specific reaction to a pathogen which the immune system recognizes from a previous encounter. The process of acquired immunity is the basis for vaccination[1]. Recent research has focused on the role of nutrition (foods and specific components of foods) in the responsiveness of the immune system to challenges. Vaccine-specific serum antibody production has been suggested as a highly suitable model to evaluate dietary intervention on the resistance to infection or to other immune system-related diseases[2].

The pneumococcal vaccine can reduce the incidence and/or severity of infections caused by *Streptococcus pneumoniae*: namely, pneumonia, otitis media, sinusitis and meningitis. The 23-valent vaccine contains 23 pneumococcal polysaccharide antigens (serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F[3]. Although there are at least 90 distinct serotypes, these 23 serotypes accounted for 85% to 90% of invasive pneumococcal infections in the US[3]. The 23-valent vaccine produces a humoral (antibody-mediated) response: inducing the production of antibody from B-lymphocytes in the absence of help from T-lymphocytes. The type and concentration of antibody produced is dependent on the site of exposure. Systemic administration results primarily in the generating of circulating immunoglobulin(Ig)G whereas mucosal antigenic challenge results in a more vigorous IgA response[1]. In contrast to the 23-valent pneumococcal vaccine, a 7-valent vaccine conjugated to a nontoxic diphtheria protein (used for children younger than 5 years) will induce a T-cell response[3].

Studies on improvement of the response to the pneumococcal vaccine by adults include revaccination, the addition on conjugates to the vaccine and alternative antigenic substances[4]. In addition, nutritional products have been tested on their effect on the response to vaccination. Supplementation with 200 mg/day vitamin E for 4 months to subjects at least 65 years of age caused a suggestive, but insignificant, increase in antibody response to the pneumococcal vaccine[5]. Another study evaluated the effects of prebiotic fructo-oligosaccharides (70% raftilose and 30% raftiline) derived from inulin on the response by an elderly population (70 years old and above). In this study the response to vaccination with the influenza B and pneumococcal vaccines was not significantly increased[6].

Arabinogalactans are high molecular weight, highly branched, water-soluble polysaccharides, which contain units of D-galactose and L-arabinose[7]. Arabinogalactans have previously demonstrated immunostimulatory activity[8,9]. They are present in several immune-enhancing herbs, including *Echinacea purpurea, Baptisia tinctoria, Thuja occidentalis, Angelica acutiloba, and Curucuma longa* and the medicinal mushroom *Ganoderma lucidum.*[10-12]. Arabinogalactans from Larch (*Larix* spp.) have been shown to stimulate natural killer cell cytotoxicity in vitro through the generation of interferon gamma and inhibit the metastasis of tumor cells to the liver in a rodent model[7,13,14]. A dog study demonstrated increases in white blood cell counts (due to increases in neutrophils and eosinophils), and no effect on serum IgG, IgM or IgA following oral administration indoses of 0.55 g/day or 1.65 g/day for 10 days [15]. A randomized, double-blind, placebo-controlled study evaluated the immunomodulating effects of a preparation of proprietary larch arabinogalactan (1.5 g/day) alone, and in combination with various preparations of Echinacea: an extract of *Echinacea purpurea* whole herb containing 4% phenolic compound (1.5 g/day), a preparation of *E. purpurea* whole herb and a preparation *E. angustifolia* root (36 to 680 mg/day)[16]. The study included 48 adult women who were divided into six groups of eight women. After 4 weeks of treatment, complement properdin increased by 18% in the group that received all four preparations and by 21% in the group given preparations of both species of Echinacea.

The current human clinical pilot study was designed to test the hypothesis that the ingestion of Resistaid™, a proprietary arabinogalactan extracted from Larch (*Larix laricina*), would selectively enhance the antibody response by adults to the 23-valent pneumococcal vaccine. Indications that the product would have immunostimulatory activity came from previous studies conducted with this proprietary product[15,16]. As there was no prior human data regarding the ability of this proprietary arabinogalactan extract to impact the immune response to the pneumococcal vaccine, a power calculation could not be performed. The sample size was set at a level consistent with prior human studies involving arabinogalactan and the immune system [16-18].

Methods

Investigational products

The proprietary arabinogalactan product ResistAid™, supplied by Lonza Ltd, Switzerland, contains

arabinogalactan extracted from Larch (*Larix laricina*). Arabinogalactan is a highly branched polysaccharide that is composed of galactose units and arabinose units in the approximate ratio of 6:1[7]. ResistAid™ is a fine, dry, light brown powder with a neutral taste that dissolves quickly in water or juice. ResistAid™ is produced via a water extraction patented process (US 5756098; EP 86608), in accordance with Hazard Analysis and Critical Control Points (HACCP) standards and in compliance with the monograph in the Food Chemicals Codex. The Good Manufacturing Practices (GMP's) used during manufacturing are audited by the American Institute of Baking. The Larch arabinogalactan used in the ResistAid™ product has been designated as Generally Recognized as Safe (GRAS) with the US FDA (GRAS Notice Nos. GRN000047 and GRN000084).

The placebo was maltodextrin (Maltrin M100, Grain Processing Corp., USA). The test product and the placebo were administered by mixing the powders into a beverage of the subject's choice. The subjects were advised to take their dosage (4.5 g) once a day in the morning with breakfast. They began taking their assigned powder on Day 1 and continued over the entire 72 day study period.

Subjects

Subjects between the ages of 18 and 65 were recruited for the study in the usual manner (subject database and community advertisements). Subjects were phone-screened prior to scheduling a screening visit.

Subjects were included if they were 18-65 years of age, had a Body Mass Index (BMI) ≥ 18 kg/m^2 and ≤ 30 kg/m^2 at screening, agreed to all study visits and visit procedures, agreed to use approved forms of birth control, and agreed not to initiate/change any exercise or diet programs during the study. Subjects were excluded if they had previously had the pneumococcal vaccine, had any major systemic, inflammatory or chronic disease, had any active infection or infection in the past month requiring antibiotics or anti-viral medication, used immunosuppressive drugs in the prior 5 years, were known to have alcohol or drug abuse, were pregnant or lactating or had any medical condition which in the opinion of the investigator might interfere with the subject's participation in the trial.

Study Design

The study was a randomized, double-blind, placebo-controlled, parallel group trial with an active investigational period of 72 days. The objective was to assess the immunomodulatory effect of the arabinogalactan product on selective markers of immune function following antigenic challenge by the pneumococcal vaccine. The primary endpoints were 7 different pneumococcal IgG antibodies. The secondary objective was to determine whether the arabinogalactan product would stimulate other arms of the immune system to which there was no direct antigenic stimulus. Secondary endpoints included salivary IgA, white blood cell counts, complement (C3 and C4) and inflammatory cytokine levels. The study was conducted at the Staywell Research clinical research site located in Northridge, CA and was designed and managed by the Medicus Research Contract Research Organization (CRO) also in Northridge, CA. IRB approval was obtained prior to the initiation of any study activities (Copernicus Group IRB, Cary, NC).

Subjects meeting all of the inclusion criteria and none of the exclusion criteria for this study were randomly assigned to receive either the arabinogalactan or placebo. Double-blinding was ensured by the use of identical opaque sachets, outer packaging, labelling and color for both investigational products (arabinogalactan and placebo). Unblinding of the entire research team, including data analysis team did not occur until after the analysis was completed; subjects were blinded throughout the trial.

The study began in August 2008 (first subject in) and lasted until December 2008 (last subject completed). The subjects in the study came to the research clinic for a total of 5 visits (V1-V5) over 72 days. Subjects took the first dose of assigned study product at the screening visit (V1-Day 0) and continued to take them over the entire study. They received the 23-valent pneumococcal vaccine (Pneumovax® 23, Merck and Co., Inc., USA) at the vaccine visit which took place 30 days after they began taking the product or placebo (V2-Day 30). They came in for safety monitoring the day immediately following the vaccine (V3-Day 31) to observe the reaction at the vaccine administration site. Then subjects returned 21 days after vaccine (V4-Day 51) and finally 42 days after vaccine administration (V5-Day 72). On study visits, blood, urine and saliva were collected and subjects were queried regarding any change in health status. Additionally, they were assessed for compliance by interview, diary, and through the return of unused study product sachets.

The most potentially immunogenic pneumococcal antibodies (Ab) were determined in consultation with the UCLA Vaccine Center (Torrance, CA, USA) as the antibodies most likely to respond to vaccination with the 23-valent pneumococcal vaccine. These antibodies included 4, 6B, 9V, 14, 18C, 19F, and 23F. Salivary IgA was measured to monitor for non-specific effects on the adaptive immune system using immuno-array assays with a minimum sensitivity of 1.0 μg/ml. Other markers of immune function were chosen to represent the innate arm of the immune system including white blood cell counts (totals and subtypes), inflammatory cytokines,

and complement (C3 and C4) determined using immuno-turbidimetric methodology. Analysis of inflammatory cytokine levels were performed using sandwich immunoassay (Affymetrix, San Diego, CA, USA). Safety monitoring included: body temperature, blood pressure, heart rate, physical exam, urinalysis, complete blood counts (CBC) and a comprehensive metabolic panel (CMP) including kidney and liver function tests.

Analyses

Excel 2003 (Microsoft Corp, Redmond WA, USA), was used for data entry, validation, restructuring, calculating changes in variables over time, reorganizing and reformatting results, and preparing graphs. Statistical analyses were performed using SPSS Base System ver. 17 (SPSS Inc., Chicago IL, USA).

Data was analyzed using paired sample t-tests for within subject means comparisons, independent sample t-tests for between group comparisons (placebo vs. the active groups individually). Difference scores for both within and between group comparisons (placebo vs. the active groups individually) were analyzed using appropriate t-tests. Analysis was completed before the blinding code was broken.

Results

Subjects

Sixty five (65) subjects were screened in person at the research clinic and 53 qualified for randomization at the screening visit (V1). Of the 53, 8 did not return for V2 and therefore a total of 45 subjects were included in the intent-to-treat analysis. The subject baseline characteristics are given in Table 1.

Pneumococcal IgG antibodies

Pneumococcal IgG antibody subtypes 4, 6B, 9V, 14, 18C, 19F, and 23F were measured on Days 0 (V1), 51 (V4), and 72 (V5). There were no significant differences between the groups at baseline (Day 0).

Pneumococcal IgG levels increased from baseline in response to the vaccine as expected. Supplementation with the arabinogalactan product caused a significantly greater increase from baseline in pneumococcal IgG antibody subtypes 18C and 23F at both 51 and 72 days (Table 2). Mean values between groups were also significantly greater in the arabinogalactan group for both days 51 and 72 for these two subtypes (Table 3).

Table 1 Subject Demographics

	ResistAid(™)	Placebo
N	21	24
Male	9 (42.9%)	16 (66.7%)
Female	12 (57.1%)	8 (33.3%)
Age (range)	33.52 (19-62)	38.25 (20-64)

Change scores from baseline and mean values were greater in the arabinogalactan group than placebo for most time points in Ab subtypes 4, 6B, 9V, and 19F, but these differences did not reach statistical significance.

Salivary IgA

Salivary IgA levels in the placebo group were 146 ± 109 mg/dl at baseline (Day 0). There were no significant changes from Day 0 to Days 51 or Day 0 to Day 72 in either group. There were also no significant differences in the mean values between groups.

White blood cells

The mean total white blood cell count was 6.50 ± 1.46 × 1000/μl in the placebo group at baseline (Day 0). Comparisons between the arabinogalactan and placebo groups on Days 0, 30, 31, 51 or 72 found no significant differences in total white blood cell counts. The change from baseline Day 0 to Day 72 was significantly greater in the arabinogalactan group than the placebo group (0.38 ± 0.79 compared to 0.15 ± 1.33; p = 0.045).

Differential analysis of white blood cells determined that the levels at baseline were as follows: neutrophils 63.1 ± 5.3, lymphocytes 28.4 ± 6.0, monocytes 6.9 ± 1.9, eosinophils 1.6 ± 1.5 and basophils 0.33 ± 0.56 (measured as a percent of total white blood cells). There were no significant differences in lymphocyte, neutrophil, monocyte, or basophil counts when comparing mean values between groups at any time point. When comparing change from baseline at each time point, there were no differences between groups for lymphocytes, neutrophils, or monocytes. Change from baseline comparisons for basophils revealed a statistically significant, but clinically insignificant increase in numbers in the placebo group compared to the arabinogalactan group when comparisons were made between Day 0 and Day 72 (0.21 ± 0.72 placebo compared to 0.09 ± 0.54 arabinogalactan; p = 0.042).

Eosinophil counts were different between groups on Day 30 (2.81 ± 2.04 vs 1.46 ± 0.98; p = 0.006) and on Day 51 (3.24 ± 2.12 vs 1.83 ± 1.55; p = 0.014) with higher numbers in the arabinogalactan group. There was a larger increase in cell number (change) from baseline to Day 31 (0.14 ± 1.39 vs 0.83 ± 0.72; p = 0.035) and from baseline to Day 51 (0.48 ± 1.69 vs 0.20 ± 0.66; p = 0.006) in the arabinogalactan group.

Complement

The levels of complement C3 and C4 at Day 0 were 125 ± 23 and 28 ± 10 mg/dl, respectively. Comparisons of means and changes from baseline for complement (C3, C4) levels between the arabinogalactan and placebo groups were not significantly different.

Table 2 Effects of the 23-valent vaccine on Pneumococcal IgG antibodies

Antibody subtype	Day 0 Mean ± SD	Day 51 Mean ± SD	Day 72 Mean ± SD	Change Days 0-51	Change Days 0-72
Type 4	0.45 ± 0.64	2.21 ± 3.15	5.84 ± 7.35	0.023	0.042
Type 6B	0.95 ± 1.51	5.18 ± 6.64	5.19 ± 7.06	0.001	0.020
Type 9V	1.32 ± 4.10	6.07 ± 7.34	5.08 ± 5.25	0.129	0.095
Type 14	1.79 ± 2.56	9.91 ± 8.54	8.86 ± 8.59	0.000	0.006
Type 18C	0.72 ± 1.35	5.06 ± 5.80	4.93 ± 5.26	0.018	0.006
Type 19F	1.10 ± 2.94	7.02 ± 7.28	6.65 ± 7.26	0.011	0.015
Type 23F	1.08 ± 1.87	4.32 ± 4.62	4.55 ± 5.23	0.017	0.006

Increases in levels of antibody subtype as observed in the placebo group (n = 24) following inoculation with the 23-valent pneumococcal vaccine which took place on Day 30. Data are means (μg/dl) ± standard deviations on Days 0, 51 and 72. P-values for the changes between baseline and days 51 and 72 are in the right hand columns.

Cytokines

Comparison of cytokine levels between groups found no significant differences in means for epithelial neutrophil-activating peptide (ENA)-78, eotaxin, granulocyte monocyte colony stimulating factor (GM-CSF), interferon-gamma (IFNg), interleukin (IL)-10, IL-12P40, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-8, monocyte chemotactic protein (MCP)-1, MCP-3, platelet-derived growth factor (PDGF)-BB or tumor necrosis factor (TNF)-alpha. When comparing the cytokine change from baseline values between groups, only the IL-6 change from Day 30 to Day 31 showed an increase in the arabinogalactan group compared to the placebo group. The change in the arabinoglactan group was from a mean of 17.8 ± 7.7 to 19.8 ± 7.7 pg/ml (+1.9), compared to a change from 50.1 ± 113.8 to 48.7 ± 112.5 pg/ml for the placebo group (-2.4) (p = 0.046). This was most likely in response to the vaccine which was administered on Day 30.

Safety

No serious adverse events were reported during this study. There were nine mild adverse events in the placebo group (erythema at injection site (1), sore throat (2), nasal congestion (3), headache (2), and abdominal pain (1). There were no adverse events in the active group. All adverse events were followed by the medical staff at the research clinic.

Discussion

The results of this pilot study suggest that the arabinogalactan preparation had a selective immunostimulating effect on acquired or adaptive immunity, as shown in the increase in antibodies without any clinically significant effects on total white blood cells, cytokines or complement. Thus it is possible that rather than acting as a general immunostimulant, arabinogalactan acted in a specific manner. The caveat to this statement is that these results are preliminary and there are confounding variables to consider.

Variables that affect the immune response to vaccines include age, gender, race and genetic characteristics[19]. One of the goals of this pilot study was to determine the effect of the intervention on a relatively broad population. As such, the study population included males and females from age 18 to 65. The randomization scheme was sequential and therefore the gender of subjects was not matched in advance. As gender and age

Table 3 Pneumococcal IgG types 18C and 23F - Comparisons between ResistAid(TM) and Placebo Groups

	Day 0 Mean ± SD	Day 51 Mean ± SD	Day 72 Mean ± SD	Change Day 0-51	Change Day 0-72
Type 18C					
ResistAid™ (n = 21)	1.49 ± 3.00	9.57 ± 7.96	9.10 ± 7.53	8.08 ± 7.12	7.61 ± 6.81
Placebo (n = 24)	0.72 ± 1.35	5.06 ± 5.80	4.93 ± 5.26	4.34 ± 5.10	4.22 ± 4.69
Comparison (p-value)	0.061	0.006	0.008	0.033	0.012
Type 23F					
ResistAid™ (n = 21)	0.74 ± 0.93	7.07 ± 7.41	7.02 ± 7.31	6.33 ± 7.36	6.28 ± 7.17
Placebo (n = 24)	1.08 ± 1.87	4.32 ± 4.62	4.55 ± 5.23	3.24 ± 4.28	3.46 ± 4.24
Comparison (p-value)	0.059	0.002	0.041	0.001	0.003

Levels of antibody subtypes 18C and 23F in the ResistAid™ and placebo groups are given as means (μg/dl) ± standard deviations on Days 0, 51 and 72 and changes from Day 0. Inoculation with the 23-valent pneumococcal vaccine took place on Day 30. P-values are comparisons between groups and comparisons of changes from Day 0 in the two groups.

differences may affect immunity these potentially confounding variable should be looked at in future studies. This study was an exploratory investigation into the effects of arabinogalactan with the goal of determining whether further studies are warranted. The result is that further studies with larger populations are indicated to clarify and potentially expand upon the effects of arabinogalactan on antibody production.

The suggestion that arabinogalactan might have a selective effect on the immune system is preliminary but promising. The immune system entails a complex matrix of responses to protect the body from pathogens and toxins. The innate immune system involves the rapid recruitment and upregulation of neutrophils, monocytes, macrophages, complement factors, cytokines and antimicrobial peptides to the site of infection. The innate response is the first line of host defense and the adaptive response follows a few days later. While the innate and adaptogenic arms of the immune system are often described as separate, they often act together in a synergistic manner[20]. In addition to antibodies, the variables tested in this study included salivary IgA, white blood cell counts (lymphocytes, neutrophils, monocytes, basophils and eosinophils), complement C-3 and C-4 as well as numerous cytokines. Additionally, suggestions of changes were observed in IL-6 levels and in eosinophil counts. IL-6 has immunostimulatory properties and eosinophils play a role in allergic responses. The clinical significance of these findings is unknown at this time. Additional measurements for future studies could include a breakdown of lymphocytes into subtypes, measuring natural killer (NK) lymphocytes and NK-T cells. NK cells are a heterogeneous population of innate T cells that have attracted interest because of their potential to regulate immune responses to a variety of pathogens and NK-T cells function as a bridge between innate and adaptive immunity[20].

Arabinogalactan was given for 30 days prior to vaccination and administration was continued throughout the study. The 30 days time period was chosen because a previous clinical trial studying the effect of arabinogalactan and echinacea preparations on the immune system observed a positive effect following treatment for this period of time[16].

This is the first human study to demonstrate an effect by Larch arabinogalactan on immunoglobulin levels. No effect on IgG antibody levels was observed in another study wherein the subjects were administered 1.5 g larch arabinogalactan per day for four weeks[16]. This study utilized a larger dose (4.5 g per day), longer administration time (10 weeks) and the vaccine as a standardized antigenic challenge all of which appear to have been useful in delineating a difference between the proprietary arabinogalactan extract and placebo.

Conclusions

The proprietary arabinogalactan extract (ResistAid™) tested in this randomized, double-blind, placebo-controlled, parallel-group study, increased the antibody response of healthy volunteers to the 23-valent pneumococcal vaccine compared to placebo. The proprietary arabinogalactan product was administered safely in this study in a dose of 4.5 g per day for approximately 10 weeks. This was a pilot study that demonstrated promising effects and further studies with larger populations are indicated which may demonstrate additional effects of arabinogalactan on the immune system.

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Author details

[1]Medicus Research LLC, Northridge, CA 91325, USA. [2]UCLA School of Medicine, Department of Medicine, Los Angeles, CA 90024, USA. [3]Pharmacognosy Consulting, Mill Valley, CA 94941, USA.

Authors' contributions

JKU conceptualized the study and was the Principal Investigator. BBS also participated in the design of the study. BBS and VJS performed the analysis. JKU, BBS and MLB contributed to writing the manuscript. All authors have read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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Original Research

Effects of Dietary Arabinogalactan on Gastrointestinal and Blood Parameters in Healthy Human Subjects

Ramona R. Robinson, MS, RD, Joellen Feirtag, PhD, and Joanne L. Slavin, PhD, RD

Department of Food Science and Nutrition, University of Minnesota, St. Paul, Minnesota

Key words: arabinogalactan, microflora, ammonia, blood lipids, gastrointestinal transit time

Objectives: Arabinogalactan (AG) is a non-digestible soluble dietary fiber that resists hydrolytic enzyme action and enters the large bowel intact where it is fermented by resident microflora. To determine whether AG has similar physiological properties to other soluble dietary fibers, we examined the effect of 15 and 30 g per day of a commercially available AG from Western Larch on several gastrointestinal and blood parameters.

Methods: Gastrointestinal parameters included fecal microflora, fecal enzyme activity, fecal short-chain fatty acids, fecal pH, fecal weight, transit time and bowel frequency. Blood parameters included total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, Apo-A1, Apo-B, glucose and insulin. The study consisted of two three-week diet treatments with no washout period. Participants (n=20, 11 males, 9 females) consumed their usual diet in addition to 15 or 30 g AG in a beverage sweetened with aspartame as compared to their usual diet with the control beverage.

Results: Significant increases in total fecal anaerobes were observed with 15 g ($p=0.01$) and 30 g AG ($p=0.001$). A significant increase ($p=0.02$) in *Lactobacillus spp.* was observed when subjects consumed AG for a total of six weeks regardless of dose. There were no significant changes in other microflora, fecal enzyme activity, transit time, frequency, fecal weight, fecal pH and short-chain fatty acids. Fecal ammonia levels decreased with 15 g ($p=0.001$) and 30 g ($p=0.002$) AG. No significant changes in blood lipids or blood insulin were observed.

Conclusions: These data suggest that dietary AG is easily incorporated into the diet, well tolerated in subjects and has some positive effects on fecal chemistry.

INTRODUCTION

Arabinogalactan (AG) is a soluble dietary fiber, commonly consumed in such foods as carrots, tomatoes, radishes, pears, maize, wheat and red wine [1]. In addition, several herbs have been found to contain significant amounts of AG, such as *Echinacea purpurea*, *Angelica acutiloba* and *Curcuma longa* [2–4]. The Western Larch (*Larix occidentalis*) and Mongolian Larch (*Larix dahurica*) are commercial sources of AG [5]. Arabinogalactan can be extracted from a variety of purified concentrated sources, although the commercial form used in this study was extracted from the butt wood of Western Larch grown in Northern Minnesota. Arabinogalactan derived from trees of the genus *Larix* (Larch) is a unique hemicellulosic product and is easily extractable by water in a pure form from non-delignified plant tissues. Arabinogalactans have an average molecular weight between 15,000 and 25,000. AG, also known as larch gum, is similar to gum arabic because it is highly branched, extremely water soluble, and high concentrations can be produced with very low viscosities [6].

Arabinogalactan is fermented by human intestinal bacteria and can induce the enzymes necessary for its degradation [7–11]. In addition, arabinogalactan is fermented at a slower rate than other carbohydrates due to its branched structure [12]. Fermentation is evidenced by the ability of human intestinal microflora to degrade arabinogalactan and produce short-chain fatty acids [13,14]. To date, the studies conducted with arabinogalactan are mainly *in vitro*. While this work contributes to our understanding of how this substrate is degraded, it is important to remember that the human colon is a complex environment and *in vitro* studies may not accurately represent bacterial activities within the human colon.

Address reprint requests to: Joanne Slavin, Ph.D., Department of Food Science and Nutrition, 1334 Eckles Avenue, St. Paul, MN 55108. E-mail: jslavin@umn.edu
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In addition to gastrointestinal parameters, blood lipids may be affected by fiber consumption. Increased fiber consumption may decrease blood cholesterol levels. There has not been previous research conducted evaluating the effect of arabinogalactan consumption on blood lipids. Thus, the objective of this study was to examine the physiological effects of a commercially available Larch arabinogalactan on the gut environment, blood lipids and blood glucose in healthy human subjects.

METHODS

Subjects

Subjects (11 male, 11 female) were recruited from the Twin Cities community. Subjects were screened for their ability to consume a beverage with or without AG, continue their habitual diet and exercise routines and provide blood samples on four occasions and fecal samples on three occasions. Participants' baseline cholesterol levels were 196 ± 26 mg/dL (Mean ± SD). Exclusion criteria included pre-existing medical conditions, recent use of antibiotics or lipid altering medications, alcohol or drug abuse, cigarette smoking and extreme diet. The conditions and procedures of the study were reviewed, and written informed consent was obtained from each subject. Twenty subjects completed this study. One subject dropped out due to illness, and the other subject did not comply with protocol. All aspects of this research study were approved by the University of Minnesota Institutional Review Board Human Subjects Committee.

Study Design

The study utilized a crossover design with no washout period. Subjects were given a beverage containing no AG for seven days. Following this control period, subjects were randomly assigned to receive a dose of either 15 g or 30 g arabinogalactan (Larex Inc., St. Paul, MN). Each dose of AG was consumed for three weeks, and then subjects were crossed over to the other dose. AG was incorporated into 16 ounces of an aspartame-sweetened beverage (Crystal Light®). Subjects consumed one 16-ounce beverage per day in addition to their typical diet throughout the entire seven weeks of the study. They were instructed to consume each beverage given to them and to maintain their usual diet and activity level for the duration of the study. Subjects provided three-day diet records and symptom evaluation surveys once during each treatment (0g, 15g, 30g AG).

Assessment of Subjects' Habitual Diets

During the last three days of baseline and treatment periods, subjects collected detailed three-day diet records. Nutrients were determined with the Nutrition Data System for Research (NDS-R) software version 4.0, developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN, Food and Nutrient Database 28.

BIOLOGICAL SAMPLE COLLECTION

Fecal Collection

Subjects collected fecal samples for the final five days of each treatment period. On days 3, 24 and 45 of the study, each subject swallowed plastic radio opaque pellets to mark intestinal transit time. All feces were subsequently collected into individual containers, defecation times were recorded and samples were weighed and frozen immediately at −20°C until analyzed. Fecal samples were subsequently X-rayed, and pellets per stool were counted. Passage of 80% of the pellets was considered transit time. The first four days of fecal samples for each subject were composited for calculation of stool weight. A fresh fecal sample was obtained from each subject at the conclusion of the transit time collection. Subjects were asked to defecate into sterile bags and include an anaero-pouch sachet (Mitsubishi Gas Chemical Company, Inc., New York, NY), which was sealed to keep the atmosphere reduced until sample analysis. Subjects delivered fresh fecal samples to our laboratory, and within 24 hours of defecation samples were analyzed for microbiological information. Subjects were given symptom evaluation questionnaires to fill out once during each phase of the study. Subjects marked their symptoms on a 145-mm line. Lines were measured and reported as subjective changes in gastrointestinal parameters.

Microbiology

Eleven grams of fresh fecal sample were obtained from the center of each stool and homogenized in 99 mL of pre-reduced 0.1% peptone water to provide a 1% (wt/vol) fecal slurry. One mL of slurry was diluted serially in peptone water and duplicate spread plates were made using 0.1 ml of each dilution. Total anaerobes were counted using Wilkins-Chalgren agar (Difco Laboratories, Detroit, MI) and enterobacteria were counted using MacConkey agar (Difco). Total lactic acid bacteria were counted using Lactobacilli modified MRS medium (Difco) [15]. *Bifidobacterium spp.* were counted on X-α-Gal based medium as described by Chevalier and colleagues [16]. *Clostridium spp.* were isolated on sulfite-polymyxin-milk agar. Plates were incubated at 37°C in the AnaeroPack™ (Mitsubishi Gas Company) containing 20% CO_2 and read after 72 hours. Stool slurry pH was determined in each sample with a glass pH electrode.

β-Glucosidase Enzyme Assay

Samples (40 mL) of 1:10 diluted stool from microbial enumeration studies were placed in 50 mL tubes; 4 mL of Oxyrase® For Broth (Oxyrase, Inc., Mansfield, OH) was added

to each sample to maintain an anaerobic environment. Samples were stored at −20°C until analyzed. Samples were thawed, sonicated for three minutes and centrifuged for five minutes at 12,000 *x g* to pellet particulate matter. Samples were transferred to capped microfuge tubes for individual enzyme assays. β-Glucosidase activity was assayed at 37°C under atmospheric conditions by following the hydrolysis of 3 mM *p*-nitrophenyl -β-D-glucopyranoside (Sigma) after one hour and comparing the *p*-nitrophenol liberated to a standard curve at an absorbance of 405 nm. The pH of the 1 mL samples was adjusted with the addition of 100 μL 1.0 M potassium phosphate, 1.5 M NaCl, pH 5.5. The reaction was stopped with the addition of 100 μL 1M Na_2CO_3.

Short Chain Fatty Acids

After transit time calculations, four-day fecal collections were homogenized in a blender and stored at −20°C for SCFA analysis. Samples were thawed and 5 g aliquots were placed in Centriprep fluid concentrators, MWCO 30,000 kDa (Amicon Inc., Beverly, MA). Samples were centrifuged for 30 minutes at 1000 *x g*, room temperature and supernatants (total volume 0.75–1.0 mL) were placed in 15 mL polypropylene tubes; 0.3 mL of 25% *m*-phosphoric acid was added to each tube, and samples were vortexed and incubated at room temperature for 25 minutes. Samples were centrifuged at 5000 *x g* for 15 minutes at room temperature. Supernatants were decanted and frozen overnight. The following day, samples were thawed, and the pH of each sample was adjusted to 6.5 using 4 N KOH. Oxalic acid was added at a final concentration of 0.03%, and SCFA concentrations were determined by gas chromatography with use of a Hewlett-Packard 5880A gas chromatograph (Hewlet Packard, Palo Alto, CA) containing an 80/120 Carbopack B-DA/4% Carbowax 20M column (Supelco, Inc., Bellefonte, PA) [17].

Ammonia Assay

Fecal ammonia levels were assayed using the CHEMets® Ammonia-Nitrogen Kit (CHEMetrics, Calverton, VA). One-mL fecal supernatant samples were diluted with 24 mL of distilled, deionized water. Glass ampoules containing Nessler's reagent, an alkaline solution comprising mercuric iodide and sodium hydroxide, were inserted into diluted fecal samples and filled. Ampoules were mixed, allowed to react for one minute and quantified by comparing to a set of colored standards. A yellow color developed in the presence of ammonia.

Blood Parameters

Fasting blood samples were drawn on the last day of baseline diet and on the last day of each three-week feeding treatment. Blood samples were analyzed for total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, Apo-A1, Apo-B, glucose and insulin.

Statistical Analysis

Statistical evaluation of results was done by analysis of variance with repeated measures using the factors: 0 g fiber *vs.* the mean of 15 g and 30g AG treatment. Data were evaluated for the effects of treatment, order and time. Values in tables represent means ± standard error of the means (SEM). Data were analyzed using SAS [18].

RESULTS

Three-Day Diet Records

Review of the subjects' habitual diets indicated that the mean carbohydrate intake as a percentage of total kilocalories did not change significantly throughout the study. Mean protein intake as a percentage of total kilocalories increased significantly ($p=0.02$) between baseline (14.70 ± 0.68%) and 15 g AG (17.06 ± 0.68%), while there were no significant differences between baseline and treatment with 30 g AG. Mean fat gram intake decreased significantly ($p = 0.04$) between baseline (84.60 ± 4.50) and 30 g AG (70.86 ± 4.50), while there were no significant differences between baseline and treatment with 15 g AG. There were significant increases in fiber intake when baseline was compared to both the 15 g AG and 30 g AG treatment. Total dietary fiber intakes, including the dietary fiber from AG, were 17.8 g ± 9.0 g for control, 30.0 g ± 8.5 g for the 15 g AG treatment and 41.5 g ± 6.2 g for the 30 g AG treatment.

Intestinal Microflora

There were significant differences in levels of total anaerobes and *Lactobacillus species* following AG consumption (Table 1). Data are expressed in colony forming units (CFUs) on the log 10 scale. Randomization order did not significantly affect bacterial counts. There were significant increases ($p=0.01$) in total anaerobes between baseline (10.35 ± 0.10) and the two levels of treatment, 15 g AG (10.74 ± 0.10) and 30 g AG (10.74 ± 0.10) respectively. *Lactobacillus spp.* measured (9.36 ± 0.14) at baseline and for the two levels of treatment, 15 g AG (9.73 ± 0.14) and 30 g AG (9.73 ± 0.14). These increases were not statistically significant. Length of time consuming AG appeared to be more important than dose (Table 2). Mean *Lactobacillus spp.* increased significantly ($p = 0.02$) between baseline (9.36 ± 0.14) and following six weeks of AG consumption (9.82 ± 0.14), whereas three weeks of AG consumption did not produce significant increases in *Lactobacillus spp.* Levels of fecal *Bifidobacterium spp., Clostridium spp.* and Enterobacteriaceae did not differ significantly between baseline and AG treatments.

Bacterial Enzyme Activity

There were no significant differences in β-Glucosidase activity between baseline (25.32 ± 2.90) and the two levels of

Table 1. Fecal Microflora Enumeration of Study Participants during Baseline and Following Three Weeks' Consumption of either 15g or 30g Arabinogalactan

Species	n	Baseline Mean ± SEM	15g AG Treatment Mean ± SEM	*p* value	30g AG Treatment Mean ± SEM	*p* value
Total Anaerobes	20	10.35 ± 0.10	10.74 ± 0.10	0.01	10.74 ± 0.10	0.01
Lactobacillus spp.	20	9.36 ± 0.14	9.73 ± 0.14	0.07	9.73 ± 0.14	0.07
Bifidobacterium spp.	20	9.00 ± 0.17	9.12 ± 0.17	0.64	8.76 ± 0.17	0.31
Clostridium spp.	20	8.53 ± 0.20	8.54 ± 0.20	0.96	8.90 ± 0.19	0.19
Enterobacteriaceae	20	6.28 ± 0.27	6.37 ± 0.27	0.80	6.22 ± 0.27	0.88

Expressed as colony forming units/gram fresh stool (CFU/g) on log 10 scale.

Table 2. Fecal Microflora Enumeration of Study Participants during Baseline and Following Three Weeks and Six Weeks of Arabinogalactan Consumption

Species	n	Baseline Mean ± SEM	3 weeks of AG Mean ± SEM	*p* value	6 weeks of AG Mean ± SEM	*p* value
Total Anaerobes	20	10.35 ± 0.09	10.55 ± 0.09	0.12	10.93 ± 0.09	0.0001
Lactobacillus spp.	20	9.36 ± 0.14	9.63 ± 0.14	0.18	9.82 ± 0.14	0.02
Bifidobacterium spp.	20	9.00 ± 0.18	8.95 ± 0.18	0.82	8.93 ± 0.18	0.77
Clostridium spp.	20	8.53 ± 0.19	8.46 ± 0.19	0.80	8.98 ± 0.19	0.10
Enterobacteriaceae	20	6.28 ± 0.27	6.39 ± 0.27	0.77	6.20 ± 0.27	0.84

Expressed as colony forming units/gram fresh stool (CFU/g) on log 10 scale.

treatment, 15 g AG (21.47 ± 2.90) and 30 g AG (27.84 ± 2.90).

SCFA and SCFA Ratios

The SCFA and SCFA ratios did not change after AG administration (Table 3).

Fecal pH and Ammonia Levels

Mean fecal pH did not change after AG administration. Mean fecal ammonia levels significantly decreased with AG consumption. Results show significant decreased ($p=0.001$) between baseline (71.25 ± 3.81) and 15 g AG (51.50 ± 3.81). Significant decreases ($p=0.002$) were also observed when comparing baseline to 30 g AG (53.25 ± 3.81).

Bowel Habit: Composite Fecal Weight, Intestinal Transit and Frequency

Mean fecal weight, transit time and frequency did not differ significantly between baseline and both the 15g and 30g dose of AG (Table 4).

GI Symptom Surveys

Surveys were evaluated according to a symptom self-recorded hash mark on a 145mm line. Stool consistency did not differ significantly between baseline and AG treatment phases. Bloating and flatulence were not reported to be significantly different when comparing baseline to the 15 g dose of AG. Bloating was reported to be more frequent ($p=0.005$) when comparing baseline (41.15 ± 6.12 mm) to 30 g AG (67.76 ± 6.35 mm). Flatulence increased significantly ($p=0.002$) when comparing baseline (53.25 ± 5.47mm) to 30 g AG (78.93 ± 5.68mm). Midrange (72.5 mm) represented the midpoint between minimal and excessive symptoms. Flatulence was the only symptom that was reported greater than mid-range and only when AG was consumed at the 30 g dose. (Table 5)

Blood Values

AG consumption had no significant effect on total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, Apo-A1 and Apo-B (Table 6). Mean blood glucose levels increased

Table 3. Fecal Short-Chain Fatty Acids of Study Participants during Baseline and Following Three Weeks' Consumption of either 15g or 30g Arabinogalactan

Fatty Acid	n	Baseline Mean ± SEM	15g AG Treatment Mean ± SEM	*p* value	30g AG Treatment Mean ± SEM	*p* value
Acetate	20	50.47 ± 3.26	50.80 ± 3.26	0.94	55.08 ± 3.26	0.32
Propionate	20	12.22 ± 0.81	12.59 ± 0.81	0.74	13.41 ± 0.81	0.31
Isobutyrate	20	1.01 ± 0.09	1.04 ± 0.09	0.80	0.91 ± 0.09	0.45
Butyrate	20	9.78 ± 0.71	9.21 ± 0.71	0.58	8.95 ± 0.71	0.42
Valerate	20	1.10 ± 0.08	1.18 ± 0.08	0.81	1.16 ± 0.08	0.60
Total SCFAs	20	76.19 ± 4.56	76.75 ± 4.56	0.93	81.50 ± 4.56	0.41

* Expressed as μmol/mL.

Table 4. Intestinal Transit Time, 4-Day Frequency and 4-Day Composite Fecal Weight of Study Participants during Baseline and Following Three Weeks' Consumption of either 15g or 30g Arabinogalactan

	n	Baseline Mean ± SEM	15g AG Treatment Mean ± SEM	*p* value	30g AG Treatment Mean ± SEM	*p* value
Transit Time (minutes)	19	2209 ± 313.84	2732 ± 326.00	0.26	2384 ± 313.84	0.70
Frequency (Stools/day)	20	4.85 ± 0.27	4.50 ± 0.27	0.37	4.25 ± 0.27	0.13
Fecal Wet Weight (grams/5-day composite)	20	684.64 ± 38.88	650.32 ± 38.88	0.54	634.61 ± 38.88	0.37

Table 5. Gastrointestinal Symptom Survey Results of Study Participants during Baseline and Following Three Weeks' Consumption of either 15g or 30g Arabinogalactan

Symptom	n	Baseline Mean ± SEM	15g AG Treatment Mean ± SEM	*p* value	30g AG Treatment Mean ± SEM	*p* value
Bloating 0=minimal 145=excessive	18	41.15 ± 6.12	50.10 ± 6.12	0.31	67.76 ± 6.35	0.005
Flatulence 0=minimal 145=excessive	18	53.25 ± 5.47	61.40 ± 5.47	0.30	78.93 ± 5.68	0.002
Stool Consistency 0=excessively soft 145=excessively hard	18	58.83 ± 3.53	62.43 ± 3.40	0.47	56.20 ± 3.53	0.60

Measured in mm (line length = 145mm).

Table 6. Blood Lipids of Study Participants during Baseline and Following Three Weeks' Consumption of either 15g or 30g Arabinogalactan

Parameter	n	Baseline Mean ± SEM	15g AG Treatment Mean ± SEM	*p* value	30g AG Treatment Mean ± SEM	*p* value
Total cholesterol	20	195.90 ± 3.06	195.85 ± 3.06	0.99	198.10 ± 3.06	0.61
LDL-cholesterol	20	116.79 ± 2.94	121.63 ± 2.94	0.25	118.53 ± 2.94	0.68
HDL-cholesterol	20	51.85 ± 1.76	51.75 ± 1.76	0.97	48.45 ± 1.76	0.18
Triglycerides (Log 10 scale)	20	2.04 ± 0.026	2.03 ± 0.26	0.89	2.10 ± 0.26	0.13
Apo-A1	20	128.60 ± 2.18	127.10 ± 2.18	0.63	126.50 ± 2.18	0.50
Apo-B	20	100.47 ± 2.90	103.70 ± 2.79	0.43	102.40 ± 2.79	0.63

Blood values expressed in mg/dL.

significantly ($p=0.02$) between baseline (76.55 mg/dL ± 2.40) and 30 g AG (84.80 ± 2.40), while there were no significant differences between baseline and 15 g AG phase (Table 7). Mean blood insulin levels did not show statistically significant changes.

DISCUSSION

Our results demonstrate that, compared with a baseline diet, a diet supplemented with 15 g and 30 g AG increased the densities of total anaerobes and *Lactobacillus species.* Lactobacilli are believed to maintain and restore normal intestinal balance. Pfeifer and Rosat [19] report that increasing Lactobacilli populations increased acidity of the gastrointestinal environment, destroyed toxic substances and produced antimicrobial compounds. Some species and strains of Lactobacilli may have immunomodulating activities, such as enhancing phagocytic activity in the peripheral blood.

There was no increase in Bifidobacteria counts, another colonic microbe found to promote health benefits. This may be

Table 7. Blood Glucose and Insulin Levels of Study Participants during Baseline and Following Three Weeks' Consumption of either 15g or 30g Arabinogalactan

	n	Baseline Mean ± SEM	15g AG Treatment Mean ± SEM	*p* value	30g AG Treatment Mean ± SEM	*p* value
Glucose (Mg/dL)	20	76.55 ± 2.40	81.05 ± 2.40	0.19	84.80 ± 2.40	0.02
Insulin (mU/L)	20	6.80 ± 4.00	6.15 ± 4.00	0.91	14.15 ± 4.00	0.20

due, in part, to the significant increase in the Lactobacilli population. Species of *Lactobacillus* may compete with *Bifidobacterium spp.* for available substrate and adhesion sites within the colonic epithelium.

Because the majority of bacterial fermentation is thought to occur in the proximal colon, analysis of fecal instead of colonic flora probably does not best represent activities within the colon. Additionally, short-term feeding studies may not provide the necessary time to produce recognized changes in bacterial populations.

There were no statistically significant changes in fecal SCFAs or SCFA ratios. Vince and colleagues [11] also did not find increases in fecal short-chain fatty acid production following arabinogalactan consumption. However, their work as well as the work of Englyst and colleagues [12] did report increases in SCFA production following arabinogalactan supplementation of fecal incubates.

Short chain fatty acids are believed to be quickly absorbed following their production; therefore, it is difficult to determine the total amount produced in human subjects. At least 95% of SCFAs produced in the colon are absorbed and therefore can not be seen upon evaluation of fecal samples.

Fecal ammonia levels decreased significantly with both 15 g and 30 g AG. This supports the work of Vince and colleagues [11], who found that subjects fed arabinogalactan had decreased fecal ammonia concentrations following AG supplementation of fecal incubates. High colonic ammonia levels may have detrimental health implications. Studies have shown that ammonia levels as low as 5 mmol/L can have cytopathic effects on colonic epithelial cells. Ammonia has been shown to affect the intermediary metabolism and DNA synthesis of mucosal cells [20]. Ammonia is reported to be toxic toward epithelial cells, a circumstance which leads to their increased turnover. Patients with liver disease who are unable to detoxify ammonia have been successfully treated with antibiotics and lactulose. Lactulose is fermented in the colon by bacteria that utilize ammonia as a nitrogen source, thus decreasing colonic ammonia concentration [21]. AG appears to be similar to lactulose in that it decreases fecal ammonia concentrations.

In the current study, ammonia levels may have been reduced due to the significant increases in total anaerobes. Some anaerobic colonic bacteria prefer to utilize ammonia as a nitrogen source rather than amino acids or peptides when fermenting carbohydrates. A strain of *Eubacterium* species is reported to have a strict requirement for ammonia [22]. *Eubacterium* was not a bacterial species enumerated in the current study. Undetected increases in this particular bacterial species may have contributed to the increase in total anaerobes

There were no observed changes in fecal wet weight, transit time or frequency following consumption of arabinogalactan. Gum arabic, a fiber similar to AG, also does not affect fecal wet weight, but has been shown to increase transit time [23]. Soluble dietary fibers, such as AG, are largely fermented, so any increase in fecal weight is due to increases in fermentation gasses and bacterial mass resulting from the proliferation of microbes metabolizing the dietary fiber [24].

Subjects reported no significant changes in bloating, flatulence or stool consistency during the consumption of 15 g AG, although they reported increases at the 30 g AG dose. The increase in flatulence was likely due to the increase in bacterial fermentation in the colon and concomitant production of gases such as hydrogen and methane.

Significant decreases in fat consumption were observed when subjects consumed the 30 g dose of AG. A reason for this change may be explained by the increased reports of bloatedness (fullness) when subjects consumed the 30 g dose of AG. A sensation of fullness may have led subjects to avoid high fat foods.

There were no significant changes in blood lipids following AG consumption. Some soluble dietary fibers have been associated with decreases in total plasma cholesterol. There are a variety of potential cholesterol lowering mechanisms associated with the consumption of dietary fiber. These mechanisms are related to viscosity, SCFA production and bacterial proliferation. Arabinogalactan is relatively non-viscous and therefore may not decrease cholesterol levels for this reason. Another mechanism believed to be involved in the cholesterol lowering effects of dietary fibers is elevated levels of short-chain fatty acids. When dietary fibers are fermented, short-chain fatty acids are produced. There is some research to support that propionate may be the hypocholesterolemic short-chain fatty acid. Also, Lactobacilli bacteria may lower serum cholesterol levels, although the mechanisms are unclear. The microflora may be involved in the deconjugation of bile salts and subsequent inefficient cholesterol absorption, or they may possibly assimilate cholesterol and remove it from the colon [19].

Blood glucose significantly increased following the consumption of 30 g AG. Blood samples were taken from fasted subjects, and we therefore did not expect to see increases in blood glucose levels at any time. The reason for these increases during the treatment phases remains unknown, although possible explanation could be associated with its influence on the production of specific fermentation end products. Increased glucose levels might have been due to an undetected increase in the production of the fermentation end product propionate, which is believed to travel to the liver and increase gluconeogenesis.

In conclusion, a 15 g or 30 g per day supplement of AG increased total fecal anaerobes and decreased fecal ammonia concentrations. Consumption of AG for six weeks led to increased *Lactobacillus* populations. A dose of 30 g AG increased blood glucose levels. A dose of 15 g/day AG appears to be particularly well tolerated by subjects and has some positive effects on fecal chemistry.

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