



THE RESEARCH GUIDING DEVELOPMENT OF

**SPOONFULONE™ MULTIPROTEIN
BLEND FOR INFANTS & CHILDREN:**

Supporting Gentle Maintenance of Tolerance to
Foods Most Commonly Responsible for Allergies



INTRODUCTION

Food allergies are common among infants and children in the US. A large survey from 2009 to 2010 of US households, including a total of 38,480 children, found that 8% had food allergies.¹ Of those who develop food allergy, almost 40% become allergic to more than one food.² Using 2017 US population estimates, that 8% represents approximately 5.9 million children in the US under age 18 with food allergies.³ In addition, there is a trend of rising prevalence of childhood food allergies in the US. Food allergies among children aged 0-17 years increased 50% from 1997 (3.4%) to 2011 (5.1%) based on a nationwide survey.⁴ In another survey of 5,300 US households, peanut or tree nut allergy among children <18 years of age increased 250% from 1997 (0.6%) to 2008 (2.1%).⁵ Notably, while peanut is the most recognized food allergy, < 7% of those who develop a food allergy become mono-allergic to peanut.^{2,6}

Both genetics and environmental factors influence development of food allergies in children. In a study of children aged 0-21 years, 65% of the 832 children with a food allergy did not have a parent with a food allergy,⁷ indicating the importance of environmental factors in the development of food allergy.

Food is an important component of environmental exposure. A lack of diversity in the diet of children may increase the risk of food allergy development. In a study of 856 children, increased dietary diversity during the first year of life was associated with a lower risk of development of food allergies.⁸ In another study conducted in 3142 newborns in Finland, by 12 months of age, less dietary diversity was associated with an increased risk of asthma, atopic asthma, wheeze, and allergic rhinitis.⁹

Conversely, avoidance of potentially allergenic food in children appears to increase the risk of developing an allergy to that food. A randomized trial of peanut consumption in 640 infants >4 to <11 months of age without a peanut allergy who were at risk for development of peanut allergy based on the presence of severe eczema or egg allergy or both, revealed in the intent-to-treat analysis that 13.7% had developed a peanut allergy at 60 months in the group that avoided consumption of peanuts versus 1.9% in the group that consumed peanuts.¹⁰ In a follow-up study, 270 of the participants who had avoided peanuts consumption continued to do so for another year, while 280 participants from the peanut consumption group began avoiding peanuts for a year. At 72 months, 18.6% of children in the original peanut avoidance group and 4.8% in the original peanut consumption group had a peanut allergy.¹¹ This extension study strengthened the original finding that early dietary exposure to a commonly allergenic food may reduce the risk of development of an allergy to that food.

To study early introduction of potentially allergenic foods, 1303 exclusively breastfed 3-month-old children in the UK were randomly assigned to the early introduction (from 4 to 6 months of age) or standard introduction (6 months of age) of 6 commonly allergenic foods (peanut, cooked egg, cow's milk, sesame, whitefish, and wheat). Among those who were adherent to the protocol, the prevalence of any food allergy was significantly lower in the early introduction group than in the standard introduction group (2.4% vs. 7.3%), demonstrating that children can safely begin eating commonly allergenic foods before 6 months of age and that delayed introduction may increase the risk of developing food allergies.¹²

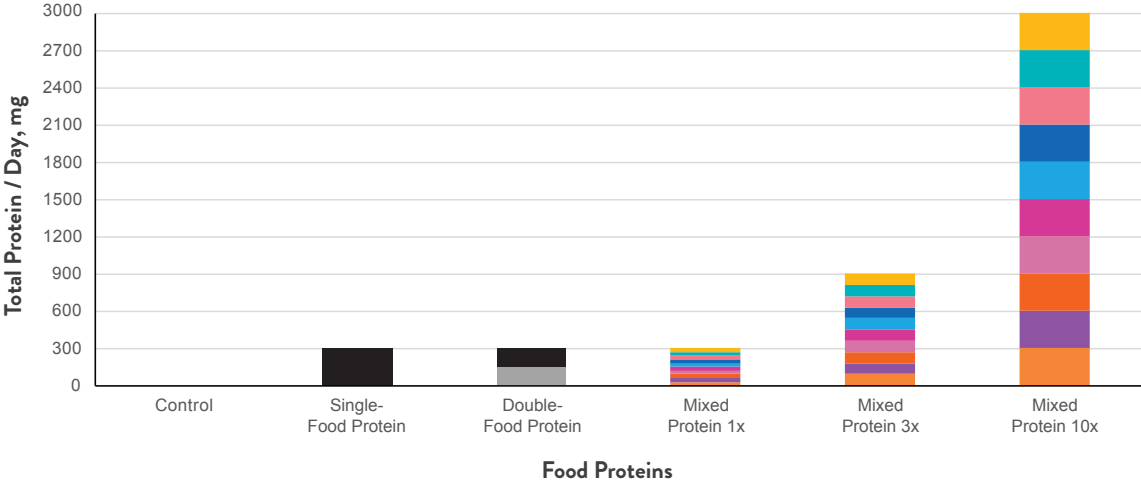
Based on the studies discussed above, early dietary exposure to commonly allergenic foods may be an effective method for reducing the risk of development of food allergy in children. SpoonfulOne, a multiprotein blend developed by a pediatric allergist using data licensed from Stanford University, is designed to gently support early maintenance of adaptive tolerance in children to the most commonly allergenic food groups (milk, egg, peanut, tree nuts, soy, wheat, fish, crustacean shellfish, and sesame), which are responsible for >90% of serious food-induced adverse reactions in people who develop food allergies.¹³ The protein levels and composition of SpoonfulOne are based on the study discussed herein.¹⁴

METHODS

To evaluate the inclusion of commonly allergenic foods in the diet of healthy infants and children, to assess the potential of such to gently reduce the risk of developing food sensitivities, and to establish an active serving size of a multiprotein formula (ten proteins) for reducing pro-allergic blood-based biomarkers, a study was conducted in 450 healthy infants and children aged 2 months to 10 years in an institutional review board–approved protocol. Participants were randomized equally to one of the following 18 cohorts for daily feeding for one year: control (no additional protein), nine groups consuming a single additional protein (egg, milk, peanut, shrimp, white fish [cod], almond, hazelnut, walnut, or cashew; in all cases, protein was fed daily at equal fixed amounts as measured by protein weight), five groups consuming a pair of proteins per day (peanut plus soy, shrimp plus crab, white fish [cod] plus pink fish [salmon], walnut plus pecan, or cashew plus pistachio; in all cases, all the pairs were combined at the same equal fixed ratio as measured by protein weight), and three groups consuming a ten-protein blend (each group included all of the following: egg, milk, peanut, shrimp, pink fish [salmon], almond, hazelnut, walnut, cashew, wheat; foods were combined at the same fixed ratio as measured by protein weight). Three different total protein concentration levels were then assessed: 1x, 3x, or 10x overall. Total protein levels were as low as 300 mg/d and as high as 3 g/d (Figure 1).

Each cohort was comprised of 25 participants divided equally among age groups. At baseline and one year, peripheral blood mononuclear cells (PBMCs) and plasma were extracted from blood. Allergen-specific IgE and IgG4 were measured using standard ImmunoCAP assay (Phadia, Uppsala, Sweden). In addition, Th2 cell proliferation indices were measured following labeling, stimulation, and enumeration via flow cytometry (BD Biosciences). In all cases, measurements represent the average of the specific IgE, IgG4, or Th2 cell proliferation indices for all relevant proteins. Results were compared with the control group and baseline. Results were interpreted observing that elevated food-specific IgE antibody and Th2 proliferation are associated with a sensitive or pro-allergic state and that, in contrast, higher levels of specific IgG4 are associated with an anti-allergic state and considered beneficial for potential protective activity from the effects of IgE.¹⁵

Figure 1. Daily Protein Levels of Included Foods by Group



Single protein sourced from either: egg, milk, peanut, shrimp, white fish, almond, hazelnut, walnut, or cashew.

Double-protein combinations sourced in equal parts by protein weight from: peanut plus soy, shrimp plus crab, cod plus salmon, walnut plus pecan, or cashew plus pistachio.

Mixed groups included protein in equal parts by protein weight from all of the following: egg, milk, shrimp, salmon, almond, hazelnut, walnut, peanut, cashew, and wheat.

RESULTS

BASELINE CHARACTERISTICS

A significant portion of children in the study were infants; 40% of the children were <12 months of age. All were breastfed in infancy for >4 months. Babies and children at both high and typical risk for food allergy were equally represented. High risk was defined as those born to a family with one first degree relative with food allergy/atopic dermatitis or two first degree relatives with atopic disease.

180 INFANTS

<12 MONTHS OF AGE

EQUAL SPLIT BETWEEN HIGH RISK
AND TYPICAL RISK



SAFETY OBSERVATIONS

All proteins, including the most complex protein mixtures, were effectively and safely incorporated into the diets of infants and children at all age ranges. No serious adverse events were reported. Mild skin rash was observed in <0.01% of protein feedings. There were no other documented reactions in the study.

ACTIVITY LEVELS

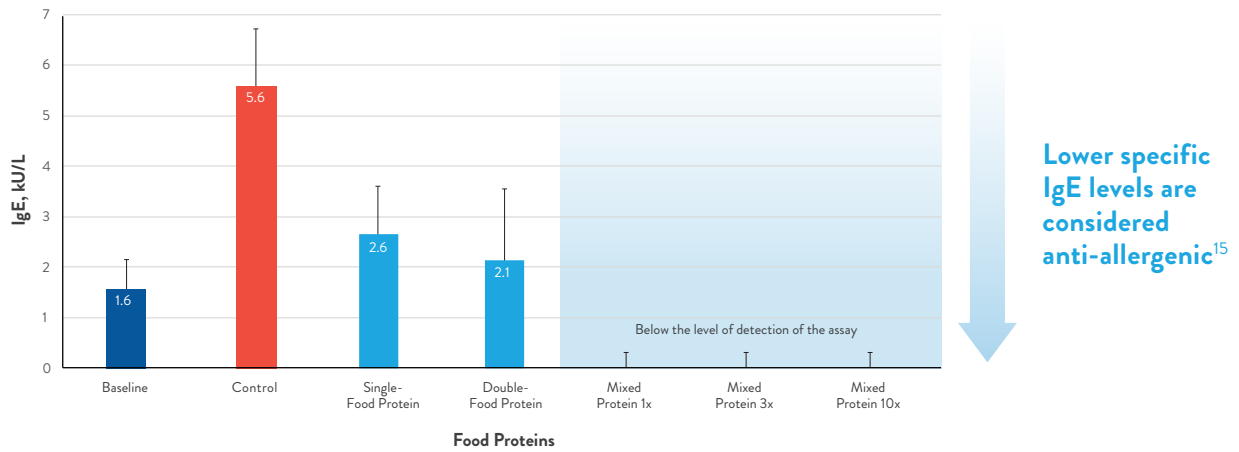
The average food-specific IgE measurements at 1 year were lower compared with the control for all cohorts receiving protein supplementation. All three multiprotein preparations reduced average specific IgE versus baseline and reduced average specific IgE more dramatically than single- or double-protein preparations. The multiprotein preparations were approximately equally active at all three protein levels: 1x, 3x, and 10x. In fact, all three multiprotein preparations resulted in IgE levels below the level of detection of the assay (Figure 2A).

Dietary supplementation with any food protein lowered the Th2 cell proliferation index compared to baseline and compared to the control. Again, the multiprotein blends reduced Th2 cell proliferation more than one- or two-protein preparations. As with IgE measurements, the multiprotein preparations were approximately equally active at all three protein levels: 1x, 3x, and 10x (Figure 2B).

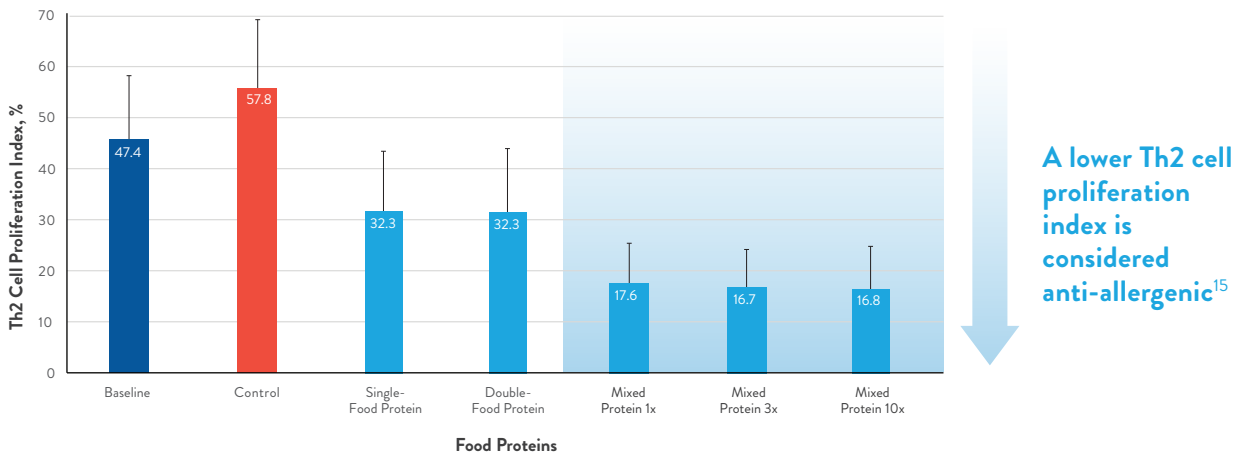
Finally, beneficial increases in IgG4 versus baseline were seen in all protein-supplemented groups and all were superior compared with the control group. Once again, multiprotein groups generated substantially higher IgG4 levels than single proteins and dual-protein combinations. Likewise, the multiprotein preparations were approximately equally active at all three protein levels: 1x, 3x, and 10x (Figure 2C).

Figure 2. Biomarker Results After One Year of Feeding by Cohort

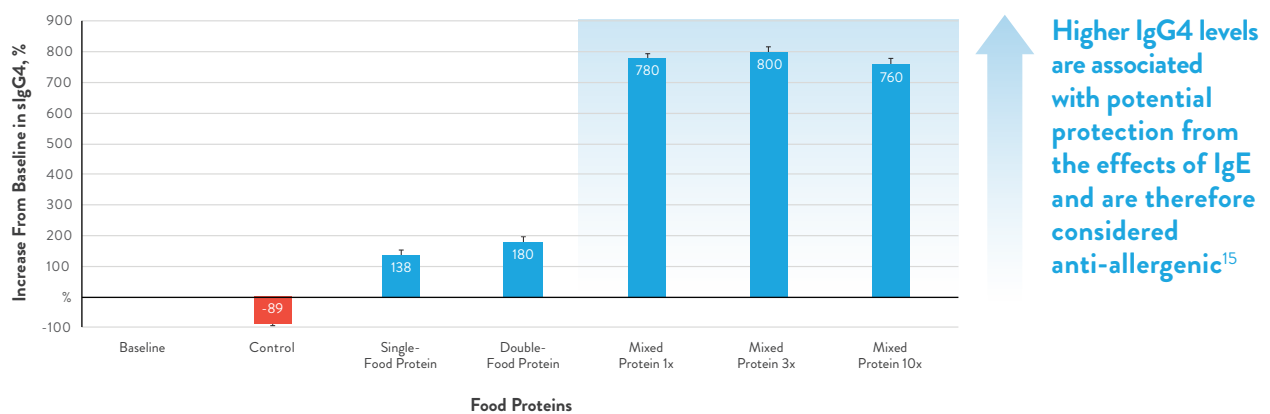
A Average of Specific IgE Levels for All Included Foods



B Th2 Cell Proliferation Index



C Percent Change in Average of Specific IgG4 Levels for All Included Foods Versus Baseline

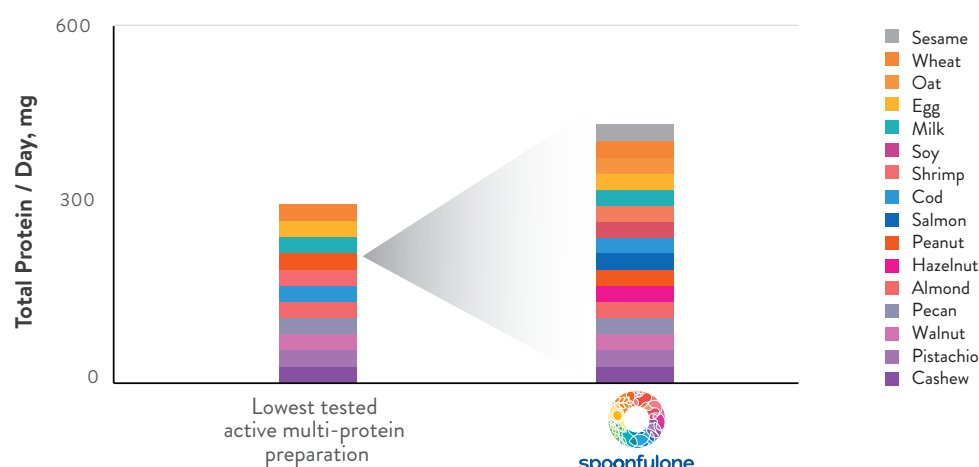


Error bars represent the standard deviation.

DISCUSSION

The biomarker data suggest that a year of daily feeding of SpoonfulOne's complex mix of potential food allergens starting at a very young age may reduce the likelihood of becoming sensitized to commonly allergenic foods compared to single protein or dual-protein combinations. Single proteins, and, dual-, and multiprotein blends were well accepted and included in the diets of healthy infants and children at the levels tested. Based on the biomarker data, supplementation with multiple proteins simultaneously resulted in greater benefits than feeding single or even paired proteins to support tolerance to those foods. That is to say, immune responses were superior to individual foods, such as peanut, when the individual food was fed in the presence of all the other potential allergens. Notably, the minimal active dietary exposure level was not determined in this study. The findings from this study, including the protein levels of individual foods included in the multiprotein blend, established the foundation for the SpoonfulOne formula (Figure 3).

Figure 3. SpoonfulOne Formula: Expanded to Include 6 Additional Proteins for More Complete Coverage



CONCLUSION

Multiprotein mixes of potential allergens were an easily accepted addition to the diet of healthy babies and children. After a year of daily feeding of multiprotein preparations, participants showed a shift towards a less allergy-prone immune profile, evidenced by changes in the measured levels of three biomarkers. Superiority of multiprotein mixes compared to single- and dual-protein formulas further supports the possibility of a “protein synergy” effect. Equivalent biomarker data generated by the multiprotein formulation at different protein levels enabled the selection of the lowest active level.

While this study identified an active protein level, a lower exposure level than those tested may be equally adequate for immunomodulation. Supporting previous research, diverse foods included in the diet of infants and children as early as four months of age and fed consistently over time may be an effective way to reduce the risk of developing a food allergy. Of note, the convenience and simplicity of the premixed multiprotein preparation may have contributed to the activity by supporting consistent daily use.



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