

A DOUBLE BLIND STUDY ON THE EFFICACY OF A COLOSTRUM AND
EGG YOLK SUPPLEMENT VS. PLACEBO TO REDUCE FREQUENCY
AND DURATION OF UPPER RESPIRATORY TRACT
INFECTIONS IN HEALTHY ADULTS

by

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STATEMENT OF THESIS APPROVAL

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ABSTRACT

Previous colostrum trials have been conducted with serum IgA levels and upper respiratory tract infections and reports document beneficial immunomodulatory effects after colostrum supplementation and no adverse effects. Having previously demonstrated that colostrum supplementation in athletes increased S-IgA levels, it was hypothesized that colostrum and egg yolk supplementation would decrease upper respiratory tract infections (URTIs). Twenty-five female subjects and 24 male subjects volunteered for the study and were randomly assigned into a placebo and a Transfer Factor group ($n = 22$ placebo, $n = 26$ Transfer Factor) to take two capsules every day for 6 weeks. After 6 weeks, there was no difference between the placebo and Transfer Factor groups in S-IgA levels, days sick, severity of illness, and severity of symptoms. In conclusion, during a 6-week supplementation protocol with 600 mg/d (420 mg of colostrum, 180 mg of egg yolk) of Tri-Factor Transfer Factor, there was no increased salivary IgA and reduced frequency and duration of cold and flu symptoms compared to placebo in a sample of healthy young adults.

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CHAPTER 1

INTRODUCTION

Influenza spreads around the world in seasonal epidemics, resulting in loss of productivity in the work force, loss of income by the patient, and the intangible cost of pain, grief, and social disruption every year. In the United States, influenza is responsible for a total cost of over \$10 billion per year, while it has been estimated that a future pandemic could cause hundreds of billions of dollars in direct and indirect costs (1).

Influenza, commonly referred to as the flu, is an infectious disease caused by Ribonucleic acid (RNA) viruses. RNA viruses generally have very high mutation rates compared to Deoxyribonucleic acid (DNA) viruses so it is difficult to make effective vaccines to prevent diseases caused by RNA viruses. The most common symptoms of the disease are chills, fever, sore throat, muscle pains, severe headache, coughing, weakness/fatigue, and general discomfort.

An upper respiratory tract infection (URTI) involves the upper respiratory tract (nose, sinuses, pharynx, or larynx) and can be caused by bacterial and/or viral infections. Viral URIs are more common than bacterial URIs. In the United States, viral URIs are the most common infectious illness in the general population and are the leading reasons for people missing work and school (2).

The mucosal immune system represents the first line of immunological defense against pathogens encountering the mucosal surfaces of the body, like those lining the upper respiratory tract. Immunoglobulin A (IgA) is the primary immunoglobulin isotype induced at the mucosal surface. Mucosal secretory IgA provides protection against bacterial and viral pathogens and neutralizes microbial toxins (3, 4). IgA is the principle mucosal antibody class and is synthesized locally by white blood cells.

Although the flu vaccine is the best flu prevention method, antiviral flu medication is also available by prescription for products such as Tamiflu® (oseltamivir), Flumadine® (rimantadine), Symmetrel® (amantadine), and Relenza® (zanamivir). In addition, many dietary supplements such as Vitamin C and various Zinc containing products have been developed and marketed for the prevention and treatment of cold and flu. Bovine colostrum has been proposed as another dietary supplement that may help in the prevention and treatment of cold and flu.

Bovine colostrum is cow milk secreted during the first few days after calving. Its importance for the development and maintenance of immune health in calves has been known for decades (5). Bovine colostrum is an extremely rich source of immunoglobulins. The concentration of immunoglobulin G (IgG) and immunoglobulin A (IgA) in bovine colostrum is 100-fold higher than in normal milk (6). Along with IgA secreted in the breast milk, residual IgG absorbed through the placenta provides the human fetus with humoral immunity before its own immune system develops. IgG can bind to many kinds of pathogens, for example viruses, bacteria, and fungi, and protects the body against them. Immunoglobulin Y (IgY) is the major immunoglobulin in birds. It

is also found together with IgA in chicken egg yolk. Avian IgY is the functional equivalent to mammalian IgG.

Recently, egg yolk-derived IgY has been combined with bovine colostrum-derived IgA to produce a dietary supplement called Tri-Factor Transfer Factor. The supplement has been marketed as a tool for boosting immune competence and reducing the incidence, duration, and/or severity of colds and flu. The goal of this study was to determine the efficacy of Transfer Factor in this regard. The rationale for examining the potential immunological benefits of Tri-Factor Transfer Factor are four-fold. First, there is a strong correlation between circulating or salivary IgA levels and URTI (7-9). Second, there is a strong clinical utility for using salivary IgA levels as a biomarker of immune competence (10-16). Third, carbohydrate and glutamine supplementation may act as an energy source or as building blocks of immunoglobulins but do not contain whole immunoglobulins in their formulation (17-25). Fourth, 20 g/day of a Dynamic Colostrum supplement containing 74 μ g IGF-I, 4.5 g IgG, and 0.3 g IgA (total energy 340 kJ: 6 g protein, 14 g carbohydrates) yielded a 33% greater increase in salivary IgA levels than placebo (26-29).

We hypothesized that during a 6-week supplementation protocol with 600 mg/d (420 mg of colostrum, 180 mg of egg yolk) of Tri-Factor Transfer Factor, there would be an increased salivary IgA and reduced frequency and duration of cold and flu symptoms compared to placebo in a sample of healthy young adults.

CHAPTER 2

METHODOLOGY/RESEARCH DESIGN

Subjects

The inclusion criteria were healthy men and women aged 18-40 years. The exclusion criteria included the following: currently smoking, cardiovascular disease, cancer, diabetes, liver disease, renal insufficiency, any chronic disease that might interfere with study participation, BMI above 40 kg/m², consumption of > 12 alcoholic drinks weekly, unwillingness to stop current supplement intake, and any allergic response to eggs (the supplement is egg-based). Any volunteer who did not know whether he or she had an egg or milk allergy were excluded. Women who were pregnant or lactating were also excluded from the study.

Participants were volunteers from students enrolled in a Nutrition 1020 class recruited by word of mouth and flyers posted at The University of Utah. Interested volunteers filled out a medical history questionnaire. If they met inclusion/exclusion criteria, then they were scheduled for a meeting to review the consent form with study staff so that they could decide if they wished to participate. Twenty-five female subjects and 24 male subjects volunteered for the study and were randomly assigned into a placebo and a Transfer Factor group. In the placebo group, there were 12 female and 11 male participants and in the Transfer Factor group, there were 13 female and 13 male

participants. Written informed consent was obtained from each participant prior to research. Each participant filled out a medical history questionnaire (Appendix) prior to enrollment.

Experimental Design

A randomized, placebo controlled, double blind, trial was used to test the efficacy of Tri-Factor Transfer Factor formula to reduce the frequency and or duration of upper respiratory tract infection and increase salivary IgA levels compared to placebo. Participants were randomly assigned to either a placebo group or supplement group for a period of 8 weeks consisting of a 1-2 week baseline period to establish a baseline salivary IgA secretion rate followed by a 6-week supplement or placebo period. They received the pills and were instructed to take two capsules daily. Table 1 shows the composition of Transfer Factor supplement and placebo capsules. Participants were instructed to maintain their normal exercise and dietary habits for the duration of the study. Diet records were also collected at week 3 and 6. These records were analyzed to confirm that participants kept a consistent diet, and that any changes in parameters measured were due to the intervention rather than changes in diet.

Each week, saliva samples were also collected to measure salivary IgA production. Salivary IgA were used as a marker of immune function/competence. Salivary IgA secretion rates have been extensively used in previous studies as a marker of immune function in humans (9, 30).

Each week, subjects also filled out a report (Appendix) logging the days they were sick, and a brief description of symptoms. These reports were made available either

Table 1

Composition of Transfer Factor Supplement

Ingredient	Amount per 2 capsules (mg)
Bovine colostrum filtrate	420
Egg yolk concentrate	180
Maltodextrin (tableting agent)	170

electronically or on paper based on the subject's personal preference. The report used a scale of severity to rank degree of illness ranging from zero to five with the following definitions: 0; feeling fine, not sick, no symptoms, 1; little if any interference with daily activities, 2; feeling sick, but can get through the day, 3; can do a little but must rest a lot, 4; confined to bed, 5; hospitalized.

Data were collected and analyzed for number of days sick per week, severity of their illness on days sick, and number of days sick in entire experimental period.

Dietary Records

Subjects were instructed on how to keep 3-day diet records during the third and sixth week of the study. All records were analyzed using the Food Processor dietary analysis program.

Health History Survey

A detailed history with an emphasis on respiratory history were collected (Appendix) prior to enrollment in the study.

Saliva Sampling for IgA Measurement

Each week, the subjects were required to submit a saliva sample according to the following procedure:

1. Subjects must not have had any dental work or consumed alcohol 24 hrs prior to collection.
2. Subjects must not have eaten any food or beverage, other than water, for 45 min prior to collection.
3. Subjects must rinse their mouth 10 min before collection.
4. Subjects filled out a questionnaire regarding their current health, medication/supplement use, compliance to the above and other pertinent details.
5. Subjects chewed a 5 cm square of ParaFilm and allow their passive drool to collect in a graduated tube for 5 min.
6. Samples were immediately placed on ice and then frozen (-70°C) within 4 hrs.

Salivary IgA Assay

Saliva samples were analyzed through the use of commercial ELISA assay kits following the manufacturer's instructions. Salivary IgA levels were measured using a BioRad X-Mark plate reader at 450 nm detection and 490-630 nm correction, as

described in the Salimetrics assay kit instructions. Reagents were reconstituted 10 min before assay. Plate layout was set up. Five final concentrations of standards (600 $\mu\text{l/ml}$, 200 $\mu\text{l/ml}$, 66.7 $\mu\text{l/ml}$, 22.2 $\mu\text{l/ml}$, 7.4 $\mu\text{l/ml}$, and 2.5 $\mu\text{l/ml}$) were made. One hundred μl of 1 X S-IgA diluents were added to each tube. Twenty-five μl of saliva were added to the appropriate tube. A separate tube for standard, control, and zero value were also made. Fifty μl of diluted antibody-enzyme conjugate were mixed gently in each tube. Then, 50 μl of solutions were added to each well according to plate layout. Fifty μl of 1 X S-IgA diluents were added to the NSB wells. The sealed plate was incubated at room temperature for 90 min with continual mixing at 400 rpm. Next, the plate was washed 6 times with 1 X wash buffer. Fifty μl of TMB solution were added to each well and were mixed on plate rotator for 5 min at 500 rpm. Then the plate was incubated in the dark at room temperature for 40 min. Fifty μl stop solution was added to each well and was mixed on plate rotator for 3 min at 500 rpm until all wells turned yellow. Plates were read in micro plate reader at 450 nm within 10 min of adding stop solution. The average OD was calculated for all wells. The average OD for the NSB wells was subtracted from the average OD of the other wells. The percent bound (B/Bo) for each standard, control, and unknown was calculated by dividing the average OD for the zero (Bo). Concentration of controls and unknowns were determined by fitting to a 4-parameter sigmoid minus curve. Concentrations were multiplied by 5 to obtain final S-IgA in $\mu\text{l/ml}$.

Statistical Analyses

A Chi square test (SPSS v.19.0.0) was used to detect significant differences between Transfer Factor and placebo groups regarding the following parameters: total

days with cold and flu-like symptoms, duration of symptoms with each illness reported. A repeated-measures ANOVA was used to analyze weekly salivary IgA secretion between Transfer Factor and placebo groups. Significance was accepted at $p < 0.05$. One male participant from placebo group left the study after 3 weeks because he did not like to continue taking the pills. Data from 48 were used for analysis. Any participants with one or more missing saliva samples were excluded from S-IgA data analysis. Three subjects from the Transfer Factor group did not show up for at least one of the saliva collection sessions. Therefore, the data analysis for S-IgA included 22 participants in the placebo group and 19 participants in the Transfer Factor group. Variability is expressed as Standard Deviation throughout unless otherwise indicated. Wherever the data were not normally distributed, data were successfully normalized using a square root transformation.

CHAPTER 3

RESULTS

Demographic Characteristics

The placebo and Transfer Factor group had similar demographic characteristics in average height, weight, and BMI ($p \geq 0.496$, Table 2). However the placebo group was slightly older on average than the Transfer Factor group (23.0 ± 4.2 vs. 21.5 ± 2.0 , $p = 0.020$). There were no differences in the percentage of females or in the percentage of those inoculated with either the flu or H1N1 vaccine ($p \geq 0.307$). In addition, there were no significant differences in energy intake, carbohydrate intake, protein intake, fat intake, and micronutrients intake at weeks 3 and 6 between the placebo and Transfer Factor groups ($p \geq 0.569$).

S-IgA

Overall, the linear, quadratic, cubic, quartic, and fifth order polynomial treatment group by time interactions were all nonsignificant ($p \geq 0.330$, Figure 1). By contrast, there was a significant sixth order polynomial treatment group by time interaction ($p = 0.005$). Because five out of six of the polynomial treatment group by time

Table 2

Demographic Characteristics of the Subjects in Experimental Groups

Subject Characteristics	Placebo (n = 22)	Transfer Factor (n = 26)	P Value
Height (cm)	173.47 ± 8.91	171.45 ± 11.48	0.496
Weight (kg)	66.41 ± 9.86	66.69 ± 13.58	0.937
BMI (kg/m ²)	22.05 ± 2.68	22.67 ± 3.97	0.526
Gender	Female n = 12 (54.5%)	Female n = 13 (50.0%)	0.981
Age (yrs)	23	20.5	0.02
Flu Shot (% inoculated)	n = 7 (31.8%)	n = 4 (15.4%)	0.315
H1N1 (% inoculated)	n = 6 (27.3%)	n = 3 (11.5%)	0.307

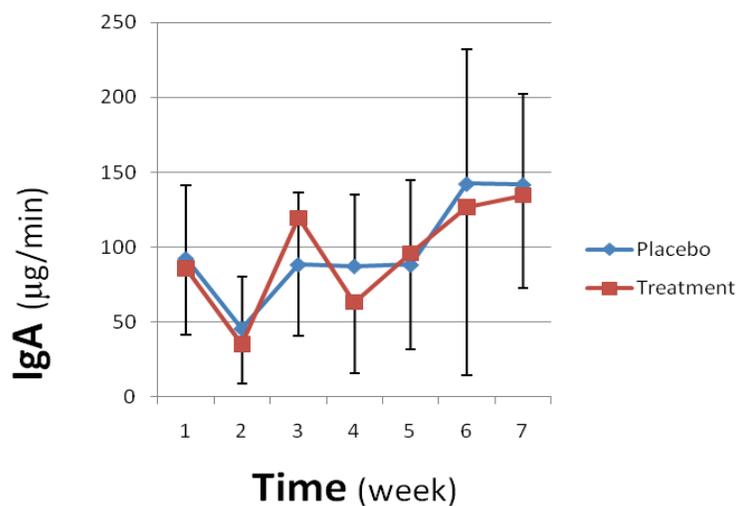


Figure 1: The average in S-IgA secretion between experimental groups (6th order polynomial treatment group X time interaction, $p = 0.005$; all other treatment group X time interactions, $p \geq 0.330$).

interactions were nonsignificant, there were no meaningful differences in the magnitude of the S-IgA changes over time between the placebo and the Transfer Factor groups.

Days Sick

Average total days sick per week are shown in Figure 2. There was no significant differences in average number of total days sick per week between the placebo and Transfer Factor group ($p = 0.411$, Table 3).

Severity of Illness

The average severity of illness between experimental groups is shown in Figure 3. There was no significant difference in severity of illness between the placebo group and the Transfer Factor group ($p = 0.407$, Table 4).

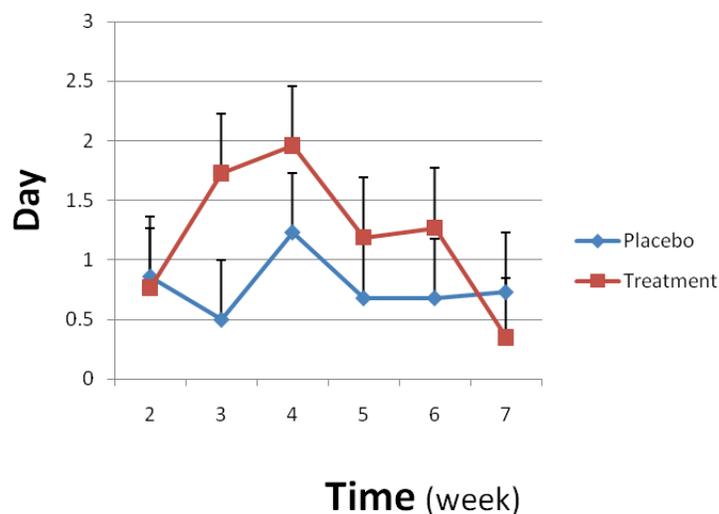


Figure 2: The average number of total days sick per week between experimental groups.

Table 3

Average Number of Total Days Sick from Week 2 to 7 between Experimental Groups
($p = 0.411$)

Placebo	Transfer Factor
4.68 ± 5.13	7.27 ± 8.26

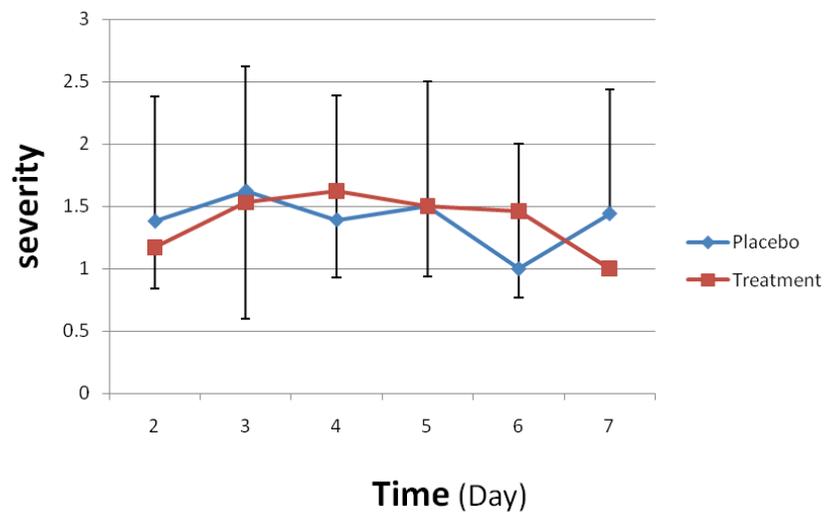


Figure 3: The average weekly severity of illness between experimental groups.

Table 4

Average Severity of Illness from Week 2 to 7 between Experimental Groups
($p = 0.407$)

Placebo	Transfer Factor
1.39 ± 0.34	1.52 ± 0.55

Severity of Symptoms

Figure 4 describes the severity of symptoms for each experimental group. This figure shows the average severity range from a low of 1.0 ± 0.0 in week 6 and a high of 1.72 ± 0.9 in week 3 in the placebo group and a low of 1.0 ± 0.0 in week 7 and 1.47 ± 0.8 in week 3 in the Transfer Factor group. There was no difference in severity of symptoms between the experimental groups ($p = 0.407$, Table 5).

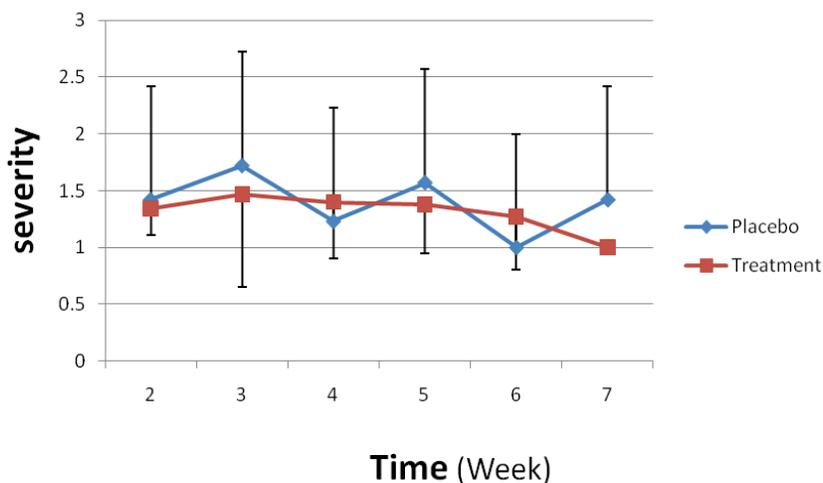


Figure 4: The average weekly severity of symptoms between experimental groups.

Table 5

Average Severity of Symptoms from Week 2 to 7 between Experimental Groups
 ($p = 0.407$)

Placebo	Transfer Factor
1.41 ± 0.34	1.32 ± 0.31

CHAPTER 4

DISCUSSION

Findings of this trial suggest that there was no difference between the placebo and Transfer Factor groups in S-IgA levels (Figure 1), days sick (Figure 2), severity of illness (Figure 3), and severity of symptoms (Figure 4). Although there was no difference in S-IgA secretion between the placebo and Transfer Factor groups, there was a significant sixth order polynomial treatment group by time interaction (Figure 1). However, 5 out of 6 of the polynomial treatment group by time interactions were nonsignificant. Moreover, there were no clinically meaningful differences in the magnitude of the S-IgA changes over time between the placebo and the Transfer Factor groups. There was no difference in the average number of total days sick from week 2 to 7 between the placebo and Transfer Factor groups (Table 3). There was no difference in average severity of illness from week 2 to 7 between the placebo and the Transfer Factor groups (Table 4). Finally, there was no difference in the average severity of symptoms from week 2 to 7 between the placebo and the Transfer Factor groups (Table 5).

The efficacy of colostrum but not Transfer Factor itself has been previously reported in modulation of IgA levels and upper respiratory tract infections in non-English language journals. These reports document beneficial immunomodulatory effects after Transfer Factor supplementation and no adverse effects. Because these studies have not

been published in peer reviewed English language journals, the results are not easily accessible to English speaking health care practitioners. However, Transfer Factor has yet to be evaluated in a healthy population with the intent of determining if this supplement can reduce the frequency or the duration of colds and flu. Transfer Factor is comprised of colostrum (bovine milk secretion during the early days of lactation) and chicken egg yolk.

Several colostrum but not Transfer Factor supplementation trials have been conducted with serum IgA levels and upper respiratory tract infections as study outcomes (26-29). Almost all of these trials were conducted in athletic samples. For example, 2 weeks of 20 g/d colostrum supplementation increased S-IgA levels by 33%, on average, in a sample of 30 adult athletes (27). Another study suggests five weeks supplementation of 10 g/d bovine colostrum increases S-IgA concentration in athletes during training (29). In addition, in two studies, fewer athletes reported Upper Respiratory Syndrome (URS) when consuming 20 g/d bovine colostrum for 2 weeks (27, 28). Though the aforementioned studies have all reported positive changes in salivary IgA, our study found that the colostrum cocktail found in Transfer Factor did not affect salivary IgA concentrations or production rate. Possible explanations for the above benefits of colostrum supplementation as compared to the present null findings of Transfer Factor supplementation include the different supplement compositions, different supplement dosages, different demographic characteristics of the participants, and different durations of follow-up. In the colostrum supplementation studies above, colostrum is the only active ingredient, and the study durations were longer than the present Transfer Factor trial. In addition, the colostrum supplementation studies were primarily conducted in

athletic men who were slightly older than the participants in the present trial. We also used a twice daily supplement of both colostrum (210 mg) and egg yolk (90 mg), and we followed our participants for only 7 weeks.

Our trial has some notable strengths and weaknesses. For instance, our trial used a double blind placebo-controlled randomized experimental design. In addition, we assessed S-IgA secretion. However, the sample size of each experimental group was small ($n = 22$ placebo, $n = 26$ Transfer Factor). Moreover, our sample size estimate was based only on the ability to detect the difference in S-IgA levels based on the above colostrum supplementation trials. By contrast, our trial was not adequately powered to detect experimental group differences in illness, severity of illness, and severity of symptoms. Finally, S-IgA was our only marker of immune competency, and we only followed the participants for 7 weeks.

Given the limitations discussed, suggested modifications for future studies would address sample size, duration of study, and number of immune markers measured. For example, bigger sample size (at least 50 subjects per experimental group), longer duration of follow up (a whole winter season), and more than one marker of immune competence (IgG) are recommended for future studies. The other possibility is the dosage of the supplements. In this study, all subjects in the Transfer Factor group took 420 mg of colostrum and 180 mg of egg yolk every day. Because the active ingredient of the supplements gets cleared out by the body and its clearance is under influence of fat free mass, maybe a metabolic clearance study has to be done to refine and individualize the dosage of the supplement based on the body weight. In addition, future studies should also exclude participants who have received flu and H1N1 vaccines.

We found no significant difference in S-IgA levels, number of days sick, severity of illness, and severity of symptoms in a sample of healthy young adults. We conclude that during a 6-week supplementation protocol with 600 mg/d (420 mg of colostrum, 180 mg of egg yolk) of Tri-Factor Transfer Factor, there was no increased salivary IgA and reduced frequency and duration of cold and flu symptoms compared to placebo in a sample of healthy young adults.

APPENDIX

Medical History Questionnaire

Personal Information

Subject ID# _____ Date of Birth _____
 Name _____ Address _____
 Home phone _____ Work phone _____
 Ht: _____ Wt: _____ BMI: _____

Health History

Do you have a history any of the following conditions? Please check all that apply and provide a brief explanation below:

_____ pneumonia	_____ musculoskeletal problems
_____ asthma (mild, mod., severe)	_____ cancer
_____ recurrent bronchitis	_____ diabetes
_____ allergies (airway)	_____ hypertension
_____ kidney problems/disease	_____ liver problems
_____ thyroid abnormalities	_____ blood disorders
_____ malabsorption disorders	_____ anemia
_____ cardiac abnormalities/disease	_____ other, please explain below

Flu shot Yes No H1N1 shot Yes No

Do you follow a special diet? (vegetarian, medical, etc.) _____

Are you currently trying to lose or gain weight? (please specify if applicable)

Please list any dietary supplements including vitamins, minerals, protein shakes, diet shakes, and herbs you currently take, or have taken in the last 2 weeks.

Please list any medications you are currently taking.

Please list any known food or drug allergies.

Transfer Factor Study Weekly Report

Name _____ Subject # _____

Date _____ Week no. _____

Please circle the days you were sick this week (if any). Use the scale below to rate the severity of illness. If you were not sick then circle "0" on the scale at each day.

0 – Feeling fine, not sick, no symptoms

1 – Little if any interference with daily activities

2 – Feeling sick, but can get through the day

3 – Can do a little but must rest a lot

4 – Confined to bed

5 – Hospitalized

Sunday	0	1	2	3	4	5
Monday	0	1	2	3	4	5
Tuesday	0	1	2	3	4	5
Wednesday	0	1	2	3	4	5
Thursday	0	1	2	3	4	5
Friday	0	1	2	3	4	5
Saturday	0	1	2	3	4	5

Did you receive medical care? (please circle)

Yes

No

If so, then what was the diagnosis?

What medication, if any, did you take, or were prescribed?

Antibiotics? Name and dose / duration _____

Antiviral? Name and dose / duration _____

Antihistamine? over the counter/prescription

Decongestant? over the counter/prescription

Anti-nausea? over the counter/prescription

Pain reliever? Please list (aspirin, Tylenol, Motrin, etc.) _____

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