Immunoglobulin Supplementation in Clinical Studies

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Ankeny, IA
Summary

- Safety and Acceptability of Bovine Serum Concentrate (BSC) in Malnourished Peruvian Infants (1997)
- **2000 – Began production and marketing of immunoglobulin isolate**
- Effects on clinical chemistry parameters in healthy adults (Internal study) (2001)
- Efficacy of Bovine Serum Globulin in Diarrhea-Predominant IBS (2003-2005)
**Intestinal effects on specific immune functions**

**Gut associated lymphoid tissue (GALT)**

Bacterial, food or other antigens

Bacteria

FAE

M cell

SEB

Peyer's patch

DC

GC

ILF

Lamina propria

Gut lumen

IgA

Plasma cell

**ProLium**
Gut level immune stimulation affects inflammatory status
The Interaction Between TNF-α and IGF-I on Protein Synthesis

Fig. 8. A hormone (IGF-I) and a proinflammatory cytokine (TNFα) interact to regulate protein synthesis in murine myoblasts. Addition of IGF-I (50 ng/ml) increases protein synthesis, whereas TNFα (1 ng/ml) alone has little effect. However, when IGF-I and TNFα are added together, TNFα greatly impairs the ability of IGF-I to promote protein synthesis (Broussard et al., 2003).
Inflammation Leads to a Loss of Lean Muscle Mass

Interactions between immune system and GH/IGF-I axis

• Decreases sensitivity to insulin and IGF-I

Interactions between nutrient supply and GH/IGF-I

• Reduction in food consumption reduces plasma IGF-I
Effects of Bovine Serum on Intestinal Restitution

Background:

Bovine serum contains IGF-I (∼3,000 ng/g) and TGF-β1 and 2. We have studied the effects of bovine serum as a source of these natural growth factors on cells in vitro and in animal models.

Cell migration assay:

Monolayer of IPEC-J2 (jejunal porcine epithelial cells)

Cells are “wounded” and then cultured with the test solution

Migration is measured and area is calculated.

Rhoads et al., 1999. Unpublished
Effects of Bovine Serum on Cell Migration in a Wounding Model
Effects of BSC (NG20) on intestinal epithelial cell migration

Migration, $\text{um}^2 / \text{um}$

- **Control**
- **Gln**
- **NG20**
- **NG20+Gln**
Effects of Bovine Serum on intestinal epithelial cell migration

Migration, $\text{um}^2 / \text{um}$

- Control
- Serum
- IGF-Ab
- TGF-Ab
Effects of BSC (NutraGammax) on intestinal epithelial cell migration

Migration, $\text{um}^2 / \text{um}$

NutraGammax, mg %
Effects of BSC on Cellular Polyamine Levels

C

Cellular Polyamine levels After Wounding
Fold Above Nonwounded (NW)

CTRL
ARG 4 mM
BSC 0.1%

NW PUT 0.13 ± 0.04 nmol/mg protein
NW SPD 2.6 ± 0.9 nmol/mg protein
NW SPM 4.5 ± 0.9 nmol/mg protein

* statistical significance
The Effects of BSC and Arginine on Epithelial Cell Migration

IPEC-J2 Cell Migration ($\mu m^2/\mu m$)

- CTL
- ARG
- DETA NONOate
- BSC
- ARG+BSC
- ARG+DETA NONOate

* and + indicate statistical significance.
Growth Factors in Serum

- Shown to be bio-active after spray-drying/processing
- Promote epithelial recovery from wounding
- Promote healing via a complementary pathway to glutamine and arginine
The Biological Roles of Serum Proteins in the Lumen

1) Immune exclusion
2) Lowers pro-inflammatory cytokine production
3) Protects against over-activation of GALT
4) Improves barrier function
5) Improves nutrient utilization

The effects combine to improve metabolism and the retention of body protein
Safety of blgG in Healthy Adults

• Two Studies
  – Double-blind cross-over design
    • Healthy adults supplemented with 10 g BlgG
    • Blood samples collected 15, 30, 45, 60, 90 and 120 min to assess post-prandial plasma amino acids and circulating blgG
  – 14 d evaluation of acceptance and gastrointestinal tolerance of 5 g of blgG daily
    • Analyzed stool samples for blgG
Amino Acid Concentrations

<table>
<thead>
<tr>
<th>Time in Minutes</th>
<th>Control</th>
<th>IgG</th>
<th>Poly. (IgG)</th>
<th>Poly. (Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>6.13</td>
<td>3.90</td>
<td>4.70</td>
<td>-1.20</td>
</tr>
<tr>
<td>30</td>
<td>5.71</td>
<td>9.30</td>
<td>16.50</td>
<td>7.60</td>
</tr>
<tr>
<td>45</td>
<td>10.69</td>
<td>8.24</td>
<td>23.00</td>
<td>15.50</td>
</tr>
<tr>
<td>60</td>
<td>0.69</td>
<td>11.74</td>
<td>29.50</td>
<td>23.00</td>
</tr>
<tr>
<td>90</td>
<td>-0.92</td>
<td>18.70</td>
<td>35.00</td>
<td>30.50</td>
</tr>
<tr>
<td>120</td>
<td>-6.55</td>
<td>13.33</td>
<td>41.00</td>
<td>37.00</td>
</tr>
<tr>
<td>150</td>
<td>-11.81</td>
<td>-10.34</td>
<td>47.00</td>
<td>44.50</td>
</tr>
</tbody>
</table>

% of Baseline

Graph showing the amino acid concentrations over time for Control, IgG, Poly. (IgG), and Poly. (Control).
Summary

- blgG was not detected in plasma samples
- IgG has a relatively slow rate of digestibility
- blgG maintained post-prandial plasma leucine levels
- blgG was detected in stool samples
- No signs of intolerance
Acceptability, Safety, and Digestibility of Spray-Dried, Bovine Serum in Mixed Diets Provided to Recovering Malnourished Children

**Cooperators:**

Proliant Inc.

University of California, Dept. of International Nutrition

Instituto de Investigacion Nutricional, Lima, Peru

Lembcke et al, 1997
Study Subjects

- Ten male children (9 to 25 months) who were previously admitted to the Institute for rehabilitation following severe malnutrition.

- Children had completed 3 days of successful therapy and were gaining weight prior to entering the study.

- Dietary treatments were assigned randomly and sequentially. Diets were fed for a 3 d adaptation period followed by a 4 d data collection
Fecal Energy Output

Means with unlike letters differ, P<.05

Lembcke et al, 1997
Nitrogen Retained

Lembcke et al, 1997

% of N Intake

Control 25 % NG20 50 % NG20
Summary

- The children accepted each of the study diets. There was no evidence of dietary intolerance.
- Fecal consistency was normal through all treatments.
- Fecal wet and dry weights were less with the 50% NG20 diet compared to control (P<.05).
- Fecal fat and energy losses were significantly lower with the 50% NG20 diet (P<.05).
- There was a trend toward superior nitrogen retention and energy absorption with increasing amounts of NG20.
Conclusions

- This suggests that either the diets containing NG20 were more digestible or the NG20 enhanced intestinal recovery during rehabilitation from severe malnutrition which improved digestion, absorption and/or utilization of protein and energy.

Lembcke et al, 1997
# Studies Evaluating Oral Ig

<table>
<thead>
<tr>
<th>N</th>
<th>HI</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>76</td>
<td>103</td>
</tr>
</tbody>
</table>

- **Applications**
  - Therapeutic: 14
  - Prophylactic: 11

- **Ig Source**
  - Human: 22
  - Bovine: 48
  - Other: 18
  - Avian: 15

N - Normal Serum  HI - Hyper Immunized
## IgG Recovery After Oral Administration

<table>
<thead>
<tr>
<th>Donor</th>
<th>Recipient</th>
<th>Preparation</th>
<th>Source</th>
<th>%*</th>
<th>Reference</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine</td>
<td>Adult</td>
<td>Powder</td>
<td>IgG, Colost.</td>
<td>10-20%</td>
<td>McClead et al.</td>
<td>1988</td>
</tr>
<tr>
<td>Bovine</td>
<td>Adult</td>
<td>Powder</td>
<td>IgG, colost.</td>
<td>++</td>
<td>Lissner et al.</td>
<td>1998</td>
</tr>
<tr>
<td>Bovine</td>
<td>Adult</td>
<td>Powder</td>
<td>IgG, colost.</td>
<td>49%</td>
<td>Warny et al.</td>
<td>1999</td>
</tr>
<tr>
<td>Bovine</td>
<td>Adult</td>
<td>Powder</td>
<td>IgG, colost.</td>
<td>25%</td>
<td>Kelly et al.</td>
<td>1997</td>
</tr>
<tr>
<td>Bovine</td>
<td>Adult</td>
<td>Powder</td>
<td>IgG, serum</td>
<td>++</td>
<td>Hanning and Drew</td>
<td>1994</td>
</tr>
<tr>
<td>Bovine</td>
<td>Adult</td>
<td>Powder</td>
<td>IgG, colost.</td>
<td>21%</td>
<td>Roos et al.</td>
<td>1995</td>
</tr>
<tr>
<td>Bovine</td>
<td>In vitro</td>
<td>Powder</td>
<td>IgG, colost.</td>
<td>20-42%</td>
<td>Petschow and Talbott</td>
<td>1994</td>
</tr>
<tr>
<td>Bovine</td>
<td>In vitro</td>
<td>Powder</td>
<td>IgG, colost.</td>
<td>50%</td>
<td>McClead and Gregory</td>
<td>1984</td>
</tr>
<tr>
<td>Bovine</td>
<td>In vitro</td>
<td>Powder</td>
<td>IgG, colost.</td>
<td>25%</td>
<td>Kelly et al.</td>
<td>1997</td>
</tr>
<tr>
<td>Bovine</td>
<td>Infant</td>
<td>Powder</td>
<td>IgG, Colost.</td>
<td>13%</td>
<td>Zinkernagel et al.</td>
<td>1975</td>
</tr>
<tr>
<td>Human</td>
<td>Infant</td>
<td>Liquid</td>
<td>IgG, serum</td>
<td>~25%</td>
<td>Losonsky et al.</td>
<td>1985</td>
</tr>
<tr>
<td>Human</td>
<td>Infant</td>
<td>Liquid</td>
<td>IgG, serum</td>
<td>4-12%</td>
<td>Blum et al.</td>
<td>1981</td>
</tr>
<tr>
<td>Human</td>
<td>Rat</td>
<td>Liquid</td>
<td>IgG, Serum</td>
<td>25.70%</td>
<td>Gmoshinkski et al.</td>
<td>1998</td>
</tr>
</tbody>
</table>

* Represents % of original dose recovered in stool or in vitro sample
NutraGammaMax™ Stability

- Average IgG recovery following digestion or simulated digestion in 12 published studies is 25%.
- No evidence for absorption of IgG
Summary - Safety

- Normal component of diets in many cultures
  - Milk, whey
- Many studies have utilized bovine Ig as a source of antibody
- No adverse effects in healthy adult subjects
- No adverse effects in malnourished children
- Acute toxicology study in rats (>2000 mg/kg)
Summary - Safety

• BSE:
  – Serum, like milk, is classified as a low-risk material
  – Serum is collected from young, healthy beef animals which greatly reduces the potential for BSE.
  – **NO BSE** has been reported in the U. S. beef or dairy population.

• SPC standards similar to ingredients used in infant formula.
Safety of Oral Plasma Proteins and Immunoglobulin

- The oral application of immunoglobulins is well-documented.
- Bovine Ig has been used in both therapeutic and prophylactic roles.
- No adverse effects have been reported.
Effects of bovine serum concentrate, with or without supplemental micronutrients, in the growth, morbidity and micronutrient status of young children in low-income, peri-urban Guatemalan communities

France Bégin, PhD¹, Maria-Claudia Santizo, MD², Janet M. Peerson, MSc³, Benjamin Torún, MD², Kenneth H. Brown, MD³

- More than 55% of children less than three years of age have low length-for-age (INCAP, 1992).
- 259 breast-fed infants and children (6-14 months) in Guatemala participated
- Double-blinded study of 6 month duration
- Four treatments:
  1) Whey protein concentrate (WPC): 3.1 g
  2) WPC + micronutrient supplement (MMN)
  3) Bovine serum concentrate (BSC): 3.1 g
  4) BSC + MMN
- The objective was to evaluate the effect of protein source and MMN on linear growth, body weight and morbidity (diarrhea and respiratory illness)
Table 1 - Composition of dry supplements, by treatment group (amount of ingredient, in grams, per daily dose of supplement, infants 6-11 mo*)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Treatment group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WPC</td>
</tr>
<tr>
<td>Maize flour</td>
<td>2.9</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>1.6</td>
</tr>
<tr>
<td>Sugar</td>
<td>3.8</td>
</tr>
<tr>
<td>Lecithin, flavoring agents</td>
<td>0.9</td>
</tr>
<tr>
<td>Bovine serum concentrate</td>
<td>0</td>
</tr>
<tr>
<td>Whey protein concentrate</td>
<td>5.0</td>
</tr>
<tr>
<td>Micronutrient mix</td>
<td>0</td>
</tr>
</tbody>
</table>

Supplements prepared as semi-solid “pudding” by adding ~30 ml of water

* Children ≥12 mo received 1.5 times this dose
Table 6– Prevalence and incidence of morbidity during period of supplementation, by treatment group (mean ± SD)¹

<table>
<thead>
<tr>
<th>Variable</th>
<th>WPC (n=57)</th>
<th>WPC + MMN (n=57)</th>
<th>BSC (n=54)</th>
<th>BSC + MMN (n=51)</th>
<th>P-value ANOVA²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence of diarrhea (%)</td>
<td>10.2 ± 8.4</td>
<td>13.5 ± 13.2</td>
<td>10.4 ± 9.6</td>
<td>11.9 ± 13.0</td>
<td>0.98</td>
</tr>
<tr>
<td>Incidence of diarrhea (episodes per 100 d at risk)</td>
<td>3.6 ± 2.6</td>
<td>5.1 ± 4.8</td>
<td>3.8 ± 3.0</td>
<td>3.9 ± 4.1</td>
<td>0.76</td>
</tr>
<tr>
<td>Incidence of severe diarrhea (episodes per 100 d at risk)</td>
<td>1.9 ± 2.0</td>
<td>2.7 ± 3.0</td>
<td>1.9 ± 1.8</td>
<td>2.1 ± 2.5</td>
<td>0.96</td>
</tr>
<tr>
<td>Incidence of persistent diarrhea (episodes per 100 d at risk)</td>
<td>0.13 ± 0.34</td>
<td>0.19 ± 0.58</td>
<td>0.03 ± 0.19</td>
<td>0.15 ± 0.40</td>
<td>0.19³</td>
</tr>
<tr>
<td>Prevalence of fever (%)</td>
<td>9.4 ± 7.3</td>
<td>9.0 ± 6.2</td>
<td>10.5 ± 7.8</td>
<td>8.8 ± 6.8</td>
<td>0.86</td>
</tr>
<tr>
<td>Incidence of fever (episodes per 100 d at risk)</td>
<td>3.9 ± 2.9</td>
<td>3.6 ± 2.2</td>
<td>3.9 ± 2.7</td>
<td>3.6 ± 2.5</td>
<td>0.78</td>
</tr>
<tr>
<td>Prevalence of URI (%)</td>
<td>7.1 ± 10.2</td>
<td>6.0 ± 8.3</td>
<td>7.1 ± 9.6</td>
<td>6.9 ± 9.5</td>
<td>0.96</td>
</tr>
<tr>
<td>Incidence of URI (episodes per 100 d at risk)</td>
<td>1.4 ± 1.6</td>
<td>1.3 ± 1.7</td>
<td>1.4 ± 1.6</td>
<td>1.6 ± 2.3</td>
<td>0.53</td>
</tr>
<tr>
<td>Prevalence of LRI (%)</td>
<td>1.2 ± 3.4</td>
<td>1.1 ± 2.7</td>
<td>1.2 ± 2.7</td>
<td>1.4 ± 2.9</td>
<td>0.85</td>
</tr>
<tr>
<td>Incidence of LRI (episodes per 100 d at risk)</td>
<td>0.6 ± 1.6</td>
<td>0.5 ± 1.0</td>
<td>0.5 ± 0.9</td>
<td>0.7 ± 1.4</td>
<td>0.95</td>
</tr>
</tbody>
</table>

¹ Analyses include children with at least 60 days of observation after initiation of supplementation. Diarrhea defined as ≥3 liquid or semi-liquid stools per d.

² ANOVA, controlling for pre-treatment morbidity rates, as explained in text. Logarithm of morbidity rate was used in all analyses, except incidence of persistent diarrhea.

³ Logistic regression was used for this variable. (Because of low incidence of persistent diarrhea, logarithmic transformation was not appropriate.)
Table 8. Changes in biochemical indicators of micronutrient status during period of supplementation and % of children with low final levels of selected indicators, by treatment group (mean ± SD)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment Group</th>
<th>P-value&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WPC</td>
<td>MMN + WPC</td>
</tr>
<tr>
<td>Mean change in hemoglobin (g/dL, n=150)</td>
<td>-0.26 ± 8.8</td>
<td>-0.06 ± 10.0</td>
</tr>
<tr>
<td>% Low hemoglobin (% &lt;11.0 g/dL; n=150)</td>
<td>37.1</td>
<td>22.1</td>
</tr>
<tr>
<td>Mean change in serum ferritin&lt;sup&gt;2&lt;/sup&gt; (μg/L, n=128)</td>
<td>-33.9 ± 44.7&lt;sup&gt;a, b&lt;/sup&gt;</td>
<td>-19.4 ± 32.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>% Low ferritin (% &lt;10 μg/L; n= 128)</td>
<td>24.5</td>
<td>24.9</td>
</tr>
<tr>
<td>Mean change in serum zinc (μg/dL, n=128)</td>
<td>-2.0 ± 24.2</td>
<td>-0.6 ± 20.1</td>
</tr>
<tr>
<td>% Low serum zinc (% &lt;60 μg/dL; n= 128)</td>
<td>30.6</td>
<td>31.8</td>
</tr>
<tr>
<td>Mean change in serum copper (μg/L, n=128)</td>
<td>14.4 ± 38.2</td>
<td>13.9 ± 35.4</td>
</tr>
<tr>
<td>Mean change in serum retinol&lt;sup&gt;2&lt;/sup&gt; (μg/L, n=103)</td>
<td>6.4 ± 11.1</td>
<td>5.8 ± 8.1</td>
</tr>
<tr>
<td>% Low serum retinol (% &lt;0.70 μmol/L; n= 103)</td>
<td>8.3</td>
<td>16.0</td>
</tr>
<tr>
<td>Mean change in serum tocopherol (μmol/L, n= 103)</td>
<td>0.09 ± 0.18</td>
<td>0.16 ± 0.22</td>
</tr>
</tbody>
</table>

<sup>1</sup> Raw data presented; P-values derived from ANCOVA for continuous variables; logistic regression for proportions, controlling for baseline values.

<sup>2</sup> Analysis based on logarithm-transformed variable

<sup>3</sup> P for MMN main effect < 0.05

<sup>4</sup> P for interaction < 0.05
Table 7. Factors associated with morbidity status of Guatemalan children from

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (0=M; 1=F)</td>
<td>-0.17</td>
<td>0.04</td>
</tr>
<tr>
<td>Initial length (cm)</td>
<td>-0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>Maternal education (years)</td>
<td>-0.06</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Initial diarrhea</td>
<td>1.55</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>( R^2 ) = 0.33</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at enrollment (mos)</td>
<td>-0.22</td>
<td>0.02</td>
</tr>
<tr>
<td>Maternal education (years)</td>
<td>-0.04</td>
<td>0.003</td>
</tr>
<tr>
<td>Initial diarrhea</td>
<td>1.40</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>( R^2 ) = 0.23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial URI</td>
<td>2.44</td>
<td>0.0002</td>
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<tr>
<td>( R^2 ) = 0.09</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (0=M; 1=F)</td>
<td>-0.17</td>
<td>0.01</td>
</tr>
<tr>
<td>Initial weight (kg)</td>
<td>-0.09</td>
<td>0.02</td>
</tr>
<tr>
<td>Maternal education (years)</td>
<td>-0.02</td>
<td>0.31</td>
</tr>
<tr>
<td>Age at enrollment (months)</td>
<td>0.17</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>( R^2 ) = 0.08</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3 - Baseline characteristics of subjects included in at least one outcome assessment, by treatment group (mean ± SD or %)

<table>
<thead>
<tr>
<th>Variable</th>
<th>WPC (N = 62)</th>
<th>MMN + WPC (N = 64)</th>
<th>BSC (N = 63)</th>
<th>MMN + BSC (N = 60)</th>
<th>P-value (^\dagger)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/Female</td>
<td>30/32</td>
<td>30/34</td>
<td>31/32</td>
<td>29/31</td>
<td>0.99</td>
</tr>
<tr>
<td>Age at start of supplementation (mo)</td>
<td>6.7 ± 0.4</td>
<td>6.8 ± 0.4</td>
<td>6.8 ± 0.4</td>
<td>6.7 ± 0.4</td>
<td>0.58</td>
</tr>
</tbody>
</table>

Infant anthropometry at start of supplementation

<table>
<thead>
<tr>
<th>Variable</th>
<th>WPC</th>
<th>MMN + WPC</th>
<th>BSC</th>
<th>MMN + BSC</th>
<th>P-value (^\dagger)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>6.94 ± 0.94</td>
<td>6.91 ± 0.78</td>
<td>6.97 ± 0.85</td>
<td>6.90 ± 0.77</td>
<td>0.96</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>64.2 ± 2.5</td>
<td>64.0 ± 2.2</td>
<td>64.0 ± 2.3</td>
<td>64.1 ± 2.0</td>
<td>0.93</td>
</tr>
<tr>
<td>Arm circumference (cm)</td>
<td>13.7 ± 1.2</td>
<td>13.6 ± 1.0</td>
<td>13.9 ± 1.0</td>
<td>13.6 ± 0.9</td>
<td>0.35</td>
</tr>
<tr>
<td>Weight-for-age (z)</td>
<td>-1.02 ± 0.77</td>
<td>-1.09 ± 0.95</td>
<td>-1.15 ± 0.98</td>
<td>-0.99 ± 0.77</td>
<td>0.81</td>
</tr>
<tr>
<td>Length-for-age (z)</td>
<td>-1.51 ± 0.83</td>
<td>-1.45 ± 0.77</td>
<td>-1.62 ± 0.76</td>
<td>-1.53 ± 0.76</td>
<td>0.77</td>
</tr>
<tr>
<td>Weight-for-length (z)</td>
<td>0.22 ± 0.68</td>
<td>0.08 ± 0.91</td>
<td>0.19 ± 0.88</td>
<td>0.27 ± 0.73</td>
<td>0.68</td>
</tr>
</tbody>
</table>
## Infant morbidity during the 30 days prior to supplementation

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th></th>
<th></th>
<th></th>
<th>P-value¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WPC</td>
<td>MMN + WPC</td>
<td>BSC</td>
<td>MMN + BSC</td>
<td></td>
</tr>
<tr>
<td>Prevalence of diarrhea (≥3 liq. or semi-liq. stools) (%)</td>
<td>9.0 ± 13.7</td>
<td>16.9 ± 22.9</td>
<td>11.6 ± 19.6</td>
<td>13.6 ± 22.4</td>
<td>0.12 (K-W)</td>
</tr>
<tr>
<td>Prevalence of diarrhea (&gt;3 liq. or semi-liq. stools) (%)</td>
<td>4.9 ± 8.1</td>
<td>11.1 ± 18.8</td>
<td>7.6 ± 15.7</td>
<td>7.8 ± 14.7</td>
<td>0.30 (K-W)</td>
</tr>
<tr>
<td>Prevalence of fever (%)</td>
<td>9.4 ± 11.1</td>
<td>11.2 ± 12.0</td>
<td>11.5 ± 13.1</td>
<td>11.4 ± 13.1</td>
<td>0.77 (K-W)</td>
</tr>
<tr>
<td>Prevalence of upper respiratory infection (%)</td>
<td>8.9 ± 14.0</td>
<td>5.9 ± 11.2</td>
<td>7.5 ± 15.5</td>
<td>7.9 ± 13.4</td>
<td>0.59 (K-W)</td>
</tr>
<tr>
<td>Prevalence of lower respiratory infection (%)</td>
<td>0.1 ± 0.8</td>
<td>0.0 ± 0.0</td>
<td>0.6 ± 5.0</td>
<td>0.1 ± 0.6</td>
<td>0.79 (K-W)</td>
</tr>
<tr>
<td>Housing quality score</td>
<td>0.92 ± 0.23</td>
<td>0.96 ± 0.24</td>
<td>0.95 ± 0.24</td>
<td>0.93 ± 0.26</td>
<td>0.82</td>
</tr>
<tr>
<td>Possessions score</td>
<td>4.2 ± 1.3</td>
<td>4.3 ± 1.6</td>
<td>4.5 ± 1.4</td>
<td>4.0 ± 1.6</td>
<td>0.33</td>
</tr>
</tbody>
</table>
Effects of NutraGammmax on Measures of Growth in Breast Fed Infants and Children

MUAC: Treatment, p value = 0.14
**Safety of Plasma Proteins or Immunoglobulin**

- Ig and albumin are the same proteins found in milk and whey
- Many published studies (>50) have utilized bovine Ig as a source of antibody without reported adverse effects
- No adverse effects in healthy adult subjects
- No adverse effects in infants and children in both short- and long-term studies
- 5 different institutional review boards have approved its use for clinical studies in both children and adults
"Intestinal" Health Concerns in the US

- **Inflammatory**
  - Prevalence of inflammatory bowel disease is increasing
  - Incidence of food-borne illness is increasing
  - Diarrhea continues to be an important childhood disease
  - Traveler’s diarrhea

- **Non-inflammatory**
  - Irritable bowel syndrome affects approximately 10% of the population
  - Digestive disturbances such as lactose intolerance are common
  - Food sensitivities: Gluten, dairy, soy, insoluble fiber
  - GI dysfunction is a common medication side effect
The Burden of Selected Digestive Diseases in the United States

- Most prevalent
  - Non-food-borne gastroenteritis (135 million cases/year)
  - Food-borne illness (76 million)
  - Gastroesophageal reflux disease (GERD; 19 million)
  - Irritable bowel syndrome (IBS; 15 million)

- Direct costs: $36 billion
- Indirect costs: $22.8 billion
- Digestive health is a large opportunity for functional foods and nutraceuticals

Irritable Bowel Syndrome

- Affects 1 in 5 people in the U.S.
- Affects both genders but more prevalent in women
- Diagnosed by exclusion of other diseases of intestinal tract
- No known cause or cure
  - Only symptomatic treatment
- Three common groupings:
  - Diarrhea
  - Constipation
  - Alternating diarrhea and constipation
Dietary Factors in IBS

- Low Immunity
- Previous Infection
- Lactose
- Stimulants
- Saturated fat
- Simple CHO
- Gluten sensitivity
- Previous Infection
- Lactose
- Stimulants
- Simple CHO
- Gluten sensitivity
Objective

• Conduct a placebo-controlled pilot study to evaluate the effects of immunoglobulin isolate on general health and symptoms of subjects with diarrhea predominant IBS
Response criteria

- Validated QOL (IBS-36)
  - e.g. Question 22: Were you troubled by loose bowel movements?
- IBS Daily Symptom score (Scale of 0-3 with 0 being none)
  - Nausea, abdominal pain, flatulence, bloating, hard stool, loose stool, urgency, straining, incomplete evacuation, mucus
- Days with symptoms
Procedures

• Key baseline characteristics of subjects
  – IBS diagnosed by Rome II diagnostic criteria
  – Diarrhea-predominant
  – Study subjects:
    • 33 females, 18 males
    • Age: 47 yr
    • Body mass index: 29.1 kg/m²
• Immunoglobulin isolate (0, 5 or 10-g) was administered orally for 6 weeks
Results

• IBS-36 Questionnaire
  – Very high placebo response
    • Significant improvement in nearly every component
  – Only 2 of the 36 components were different between treatment groups

• Total symptom score
  – Numerical improvement in Ig-10 g group but no statistically significant differences.
Days With Symptoms

- Placebo
- Ig (5 g)
- Ig (10 g)

* P < 0.05
Total Days With Symptoms

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Placebo</th>
<th>Ig (5 g)</th>
<th>Ig (10 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>* P &lt; 0.05</td>
<td>* P &lt; 0.05</td>
<td></td>
</tr>
</tbody>
</table>
Conclusion

• Immunoglobulin supplementation reduced days with symptoms in subjects with diarrhea-predominant IBS in a placebo-controlled clinical study

• If the data can be extrapolated to a full year, a person suffering from IBS could have 35 more days per year without symptoms
Immunoglobulin Isolates

A Unique Dietary Protein Source With Hypocholesterolemic and Immunomodulatory Effects

Eric Weaver, Ph.D.
Proliant Health and Biologicals
Ankeny, IA
• Antibodies: Discovered in the 1900’s
• Immunoglobulins are **essential** proteins for immunity!
  – Immunoglobulin (antibodies), sIgA, is the body’s primary means of defending the body in the oral cavity and digestive tract
  – >90% of all immunoglobulin is antibodies directed against intestinal antigens
• Supplementation of immunoglobulin has not been feasible.
  – Technology and cost have been important factors
  – Dose is critical to the effect.
• New processing and sourcing technologies have now made oral supplementation feasible.
Cholesterol in the US

• The Adult Population
  – Greater than Desirable adults 50% 105 million
  – Borderline High adults 30% 63 million
  – High 20% 42 million adults
Cholesterol Market in the US

• > $20 billion market and growing
• **Products Used to Lower Cholesterol**
  – **Drugs**
    • Statins, ezetimibe, niacin, and resins
  – **Functional foods**
    • Phytosterol-enhanced
    • Soluble-fiber rich
    • Soy protein enriched
  – **Nutraceuticals**
    • Phytosterols, soy, B-glucan, red rice yeast, and many others
Cholesterol absorption
Changes in Theories of Cholesterol Absorption

• Recent discovery of active process
  – Facilitated by transmembrane proteins such as NCP1L1
• Can anti-cholesterol antibodies play a role in inhibiting absorption?
• Can plasma proteins disrupt micelle formation?
Can Dietary Protein Components Influence Cholesterol Concentrations?

- Yogurt and milk have both been shown to be hypocholesterolemic despite the hypercholesterolemic effect of casein
  - Is there a cholesterol-lowering component in milk?
  - Is casein representative of the proteins in milk?
  - Is there a factor in the whey protein fraction which can lower cholesterol?
- Immunoglobulin (Ig) is a viable candidate.
  - High molecular weight with demonstrated binding activity to cholesterol
  - Antibodies to cholesterol have been associated with a lower incidence of atherosclerosis
  - Immunomodulating: a characteristic which may reduce cholesterol production
- Two clinical studies published in early ‘90’s reported lower cholesterol concentrations when subjects were fed milk from hyperimmunized cows, with an elevated immunoglobulin (Ig) level
Structures

Immunoglobulin (IgG)

Cholesterol

C. Ophardt, c. 2003
Can Dietary Protein Source Influence Cholesterol Concentrations?

• Very little data in the literature on animal proteins other than casein

• Two animal studies using duodenally-cannulated rats demonstrated a reduction in total lipid and cholesterol absorption when plasma protein or Ig were included in the duodenal-infusion mixture.
Effects of IgG on Cholesterol Absorption

Absorption (% of dose)

Hours Post-Infusion

Hour: Chol, Alb at 4 and 8 different from IgG (P < .05)
Effects of IgG on Cholesterol Absorption

Cumulative cholesterol absorption, %

Hour Post Infusion

Hour: 6, 7 and 8 different from Hour 1 (P < .05)
Other findings

• Porcine immunoglobulin was utilized in the first two studies.
• Reducing the lipid concentration of bovine immunoglobulin negated the effect of Ig on absorption
Clinical Studies

• Initiated a small pilot study in 2002 (n=22) to measure impact of natural Ig isolate (ImmunoLin) supplementation on clinical chemistry parameters in healthy adults
Effects of ImmunoLin on Total Cholesterol

<table>
<thead>
<tr>
<th>Week of Study</th>
<th>Total Cholesterol, mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>233.1 224.9 216 210 200.9 217.6</td>
</tr>
<tr>
<td>1</td>
<td>174.4 182.6 178.6 182.1 174.6 177.6</td>
</tr>
</tbody>
</table>

HIGH group: Week 2,3, 6 different from Week 0 (P < .01)
NORMAL group: No significant differences
Effects of ImmunoLin on Change in Triglycerides

HIGH group: Week 1, 3, 6 and 12 different from Week 0 (P < .05)
NORMAL group: No significant differences
Effects of ImmunoLin on LDL

High

Normal

<table>
<thead>
<tr>
<th>Week of Study</th>
<th>High</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>152.8</td>
<td>97.2</td>
</tr>
<tr>
<td>1</td>
<td>152.1</td>
<td>107.8</td>
</tr>
<tr>
<td>2</td>
<td>136.7</td>
<td>96.7</td>
</tr>
<tr>
<td>3</td>
<td>136</td>
<td>100.7</td>
</tr>
<tr>
<td>6</td>
<td>126.2</td>
<td>90.9</td>
</tr>
<tr>
<td>12</td>
<td>145.8</td>
<td>99.4</td>
</tr>
</tbody>
</table>

HIGH group: Week 2, 3, 6 different from Week 0 (P < .05)
NORMAL group: No significant differences
Effects of ImmunoLin on HDL

<table>
<thead>
<tr>
<th>Week of Study</th>
<th>High</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>44.7</td>
<td>51.9</td>
</tr>
<tr>
<td>1</td>
<td>43.8</td>
<td>51.4</td>
</tr>
<tr>
<td>2</td>
<td>45.5</td>
<td>54</td>
</tr>
<tr>
<td>3</td>
<td>45</td>
<td>53.5</td>
</tr>
<tr>
<td>6</td>
<td>48</td>
<td>54.1</td>
</tr>
<tr>
<td>12</td>
<td>43.1</td>
<td>48.5</td>
</tr>
</tbody>
</table>

HIGH group: No significant differences
NORMAL group: No significant differences
Effects of ImmunoLin on Change in Chol:HDL Ratio

**High group:** Week 2, 3, 6 different from Week 0 (P < .05)

**NORMAL group:** No significant differences
Effects of ImmunoLin on Change in Total Cholesterol

<table>
<thead>
<tr>
<th>Study Period</th>
<th>High</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 1</td>
<td>-9</td>
<td>5.1</td>
</tr>
<tr>
<td>0 to 2</td>
<td>-17.9</td>
<td>1.1</td>
</tr>
<tr>
<td>0 to 3</td>
<td>-23.1</td>
<td>1.9</td>
</tr>
<tr>
<td>0 to 6</td>
<td>-32.2</td>
<td>-2.4</td>
</tr>
<tr>
<td>0 to 12</td>
<td>-15.5</td>
<td>-1</td>
</tr>
</tbody>
</table>

Change, mg/dl
Summary

• 5 g of ImmunoLin reduced total cholesterol and LDL-C in subjects with mild hypercholesterolemia

• On the basis of previous studies in animals and humans, the lipid-lowering effect of ImmunoLin should be investigated.
Cholesterol-lowering effects of bovine serum immunoglobulin in participants with mild hypercholesterolemia


¹Earnest, C.P., ¹Jordan, A.N, ¹Safir, M., ¹Church, T.S. and ²Weaver, E.
¹Center for Human Performance and Nutrition Research, The Cooper Institute
²Proliant Health and Biologicals
Study II. Double blind, placebo-controlled study of Ig isolate supplementation in mildly-hypercholesterolemic subjects

Procedures:

• 52 subjects of 250 screened met criteria and completed study
  – Total cholesterol (210 - 270 mg/dl)
    • Baseline cholesterol determined with two visits (3rd if >10% change)
  – HDL cholesterol (<70 mg/dl)
• Age: 51 yr (Range, 25-70)
• Weight: 80 kg
• Advised to continue normal diet and exercise habits and avoid new vitamin and mineral supplements
Double blind, placebo-controlled study of Ig supplementation in mildly-hypercholesterolemic subjects

Procedures:

• Randomly assigned to treatment groups:
  – Control: hydrolyzed gelatin (isonitrogenous to treatment)
  – Treatment: 5 g Ig isolate

• Statistical analysis:
  – Repeated measures (MANOVA) for within group effects
  – Tukey-Kramer tests used for between group effects
Results: Blood lipids in treated (Ig) and control groups

<table>
<thead>
<tr>
<th></th>
<th>Treatment (n = 26)</th>
<th>Control (n = 26)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total cholesterol, mmol/L</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>6.33 ± 0.10</td>
<td>6.16 ± 0.08</td>
</tr>
<tr>
<td>3Wk</td>
<td>5.98 ± 0.10 *</td>
<td>6.14 ± 0.12</td>
</tr>
<tr>
<td>6Wk</td>
<td>5.97 ± 0.13 *</td>
<td>6.07 ± 0.09</td>
</tr>
<tr>
<td>3Wk change vs. baseline</td>
<td>-0.35 ± 0.10 (-0.56, -0.15) **, †</td>
<td>-0.03 ± 0.09 (-0.22, 0.17)</td>
</tr>
<tr>
<td>6Wk change vs. baseline</td>
<td>-0.37 ± 0.12 (-0.61, -0.12) **, †</td>
<td>-0.10 ± 0.08 (-0.27, 0.07)</td>
</tr>
<tr>
<td><strong>LDL cholesterol, mmol/L</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>4.12 ± 0.11</td>
<td>3.95 ± 0.09</td>
</tr>
<tr>
<td>3Wk</td>
<td>3.92 ± 0.12 *</td>
<td>4.00 ± 0.12</td>
</tr>
<tr>
<td>6Wk</td>
<td>3.84 ± 0.12 *</td>
<td>3.83 ± 0.12</td>
</tr>
<tr>
<td>3Wk change vs. baseline</td>
<td>-0.20 ± 0.11 (-0.43, 0.04)</td>
<td>0.05 ± 0.08 (-0.11, 0.20)</td>
</tr>
<tr>
<td>6Wk change vs. baseline</td>
<td>-0.28 ± 0.09 (-0.46, -0.10) *</td>
<td>-0.13 ± 0.09 (-0.32, 0.05)</td>
</tr>
</tbody>
</table>
## Results: Blood lipids in treated (Ig) and control groups

<table>
<thead>
<tr>
<th></th>
<th>Treatment (n = 26)</th>
<th>Control (n = 26)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HDL cholesterol, mmol/L</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1.35 ± 0.05</td>
<td>1.49 ± 0.04</td>
</tr>
<tr>
<td>3Wk</td>
<td>1.28 ± 0.05</td>
<td>1.47 ± 0.05</td>
</tr>
<tr>
<td>6Wk</td>
<td>1.29 ± 0.06</td>
<td>1.49 ± 0.05</td>
</tr>
<tr>
<td>3Wk change vs. baseline</td>
<td>-0.07 ± 0.04 (-0.15, 0.00)</td>
<td>-0.03 ± 0.03 (-0.08, 0.03)</td>
</tr>
<tr>
<td>6Wk change vs. baseline</td>
<td>-0.06 ± 0.03 (-0.12, 0.00)</td>
<td>± 0.03 (-0.06, 0.07)</td>
</tr>
<tr>
<td><strong>VLDL cholesterol, mmol/L</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.87 ± 0.09</td>
<td>0.71 ± 0.06</td>
</tr>
<tr>
<td>3Wk</td>
<td>0.78 ± 0.07</td>
<td>0.67 ± 0.06</td>
</tr>
<tr>
<td>6Wk</td>
<td>0.83 ± 0.09</td>
<td>0.72 ± 0.05</td>
</tr>
<tr>
<td>3Wk change vs. baseline</td>
<td>-0.09 ± 0.07 (-0.23, 0.06)</td>
<td>-0.05 ± 0.05 (-0.16, 0.06)</td>
</tr>
<tr>
<td>6Wk change vs. baseline</td>
<td>-0.03 ± 0.05 (-0.14, 0.08)</td>
<td>0.02 ± 0.03 (-0.05, 0.10)</td>
</tr>
<tr>
<td><strong>Triglycerides, mmol/L</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1.84 ± 0.17</td>
<td>1.51 ± 0.12</td>
</tr>
<tr>
<td>3Wk</td>
<td>1.68 ± 0.16</td>
<td>1.43 ± 0.13</td>
</tr>
<tr>
<td>6Wk</td>
<td>1.80 ± 0.19</td>
<td>1.67 ± 0.16</td>
</tr>
<tr>
<td>3Wk change vs. baseline</td>
<td>-0.16 ± 0.13 (-0.44, 0.11)</td>
<td>-0.08 ± 0.10 (-0.29, 0.14)</td>
</tr>
<tr>
<td>6Wk change vs. baseline</td>
<td>-0.04 ± 0.11 (-0.27, 0.18)</td>
<td>0.16 ± 0.14 (-0.12, 0.44)</td>
</tr>
</tbody>
</table>
Study II. Effects of Ig supplementation on Total Cholesterol*

*Represents significant change from baseline at 3 and 6 weeks (P<.01).

<table>
<thead>
<tr>
<th>Week of Study</th>
<th>Control</th>
<th>blg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>237.8</td>
<td>244.4</td>
</tr>
<tr>
<td>3</td>
<td>237</td>
<td>230.9</td>
</tr>
<tr>
<td>6</td>
<td>234.4</td>
<td>230.5</td>
</tr>
</tbody>
</table>
Study II. Effects of Ig Supplementation on LDL Cholesterol*

<table>
<thead>
<tr>
<th>Week of Study</th>
<th>Control</th>
<th>Ig</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>152.5</td>
<td>159.1</td>
</tr>
<tr>
<td>3</td>
<td>154.4</td>
<td>151.4</td>
</tr>
<tr>
<td>6</td>
<td>147.9</td>
<td>148.3</td>
</tr>
</tbody>
</table>

*Represents significant change from baseline at 3 and 6 weeks (P < .01).
Change in control group not significant (P < .05).
Improvement in Total Cholesterol (%)

Change in Total Cholesterol, %

Baseline | Week 3 | Week 6

Placebo

Ig
Results

- No between group differences in mean total cholesterol or LDL-C were observed, resulting from numerical differences at baseline.

- Ig administration resulted in lower total and LDL-C at 3 and 6 weeks from baseline.

- Between group differences in change in total cholesterol were significant at 3 and 6 weeks (P<.05)

- No significant differences in HDL were observed.

- No changes in markers of hepatorenal or cardiovascular function
  - Study was not adequately powered for changes in C-reactive protein
Response Rate after 3 Weeks of Supplementation

These data indicate that the cholesterol lowering response is relatively rapid and large in 35% of the group studied.
Response Rate after 6 Weeks of Supplementation

Decrease in Total Cholesterol

Rate (%)

>-30 mg/dl
>-15 mg/dl
All < Baseline

Control
blg
Summary

• Ig Supplementation Lowers Total and LDL Cholesterol

• Efficacy - Pilot study (n=22)
  – 14% improvement in total cholesterol
  – 18% improvement in LDL

• Double-blind, placebo controlled clinical study (n=52)
  – 6% improvement in total cholesterol
  – 7% improvement in LDL

• Second, larger study confirmed observations of pilot study
Mode of Action?

- Lowering cholesterol absorption
  - Antibodies to cholesterol to have been shown in humans and animals
  - Whey and milk are low in immunoglobulin, utilize processes that denature proteins, and contain confounding components, such as casein. Not surprisingly, responses in clinical studies to these materials has been inconsistent.
  - Immunoglobulin isolated without denaturation is the first step in delivering binding activity
  - The response is undoubtedly diet and dose-dependent.

- Reducing cholesterol synthesis
  - TNF-α, an inflammatory cytokine, is responsive to Ig supplementation
  - TNF-α has also been shown to promote hyperlipidemia in animal models
  - Possible explanation for a continued response after dosing
The Use of Immunoglobulin Isolates for Cholesterol Management

- Administration is practical
  - 5 grams per day can be easily incorporated into protein shake or bar
- **Safety - No side effects**
  - Only contraindication is allergies to meat or dairy proteins.
- Cost
  - Competitive with other products at efficacious dose
- Efficacy comparisons - Based on two studies (pilot and controlled clinical)
  - Rapid effect!
  - As effective as phytosterols
  - More effective than soy, fiber, oat fiber, B-glucan, garlic
Future studies

• Sports nutrition
  – Evaluate effect of NutraGammmax on lean body mass, recovery, and muscle soreness vs whey or casein during intense training
Summary