The effect of bovine colostrum supplementation on levels of secretory immunoglobulin-A (S-IgA) in saliva of elite athletes, non-exercising controls and non-exercising older adults

A project completed as fulfilment of the
requirements of a doctoral thesis in clinical nutrition

Massey University
Albany Campus
New Zealand

Christine Crooks
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Abstract

Secretory immunoglobulin-A (S-IgA) in saliva may reflect levels of immune defence at other mucosal sites. Reduced levels of salivary S-IgA have been associated with an increased risk for upper respiratory symptoms (URS) in athletes. Previously, the consumption of a nutrition supplement, bovine colostrum (BC) by distance runners, was shown to significantly increase levels of salivary S-IgA compared to baseline; however the mechanism was not known. The immunomodulatory effect of BC is investigated further in these current studies.

Twenty-five swimmers (12 males [M], 13 females [F], age 14-23 years) training at an elite level, 28 lightly-exercising students (9M, 19F, age 18-27 years), and 45 healthy older adults (20M, 20F, age 65-76 years), consumed a supplement of either BC or placebo for ten weeks. Saliva samples were collected at baseline, weekly for four weeks during supplementation and post-supplementation. Blood samples were collected at baseline, monthly during supplementation and post-supplementation.

No significant changes were seen in levels of S-IgA in either BC or placebo groups within any of the cohorts. There was a trend towards a significant difference in URS reportage between BC and placebo groups in the swimmers cohort, but not in the students or older adults. There was also a trend towards a difference in the number of swimmers reporting URS. Fewer numbers of swimmers consuming BC reported URS compared the placebo (P=0.062) after consuming BC for four weeks compared to those consuming the placebo. Post-exercise plasma cortisol results were significantly reduced in the BC subgroup compared to the placebo (P=0.004).

These results do not support the findings of previous intervention studies investigating the immunomodulatory effect of BC in athletes. However the reduced reportage of URS, among swimmers consuming the BC supplement, suggested there was some benefit to their health. A possible explanation is that BC has impacted on non-infectious causes of URS. Growth factors present in

BC may enhance intestinal repair which could be advantageous to athletes recovering from bouts of prolonged intensive exercise. The effect of gastrointestinal disturbances on local and systemic immunity may be minimised which benefits immune protection. However an inconsistent effect of BC supplementation on immune protection in athletes means further research is still required. In these studies there was no benefit to immune protection in the student or older adult cohort. Further investigation into the safety of BC for all population groups is still required.

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Introduction

The aim of these current studies was to further investigate the role of a bovine colostrum supplement in mucosal immune protection in other potentially immune depressed groups.

Two studies were planned with three different cohorts (two cohorts in Study 1 and one cohort in Study 2), the sample size for each cohort was based on a power calculation carried out with the results from the previous study involving middle-aged recreational runners [1]. The study design involved saliva and blood sampling as well as the self-recording of wellness symptoms and activity or training, and the monitoring of dietary intake. A large number of saliva samples were provided by the participants at specified sampling times at their training locations from three different Auckland swimming pools, University or home. To ensure standardised sampling procedures were followed research assistants (RAs) were hired to assist with the collection of saliva, aliquotting to microtubes and freezing. Assistance was sought to centrifuge blood, aliquot and freeze the plasma. Further assistance was also sought to issue the trial supplement and collect dietary and anthropometric data. This was especially important during the study with older adults where each participant was visited at their home or place of work.

The planning, co-ordinating and project managing of the two studies was my responsibility which included:

- Development of the research proposal in conjunction with my supervisors
- Preparation of two ethics applications for the different studies
- Recruitment and training of the participants
- Employment and training of the research assistants
- Collection and storage of saliva samples and co-ordination of RAs to assist
- Co-ordination of blood collection with participants, coaches, phlebotomists (Diagnostic Medlab, Massey University Health Centre) and timely delivery of blood samples to LabPlus for analysis

- Co-ordination of blood centrifugation, storage and delivery of plasma samples to the appropriate laboratory for analysis
- Performing cytokine analysis: interleukin (IL)-6, IL-1ra and interferon-alpha at Massey University (Albany campus)
- Issuing of supplement, collection of unconsumed supplement and determining compliance
- Liaison with Fonterra for delivery of supplement and the issuing of ID codes in a double blinded manner
- Liaison with Fonterra for test results of supplement
- Performing dietary interviews, co-ordinating data entry of dietary data and performing quality control of all dietary data
- Checking and 'data-cleaning' all immunological and biochemical data
- Performing statistical analysis of data
- Providing written study summary to participants and updates to ethics committee
- Preparing regular reports for sponsor (with Frank's help)
- Presenting results at conferences which included:
    - Self-reported upper respiratory symptoms were reduced among swimmers who consumed a bovine colostrum supplement, oral presentation, 'New Image'-Colostrum seminar, Rotorua, 2008
    - Reduced reporting of upper respiratory symptoms among swimmers following bovine colostrum supplementation. Oral presentation 'Australian Association of Exercise and Sport Science', conference, September 2006.
    - The effects of ageing on the homing ability of lymphocyte subsets in older adults. Poster presentation to 'Development and Function of Secondary Lymphoid Tissues' conference, Pasteur Institute, Paris, 2006.
    - The Effect of Bovine Colostrum Supplementation on Salivary IgA in Recreational Runners. Poster presentation to 'Nutrigenomics' conference, Auckland, 2006.

- The Effect of Bovine Colostrum Supplementation on Salivary IgA in Distance Runners. Oral presentation to the 'National Sport Science' conference, Kuala Lumpur, 2004.

- Writing thesis

My PhD supervisors provided assistance during the project planning stage and gave support and feedback during the trials and the preparation of the thesis:

- Dr Frank Cross (Frank taught me how to do an enzyme-linked immunoassay and assisted with all report writing)
- Dr Clare Wall (Clare helped with saliva and dietary collection during the older adult study)

In addition:

- Dr Ajmol Ali (Aj helped with the early morning saliva sampling of the swimmers and volunteered as a participant in the student cohort)
- Professor Marlena Kruger supported me through the completion of the thesis, PhD thesis examination and emendations

The research assistants performed the following tasks:

- Janie Proctor (assisted with saliva sampling and blood centrifuging for both Study 1 and 2, performed data entry for most of the wellness and training records for Study 1 and most of the dietary data)
- Christel Dunshea-Mooij (assisted with saliva sampling, anthropometric measurements and diet interviews in Study 2)
- Catherine Murray (assisted with saliva sampling, anthropometric measurements and diet interviews in Study 2)
- Jane Fenton (assisted with saliva sampling, anthropometric measurements and diet interviews in Study 1 and 2, performed most of the data entry of wellness and activity records for Study 2)
- Pam Van Horst (assisted with saliva sampling for Study 1 and 2, anthropometric measurements and diet interviews in Study 2)
- Renee Prideaux (assisted with saliva sampling in Study 1)
- Claire Svendsen (assisted with saliva sampling in Study 1)

- Cindy Claassens (anthropometric measurements and diet interviews in Study 2)

In summary laboratory analysis of samples collected for this study were performed as listed below:

- Blood samples were analysed at LabPlus for full blood count, liver function analysis and cell phenotyping
- Plasma samples were analysed for levels of cytokines by me at the nutrition laboratory, Albany campus, Massey University
- Plasma samples were analysed for immunolgobulins, albumin, cortisol, C-reactive protein and cortisol by Dr Phil Pearce, nutrition laboratory, Palmerston North campus, Massey University
- Saliva samples were analysed for immunolgobulins, albumin and osmolality by Dr Phil Pearce, nutrition laboratory, Palmerston North campus, Massey University

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List of Abbreviations used

Abbreviation	Definition
AF	Activity factor
Alb	Albumin
APC	Antigen presenting cell
Ave	Average
BALT	Bronchial associated lymphoid tissue
Base	Baseline
BC	Bovine colostrum
B-cell	B-lymphocyte
BM	Body mass
BMR	Basal metabolic rate
CD	Cluster of differentiation number
CHO g/kgBM	Grams of carbohydrate per kilogram of bodymass
CHO/kgBM/day	Carbohydrate per kilogram body mass per day
CMIS	Common mucosal immune system
CTL	Cytotoxic T-cell
diff	Difference
Drink g/day	Grams of drink consumed per day
EDEE	Estimated daily energy expenditure
EGF	Epidermal growth factor
F	Female
GALT	Gut associated lymphoid tissue
Gen	Gender
GH	Growth hormone
IFN	Interferon
IFN-α	Interferon-alpha
Ig	Immunoglobulin
IgA	Immunoglobulin A
IGF-1	Insulin-like growth factor-1
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukin
IL-1ra	Interleukin-1receptor antagonist
IL-6	Interleukin-6
Intens	Intensity
IQ range	Interquartile range
J-chain	Polypeptide joining chain
LP	Lamina propria
LPS	Lipopolysaccharide
M	Male
MAdCAM-1	Mucosal addressin cell adhesion molecule
MBM	Mature bovine milk
M-cell	Microfold cell
Med	Median
MET	Metabolic equivalent
MHC	Major histocompatibility complex
N/A	Not applicable
ND	Not detected
No Ana.	Analysis not performed
NP	Not possible to calculate
NRV	Nutrient reference value
NSAID	Non-steroid anti-inflammatory drug
OA	Older adults
Osm	Osmolality
PAL	Physical activity level
PIgR	Receptor for polymeric immunoglobulin
Pl	Placebo

List of Abbreviations used (continued)

Abbreviation	Definition
Post	Post-supplementation
PPs	Peyer's patches
Ptn g/kgBM	Grams of protein per kilogram of body mass
RDBPC	Randomised double blind placebo controlled
RDDI	Reported daily dietary intake
RDEI	Reported daily energy intake
RDI	Reported daily intake
SEM	Standard error of the mean
S-IgA	Secretory immunoglobulin A
St	Students
Sw	Swimmers
T-cell	T-lymphocyte
TGF-β	Transforming growth factor beta
UL	Upper level of intake
URS	Upper respiratory symptoms
URT	Upper respiratory tract
URTI	Upper respiratory tract infection
VCAM-1	Vascular adhesion molecule
wks	Weeks