PILOT STUDY

Safety and Efficacy of a Multivitamin, Multimineral, Bovine Colostrum–Containing Supplement: An Open-label Pilot Intervention Trial in Healthy Adult Women and Men

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ABSTRACT

Objective • This study was undertaken to explore the effects of a bovine colostrum–containing multivitamin multimineral (MVM) supplement on healthy, adult women and men by determining blood chemistries and health parameters via serum and saliva sampling and measuring each subject's physical characteristics over a 12-week interval.

Participants • Fifty participants were screened for the study, after which twenty participants were determined eligible to enter the study. Thirteen participants (6 women and 7 men, average age 30.9 years, average BMI 27.3 kg/m^2) completed the whole study.

Results • MVM did not significantly impact serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT) levels. The MVM significantly improved serum folate levels (48.3% increase at Week 12: 23.56 ± 5.75 ng/mL versus Week 0: 15.88 ± 3.40 ng/mL, P = .0001). MVM improved serum levels of vitamin B12 (21.3% increase at Week 12: 789.38 ± 313.23 pg/mL versus Week 0: 650.54 ± 228.02 pg/mL, P = .0690) and 25-hydroxy-vitamin D levels (9.1% increase at Week 12: 32.22 ± 5.81 ng/mL versus Week 0: 29.54 ± 11.30 ng/mL, P = .3570). The salivary IgA levels showed a significant increase at Week 4 (249.85 ± 95.63 ng/ml), Week 8 (271.65 ± 133.52 ng/ml), and Week 12 (279.88 ± 128.19 ng/ml) compared to Week 0 (177.57 ± 74.81 ng/ml).

Conclusions • This study shows that MVM has a good safety and tolerability profile and can be used as a daily nutritional supplement safely. MVM may improve serum levels of vitamin D, folate, vitamin B12, and, possibly, other blood markers. The study showed that MVM may improve secretory IgA levels, a major component of oral immunity. These findings suggest an overall improvement in several aspects of health and need to be confirmed in a larger, placebo-controlled study. (*Altern Ther Health Med.* [E-pub ahead of print.])

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INTRODUCTION

It is estimated that at least one-third of Americans take a multivitamin multimineral supplement (MVM) regularly.[1] Though people might take an MVM for different reasons, studies have shown that its use improved not only nutrient intake but also overall health. MVM use increases nutrient intake and helps people obtain their recommended intake of vitamins and minerals especially when they cannot meet these needs from food alone. In one study, researchers evaluated the diets and MVM usage of a large multiethnic cohort from Los Angeles and Hawaii[2] and found that MVM usage increased the prevalence of nutrient adequacy by 8% on average for men and women, with the greatest improvements being in vitamin E, vitamin A, and zinc intake. In another clinical study with healthy participants, researchers found that two months of MVM usage significantly improved the participants' HDL cholesterol levels, LDL/HDL cholesterol ratios, fasting insulin, homocysteine, serum vitamin E, EPA, and the AA/EPA ratio.[3] A randomized controlled trial in adults with diabetes found that MVM consumption with zinc for four months led to a significant reduction in fasting blood sugar and glycosylated hemoglobin in comparison to the placebo.[4] Physicians Health Study II was the longest clinical trial to investigate whether an MVM might help prevent chronic disease. The study randomly assigned 14 641 male physicians in the United States, aged 50 and older, to an intervention group or a control group. The intervention group had to take an MVM daily and the control group took a placebo daily.

Both groups were followed for a median of 11.2 years. The study concluded that MVM supplementation reduced the subjects' risk of developing cancer modestly but significantly, by 8% on average.[5]

Among the various MVMs available on the market, RiteStart® (RS hereafter) is a comprehensive multivitamin, multimineral, and all-in-one daily supplement. Besides having comprehensive and balanced amounts of essential vitamins and minerals that the human body needs, RS includes many other health-promoting ingredients. It contains potent antioxidants, including vitamin A, C, and E, oligomeric proanthocyanidins from pine bark and grapeseed extracts, lutein, CoQ10, alpha-lipoic acid, and green tea. It is also a source of essential fatty acids from fish and plant oils. RS contains transfer factor (bovine colostrum and egg yolk extract), which supports immunity.[6-8] RS also includes a blend of maitake mushrooms, shiitake mushrooms, cordyceps, inositol hexaphosphate, olive leaf extract, and other ingredients that support immune function and overall health.

The current study was designed to explore the effects of RS supplementation on different health aspects, by quantitatively measuring relevant serum and saliva biomarkers in healthy participants who had not previously taken the supplement over 12 weeks. It is hypothesized that several serum biomarkers would be significantly improved as per the designed health benefits of RS, such as immune health, brain health, and heart health. It is also hypothesized that many serum biomarkers would be unaffected by RS supplementation, indicating good safety and tolerability of RS for daily use.

MATERIALS AND METHODS

Study Materials

The study product, RS, is manufactured and provided by 4Life Research, Utah, USA. Participants were instructed to take the product twice daily as a comprehensive all-in-one dietary supplement. RS contains well-studied vitamins, minerals, herbal extracts, omega-3 fatty acids, and transfer factors. The study product comes in two versions: one is tailored to the nutritional needs of women and the other to those of men. The two versions are largely identical, except for the following nutrients (daily amount): folic acid, 1360 µg for women versus 600 µg for men; calcium, 1108 mg for women versus 334 mg for men; iron, 15 mg for women versus 10 mg for men; magnesium, 400 mg for women versus 125 mg for men; zinc, 15 mg for women versus 20 mg for men; selenium, 35 µg for women versus 70 µg for men. More information on the product can be found on the RiteStart website (RiteStart Women [4life.com] and RiteStart Men [4life.com]).

Study Procedure

This study was reviewed and approved by an ethics committee before its commencement. The study was registered on ClinicalTrials.gov (NCT05130905). The study started on September 1, 2017, and ended on December 20, 2017. The participants were employees of 4Life or friends or family of 4Life employees. They were screened using a health questionnaire to determine eligibility. Participants were required to be in self-assessed good health with no diagnosis of any disease. Participants were between 18 and 45 years old, non-smokers, not sensitive or allergic to dairy products, and with a BMI between 19 and 30 kg/m^2. Those who had previously taken RS (as early as one month before the first study visit) were excluded. Participants were asked to stop the use of all other supplements before and during the study. In all other respects, they were encouraged to continue with their normal lifestyles. Pregnant and lactating women were excluded from the study.

Once enrolled in the study, participants were instructed to take RS daily for 12 weeks. To ensure data consistency, participants were asked to fast overnight before their study visits. Blood and saliva samples and blood pressure, heart rate, weight, height, and body composition measurements were collected during Visits 1 (Week 0), 2 (Week 4), 3 (Week 8), and 4 (Week 12).

Blood samples were drawn by a certified phlebotomist, prepared, and then analyzed by a Clinical Laboratory Improvement Amendments (CLIA)–certified, independent laboratory (Quest Diagnostics, Draper, Utah). The following components were assessed in sera from the participants: alanine aminotransferase (ALT); aspartate aminotransferase (AST); gamma-glutamyl transpeptidase (GGT); red blood cell count (RBC); hematocrit (HCT); mean cell volume (MCV); mean cell hemoglobin (MCH); mean cell hemoglobin concentration (MCHC); red cell distribution width (RDW); mean platelet volume (MPV); monocytes; neutrophils; lymphocytes; eosinophils; basophils; hemoglobin; ferritin; total iron binding capacity (TIBC); iron; transferrin saturation (TS); folate; vitamin B12; calcium; vitamin D; magnesium; red blood cell magnesium; potassium; sodium; creatine kinase (CK); testosterone; free testosterone; dehydroepiandrosterone-sulfate (DHEAS); cortisol; sex hormone binding globulin (SHBG); albumin; glucose; total cholesterol; high-density lipoprotein cholesterol (HDL); low-density lipoprotein cholesterol (LDL); triglycerides; white blood cell count (WBC); high sensitivity C-reactive protein (hsCRP). Secretory immunoglobulin A (sIgA) was measured from the saliva samples collected from the participants.

These biomarkers are generally thought to provide a good assessment of one's overall health, including liver, brain, heart, bone, and muscle health, glucose and lipid metabolism, inflammation, and immunity.

Data Analysis

The results were analyzed using two-tailed, paired t-tests. To control for multiple comparisons, we limited the reporting of statistical differences to only those items that had a *P* value of ≤.01.

RESULTS

Recruitment and Dropout

Fifty volunteers expressed an interest in participating in the study and were screened upon consent. A total of twenty participants were determined to be eligible for the study and completed Visit 1. The results from Visit 1 showed that four participants presented high blood iron levels. The study product contains iron, thus, for safety reasons, these four participants were excluded from the study and were recommended to visit a physician. Two participants did not show up for Visit 2. One participant did not show up for Visit 3. Therefore, the results are based on data from 13 participants (six women and seven men) who completed the study.

Participants Characteristics

The participants had an average age of 30.9 years and an average BMI of 27.3 kg/m^2 at the commencement of the study. Body composition, blood pressure, and heart rates were measured before and after the supplementation period. There were no statistically significant differences in these parameters before and after the supplementation period. It should be noted that the body weight, fat percentage, and fat mass of participants showed moderate increases during the supplementation period though such increases were not considered statistically significant. These data are presented in Table 1.

The data of men and women were stratified and analyzed separately. No significant differences were observed between men and women. Therefore, all data – except testosterone (men and women), free testosterone (men), dehydroepiandrosterone-sulfate (DHEAS, women), and SHBG – were presented by combining data from men and women.

Salivary Biomarker Analysis

Salivary IgA levels of participants showed a significant increase in Week 4 (249.85 ± 95.63 ng/ml), Week 8 (271.65 ± 133.52 ng/ml), and Week 12 (279.88 ± 128.19 ng/ml) in comparison to Week 0 (177.57 ± 74.81 ng/ml) (Table 2).

Serum Biomarkers Analysis

The results of the serum biomarkers analysis are summarized in Table 3. The ALT, AST, and GGT levels of participants showed no significant change during the supplementation period.

Most CBC measures–including HCT, MCH, MCHC, platelets, MPV, monocytes, neutrophils, lymphocytes, eosinophils, and basophils–showed no significant change before and after the supplementation period. RBC showed a small but significant decrease after the supplementation period (Week 12: 5.18 ± 0.48 10^6/μL versus Week 0: 5.34 ± 0.50 10^6/μL, $P = .0088$). MCV showed a small but significant increase (Week 12: 89.98 ± 4.50 fL versus Week 0: 87.32 ± 4.10 fL, $P = .0011$). RDW showed a small but significant increase (Week 12: 13.68 ± 1.00 % versus Week 0: 13.08 ± 0.74 %, $P = .0013$).

Table 1. Participants' characteristics before Week 0 and after Week 12 of the study

	Week 0	Week 12	Paired *t* Test
Study Size	13		
Men	7		
Women	6		
Ethnicity	1 Asian, 8 Hispanic, 4 White		
Age (years)	30.9 ± 8.4	30.9 ± 8.4	
Height (inch)	66.5 ± 5.1	66.5 ± 5.1	
Weight (lb)	166.7 ± 35.9	168.4 ± 35.6	.074
BMI (kg/m^2)	27.0 ± 4.5	27.3 ± 4.4	.074
Fat (%)	25.8 ± 12.3	26.8 ± 11.2	.029
FM (lb)	43.3 ± 24.6	45.3 ± 23.2	.016
FFM (lb)	123.5 ± 33.6	123.1 ± 32.4	.719
TBW (lb)	90.4 ± 24.6	90.1 ± 23.7	.676
TFAT (%)	24.0 ± 12.0	25.5 ± 10.3	.019
TFM (lb)	22.2 ± 13.8	23.8 ± 12.5	.016
TFFM (lb)	68.5 ± 18.1	68.0 ± 17.2	.336
SBP (mmHg)	120.0 ± 11.5	119.2 ± 10.3	.621
DBP (mmHg)	80.4 ± 6.2	79.6 ± 7.9	.683
HR (bpm)	65.9 ± 12.1	66.0 ± 8.1	.981

Note: Data were presented as mean ± standard deviation (SD).

Abbreviations: BMI, body mass index; FM, fat mass; FFM, fat free mass; TBW, total body water; TFAT, trunk fat; TFM, trunk fat mass; TFFM, trunk fat free mass; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; bpm, beat per minute.

Table 2. Secretory Immunoglobulin A (sIgA) levels at Weeks 0, 4, 8, and 12

	Week 0	Week 4	Week 8	Week 12
sIgA (ng/ml)	177.57 ± 74.81	249.85 ± 95.63	271.65 ± 133.52	279.88 ± 128.19
P value	n/a	.003	.012	.010

Note: Data were presented as mean ± SD, *P* values and differences compared to Week 0.

Folate levels showed a significant increase after the supplementation period (Week 12: 23.56 ± 5.75 ng/mL versus Week 0: 15.88 ± 3.40 ng/mL, $P = .0001$). At Week 8, folate levels showed an even bigger increase (Week 8: 26.27 ± 11.33 ng/mL versus Week 0: 15.88 ± 3.40 ng/mL, $P = .0001$). B12 levels also showed a big but insignificant increase after the 12-week supplementation (789.38 ± 313.23 pg/mL versus 650.54 ± 228.02 pg/mL, $P = .0690$). Hemoglobin, ferritin, TIBC, iron, and TS showed no significant change before and after the supplementation period.

Vitamin D levels showed an increase over the course of the study (Week 12: 32.22 ± 5.81 ng/mL versus Week 0: 29.54 ± 11.30 ng/mL, $P = .3570$). Calcium levels were not impacted significantly. Magnesium levels showed a small but significant

Table 3. Serum Biomarkers at Weeks 0, 8, and 12

	Week 0	Week 8		Week 12	
Biomarker (unit)	**Mean ± SD**	**Mean ± SD**	***P* value**	**Mean ± SD**	***P* value**
ALT (U/L)	27.08 ± 19.03	26.08 ± 18.00	.745	28.46 ± 22.10	.781
AST (U/L)	21.62 ± 10.60	20.00 ± 10.11	.298	21.77 ± 14.11	.952
GGT (U/L)	19.85 ± 9.58	21.85 ± 11.03	.018	21.31 ± 10.09	.153
RBC (10^6/μL)	5.34 ± 0.50	5.18 ± 0.41	.005	5.18 ± 0.48	.008
Hematocrit (%)	46.65 ± 4.57	48.02 ± 3.41	.008	46.52 ± 4.31	.812
MCV (fL)	87.32 ± 4.10	93.00 ± 4.83	.000	89.98 ± 4.50	.001
MCH (pg)	29.54 ± 1.67	29.92 ± 1.36	.080	29.96 ± 1.44	.146
MCHC (g/dL)	33.83 ± 0.54	32.22 ± 0.84	.000	33.36 ± 0.89	.161
RDW (%)	13.08 ± 0.74	13.85 ± 1.09	.002	13.68 ± 1.00	.001
PLT (thousands/uL)	269.31 ± 74.53	264.23 ± 55.85	.705	259.69 ± 59.29	.444
MPV (fL)	10.25 ± 1.05	10.85 ± 0.91	.000	10.26 ± 1.42	.976
Neutrophils (cells/μL)	3645.15 ± 1207.17	3453.85 ± 1016.21	.647	3586.23 ± 1422.47	.889
Basophils (cells/μL)	40.92 ± 13.09	42.31 ± 14.23	.737	39.23 ± 16.74	.661
Eosinophils (cells/μL)	140.23 ± 91.74	149.23 ± 58.66	.649	141.08 ± 74.26	.969
Lymphocytes (cells/μL)	2309.46 ± 562.44	2461.54 ± 335.51	.228	2364.85 ± 437.71	.692
Monocytes (cells/μL)	502.92 ± 125.88	523.08 ± 123.52	.501	504.00 ± 148.93	.954
Neutrophils (%)	53.95 ± 9.37	51.28 ± 6.38	.420	52.77 ± 8.71	.683
Eosinophils (%)	2.22 ± 1.59	2.38 ± 1.22	.682	2.24 ± 1.27	.954
Basophils (%)	0.62 ± 0.16	0.65 ± 0.22	.625	0.60 ± 0.24	.759
Lymphocytes (%)	35.58 ± 8.44	37.64 ± 5.10	.493	36.46 ± 7.10	.738
Monocytes (%)	7.64 ± 1.92	7.82 ± 1.53	.476	7.80 ± 2.24	.476
Hemoglobin (g/dL)	15.78 ± 1.66	15.48 ± 1.36	.067	15.52 ± 1.60	.044
Ferritin (ng/mL)	101.46 ± 106.08	100.77 ± 88.78	.951	88.08 ± 86.00	.250
TS (%)	31.46 ± 11.22	31.00 ± 12.14	.897	27.69 ± 8.27	.139
Iron (ug/dL)	117.31 ± 41.54	113.31 ± 41.17	.757	101.31 ± 29.33	.089
TIBC (ug/dL)	384.69 ± 72.78	372.31 ± 49.47	.194	369.38 ± 43.18	.227
Folate (ng/mL)	15.88 ± 3.47	26.27 ± 11.33	.009	23.56 ± 5.75	.000
Vitamin B12 (pg/mL)	650.54 ± 228.02	650.92 ± 205.16	.994	789.38 ± 313.23	.069
Vitamin D (ng/mL)	29.54 ± 11.30	31.69 ± 6.41	.288	32.22 ± 5.81	.357
Calcium (mg/dL)	9.67 ± 0.45	9.78 ± 0.46	.154	9.62 ± 0.44	.569
Magnesium (mg/dL)	2.14 ± 0.19	2.02 ± 0.11	.005	2.00 ± 0.14	.003
RBC Mg (mg/dL)	5.41 ± 0.45	4.77 ± 0.37	.000	4.48 ± 0.84	.004
Potassium (mmol/L)	4.11 ± 0.19	4.15 ± 0.20	.536	4.00 ± 0.23	.126
Sodium (mmol/L)	139.08 ± 1.26	136.77 ± 1.92	.000	138.31 ± 2.02	.096
CK (U/L)	149.46 ± 151.74	131.15 ± 99.66	.531	158.31 ± 161.69	.471
Testosterone (ng/dL)	288.23 ± 281.48	274.38 ± 249.12	.650	242.54 ± 218.41	.247
Men, n = 7	512.29 ± 177.06	472.00 ± 158.84	.492	424.29 ± 107.99	.242
Women, n = 6	26.83 ± 16.07	43.83 ± 14.40	.007	30.50 ± 17.24	.234
Free testosterone (ng/dL) Men only, n = 7	9.17 ± 3.69	7.53 ± 2.95	.196	6.70 ± 2.05	.150
Cortisol (μg/dL)	10.31 ± 3.72	11.65 ± 3.81	.213	10.32 ± 3.74	.988
DHEAS (μg/dL) Women only, n = 6	145.00 ± 89.37	115.50 ± 71.26	.013	132.67 ± 67.55	.333
SHBG (nmol/L)	27.92 ± 6.17	40.69 ± 9.01	.000	36.77 ± 11.79	.003
Men, n = 7	29.43 ± 6.00	42.14 ± 9.08	.000	39.43 ± 8.81	.003
Women, n = 6	26.17 ± 6.43	39.00 ± 9.47	.000	33.67 ± 14.80	.182
Albumin (g/dL)	4.77 ± 0.26	4.62 ± 0.28	.004	4.57 ± 0.28	.001
Fasting glucose (mg/dL)	77.69 ± 7.11	93.69 ± 5.95	.000	94.08 ± 6.92	.000
Cholesterol (mg/dL)	192.62 ± 29.11	192.38 ± 27.61	.967	194.08 ± 29.51	.816
HDL (mg/dL)	43.85 ± 8.32	44.00 ± 9.14	.902	45.85 ± 9.75	.134
LDL (mg/dL)	121.31 ± 23.54	111.31 ± 20.79	.177	113.62 ± 19.47	.104
Triglycerides (mg/dL)	160.85 ± 78.33	198.23 ± 166.65	.353	177.62 ± 110.40	.524
WBC (thousands/uL)	6.64 ± 1.21	6.61 ± 1.28	.935	6.64 ± 1.50	1.000
hsCRP (mg/L)	1.88 ± 1.98	2.26 ± 2.25	.446	2.07 ± 2.31	.698

Note: Data were presented as Mean ± SD, *P* values and differences compared to Week 0.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase; RBC, red blood cell count; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; RDW, red cell distribution width; MPV, mean platelet volume; TIBC, total iron binding capacity; TS, transferrin saturation; RBC Mag, red blood cell magnesium; CK, creatine kinase; DHEAS, dehydroepiandrosterone-sulfate; SHBG, sex hormone binding globulin; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; WBC, white blood cell count; hsCRP, high sensitivity C-reactive protein.

decrease after the supplementation period (Week 12: 2.00 ± 0.14 mg/dL versus Week 0: 2.14 ± 0.19 mg/dL, $P = .0036$). RBC-magnesium levels also decreased after the supplementation period (Week 12: 4.48 ± 0.84 mg/dL versus Week 0: 5.41 ± 0.45 mg/dL, $P = .0041$). Potassium and sodium levels were not impacted significantly by supplementation.

CK, testosterone, free testosterone, DHEAS, and cortisol levels were not impacted significantly by RS supplementation. SHBG showed a significant increase after supplementation (Week 12: 36.77 ± 11.79 nmol/L versus Week 0: 27.92 ± 6.17 nmol/L, $P = .0034$) but was still within the normal ranges (women: 18–144 nmol/L, men: 10–57 nmol/L). Albumin saw a small but significant decrease after supplementation (Week 12: 4.57 ± 0.28 g/dL versus Week 0: 4.77 ± 0.26 g/dL, $P = .0016$) but was still within the normal range (3.4 g/dL–5.4 g/dL).

Total cholesterol, HDL, LDL, and triglyceride levels showed no significant changes before and after supplementation. Glucose levels showed a significant increase after the supplementation period (Week 12: 94.08 ± 6.92 mg/dL versus Week 0: 77.69 ± 7.10 mg/dL, $P = .0000$). WBC and hsCRP levels were not significantly impacted by RS supplementation.

DISCUSSION

Safety and Tolerability of RS

No adverse events were reported during the 12 weeks of RS supplementation or within 1 month after the study closure, demonstrating the product's good safety and tolerability.

ALT, AST, and GGT are important biomarkers for liver function and are commonly used for safety assessments.[9] RS did not significantly impact ALT, AST, or GGT levels, indicating its good safety and tolerability.

Effect of RS on the CBC

CBC is a blood test that is commonly used to evaluate one's overall health and detect a wide range of disorders including anemia, infection, and leukemia. RS supplementation did not significantly affect most CBC components such as hemoglobin, HCT, MCH, MCHC, platelets, MPV, monocytes, neutrophils, lymphocytes, eosinophils, or basophils, further supporting the good safety and tolerability of RS. RS supplementation did not significantly affect serum levels of ferritin, TIBC, iron, or TS, which are important biomarkers for oxygen and iron status.

Effect of RS on Red Blood Cells

RBC, HCT, and hemoglobin are closely related as they each measure different aspects of red blood cells.[10] Higher than normal RBC levels (erythrocytosis) or high hemoglobin or HCT levels could point to an underlying medical condition such as polycythemia vera or heart disease. In contrast, if these three measures are lower than normal, it might be an indication of anemia, which often causes fatigue and weakness.[11] RS supplementation did not significantly affect HCT or hemoglobin levels, which were already within normal healthy ranges. It is noteworthy that RS supplementation slightly decreased RBC (Week 12: 5.18 ± 0.48×10^6/μL versus Week 0: 5.34 ± 0.50×10^6/μL) and brought it closer to the optimal level (women: 3.8×10^6/μL–5.1×10^6/μL, men: 4.2×10^6/μL–5.8×10^6/μL), suggesting that it has a positive effect on the health of red blood cells.

MCV is a measure of the average volume of red blood cells and is an important indicator of their health and how well they carry oxygen. RDW measures variations in the size and volume of red blood cells. Since RDW is calculated using MCV, the two measures are closely related and can provide insights into the health of red blood cells.[12] RS increased MCV (89.98 ± 4.50 fL versus 87.32 ± 4.10 fL, $P = .0011$) and RDW (13.68 ± 1.00 % versus 13.08 ± 0.74 %, $P = .0013$), further indicating a potential positive effect on the health of red blood cells.

RS significantly improved the serum folate levels of the participants (48.3% increase at Week 12: 23.56 ± 5.75 ng/mL versus Week 0: 15.88 ± 3.40 ng/mL, $P = .0001$). RS improved vitamin B12 serum levels by 21.3% (Week 12: 789.38 ± 313.23 pg/mL versus Week 0: 650.54 ± 228.02 pg/mL, $P = .0690$), though the improvement was not considered statistically significant. RS also improved the vitamin D levels of the participants by 9.1% (Week 12: 32.22 ± 5.81 ng/mL versus Week 0: 29.54 ± 11.30 ng/mL, $P = .3570$). Although this increase appears moderate and statistically insignificant, its impact can be robust considering that vitamin D levels in the human body tend to drop towards the onset of fall and be lowest in winter, primarily due to less exposure to sunlight.[13,14] The study was conducted in Salt Lake City, Utah, which is situated along the 40th parallel. The study started in early September and ended in late December. These results can be partially explained by the fact that RS delivers adequate amounts of vitamin D (3000 IU), folate (800 mcg), and vitamin B12 (18 mcg) daily.

In addition, folate and vitamin B12 are necessary to produce healthy red blood cells, specifically to ensure the adequate synthesis of DNA and RNA and the integrity of DNA. Researchers have long hypothesized that vitamin D levels not only impact the absorption of folate and vitamin B12 but also red blood cell parameters. Doudin and colleagues found that serum vitamin D was positively correlated with the MCV of red blood cells ($r = 0.08$, $P < .001$) but negatively correlated with RBC.[15] Another recent clinical study found that serum vitamin D levels are positively associated with folate and B12 levels in adolescents.[16] The results from this study are consistent with these hypotheses and studies conducted by other researchers.

Studied together, these results show that RS improves the size and health of red blood cells, at least partially by increasing serum levels of vitamin D, folate, and B12. The improved size and health of red blood cells are important for the overall health and function of the human body because these cells carry oxygen throughout the whole system. Healthy red blood cells are especially critical for brain health as the brain consumes a disproportionally high amount of oxygen and energy. Improved levels of vitamin D, folate, and

vitamin B12 are also important factors for brain, cardiovascular, and heart health. Therefore, this study suggests that RS supplementation might improve overall health and, likely, brain, cardiovascular, and heart health, by improving serum levels of vitamin D, folate, and vitamin B12 and, thus, the size and health of red blood cells.

Effect of RS on Bone and Muscle Health

Among the many roles vitamin D plays in the body, promoting bone and muscle health is prominent.[17] It promotes calcium absorption in the gut and helps maintain adequate serum calcium and phosphate concentrations to enable normal bone mineralization and helps prevent hypocalcemic tetany. It is also critical for bone growth and bone remodeling by osteoblasts and osteoclasts. Vitamin D sufficiency prevents rickets in children and osteomalacia in adults. Minerals such as calcium and magnesium are also important nutrients for optimal bone health. Potassium and sodium are important electrolytes for optimal muscle function.

RS supplementation did not significantly impact serum levels of calcium, potassium, and sodium, which were already within normal ranges. Unexpectedly, RS supplementation decreased magnesium levels in participants (Week 12: 2.00 ± 0.14 mg/dL versus Week 0: 2.14 ± 0.19 mg/dL, $P = .0036$), but these levels were still within the normal range (1.7 mg/dL–2.5 mg/dL).

Assessing the true magnesium status is difficult, as most of the magnesium found in our body is inside cells or bones. The total serum magnesium concentration indicates the level of readily available magnesium, but it is not the best option to evaluate magnesium status, as changes in serum protein concentrations may affect the total concentration without necessarily affecting the ionized fraction or total body magnesium status. Therefore, the correlation between serum total magnesium and the total body magnesium status is poor.[18,19] Red cell magnesium concentration can be determined easily but does not seem to correlate well with total body magnesium status or with other measures of magnesium status.[20] The magnesium tolerance test has been used for many years, and it appears to be an accurate means of assessing magnesium status,[21] and, thus, can be a reliable method for measuring magnesium status in follow-up studies.

RS did not significantly affect CK, testosterone, free testosterone, DHEAS, and cortisol levels, suggesting the overall good safety and tolerability of RS for daily use. Albumin saw a small but significant decrease after supplementation (Week 12: 4.57 ± 0.28 g/dL versus Week 0: 4.77 ± 0.26 g/dL, $P = .0016$) but was still within the normal range (3.4 g/dL–5.4 g/dL).

SHBG is a protein that transports sex hormones, including testosterone, throughout the body, and which impacts the bioavailability of testosterone. Though RS did not impact testosterone levels, it significantly increased SHBG levels (Week 12: 36.77 ± 11.79 nmol/L versus Week 0: 27.92 ± 6.17 nmol/L, p = 0.0034) but within the normal range (women: 18 nmol/L–144 nmol/L, men: 10 nmol/L–57 nmol/L). An abnormally high SHBG level is correlated with lower bone mineral density, increased risk of osteoporosis/osteopenia, and fracture risk.[22,23,24] Further investigation is needed to confirm the impact of RS on SHBG and its subsequent effects on bone and muscle health.

These results suggest that RS supplementation might improve overall bone and muscle health, partially by improving the vitamin D levels of the participants.

Effect of RS on Glucose and Lipid Metabolism

RS did not significantly impact total cholesterol, HDL, LDL, and triglyceride levels. Unexpectedly, RS increased fasting glucose levels. The increase in the glucose level is likely a result of the increased body weight, fat mass, fat percentage, and, thus, the increased BMI of participants during the study duration, as it has been shown that the fasting glucose level is positively correlated with BMI,[25] fat mass,[26] and fat percentage.[27] Ideally, if participants had followed the study instructions and maintained their lifestyles, including eating and exercise routines, their body weight and body composition would have remained stable during the study period.

Further, it should be noted that the study began in September and ended in December, during winter in the study location (Salt Lake City). It is well documented that people tend to gain weight and fat mass during the winter holiday months,[28] mostly due to an increase in food intake either unconsciously or subconsciously.[29] It has also been observed that glucose, cholesterol, triglyceride, and HbA_{1c} levels are significantly higher in the winter months.[30] Despite study participants gaining weight and fat during the study period, their lipid profiles remained relatively stable and even demonstrated a slight improvement. RS improved the HDL levels of participants from Week 0 (43.85 ± 8.32) to Week 12 (45.85 ± 9.75) ($P = .134$). Though the improvement is not statistically significant, it suggests that RS supplementation has a moderately positive effect on lipid metabolism.

Effect of RS on Inflammation and Immunity

RS supplementation did not significantly impact WBC and hsCRP levels. However, it significantly improved the salivary IgA levels of participants in Week 4 (249.85 ± 95.63 ng/ml), Week 8 (271.65 ± 133.52 ng/ml), and Week 12 (279.88 ± 128.19 ng/ml) in comparison to Week 0 (177.57 ± 74.81 ng/ml).

The increase in sIgA levels might be a combinatory effect of transfer factor, vitamin D, and other immunity-promoting ingredients present in the MVM. Transfer factor contains extracts from bovine colostrum, which has been shown to improve the sIgA level in athletes.[31,32] A study [unpublished data] demonstrates that daily supplementation of 1200 mg of transfer factor improved the sIgA levels of participants on average by 73% within four weeks. RS delivers 300 mg of transfer factor per serving, and the impact of RS

supplementation on sIgA at Week 4 is largely consistent with the results from a prior study. RS also has a sufficient amount of vitamin D (3000 IU), which was shown to significantly improve sIgA level.[33]

IgA is the most abundant antibody in human saliva[34] and is associated with stronger immunity, better oral health, and other aspects of health.[35,36] Mucosal immunity, including sIgA as the major antibody, plays an important role in early defenses against respiratory pathogens[37] and improves resistance to respiratory infections. Therefore, RS may improve mucosal immunity by improving oral salivary IgA levels.

Salivary IgA is a valid and reliable point-of-care test (POC) biomarker to indicate a person's immunity status in just a few minutes.[38, 39] Saliva sample collection is non-invasive, quick, painless, and convenient. Collecting saliva samples is possible any time, day or night, because of the overall convenience and can be accomplished in circumstances where blood collection is difficult or inadvisable. Considering the validity of sIgA level as a POC biomarker of immunity, the convenience of sample collection, and the significant impact of RS supplementation on sIgA, it is worth exploring the incorporation of POC technology into one's daily RS supplementation and even one's lifestyle management. Such an incorporated technology could guide people's dietary and behavioral habits for improved immunity and better health, since the test results can be measured and reported in real-time, and, thus, can be acted upon immediately.

Limitations

The present study had multiple limitations. The participants were employees, family, or friends of 4Life employees and, as such, this may represent a bias towards the study product. It was an open-label, exploratory clinical trial and lacked a formal placebo-controlled group. The study had a small subject size of 13 and lasted only 12 weeks. The fact that the study started in early September and ended in late December might have contributed to the confounding of some of the study results, to some extent. A longer, better designed, randomized, placebo-controlled, and double-blinded study will likely provide more useful and conclusive answers to the specifics of RS supplementation efficacy.

CONCLUSION

This study shows that RS has a good safety and tolerability profile and can be safely used as a daily nutritional supplement. The study also indicates that RS may moderately improve serum levels of vitamin D, folate, vitamin B12, and, possibly, other blood biomarkers. The study also shows that RS may improve the level of secretory IgA. These findings suggest an overall improvement in several aspects of health and should be confirmed in a more extensive, placebo-controlled study.

AUTHORS' DISCLOSURE STATEMENT

The authors are employees of 4Life Research, LLC, Sandy, Utah.

REFERENCES

1. Bailey RL, Gahche JJ, Lentino CV, et al. Dietary supplement use in the United States, 2003–2006. *J Nutr*. 2011;141(2):261–266. doi: 10.3945/jn.110.133025
2. Murphy SP, White KK, Park SY, Sharma S. Multivitamin-multimineral supplements' effect on total nutrient intake. *Am J Clin Nutr*. 2007;85(1):280S–284S. doi: 10.1093/ajcn/85.1.280S
3. Han X, Eggett DL, Parker TL. Evaluation of the health benefits of a multivitamin, multimineral, herbal, essential oil-infused supplement: a pilot trial. *J Diet Suppl*. 2018;15(2):153–160. doi: 10.1080/19390211.2017.1331943
4. Gunasekara P, Hettiarachchi M, Liyanage C, Lekamwasam S. Effects of zinc and multimineral vitamin supplementation on glycemic and lipid control in adult diabetes. *Diabetes Metab Syndr Obes*. 2011;4:53–60. doi: 10.2147/DMSO.S16691
5. Gaziano JM, Sesso HD, Christen WG, et al. Multivitamins in the prevention of cancer in men: the physicians' health study II randomized controlled trial [published correction appears in JAMA. 2014 Aug 6;312(5):560]. *JAMA*. 2012;308(18):1871–1880. doi: 10.1001/jama.2012.14641
6. Bagwe S, Tharappel LJ, Kaur G, Buttar HS. Bovine colostrum: an emerging nutraceutical. *J Complement Integr Med*. 2015;12(3):175–85. doi: 10.1515/jcim-2014-0039
7. Menchetti L, Traina G, Tomasello G, et al. Potential benefits of colostrum in gastrointestinal diseases. *Front Biosci (Schol Ed)*. 2016;8(2):331–351. doi: 10.2741/s467
8. Główka N, Woźniewicz M. Potential use of colostrum bovinum supplementation in athletes - a review. *Acta Sci Pol Technol Aliment*. 2019;18(2):115–123. doi: 10.17306/J.AFS.0654
9. Pastori D, Pani A, Di Rocco A, et al. Statin liver safety in non-alcoholic fatty liver disease: a systematic review and metanalysis. *Br J Clin Pharmacol*. 2022;88(2):441–451. doi: 10.1111/bcp.14943
10. Higgins JM. Red blood cell population dynamics. *Clin Lab Med*. 2015;35(1):43–57. doi: 10.1016/j.cll.2014.10.002
11. Ford J. Red blood cell morphology. *Int J Lab Hematol*. 2013;35(3):351–357. doi: 10.1111/ijlh.12082
12. Maner BS, Moosavi L. Mean corpuscular volume. In: *StatPearls* [Internet]. Treasure Island (FL): StatPearls Publishing; July 10, 2021.
13. Thieden E, Philipsen PA, Heydenreich J, Wulf HC. Vitamin D level in summer and winter related to measured UVR exposure and behavior. *Photochem Photobiol*. 2009;85(6):1480–4. doi: 10.1111/j.1751-1097.2009.00612.x
14. Watad A, Azrielant S, Bragazzi NL, et al. Seasonality and autoimmune diseases: the contribution of the four seasons to the mosaic of autoimmunity. *J Autoimmun*. 2017;82:13–30. doi: 10.1016/j.jaut.2017.06.001
15. Doudin A, Becker A, Rothenberger A, Meyer T. Relationship between serum 25-hydroxyvitamin D and red blood cell indices in German adolescents. *Eur J Pediatr*. 2018;177(4):583–591. doi: 10.1007/s00431-018-3092-3
16. Rahman A, Al-Taiar A, Shaban L, Al-Sabah R, Mojiminiyi O. Plasma 25-hydroxyvitamin D is positively associated with folate and vitamin B_{12} levels in adolescents. *Nutr Res*. 2020;79:87–99. doi: 10.1016/j.nutres.2020.06.003
17. Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium. Dietary reference intakes for calcium and vitamin D. Ross AC, Taylor CL, Yaktine AL, Del Valle HB, eds. *Dietary Reference Intakes for Calcium and Vitamin D*. Washington (DC): National Academies Press (US); 2011.
18. Saris NE, Mervaala E, Karppanen H, Khawaja JA, Lewenstam A. Magnesium. An update on physiological, clinical and analytical aspects. *Clin Chim Acta*. 2000 Apr;294(1-2):1-26. doi: 10.1016/s0009-8981(99)00258-2. PMID: 10727669.
19. Fox C, Ramsoomair D, Carter C. Magnesium: its proven and potential clinical significance. *South Med J*. 2001;94(12):1195–1201.
20. Elin RJ, Hosseini JM, Gill JR Jr. Erythrocyte and mononuclear blood cell magnesium concentrations are normal in hypomagnesemic patients with chronic renal magnesium wasting. *J Am Coll Nutr*. 1994;13(5):463–466. doi: 10.1080/07315724.1994.10718435
21. Saris NE, Mervaala E, Karppanen H, Khawaja JA, Lewenstam A. Magnesium. An update on physiological, clinical and analytical aspects. *Clin Chim Acta*. 2000;294(1-2):1–26. doi: 10.1016/s0009-8981(99)00258-2
22. Hoppé E, Bouvard B, Royer M, Audran M, Legrand E. Sex hormone-binding globulin in osteoporosis. *Joint Bone Spine*. 2010;77(4):306–12. doi: 10.1016/j.jbspin.2010.03.011.
23. Cawthon PM, Schousboe JT, Harrison SL, et al. Sex hormones, sex hormone binding globulin, and vertebral fractures in older men. *Bone*. 2016;84:271–278. doi: 10.1016/j.bone.2016.01.009. PMID: 26778261; PMCID: PMC4755786.
24. Jing Y, Wang X, Yu J, et al. Associations of serum sex hormone binding globulin with bone mineral densities and higher 10-year probability of fractures in postmenopausal women with type 2 diabetes mellitus. *Ann Transl Med*. 2019;7(18):457. doi:10.21037/atm.2019.08.46
25. Agrawal N, Agrawal MK, Kumari T, Kumar S. Correlation between body mass index and blood glucose levels in Jharkhand population. *Int J Contemp Med Res*. 2017;4(8):1633-1636.
26. Mehdad S, Hamrani A, El Kari K, et al. Body mass index, waist circumference, body fat, fasting blood glucose in a sample of Moroccan adolescents aged 11-17 years. *J Nutr Metab*. 2012;2012:510458. doi:10.1155/2012/510458

27. Kang TS, Lee WS, Han MK. Correlation between percent body fat and fasting blood sugar in Korean adult women under the age of 40. *Korean J Fam Pract*. 2017;7(3):353–357. https://doi.org/10.21215/kjfp.2017.7.3.353
28. Roberts SB, Mayer J. Holiday weight gain: fact or fiction? *Nutr Rev*. 2000;58(12):378–9. doi: 10.1111/j.1753-4887.2000.tb01839.x
29. Bhutani S, Wells N, Finlayson G, Schoeller DA. Change in eating pattern as a contributor to energy intake and weight gain during the winter holiday period in obese adults. *Int J Obes (Lond)*. 2020;44(7):1586–1595. doi: 10.1038/s41366-020-0562-2
30. Jones AG, McDonald TJ, Hattersley AT, Shields BM. Effect of the holiday season in patients with diabetes: glycemia and lipids increase postholiday, but the effect is small and transient. *Diabetes Care*. 2014;37(5):e98-e99. doi:10.2337/dc13-2353
31. Crooks CV, Wall CR, Cross ML, Rutherfurd-Markwick KJ. The effect of bovine colostrum supplementation on salivary IgA in distance runners. *Int J Sport Nutr Exerc Metab*. 2006;16(1):47-64. doi:10.1123/ijsnem.16.1.47
32. Appukutty M, Radhakrishnan A, Ramasamy K, et al. Salivary immunoglobulin A (sIgA) responses to bovine colostrum supplementation during regular training in physically active young healthy adolescents. *Br J Sports Med*. 2010;44:i44.
33. He CS, Fraser WD, Tang J, et al. The effect of 14 weeks of vitamin D3 supplementation on antimicrobial peptides and proteins in athletes. *J Sports Sci*. 2016;34(1):67-74. doi:10.1080/02640414.2015.1033642
34. Brandtzaeg P. Secretory immunity with special reference to the oral cavity. *J Oral Microbiol*. 2013;5:10. 3402/jom.v5i0.20401. doi: 10.3402/jom.v5i0.20401
35. Costalonga M, Herzberg MC. The oral microbiome and the immunobiology of periodontal disease and caries. *Immunol Lett*. 2014;162(2 Pt A):22–38. doi: 10.1016/j.imlet.2014.08.017
36. Javed F, Akram Z, Binshabaib MS, ALHarthi SS, Kellesarian SV, Vohra F. Is salivary IgA level a potential biomarker for immunosuppression in HIV-positive children? A systematic review and meta-analysis. *Rev Med Virol*. 2017;27(4):10.1002/rmv.1933. doi: 10.1002/rmv.1933
37. Varadhachary A, Chatterjee D, Garza J, et al. Salivary anti-SARS-CoV-2 IgA as an accessible biomarker of mucosal immunity against COVID-19. Preprint. *medRxiv*. 2020;2020.08.07.20170258. doi: 10.1101/2020.08.07.20170258
38. Dunbar J, Hazell G, Jehanli A. Evaluation of a new point of care quantitative reader for salivary analysis in premier league soccer clubs. *Br J Sports Med*. 2015;49:A2–A3.
39. MacDonald LA, Bellinger PM, Minahan CL. Reliability of salivary cortisol and immunoglobulin-A measurements from the IPRO® before and after sprint cycling exercise. *J Sports Med Phys Fitness*. 2017;57(12):1680–1686. doi: 10.23736/S0022-4707.16.06785-2