

Effects on the Immune System of a Three-Month Consumption of an Extremely Diverse Probiotic Yogurt: Decrease of Serum Alpha-N-Acetylgalactosaminidase Activity, Detoxification and Gut Microbiota Normalization

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Abstract: In this study, we describe the changes associated with the consumption of an extremely biodiverse probiotic yogurt in a 55-year-old female from South Vietnam. In August 2019, the subject voluntarily embarked on a three-month nutritional experience and decided to share her results with the goal of advancing scientific knowledge in the field of nutritional health. Consumption of this biodiverse probiotic yogurt was associated with a decrease in serum alpha-N-acetylgalactosaminidase (nagalase) activity, increased elimination of toxic metals and non-metal toxicants, a trend toward normalization of the lipid profile and a trend toward a rebalance of the gut microbiota.

Keywords: Probiotic Yogurt, Nagalase, Detoxification, Toxic Metals, Microbiome

Introduction

We have previously observed and reported in two recent articles (Pacini and Ruggiero, 2019; 2020) that the consumption of an extremely biodiverse probiotic yogurt with a unique microbial composition is associated with detoxification of non-metal toxicants (Blythe *et al.*, 2017), decrease of serum alpha-N-acetylgalactosaminidase (nagalase) activity (Blythe *et al.*, 2017; Carter *et al.*, 2020), decrease of serum C-Reactive Protein (CRP) (Carter *et al.*, 2020), decrease of markers specific for multiple myeloma (Antonucci *et al.*, 2019) and a dramatic decrease of viral load in Hepatitis B (Zunaid *et al.*, 2020).

In this report, we describe the changes associated with consumption of an extremely biodiverse probiotic yogurt in a 55-year-old female from South Vietnam who presented in August 2019 feeling sometimes feverish at night with morning fatigue for several months.

Subject Information

The subject grew up in South Vietnam during the Agent Orange era and left Vietnam at age 17 as part of the "Boat Migration" in the 1970s. Settling in the United States, she earned a college degree in mathematics and computer sciences and has worked in the Chicago area the past 35 years. The subject is a

Buddhist minister, active in her Temple and has several outside business interests. She described hour-long commutes for years to and from downtown Chicago to her alternative medical clinic.

She is not a runner but ran the 2019 Peachtree 10 Km road race in Atlanta on July 4 in 90 degree Fahrenheit weather without incident. She is married and raised three children. As a teenager she suffered from severe nutritional anemia with delayed menses until 17 (hemoglobin reported 6 g/dL requiring hospitalization and units of blood). The subject returned to South Vietnam in 2016 and again in 2018. Upon returning from her last trip, she described vague abdominal symptoms and not feeling well.

She reported mold exposure, a common finding living in the midwestern part of the United States. She also reported a persistent mild dry cough, sneezing and sinus congestion, along with fatigue and a poor sleep cycle.

In 2017 the subject had the flu and was bedridden for 6 weeks. It is worth mentioning that 2017 was a record year for the flu in the United States with a significant number of deaths (Harben *et al.*, 2020). The subject reported few other lifetime illnesses or problems. In August 2019, the subject voluntarily embarked on a three-month nutritional experience that included consumption of an extremely biodiverse probiotic yogurt, as described in the references quoted above.

Study Design and Methodology

The subject provided one of the authors, Dr. JB, selected laboratory studies from 2016 through 2019 to go along with her current studies with the goal of advancing scientific knowledge in the field of nutritional approaches to health. In addition to using the biodiverse probiotic yogurt during her three-month experience, the subject reported taking several supplements including a multi-vitamin, 2000 to 5000 IU vitamin D₃ daily, Omega-3, glucosamine and recently a non-prescription thyroid supplement containing vitamins and minerals to assist her morning fatigue. It is worth noting that the supplements were consumed both before and during the experience. She consumed the probiotic yogurt only during the three-month experience. It is therefore plausible that the observed changes are attributed primarily to the consumption of the probiotic yogurt.

Blood and urine analyses were performed at different times before this study and after the three-month experience. Determination of serum alpha-N-acetylgalactosaminidase (nagalase) activity was performed at the Health Diagnostics and Research Institute (South Amboy, NJ, USA), a consociate of European Laboratory of Nutrients, (Bunnik, The Netherlands); the results are expressed as nMol/mL/min. Blood analyses were performed at Lab Corp (Laboratory Corporation of America) and Quest Diagnostics. Analyses of metal and non-metal toxicants in early morning urine samples were performed at The Great Plains Laboratory Inc. (Lenexa, KS, USA) and were normalized per grams of creatinine. Copies of the original records are conserved at the office of Dr. JB. In this article we describe and discuss only the values that changed during the three-month experience and, for the sake of brevity, we omit discussing the values that did not change.

Consent

Since this is a single case report that does not produce generalizable knowledge, nor is it an investigation of an FDA regulated product, it is accepted that Institutional Review Board (IRB) review is not required for this activity (JHM, 2020). Written informed consent for publication of clinical details and laboratory data was obtained from the subject and it is conserved at the office of Dr. JB.

Results

Serum Lipid Profile

Comprehensive metabolic profile studies by Lab Corp were performed prior to this experience in 2018 and 2019. A lipid profile in July 2018 showed high triglycerides (375 mg/dL; reference range: 30-150 mg/dL), mildly elevated total cholesterol (231 mg/dL: Reference range: <199 mg/dL) and normal blood sugar. Triglyceride values

improved to 232 mg/dL over the three-month experience with the probiotic yogurt and cholesterol fell to 209 mg/dL, only slightly elevated, Fig. 1. These results seem to indicate that consumption of the probiotic yogurt was associated with a trend toward normalization of the lipid profile and are consistent with previous observation concerning probiotic yogurts and serum lipids (Cho and Kim, 2015).

Serum Vitamin D₃

Vitamin D₃ was quite low in 2017 at 15 ng/mL (reference range: 30-100 ng/mL). Levels improved in 2018 to 37 ng/mL and to 43.5 ng/mL in August 2019 and to 100 ng/mL in December 2019 following the three-month experience with the biodiverse probiotic yogurt. It is worth mentioning, however, that, since beginning the experience in 2019, the subject began walking outdoors in the sun 30 to 45 min two to three days per week and consumed a 2000 to 5000 IU vitamin D₃ supplement. It is plausible, therefore, that the normalization of vitamin D₃ values may be ascribed primarily to consumption of the vitamin supplement and exposure to sunlight with possible contribution from the probiotic yogurt (Rizzoli and Biver, 2020).

Serum Nagalase Activity

Serum nagalase activity was elevated prior to the three-month experience at 1.94 nMol/mL/min (reference range: 0.32-0.95 nMol/mL/min). Nagalase activity fell 27.8% after the three-month experience, Fig. 2. Nagalase is a marker for inflammation often found elevated in viral infections, cancer and other chronic conditions (Carter *et al.*, 2020; Yamamoto and Urade, 2005; Yamamoto, 2006). These results are consistent with previous observations (Blythe *et al.*, 2017; Carter *et al.*, 2020); we hypothesize that decrease of serum nagalase activity is due to the presence in the probiotic yogurt of high levels of naturally formed Gc protein-derived Macrophage Activating Factor that binds and neutralizes nagalase (Carter *et al.*, 2020; Pacini *et al.*, 2011).

Toxic Metals in Urine

Studies of toxic metals in urine, before and after intravenous (IV) chelation, demonstrated significant elimination associated with consumption of the probiotic yogurt, Fig. 3.

Cadmium

Cadmium is a ubiquitous metal toxicant that can have adverse effects on the kidneys. The International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) considers cadmium a Group 1 human carcinogen with prolonged exposure usually through the oral route (food or water). Cadmium is typically found in mining, paints, dyes, metallurgy, soils and foods, but specifically mentioned is that it can enter

rice through the soil and water. It is noted in the subject's history that she consumes rice on a daily basis and has managed a garment factory for a number of years where she was exposed to dyes.

We previously demonstrated that cadmium may be responsible for the neurological signs and symptoms of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome and for abnormalities of breast cancer cell-induced angiogenesis (Pacini *et al.*, 2012; 2009). In the

subject, cadmium in the urine exceeded twice the maximum expected value at baseline in August. Pre-provocative (*i.e.*, before IV chelation) baseline levels were high at 2.1 ug/g creatinine (reference interval high: <1 ug/g creatinine). Post-provocative cadmium remained high at 1.7 ug/g creatinine, using the IV chelating agent EDTA, as cadmium was being removed and is suggestive of a fair amount of the metal present in the body of the subject.

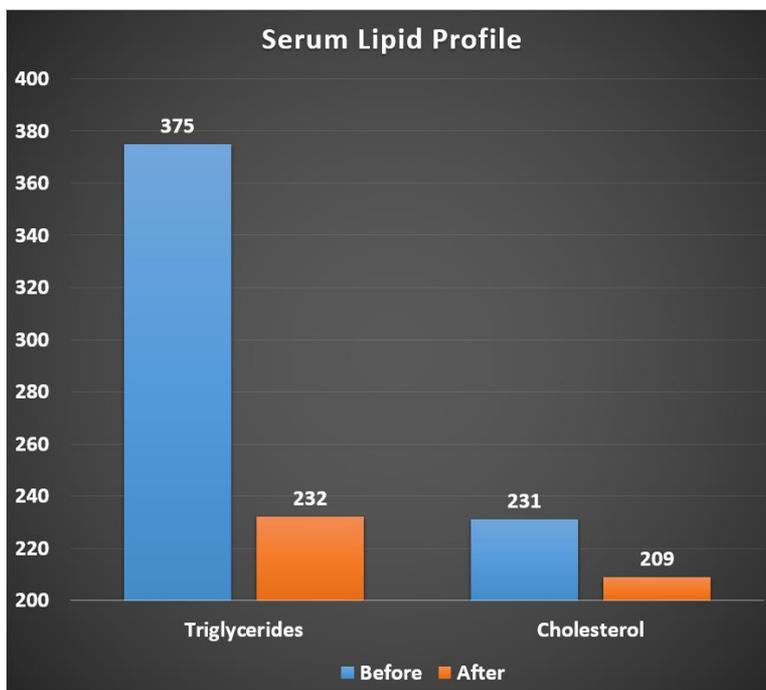


Fig. 1: Serum lipid profile

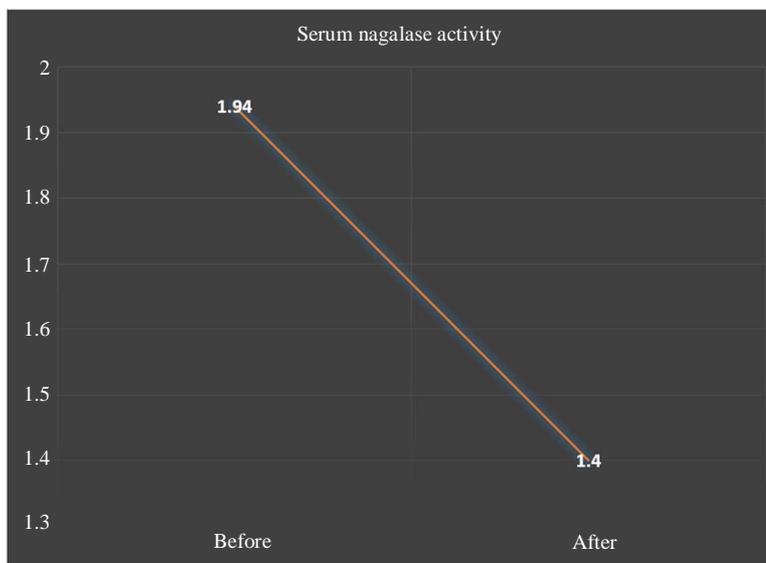


Fig. 2: Serum nagalase activity

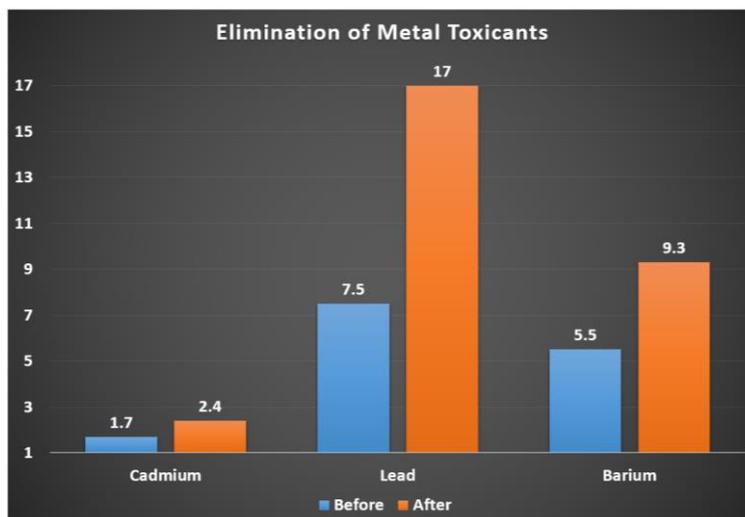


Fig. 3: Elimination of metal toxicants

In December, after completing the three-month experience with the probiotic yogurt, pre-provocative cadmium was 1.8 ug/g creatinine, still high and little changed from post-provocative cadmium in August (1.7 ug/g creatinine). However, after the three-month experience, post-provocative cadmium was 2.4 ug/g creatinine, suggesting increased removal of cadmium, Fig 3. Such a removal could be explained considering that probiotics, such as those present in the product consumed by the subject, inhibit absorption of cadmium by protecting the intestinal barrier (Zhai *et al.*, 2016).

Lead

Lead levels pre-provocative in August were within a reference interval considered low risk at 0.5 ug/g creatinine. Post-provocative lead was high at 7.5 ug/g creatinine (reference range: < 2 ug/g creatinine), over three times the upper limit of the reference interval, suggesting the presence of substantial amounts of lead in the subject's body that chelation was helping remove. In December, after the three-month experience with the probiotic yogurt, pre-provocative lead was again low risk at 0.7 ug/g creatinine, but post-provocation using a chelating agent, urine lead excretion significantly increased to 17 ug/g creatinine, which is 8.5 times the upper limit, considerably higher than in August and suggesting substantial elimination using both the probiotic yogurt and chelation, Fig 3. These results can be interpreted considering that probiotics, such as those present in the product consumed by the subject, are known to protect against lead toxicity (Zhai *et al.*, 2018) and may enhance lead elimination (Yi *et al.*, 2017).

Barium

Barium is another toxic metal whose elimination was enhanced concomitant with consumption of the probiotic yogurt. This metal can interfere with calcium and

potassium metabolism leading to hypokalemia with tingling of the extremities, loss of tendon reflexes and cardiac stimulation. Baseline pre-provocative barium in August was normal at 1.8 ug/g creatinine (reference range: <7 ug/g creatinine); post-provocative barium was 5.5 ug/g creatinine, suggesting some elimination of barium from the body with chelation. In December, after the three-month experience with the probiotic yogurt, pre-provocative barium remained normal at 3 ug/g creatinine. Post-provocative barium however was elevated at 9.3 ug/g creatinine, suggesting further elimination, Fig. 3. The subject remembered swallowing some material before an X-ray a number of years ago, presumably containing barium as a contrast agent, but could not fill in further details. Barium is also found in Brazil nuts, peanuts and peanut butter and certain fast foods and is also found in seaweed and fish, all foods the subject eats. The working hypothesis is that, after the three-month nutritional program with the probiotic yogurt, barium was being eliminated. These results can be interpreted considering that removal of toxic metals is a common feature of probiotics, such as those present in the product consumed by the subject (Monachese *et al.*, 2012).

Non-Metal Toxicants in Urine

Studies of non-metal toxicants in urine, before and after the three-month experience with the biodiverse probiotic yogurt, demonstrated significant changes for a number of toxicants as shown in Table 1 and described in detail for each toxicant.

Glyphosate

Glyphosate is the world's most widely produced herbicide and is found in runoff waters in agricultural areas. The WHO IARC considers glyphosate a probable Group 1 human carcinogen implicated in diseases as non-

Hodgkin lymphoma, renal tubule carcinoma and other pathologies. Glyphosate disrupts the microbiome in the intestine, decreasing the ratio of beneficial to harmful microbes. Consistent with these effects of glyphosate, the subject's stool sample showed a dysbiotic pattern with the absence of the usually present *Lactobacillus* strains.

Glyphosate in the subject's urine showed a moderate presence at 1.35 ug/g creatinine in August. However, glyphosate fell to 0.8 ug/g creatinine, Table 1 and Fig. 4, a 40% decline following the three-month experience with the probiotic yogurt. Beneficial *Lactobacillus* strains were believed added by the probiotic yogurt with the ratio of expected/beneficial flora registering a two-fold increase for *Lactobacillus*. These results can be interpreted considering that removal of non-metal toxicants is a well-established feature of probiotics, such as those present in the product consumed by the subject (Blythe *et al.*, 2017; He *et al.*, 2017).

2-Hydroxyisobutyric Acid

2-Hydroxyisobutyric acid (2HIB) in urine was within the reference values at 4,082 ug/g creatinine. 2HIB is an additive to improve vehicle octane ratings and is found in exhaust fumes. 2HIB has been shown to cause liver and kidney toxicity and cancer in animals. After completing the three-month experience with the probiotic yogurt, urinary excretion of 2HIB rose 94% to 7,912 ug/g creatinine, thus suggesting elimination 2HIB from the body at an increased rate, consistent with well-established features of probiotics.

Perchlorate

Perchlorate (PERC) in urine in August was non-detectable; this result may be interpreted in two ways: (a) The subject had not been exposed to PERC and no PERC had accumulated in her body; (b) the subject was unable to excrete PERC. The first hypothesis seems less plausible because PERC is a ubiquitous pollutant. In addition to being used in rocket fuel and explosives, it is used in fertilizers and ordinary bleach and often found contaminating food and water supplies. The second hypothesis was confirmed by the observation that, after completing the three-month experience with the probiotic yogurt, PERC was being excreted in December at 2.7 ug/g creatinine. Therefore, these results may be interpreted as increased excretion of PERC in association with consumption of the probiotic yogurt.

Table 1: Non-metal toxicants in urine

Toxicant	Before	After
Glyphosate	1.35	0.8
2-hydroxyisobutyric acid	4,082	7,912
Perchlorate	ND	2.7
N-acetyl-S-(2-carbamoyl)ethyl cysteine	22	115
N-acetyl(propyl)cysteine	19	ND
2-hydroxyethyl mercapturic acid	0.55	ND
Diethylphosphate	3.7	ND
3-Phenoxybenzoic acid	2.1	ND

Non-Metal Toxicants in urine are expressed as ug/g creatinine before and after the three-month experience with the probiotic yogurt. ND: Non detectable

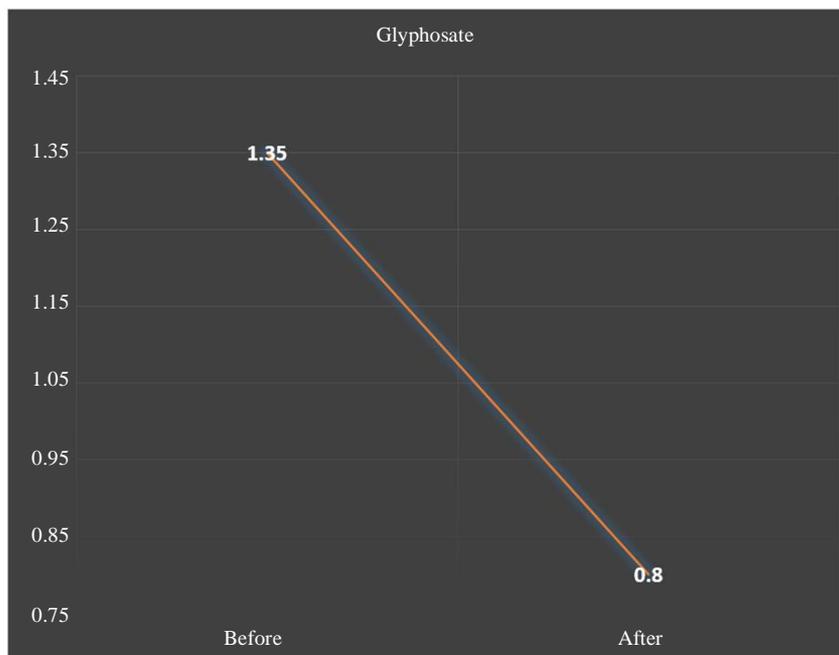


Fig. 4: Glyphosate in urine

N-Acetyl-S-(2-Carbamoylethyl) Cysteine

N-Acetyl-S-(2-carbamoylethyl) cysteine (NAE) in urine was 22 ug/g creatinine in August. Acrylamide is the parent polymer used in industrial processes as plastics, food packaging, cosmetics and dyes. Foods like potato chips and French fries and cigarette smoke exposure are major sources. The toxicity occurs because asparagine, an amino acid important for central nervous system function, can produce acrylamide when cooked at high temperatures in the presence of sugars. High levels of acrylamide can elevate a patient's risk of cancer and cause neurological damage. Foods rich in asparagine include asparagus, potatoes, legumes, nuts, seeds, beef, eggs and fish. After completing her three-month experience with the probiotic yogurt, NAE excretion levels increased substantially to 115 ug/g creatinine, well above the 75th percentile, indicating increased removal of this toxicant.

N-Acetyl (Propyl) Cysteine

N-Acetyl (Propyl) cysteine (NAPR) in urine was moderately elevated in August at 19 ug/g creatinine, well above the 75th percentile. NAPR is an organic solvent and metabolite of 1-bromopropane used in dry cleaning, foam gluing and metal cleaning. It is a neurotoxin associated with sensory and motor deficits, decreased cognitive function and with acute exposures, headaches. After completing the three-month experience with the probiotic yogurt in December, NAPR was no longer detectable, suggesting that substantial removal of the toxicant through urinary excretion had occurred.

2-Hydroxyethyl Mercapturic Acid

A similar situation occurred with 2-Hydroxyethyl Mercapturic Acid (HEMA), a toxicant that was detectable in the subject's urine in August at 0.55 ug/g creatinine. HEMA comes from exposure to ethylene oxide used in agrochemicals, detergents, pharmaceuticals and personal care products. Ethylene oxide is also a sterilizer on rubber, plastics and electronics. Chronic exposure is mutagenic and ethylene oxide is considered a carcinogen with increased incidence of breast cancer and leukemia. HEMA may also develop from exposure to vinyl chloride, an intermediate in the synthesis of these chemicals. These exposures have been associated with autism, headache, dizziness, liver damage and cancer. After completing the three-month experience with the probiotic yogurt in December, HEMA was no longer detectable, suggesting that substantial removal of the toxicant through urinary excretion had occurred.

Diethylphosphate

Diethylphosphate (DEP) in urine was moderately elevated in August at 3.7 ug/g creatinine. Organophosphates such as DEP are among the most

toxic groups of substances in the world and are primarily found in pesticide formulations. 85% of households in United States store at least one pesticide. These cholinesterase inhibitors cause sweating, salivation, diarrhea and both depression and aggressive behavior. Autism spectrum disorder is associated with organophosphate exposure (Sagiv *et al.*, 2018). It may be worth noting that the subject's house was sprayed with pesticides in 2019. After completing the three-month experience with the probiotic yogurt in December, DEP was no longer detectable, suggesting that substantial removal of the toxicant through urinary excretion had occurred.

3-Phenoxybenzoic Acid

3-Phenoxybenzoic Acid (3BPA) in urine was moderately elevated in August at 2.1 ug/g creatinine, well above the 75th percentile. 3BPA is a metabolite of six different pyrethroid insecticides. They affect neurological development, disrupt hormones, induce cancers and suppress the immune system. After completing the three-month experience with the probiotic yogurt in December, 3BPA was no longer detectable, suggesting that substantial removal of the toxicant through urinary excretion had occurred.

Stool Analysis and Microbiota Status

Comprehensive stool analysis showed the absence of beneficial Lactobacillus strains at baseline in August. There were no parasites. Bacteria that are indicators of dysbiosis were present including Klebsiella oxytoca. As described above, toxicology testing found toxicants (Glyphosate) which disrupt the balance of the intestinal microbiota and may have contributed to the observed alterations. After completing the three-month experience with the probiotic yogurt in December, marked improvements were seen with the presence of Lactobacillus strains significantly repopulating the colon in high numbers with positive consequences for immune and other biological functions. Consistent with this observation, Klebsiella oxytoca, an indicator of dysbiosis, significantly decreased after completing the three-month experience.

As far as Short Chain Fatty Acids (SCFA) are concerned, significant results were observed for butyrate that increased from 3.7 to 6.7% of total SCFA after completing the three-month experience, Fig. 5. It is worth noting that butyrate is produced by microbial fermentation in the colon of humans and animals. It is utilized as not only a primary nutrient that is the source of energy for epithelial cells of the colon, but also as a signaling molecule that regulates multiple functions of cells in the colon and other organs by optimizing gene expression, cell differentiation and tissue development, modulation of the immune system and reduction of oxidative stress (Bedford and Gong, 2018).

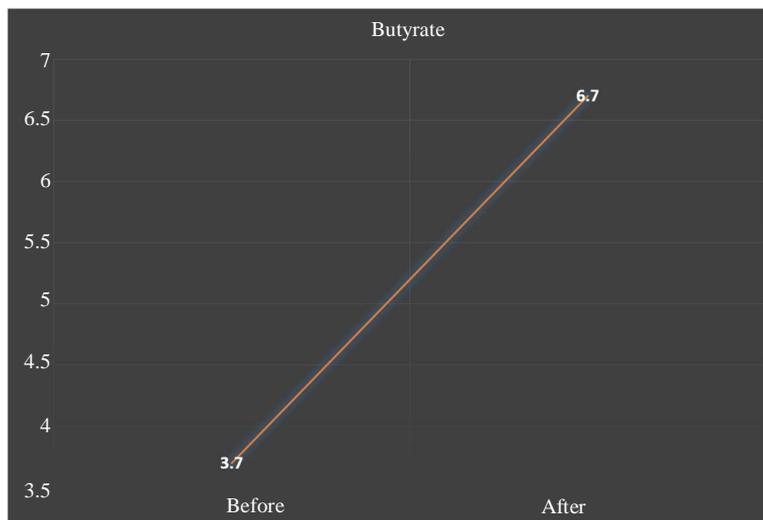


Fig. 5: Butyrate in stool

Discussion

Since this is an open-label, non-controlled, retrospective analysis, caution must be used when ascribing cause and effect to any nutritional approach outcome. However, the response associated with consumption of the probiotic yogurt was robust with regard to nagalase activity reduction, as well as to detoxification and microbiota reconstitution. Despite the short observational time period, the results were substantial and confirm and expand previous observations (Blythe *et al.*, 2017; Antonucci *et al.*, 2019; Carter *et al.*, 2020; Zunaid *et al.*, 2020).

Reduction of serum nagalase activity is consistent with the observation that the extremely biodiverse probiotic yogurt described in this article has high Gc protein-derived macrophage activating factor activity as demonstrated in recent studies (Pacini and Ruggiero, 2020; Carter *et al.*, 2020). In addition to the effects on modulation of macrophage activity, it is worth noting that the probiotic yogurt used in this experience is highly fermented. It is known that the acidity associated with fermentation as well as the proteolytic activity of fermenting probiotics lead to formation of molecules, mainly derived from the hydrolysis of caseins, that have immune-modulatory and anti-oxidant properties (Ebner *et al.*, 2015). These may play an additional role in decreasing the subclinical inflammation associated with the presence of toxicants that in turn might have been responsible for the elevated nagalase activity observed prior to the experience described here. Also, the probiotic yogurt used in this study has the unique peculiarity of fermenting bovine colostrum; colostrum is endowed with intrinsic immune-modulating properties (Shing *et al.*, 2009) and has high levels of vitamin D-binding protein that is the precursor of Gc protein-derived Macrophage Activating Factor (Senda *et al.*, 2011).

Nagalase is considered an indicator of cancer cell proliferation, viral infections as well as a marker of systemic inflammation, (Carter *et al.*, 2020; Yamamoto and Urade, 2005; Yamamoto, 2006; Thyer *et al.*, 2013). Since many of the toxicants described in this article are associated with increased cancer risk, immune dysfunction and chronic inflammation, it is tempting to speculate that the decrease of nagalase activity represented a reduction of the risks associated with exposure to those toxicants. In other words, we hypothesize that there are two factors involved in the observed decrease of serum nagalase activity. First is the Gc protein-derived Macrophage Activating Factor activity of the probiotic yogurt, a mechanism thoroughly described in previous studies (Carter *et al.*, 2020; Thyer *et al.*, 2013); second is the removal of toxicants that might have been responsible for elevated nagalase activity (Blythe *et al.*, 2017).

As far as the observed increased urinary excretion of toxic metals and other toxicants is concerned, the present observations extend and expand the results we first described in 2017 (Blythe *et al.*, 2017). In that article, we described increased urinary excretion of lipophilic toxicants associated with intermittent fasting, weight loss and consumption of the probiotic yogurt; in the present article, we are able to add that consumption of this peculiar probiotic yogurt is associated also with increased excretion of toxic metals and non-lipophilic toxicants such as Glyphosate. It is therefore plausible that the detoxifying effect of a biodiverse probiotic yogurt is a general one and is not limited to a particular class of toxicants.

It is well assessed that probiotic strains help against metal toxicity by reducing the amount of toxicants in the liver and kidney and by counteracting alterations in the levels of glutathione peroxidase and superoxide dismutase (Majlesi *et al.*, 2017). In addition, probiotic

strains may help eliminate toxicants by physical binding (Zoghi *et al.*, 2014) as it is the case for lactic acid bacteria in the removal of Aflatoxin B1 (Haskard *et al.*, 2001). Furthermore, the present observations support the hypothesis that detoxification associated with probiotic yogurt consumption may be independent of mobilization of lipophilic toxicants from fat cells as a consequence of intermittent fasting or other weight loss protocols. In our previous article, we had postulated that mobilization of lipophilic toxicants from fat cells as a consequence of weight loss protocols was pre-requisite for their urinary excretion and, in that context, the probiotic yogurt helped by increasing the rate of urinary excretion (Blythe *et al.*, 2017). However, the subject described in this article did not lose any weight. Her body weight remained constant during the three-month experience. Therefore, we can deduce that consumption of the probiotic yogurt mentioned in this study helps with detoxification of all types of toxicants without the need of any further nutritional intervention.

The observed trend toward normalization of the gut microbiota and correction of dysbiosis is a well-known phenomenon associated with consumption of probiotics (Bull and Plummer, 2015). In the case reported in this article, it may be argued that a reduction of Glyphosate,

a known disruptor of the healthy gut microbiota, contributed to the trend toward normalization.

Conclusion

The experience of the subject suggests that the consumption of an extremely biodiverse probiotic yogurt is associated increased urinary excretion of toxicants, modulation of the immune system and rebalance of the gut microbiota with overall positive effects on health. The authors wish to emphasize that the data in this case report are from one individual and not part of a larger study even though they confirm and expand the results observed in other, unrelated, articles (Blythe *et al.*, 2017; Carter *et al.*, 2020; Antonucci *et al.*, 2019; Zunaid *et al.*, 2020).

It is worth noticing, however, that the observations described in this study are consistent with a number of other observations where the same probiotic yogurt, integrated in with specific therapies as a complementary nutritional support, yielded similar effects with particular reference to supporting immune system function. Table 2 shows the number of subjects observed in each of those studies starting from 2011.

Table 2: Studies and subject

Title	Number	Reference
Macrophages of the Mucosa-Associated Lymphoid Tissue (MALT) as key elements of the immune response to vitamin D binding protein-macrophage activating factor.	4	Pacini <i>et al.</i> (2011 It J Anat Embryol (2011) 116(S1):136.
Clinical experience of cancer immunotherapy integrated with oleic acid complexed with de-glycosylated vitamin D binding protein.	7	Emma Ward <i>et al.</i> Am J Immunol (2014) 10(1):23-32.
Oleic acid, deglycosylated vitamin D-binding protein, nitric oxide: A molecular triad made lethal to cancer.	2	Ruggiero <i>et al.</i> Anticancer Res (2014) 34:3569-3578.
Effects of Pre-Surgical vitamin D supplementation and ketogenic diet in a patient with recurrent breast cancer.	1	Branca <i>et al.</i> Anticancer Res (2015) 35:5525-5532.
Clinical observation of a novel, complementary, immunotherapeutic approach based on ketogenic diet, chondroitin sulfate, vitamin D3, oleic acid and a fermented milk and colostrum product.	6	Schwalb <i>et al.</i> (2016 Am J Immunol (2016) 12(4):91-98.
Intermittent fasting and probiotic yogurt consumption are associated with reduction of serum alpha-N-acetylgalactosaminidase and increased urinary excretion of lipophilic toxicants.	1	Blythe <i>et al.</i> Madr J Immunol (2017) 1(1):23-27.
Use of an extremely biodiverse probiotic and supplement based on microbial chondroitin sulfate is associated with a significant decrease of serum free kappa light chains as well as a trend toward normalization of kappa/lambda ratio and of plasma cell bone marrow infiltration in a case of multiple myeloma.	1	Antonucci <i>et al.</i> Am J Immunol (2019) 15(1):5-9.
Significance of hydrophobic and charged sequence similarities in sodium-bile acid cotransporter and vitamin D-binding protein macrophage activating factor.	1	Zunaid <i>et al.</i> bioRxiv (2020)
Consumption of an Extremely Biodiverse Probiotic and a Supplement based on Microbial Chondroitin Sulfate is Associated with Very Low Serum Alpha-N-acetylgalactosaminidase (Nagalase) Activity and Decrease of C-reactive Protein Values	1	Carter <i>et al.</i> Am J Immunol (2020) 16(1):8-18.

Scientific articles describing the effects observed in subjects who consumed the extremely diverse probiotic yogurt described in this study. The probiotic yogurt was consumed in these studies as part of a complementary nutritional approach in the context of integrated strategies using specific therapies as described in each article. Number refers to the number of subjects observed in each study.

Without doubt, the observations described here can be classified as anecdotes. Anecdotes, or informal stories, once were the primary instrument for the advancement of medical knowledge; however, in recent times, anecdotes have less standing. According to this interpretation of the process of knowledge in medicine today, controlled experiments such as randomized controlled trials have standing and provide medical knowledge while anecdotes do not share the same standing (Nunn, 2011). However, the authors share the convincing argument of the philosopher of science, (Nunn, 2011) when he challenged the deprecation of anecdotes in medicine by positing that anecdotes contribute to the advancement of knowledge in medicine with the same significance of randomized controlled trials with impressive statistics or systematic reviews because "*All of these stories become evidence of what works in medicine*" (Nunn, 2011).

Acknowledgement

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Authors' Contributions

Jerry Blythe: Observed the experience described in this article, provided critical input and assisted in revising and improving the article.

Marco Ruggiero: Wrote the first draft of this article, provided critical input and assisted in revising and improving the article.

Disclosures

Jerry Blythe discloses no conflict of interest. The subject described in this study bought all the foods and supplements used during her experience and paid for the analyses reported in this article.

Marco Ruggiero is the founder of Silver Spring Sagl, the company producing the probiotic yogurt used in this experience and has served as CEO of the company until his retirement in 2020. However, he had no prior knowledge of the nutritional plan followed by the subject of this article nor of the results of the analyses that were communicated by Dr. JB only after completion of the experience. Marco Ruggiero is member of the Editorial Board of the American Journal of Immunology and is waived from the Article Processing fee for this contribution; he receives no remuneration for his editorial work.

Ethics

This article is original and contains material that has not been submitted or published in any other scientific journal.

References

- Antonucci, N., Pacini, S., & Ruggiero, M. (2019). Use of an extremely biodiverse probiotic and a supplement based on microbial chondroitin sulfate is associated with a significant decrease of serum free kappa light chains as well as a trend toward normalization of kappa/lambda ratio and of plasma cell bone marrow infiltration in a case of multiple myeloma. *Am J Immunol*, 15, 5-9.
- Bedford, A., & Gong, J. (2018). Implications of butyrate and its derivatives for gut health and animal production. *Animal Nutrition*, 4(2), 151-159.
- Blythe, J., Ruggiero, M., & Pacini, S. (2017). Intermittent fasting and probiotic yogurt consumption are associated with reduction of serum alpha-N-acetylgalactosaminidase and increased urinary excretion of lipophilic toxicants. *Madridge J Immunol*, 1(1), 23-27.
- Branca, J. J., Pacini, S., & Ruggiero, M. (2015). Effects of pre-surgical vitamin D supplementation and ketogenic diet in a patient with recurrent breast cancer. *Anticancer Research*, 35(10), 5525-5532.
- Bull, M. J., & Plummer, N. T. (2015). Part 2: Treatments for chronic gastrointestinal disease and gut dysbiosis. *Integrative Medicine: A Clinician's Journal*, 14(1), 25.
- Carter, M., Pacini, S., & Ruggiero, M. (2020). Consumption of an Extremely Biodiverse Probiotic and a Supplement based on Microbial Chondroitin Sulfate is Associated with Very Low Serum Alpha-N-acetylgalactosaminidase (Nagalase) Activity and Decrease of C-reactive Protein Values. *Am J Immunol*, 16, 8-18.
- Cho, Y. A., & Kim, J. (2015). Effect of probiotics on blood lipid concentrations: A meta-analysis of randomized controlled trials. *Medicine*, 94(43).
- Ebner, J., Arslan, A. A., Fedorova, M., Hoffmann, R., Küçükçetin, A., & Pischetsrieder, M. (2015). Peptide profiling of bovine kefir reveals 236 unique peptides released from caseins during its production by starter culture or kefir grains. *Journal of proteomics*, 117, 41-57.
- Emma Ward, R., Branca, J. V., Noakes, D., Morucci, G., & Thyer, L. (2014). Clinical experience of cancer immunotherapy integrated with oleic acid complexed with de-glycosylated vitamin d binding protein. *Am. J. Immunol.*, 10(1), 23-32.
- Harben, K., Schuchat, A., & Jenrigan, D. (2020). Transcript for CDC Update on Flu Activity. 2020. <https://www.cdc.gov/media/releases/2018/t0202-flu-update-activity.html>

- Haskard, C. A., El-Nezami, H. S., Kankaanpää, P. E., Salminen, S., & Ahokas, J. T. (2001). Surface binding of aflatoxin B1 by lactic acid bacteria. *Applied and environmental microbiology*, 67(7), 3086-3091.
- He, F., Zuo, L., Ward, E., & Arciero, P. J. (2017). Serum polychlorinated biphenyls increase and oxidative stress decreases with a protein-pacing caloric restriction diet in obese men and women. *International journal of environmental research and public health*, 14(1), 59.
- JHM. (2020). 102.3 Organization Policy on Single Case Reports and Case Series. "It is the policy of the Organization that a "single" case report (three or fewer cases) does not require review by the JHM IRB."
https://www.hopkinsmedicine.org/institutional_review_board/guidelines_policies/organization_policies/102_3.html
- Majlesi, M., Shekarforoush, S. S., Ghaisari, H. R., Nazifi, S., Sajedianfard, J., & Eskandari, M. H. (2017). Effect of probiotic *Bacillus coagulans* and *Lactobacillus plantarum* on alleviation of mercury toxicity in rat. *Probiotics and Antimicrobial Proteins*, 9(3), 300-309.
- Monachese, M., Burton, J. P., & Reid, G. (2012). Bioremediation and tolerance of humans to heavy metals through microbial processes: A potential role for probiotics? *Applied and Environmental Microbiology*, 78(18), 6397-6404.
- Nunn, R. (2011). Mere anecdote: Evidence and stories in medicine. *Journal of Evaluation in Clinical Practice*, 17(5), 920-926.
- Pacini, S., & Ruggiero, M. (2019). Natural Plasmids in a Swiss Fermented Milk and Colostrum Product assessed by Microbiome Array. *Madridge J Immunol*, 3(2), 100-108.
- Pacini, S., & Ruggiero, M. (2020). Phage composition of a fermented milk and colostrum product assessed by microbiome array; putative role of open reading frames in reference to cell signaling and neurological development. *J Neurol Stroke*. 2020;10(2):80-90.
- Pacini, S., Fiore, M. G., Magherini, S., Morucci, G., Branca, J. J., Gulisano, M., & Ruggiero, M. (2012). Could cadmium be responsible for some of the neurological signs and symptoms of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. *Medical hypotheses*, 79(3), 403-407.
- Pacini, S., Punzi, T., Morucci, G., Gulisano, M., & Ruggiero, M. (2009). A paradox of cadmium: A carcinogen that impairs the capability of human breast cancer cells to induce angiogenesis. *Journal of Environmental Pathology, Toxicology and Oncology*, 28(1).
- Pacini, S., Ruggiero, M., Morucci, G., & Punzi, T. (2011). Macrophages of the Mucosa-Associated Lymphoid Tissue (MALT) as key elements of the immune response to vitamin D binding protein-macrophage activating factor, 136-136.
- Rizzoli, R., & Biver, E. (2020). Are Probiotics the New Calcium and Vitamin D for Bone Health? *Current Osteoporosis Reports*.
- Ruggiero, M., Ward, E., Smith, R., Branca, J. J., Noakes, D., Morucci, G., ... & Pacini, S. (2014). Oleic Acid, deglycosylated vitamin D-binding protein, nitric oxide: A molecular triad made lethal to cancer. *Anticancer research*, 34(7), 3569-3578.
- Sagiv, S. K., Harris, M. H., Gunier, R. B., Kogut, K. R., Harley, K. G., Deardorff, J., ... & Eskenazi, B. (2018). Prenatal organophosphate pesticide exposure and traits related to autism spectrum disorders in a population living in proximity to agriculture. *Environmental Health Perspectives*, 126(4), 047012.
- Schwalb, M., Taubmann, M., Hines, S., Reinwald, H., & Ruggiero, M. (2016). Clinical observation of a novel, complementary, immunotherapeutic approach based on ketogenic diet, chondroitin sulfate, vitamin D3, oleic acid and a fermented milk and colostrum product. *Am J Immunol*, 12(4), 91-98.
- Senda, A., Fukuda, K., Ishii, T., & Urashima, T. (2011). Changes in the bovine whey proteome during the early lactation period. *Animal Science Journal*, 82(5), 698-706.
- Shing, C. M., Hunter, D. C., & Stevenson, L. M. (2009). Bovine colostrum supplementation and exercise performance. *Sports Medicine*, 39(12), 1033-1054.
- Thyer, L., Ward, E., Smith, R., Branca, J. J., Morucci, G., Gulisano, M., ... & Pacini, S. (2013). GC protein-derived macrophage-activating factor decreases α -N-acetylgalactosaminidase levels in advanced cancer patients. *Oncoimmunology*, 2(8), e25769.
- Yamamoto, N. (2006). Pathogenic significance of α -N-acetylgalactosaminidase activity found in the envelope glycoprotein gp160 of human immunodeficiency virus type 1. *AIDS Research & Human Retroviruses*, 22(3), 262-271.
- Yamamoto, N., & Urade, M. (2005). Pathogenic significance of α -N-acetylgalactosaminidase activity found in the hemagglutinin of influenza virus. *Microbes and infection*, 7(4), 674-681.
- Yi, Y. J., Lim, J. M., Gu, S., Lee, W. K., Oh, E., Lee, S. M., & Oh, B. T. (2017). Potential use of lactic acid bacteria *Leuconostoc mesenteroides* as a probiotic for the removal of Pb (II) toxicity. *Journal of Microbiology*, 55(4), 296-303.
- Zhai, Q., Tian, F., Zhao, J., Zhang, H., Narbad, A., & Chen, W. (2016). Oral administration of probiotics inhibits absorption of the heavy metal cadmium by protecting the intestinal barrier. *Applied and environmental microbiology*, 82(14), 4429-4440.

- Zhai, Q., Yang, L., Zhao, J., Zhang, H., Tian, F., & Chen, W. (2018). Protective effects of dietary supplements containing probiotics, micronutrients and plant extracts against lead toxicity in mice. *Frontiers in Microbiology*, 9, 2134.
- Zoghbi, A., Khosravi-Darani, K., & Sohrabvandi, S. (2014). Surface binding of toxins and heavy metals by probiotics. *Mini reviews in medicinal chemistry*, 14(1), 84-98.
- Zunaid, I. R., Pacini, S., & Ruggiero, M. (2020). Significance of hydrophobic and charged sequence similarities in sodium-bile acid cotransporter and vitamin D-binding protein macrophage activating factor. *bioRxiv*.