ENDOCRINE DISRUPTOR COMPOUNDS (EDCs) and PROTEOLYTIC ENZYMES

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Endocrine disruptor compounds, also commonly referred to as EDCs, are organic compounds that can mimic and/or inhibit endogenous hormones. These man-made compounds are widely found in everyday living conditions and can infiltrate the body's barriers via skin, inhalation, or digestion. They are specifically found in cosmetics, personal care products, packaging materials, foods, pharmaceuticals, and many other sources. It is almost impossible not to be exposed to them one way or another, and once they penetrate any of our barriers, the chemical may elicit a systemic reaction leading to the production of antibodies which can ignite autoimmune processes and even cancer.

Some of the most common EDCs and their effects on the body are:

- Bisphenol-A (BPA), a plasticizer found in plastic products, canned foods, etc, which may cause many types of cancer and reproductive problems
- Atrazine, used in agriculture, which can feminize males and impair reproduction
- Dioxin, mostly found in animal products, which can permanently lower sperm count and quality
- Phthalates, used in cosmetics, plastic containers/wraps, toys, etc, which can destroy testicular cells and cause obesity, diabetes, thyroid problems, etc
- Perchlorate, used in water systems, which impairs thyroid function, affecting metabolism, proper infant development, etc
- Fire retardants such as polybrominated diphenyl ethers found in carpets, furniture, etc, which can impair thyroid function and lower IQ
- Perfluorinated chemicals (PFCs) in coating of non-stick cookware which cause low birth weight, infertility, low sperm quality, and damage to many organs
- Organophosphates, potent neurotoxic pesticides which lower testosterone and impair reproductve and thyroid functions
- Glycol ethers found in paints, cleaning products, brake fluid, and cosmetics which can shrink testicles, lower sperm count, and cause asthma and allergies

The above are just some examples of man-made chemicals with harmful effects on the human body even at nano concentrations. As stated above, these chemicals are found everywhere. It is almost impossible to avoid them in today's modern and industrial society. Yet, once introduced in the body, they are bound to proteins such as steroid hormone binding globulins (SHBG), albumin, and/or other molecules (proteins or lipids) within the body. These carrier molecules, as well as tissues to which these EDCs are bound to, help maintain their persistence in the body. As with normal hormones, when they are released from the carrier proteins or tissues, they can bind to endogenous hormone receptors in the body and elicit metabolic and physiological disorders as outlined above. Their persistent presence in the tissues creates serious health risks, including reproductive and sexual disorders, developmental abnormalities in babies, cancer, neurological disorders including lowering intelligence and attention deficits, and many others. In reality, the action of any hormones could be undermined directly or indirectly.

As these compounds are so prevalent and no concrete actions are being taken to effectively eliminate their presence in human lives and exposure, it is imperative to continue to investigate ways to eliminate and/or alleviate their physiological impact on the human body. Fortunately, once the immune system is able to recognize them, the body is able to develop antibodies against them. But although the detoxification processes of the body via the liver can help remove many of these chemicals, it is important to find ways to enhance their removal from the body. Proteolytic enzymes (proteases and peptidases) have been shown to play important functions in improving blood rheology, helping control inflammation and modulate the immune system. As part of these functional benefits, these enzymes help support the body's detoxification processes.

OBJECTIVES

The objective of this exploratory study is to investigate the role of proteolytic enzymes in detoxifying the body. More specifically, the study will investigate and analyze the effect of a proteolytic enzyme supplement (Transformation Enzyme Corporation's Professional Protocol™ Protease) on the removal and/or reduction of EDCs from human volunteers. The focus will be on agricultural toxins such as pesticides and herbicides along with industrial, commercial, and other various man-made chemicals found in plastics, cosmetics, cleaning products, etc. Exposure to these EDCs is not in question—they are practically found in every household, and everyone is exposed to them in one way or another. It is rather the level of exposure and how Protease can impact the degree of detoxification when compared to control volunteers who are not taking the enzymes.

Materials & Methods

This observatory study had participants placed in two groups: a treatment group and a placebo group. We started with twenty participants and ended with eighteen due to two of the participants being excused for personal reasons.

- Group A with age range 27-63 consisted of four men with mean age of 46 and seven women with mean age of 42.
- Group B with age range 42-79 consisted of three men with mean age of 49 and four women with mean age of 62.

Exclusion criteria were people who had liver disease, ulcers, women who were pregnant, and anyone with any serious health challenge requiring constant medical supervision or administration of medication.

Participants were asked to stop taking other supplements during the 12 weeks of the study, present to our office three times for testing, and keep in constant communication with our staff on a weekly basis for the length of the study. The protocol consisted of taking four capsules three times daily on an empty stomach for 12 weeks.

- **Protease group:** Group A, the treatment group, was on a healthy, all natural, anti-inflammatory diet and took a Protease supplement yielding >4.26 million HUT of proteolytic enzyme activity per day.
- **Control group:** Group B was on a healthy, all natural, anti-inflammatory diet and took a starch-filled placebo.

The study has a limitation in the selection of participants. Baselines were taken after they were randomly assigned to groups, and participants did not know if they were in the Protease group or the control group. The following list of labs along with their respective markers includes antigens, inflammatory markers, and immunoglobulins to different environmental toxins including EDCs which were measured in blood and urine at the beginning, at 6 weeks, and finally at 12 weeks of the study.

- **Blood Markers:** CRP, Fibrinogen, Homocysteine, and Sed Rate
- **Pesticides:** Organochlorine pesticides (DDT/DDA), Organophosphate pesticides (DEDTP, DMDTP, DETP, DMTP), and Pyrethroid pesticides (3PBA)
- **Herbicides:** Glyphosate and Atrazine
- **Volatile organic compounds:** Xylene (2MHA), Styrene (PGO), Benzene (NAP), 1-Bromopropane (NAPR), Propylene Oxide (NAHP), Ethylene Oxide (HEMA), and MTBE (2HIB)
- **Parabens:** Propylparaben and Butylparaben
- **Phthalates:** MEHP
- **Environmental phenols:** BPA and Triclosan
- **Antigens:** Formaldehyde & Glutaraldehyde IgM, Isocyanate IgM, BPA Binding Protein IgM, Tetrabromobisphenol A IgM, Tetrachloroethylene IgM, Parabens IgM, and Mixed Heavy Metals IgM (Nickel, Cobalt, Cadmium, Lead, and Arsenic)

Blood panels were through Labcorp, environmental toxin testing was through Vibrant Wellness Labs, and the antigen array was through Cyrex™ Laboratories, LLC.

RESULTS & DISCUSSION

Inflammatory Markers

The inflammatory markers C-Reactive Protein (CRP), Homocysteine, Sed Rate, and Fibrinogen were chosen as a representative set of blood markers for assessing changes in the level of inflammation in the participants. Participants completed baseline (week 1), midpoint (week 6), and final (week 12) testing.

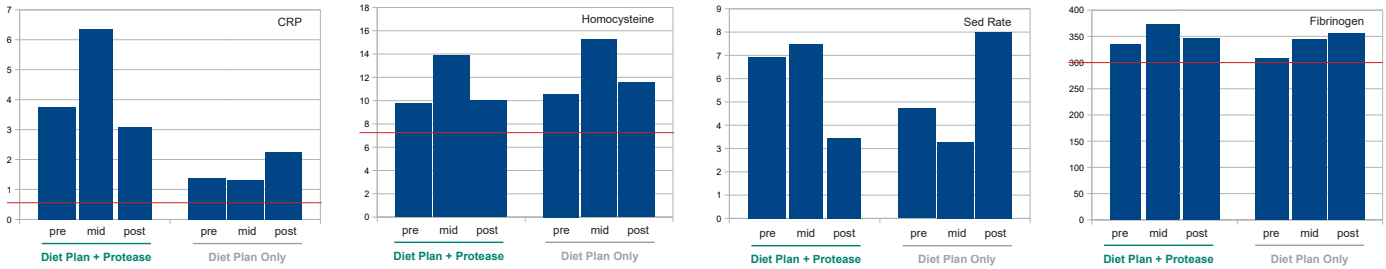

Figure 1 (A,B,C,D). Relative comparison of blood markers between Protease group and control group

CRP is an acute phase reactant which increases sharply in response to inflammation. It binds to phosphocholine expressed on the surface of dead or dying cells (and some types of bacteria) to activate the complement system. It is synthesized by the liver in response to cytokines such as IL1β, IL6, IL12, and TNFα released by macrophages and fat cells. Healthy CRP levels are less than 0.55 mg/L.

In the present study, although the baseline CRP level in the Protease group appeared to be high, by the end of the 12-week period there was noticeable decrease (see ***Fig. 1A***). Meanwhile, in the control group, there was an increase in the CRP levels from the baseline to the 12-week study end period. Inflammation is not necessarily a bad or negative thing. In fact, it is a vital biological process. Inflammation becomes negative when it is chronic and unmanaged or uncontrolled. The initial increase in CRP in the Protease group could be attributed to the presence of the proteolytic enzymes and their association with alpha-2 macroglobulin, a process that could trigger a temporary inflammatory response.

Homocysteine is a non-protein amino acid which can be recycled into methionine or converted into cysteine with the aid of B12, B6, Betaine, or Folic acid. It accumulates in circulation when there is a deficiency of methyl donors. When there is too much homocysteine in the blood it sets the stage for the formation of atherosclerotic plaques. It does this by stopping the production of nitric oxide (vasodilator), increasing blood viscosity, and facilitating oxidation of LDL cholesterol. Thus, high uncontrolled homocysteine levels are not good. Homocysteine values should be less than 7.2 umol/L.

In this study, we observed relatively high homocysteine levels from both the Protease and the control groups (see ***Fig. 1B***). But note the relatively lower values and decrease of the homocysteine in the Protease group when compared to the control group.

Sed Rate (erythrocyte sedimentation rate) shows the rate of settlement of whole blood against gravity in a 1-hour period. It is a rough measure of abnormal concentration of acute phase proteins. This marker is a non-specific indicator of tissue damage and inflammation. It is mainly influenced by fibrinogen and less by globulins. Healthy ESR levels are below 10 mm/h.

In this study, the Sed Rate appeared to decrease in the Protease group compared to the control group (see ***Fig. 1C***). This could indicate a reduced damage of tissues over the 12-week period.

Fibrinogen is an acute phase reactant which increases sharply in states of inflammation and the presence of IL6. It is a key factor in blood viscosity and promotes the formation of blood clots inside coronary arteries. The optimal fibrinogen range is 200-300 mg/dL.

In this study, the profile of the two groups appeared similar (see ***Fig. 1D***). However, it is expected that the Protease group would have less fibrin, the product of fibrinogen, that prevents blood clots. It should also be reiterated that both groups in the study were on a healthy diet, which should contain beneficial antioxidants and other phytonutrients that could alleviate the impact of the negative blood markers.

Two participants in the Protease group—a 63-year-old, 223-lb Caucasian male and a 46-year-old, 209-lb Hispanic male—responded to the detoxification protocol in the most statistically significant way.

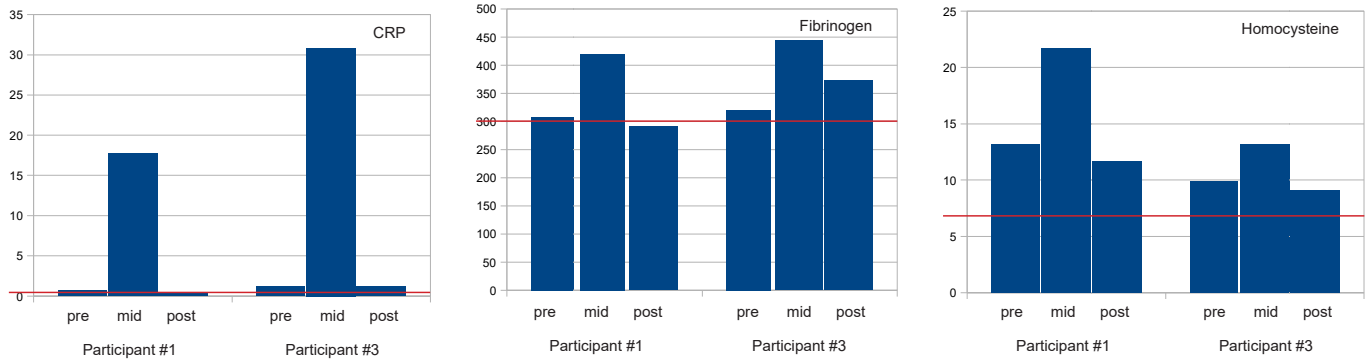

Figure 2 (A,B,C). Spotlight: blood panel markers for two selected participants

Their CRP inflammation levels rose into pathological "red-line" ranges during the course of their first 6 weeks on the program (see ***Fig. 2A***). They had the biggest midpoint increases in Fibrinogen in the Protease group as well (see ***Fig. 2B***), and their Homocysteine levels also become elevated at midpoint (see ***Fig. 2C***). However, all three of these areas had resolved by the end of the study. Again the initial increase in CRP in the Protease participants could be attributed to the presence of the proteolytic enzymes and their association with alpha-2 macroglobulin, a process that could trigger a temporary inflammatory response.

Environmental Toxins

A variety of chemicals were chosen as markers for assessing environmental toxins including EDCs in the participants.

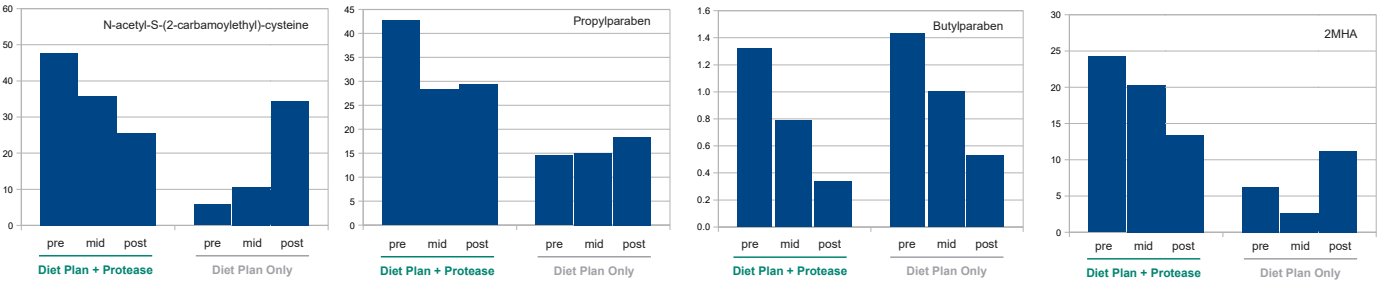

Figure 3 (A,B,C,D). Acrylamide (N-acetyl-S)), paraben (Propylparaben, Butylparaben), and xylene (2MHA) toxicity

N-acetyl-S-(2-carbamoylethyl)-cysteine (AAMA, or NAE) is the metabolite of Acrylamide, a human carcinogen present in processed foods and environmental smoke. Whereas all control group participants had increased N-acetyl-S over the course of the study, the Protease group had a downward trend that points to a more efficient clearance (see ***Fig. 3A***).

Parabens are chemicals that are commonly used as preservatives in cosmetic and pharmaceutical products. Propylparaben is a chemical compound used as a preservative in many products including cosmetics, drugs, and foods. The Protease group experienced a significant decrease of Propylparaben by the midpoint of the study which was maintained through to the end of the study (see ***Fig. 3B***).

Butyl-p-hydroxybenzoate (Butylparaben) is an antimicrobial preservative used in cosmetics, medication suspensions, and in food as a flavoring additive or to prevent growth of bacteria and fungi. The study's paraben decrease trend is most noteworthy with butylparaben (see ***Fig. 3C***).

2-Methylhippuric acid (2MHA) is a metabolite of the organic solvent xylene, a colorless, flammable, slightly greasy liquid of great industrial value. It has been found in the urine of industrial workers exposed to xylene. Tobacco smoke is also a significant source of xylene exposure as measured by urinary 2MHA levels. The Protease group's 2MHA levels decreased consistently throughout the course of the study (see ***Fig. 3D***).

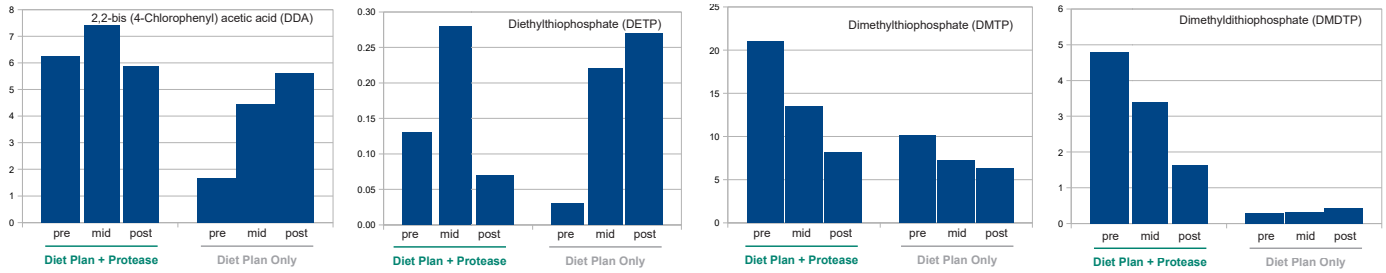

Figure 4 (A,B,C,D). Organochlorine (DDA) and organophosphate (DETP, DMTP, DMDTP) pesticides in urine

2,2-bis (4-chlorophenyl) acetic acid (DDA) is the primary urinary metabolite and potential exposure biomarker for DDT, a persistent organic pollutant once commonly used as a pesticide for controlling malaria and typhus that poses significant health risks. While the DDA scores here aren't statistically significant in themselves from pre-study to post-study, there is an observable trend due to the midpoint reading in this test period (see ***Fig. 4A***). Because the Protease group had an increase followed by a decrease whereas the control group had a steady increase, we can observe a better clearance from that higher level in the Protease group vs the control group, allowing the Protease participants to release the pesticide within the timeframe whereas the control group only went up.

In the case of diethylthiophosphate (DETP), the Protease group showed an initial increase in the pesticide at the 6-week time point followed by a notable decrease (clearance) at 12 weeks (see ***Fig. 4B***). The diet-only group showed a progressive clearance of the pesticide from the beginning of the study to the 12-week study end. It should be noted that the values shown are the levels of the pesticides in urine over a period. This indicates that the pesticides were processed by the liver, and the resulting conjugated metabolites were taken to the kidneys for elimination via the urine. So, the higher the values indicate elimination of the harmful metabolites. In the case of the Protease group, it appears that by 6-week, the body has eliminated most of the pesticide through the urine whereas in the diet group the elimination was slow and continued at higher levels even at 12 weeks.

In fact, most of the benefits that could be attributed to the proteolytic enzymes are due to its improved elimination capacities. The Protease group had generally cleared more efficiently. This could be due to the action of the protease on releasing the pesticides from the tissues, enhancing blood circulation to deliver pesticides to the liver, and supporting the detoxification process of the liver. Although the clean diet in the placebo group resulted in an overall decrease in the pesticide dimethylthiophosphate (DMTP), the addition of Protease supplementation to a clean diet created the environment for additional detoxification which brought high baseline levels down significantly (see ***Fig. 4C***). Although both groups expressed a clearance of these pesticides, the Protease-treated group showed a relatively larger release of the pesticides.

Over the course of the study, especially in urine samples, we generally observed a higher release of the toxins from the tissues and carrier proteins and their elimination. In the case of dimethyldithiophosphate (DMDTP) we noted a gradual decrease of the pesticide in the Protease-treated participants over the 12-week period of the study (see ***Fig. 4D***). However, the trend in the diet-only group was constant, i.e., no change in the first 6 weeks with a slight increase at the 12-week time point. Here we observed a gradual decrease of the levels of the pesticides in the protease-treated groups. The diet-only group showed a rather gradual increase in metabolite. From the various graphs it could be deduced that there is faster clearance from the Protease group as compared to the diet-only or control group.

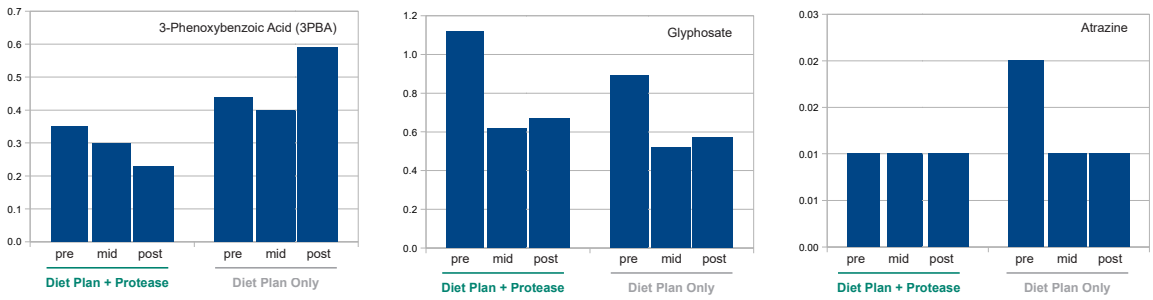

Figure 5 (A,B,C). Pyrethroid pesticide (3PBA) and Herbicides (Glyphosate, Atrazine) in urine

For the pyrethroid pesticide 3-Phenoxybenzoic Acid (3PBA), it appears there is more release in the diet-only group compared to the Protease group (see ***Fig. 5A***). In this case, the mechanism of detoxification by the proteolytic enzymes may be through another route, possibly via the gastrointestinal tract in the stool as compared to the toxins released through the urine. It should be noted that many toxins could be eliminated via the bile and released in the digestive tract to be eliminated in the feces. This alternative route of detoxification could explain some of the results seen with pyrethroid pesticides as well as other pesticides and toxins. In future studies it may be suggested to do stool analysis for the various toxins. From these graphs, we can observe a similar trend of elimination of the specific herbicides Glyphosate and Atrazine (see ***Fig. 5B, Fig. 5C***). Again, the alternate route, i.e., via the stool, may also be part of the elimination process.

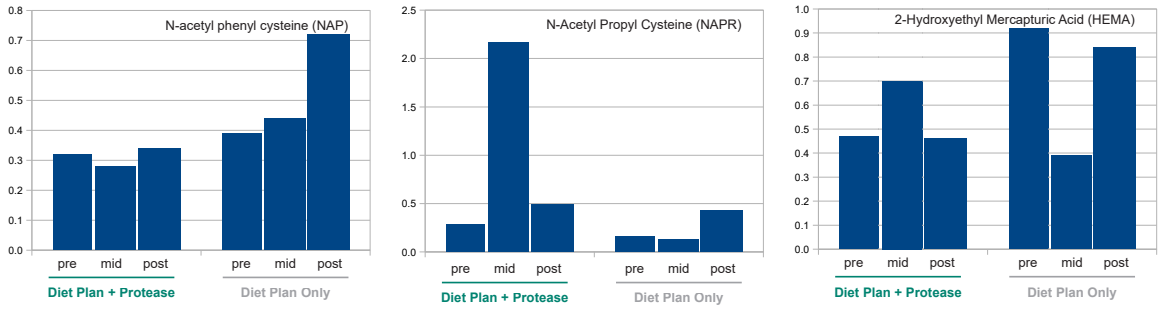

Figure 6 (A,B,C). Benzene (NAP), Bromopropane (NAPR), and Ethylene Oxide (HEMA) in urine

Although both groups expressed a clearance of benzene in the form of N-acetyl phenyl cysteine (NAP), we can make the observation in these graphs that there is faster clearance from the Protease group versus the diet-only group (see ***Fig. 6A***). The control group is releasing more at 12 weeks.

Although both groups expressed a clearance of 1-bromopropane in the form of N-Acetyl Propyl Cysteine (NAPR), we can make the observation in these graphs that there is faster clearance from the Protease group versus the diet-only group (see ***Fig. 6B***). The control group is releasing more at 6 weeks.

Although both groups expressed a clearance of ethylene oxide in the form of 2-Hydroxyethyl Mercapturic Acid (HEMA), we can make the observation in these graphs that there is faster clearance from the Protease group versus the diet-only group (see ***Fig. 6C***). In this case, it is possible as mentioned above that the toxin was carried by the bile into the digestive system for elimination via the colon with the stool.

The mechanism by which the Protease may lead to elimination via the bile is not well understood. However, it is known that depending on the conjugation enzymes, some toxins will be taken to the liver for elimination while others could be taken along with the bile to the digestive tract.

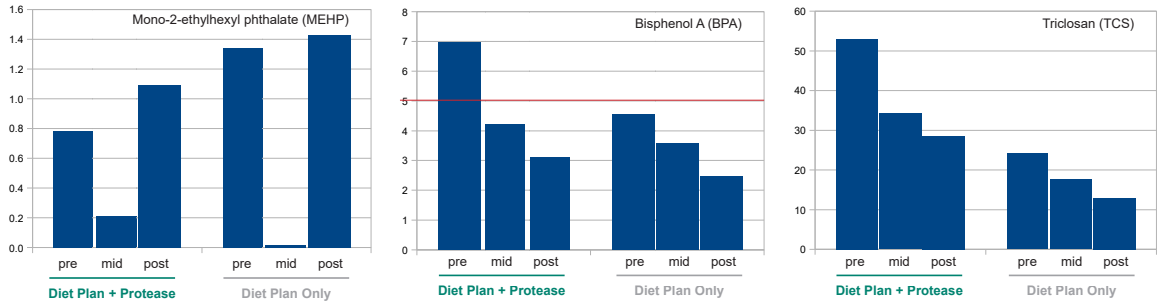

Figure 7 (A,B,C). Phthalates (MEHP) and Environmental Phenols (BPA, TCS) in urine

With the phthalate Mono-2-ethylhexyl phthalate (MEHP), the Protease group started the elimination process at 6 weeks, which is why the 12-week score for protease is less than the 12-week score for placebo (see ***Fig. 7A***).

Although both groups experienced a clear downward trend in the Environmental Phenols Bisphenol A (BPA) and Triclosan (TCS), the Protease group would be considered a very efficient reduction, especially for BPA which was a reduction from "red-line" (out of range) levels into acceptable levels (see ***Figs. 7B, 7C***). By 12 weeks the Protease group has excreted most of it so there is not much there.

INDIVIDUAL CHART REVIEW SPOTLIGHT | GASOLINE POISONING

2-Hydroxyisobutyric Acid (2HIB) is most often the result of exposure to Methyl Tertiary-Butyl Ether (MTBE) or Ethyl Tertiary Butyl Ether (ETBE), which are gasoline additives used as octane enhancers. MTBE has been found to pollute large quantities of groundwater when gasoline with MTBE is spilled or leaked at gas stations. In addition, MTBE and ETBE are volatile and may be inhaled or absorbed through the skin by drivers during fueling or from exhaust exposure. MTBE and its metabolites have been shown to cause hepatic, kidney, and central nervous system toxicity, peripheral neurotoxicity, and cancer in animals.

2HIB has therefore become a common pollutant in our industrialized age, especially in an urban setting like the Greater Houston area where the majority of this study's participants live and work. In fact, nine out of the original group of twenty applicants (45%) who took baseline testing measured as having dangerously elevated 2HIB levels (scores above 1,215.72 µg/g creatinine). Of those nine, five in the Protease group (see ***Figs. 8A-8E***) and three in the Placebo group (see ***Figs. 8F-8H***) continued with the study through to midpoint and final testing.

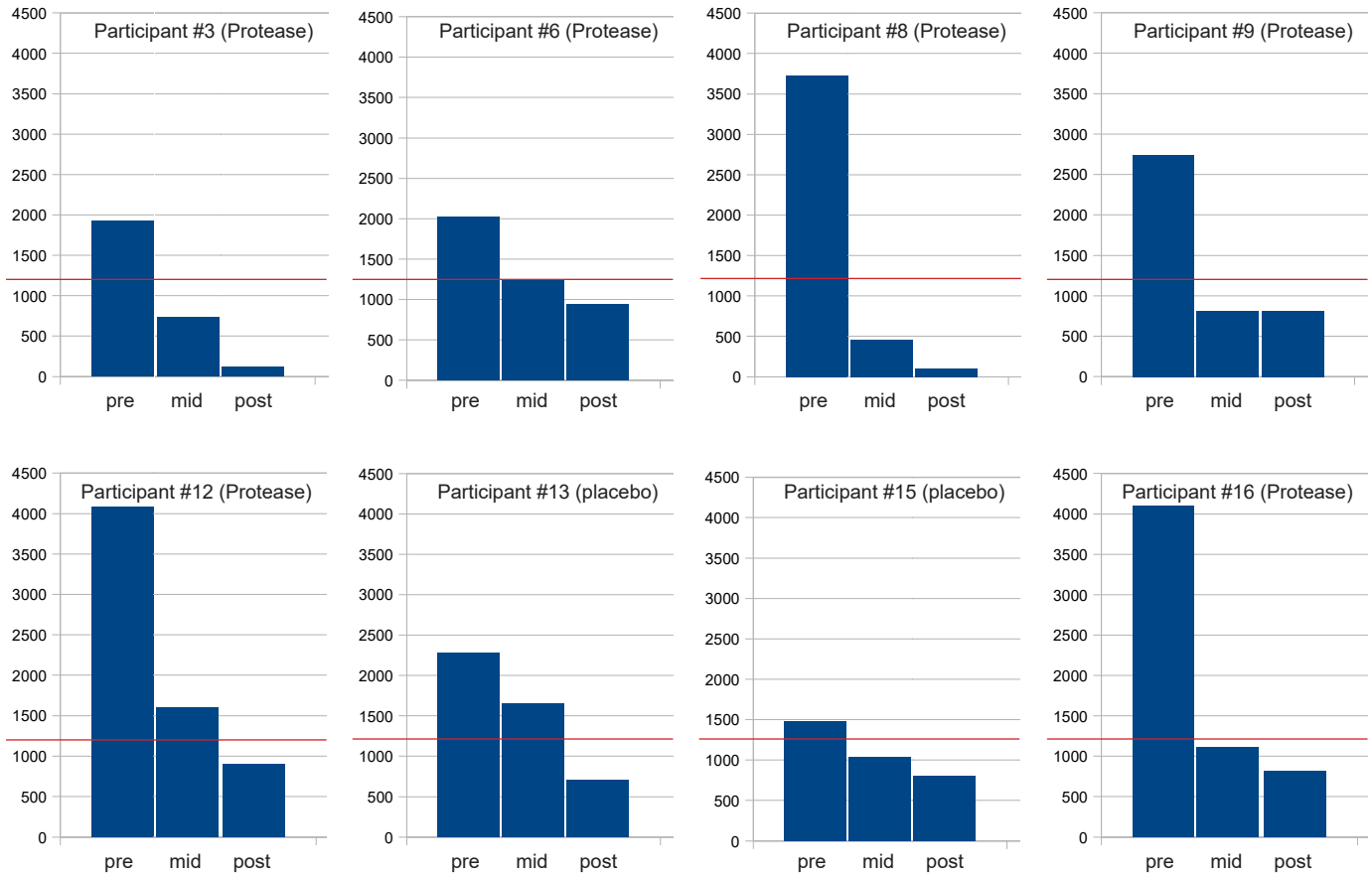

Figure 8 (A,B,C,D,E,F,G,H). Baseline out-of-range 2HIB scores

As seen here, all but three of those with baseline gasoline poisoning in both groups were within range after 6 weeks (see ***Figs. 8A***, ***8C***, ***8D***, ***8G***, ***8H***), and all had normal levels by the completion of the detox program (see ***Fig. 8A-8H***). Furthermore, although only seven of the twenty total baseline tests (35%) had optimal 2HIB levels (<200 µg/g creatinine) before the program began, twelve of the final eighteen participants (67%) had optimal 2HIB levels in their tests at the completion of the program at 12 weeks.

So although both groups benefited from the diet and lifestyle portion of the program, the Protease group still outperformed the control group. Eight out of the eleven Protease group participants (73%) had optimal 2HIB levels at the end of 12 weeks. By comparison, a smaller ratio of the control group participants had optimal 2HIB levels at the end of 12 weeks, counting four out of the seven in the control group (57%) who followed the diet plan but were taking placebo.

INDIVIDUAL CHART REVIEW SPOTLIGHT | COMMERCIAL TOXINS

There are also meaningful results that can be gleaned when tracking an individual participant's scores across several tests. This approach reveals at a glance the type of broad physiological impact of Protease supplementation combined with diet and lifestyle changes on environmental toxicity and inflammatory markers. For example, a 39-year-old, 153-lb Caucasian female participant in the Protease group experienced the following:

- The largest drop among all participants in Phenylglyoxylic acid (PGO), a chemical used in the production of rubber and other materials (see ***Fig. 9A***).
- The second biggest drop among all participants in Propylparaben, a chemical compound used as a preservative in many products, including cosmetics, drugs, and foods (see ***Fig. 9B***).
- A tremendous and sustained drop in Triclosan (TCS), an antibacterial chemical used in many consumer products (see ***Fig. 9C***).
- A remarkable drop in DMDTP pesticide levels (see ***Fig. 9D***).
- A decrease in DDA pesticide levels within the first 6 weeks of the detox program which fell even more dramatically by the end of the program (see ***Fig. 9E***).
- A dramatic increase in N-Acetyl (2,Hydroxypropyl) Cysteine (NAHP), a metabolite of propylene oxide used in the production of plastics and polyester resins and as a fumigant. These levels resolved by the end of the study (see ***Fig. 9F***).
- The second biggest drop overall by the end of the study in the inflammatory marker Fibrinogen after an initial increase (see ***Fig. 9G***).

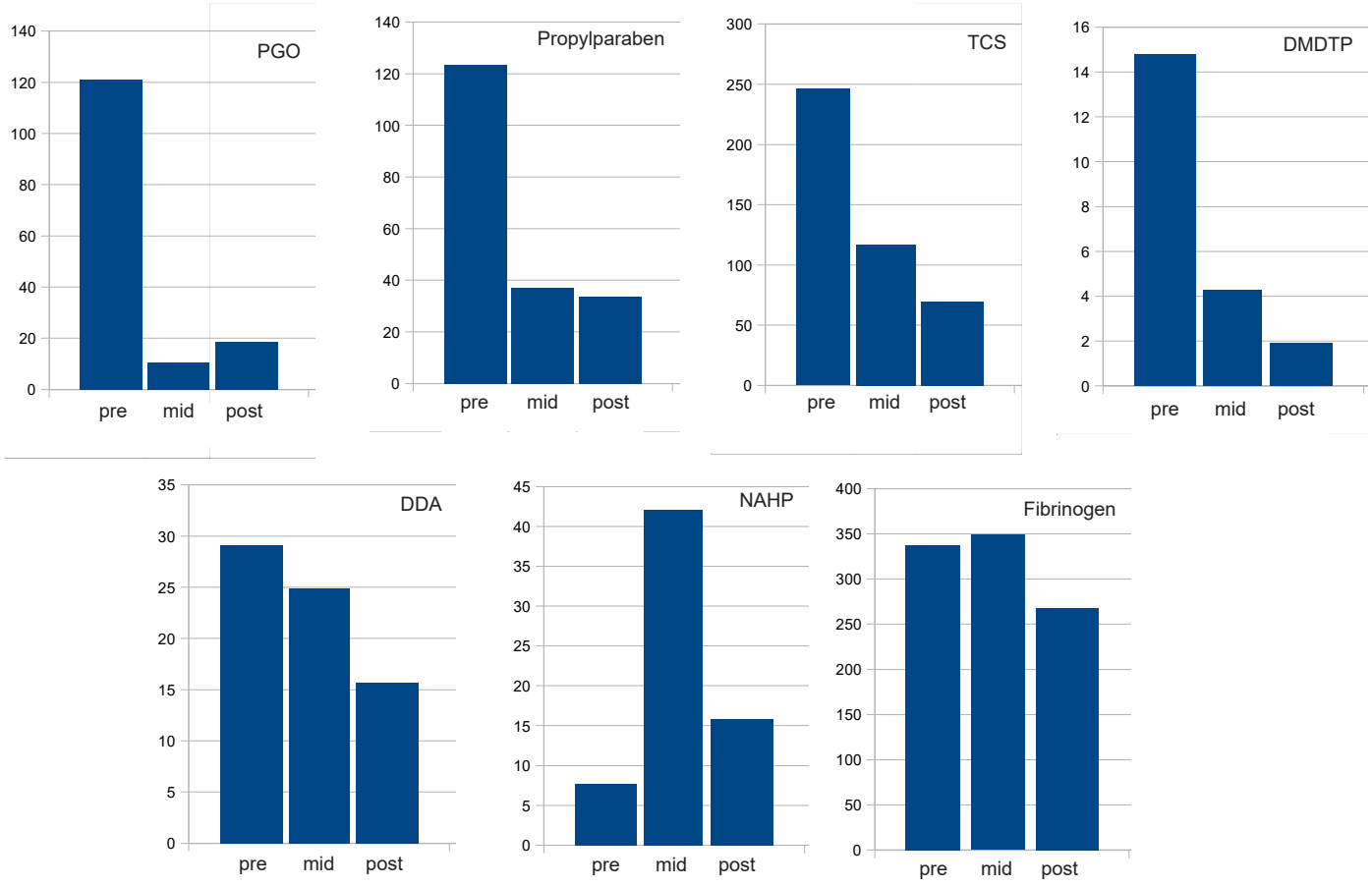

Figure 9 (A,B,C,D,E,F,G). Baseline out-of-range 2HIB scores

A 30-year-old, 220-lb Hispanic female in the Protease group described herself in her program intake form as a stay-at-home mom who went out to eat a lot, used several plastic/paper containers a day, and regularly wore a variety of cosmetics (mascara, countour, liner, lash glue, concealer, lipstick). She stuck with the program and displayed some of the most striking results when it came to eliminating environmental toxicity from the body. The following set of graphs highlight this participant's baseline, midpoint, and final levels of the toxins BPA, Butylparaben, and N-acetyl-S along with the pesticides DDA, DEDTP, and DMDTP.

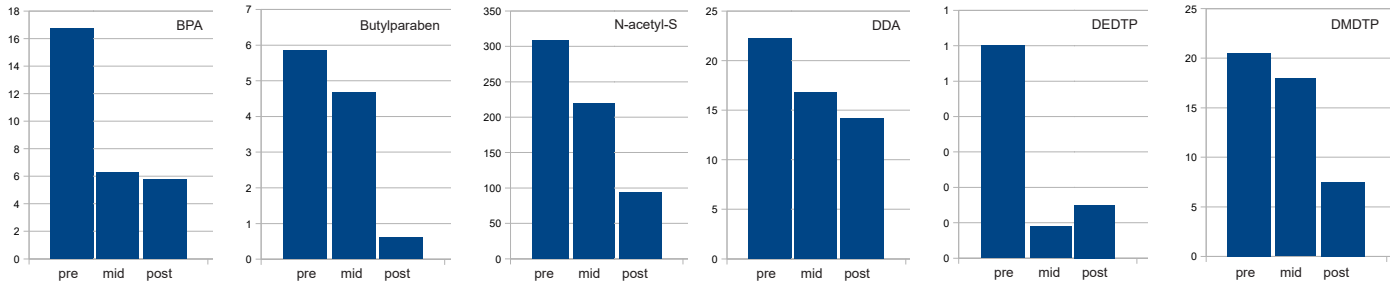

Figure 10 (A,B,C,D,E,F). Selected charts for Paricipant #4 in the Protease group

- Environmental phenols are a complex class of EDC compounds found widely in personal care and consumer products such as lotions and creams. BPA, tested for here, is an industrial chemical that is used to make plastics. It is even used in the thermal paper receipts that are printed when these items are sold! This participant's BPA levels fell dramatically within the first 6 weeks of the detox program and remained low through the remainder of the program (see ***Fig. 10A***).
- Butylparaben is used in cosmetics, medication suspensions, and as a food additive. This participant's levels decreased within the first 6 weeks of the detox program and then fell dramatically by the end of the program (see ***Fig. 10B***).
- N-acetyl-S is the metabolite of a probable human carcinogen present in high-temperature-processed foods and environmental smoke. This participant's levels decreased noticeably within the first 6 weeks of the detox program and then fell even more dramatically by the end of the program (see ***Fig. 10C***). Their extremely high baseline level of 309 came down to 220 at the midpoint and resolved to 94 at the end, which contributed to such a clear downward trend for this marker in the Protease group (see ***Fig. 7B***).
- DDA is the primary biomarker for DDT exposure, a persistent and dangerous organic pollutant. This participant's DDA levels decreased noticeably within the first 6 weeks of the detox program and continued decreasing through to the end of the program (see ***Fig. 10D***).
- DEDTP is a product of certain pesticides. This participant's DEDTP levels fell dramatically within the first 6 weeks of the detox program and remained low through the remainder of the program (see ***Fig. 10E***).
- Dimethyldithiophosphate (DMDTP) is another member of the group of organophosphate pesticides, metabolic products of dimethylphosphorothionate insecticides. This participant's DMDTP levels decreased within the first 6 weeks of the detox program and then fell dramatically by the end of the program (see ***Fig. 10F***).

Chemical Immune Reactivity Screen

Rather than simply detecting the level of chemical exposure in the body, the Chemical Immune Reactivity Screen by Cyrex™ Laboratories, LLC, measures the immune response to various environmental chemicals that bind to human tissues, specifically the loss of immune tolerance in the form of antibody production. Environmental toxins enter the body in a variety of ways. Depending upon the chemical and its use, infiltration of the body by a toxin can occur by contact (through the skin), inhalation (through the lungs), and ingestion (through the GI tract). These chemicals and/or their metabolites bind to human tissue proteins forming a neo-antigen. This neo-antigen is picked up by the antigen-presenting cell (APC) which facilitates the production of immunoglobulins. Immunoglobulins may be created to target the chemical bound to human tissue, the chemical/metabolite bound to human tissue, and/or human tissue. High levels of antibodies indicate a breakdown in immunological tolerance or disruption in immune homeostasis. Again, the detection of antibodies to chemicals bound to human protein in serum indicates a breakdown in immunological tolerance and induction of chemical intolerance.

Each person has an individual response to chemicals. Some may have low-level exposure but high body-burden (elevated antibodies), while others may have high or acute-level exposure but no measurable body burden (normal antibody levels). In general, however, high levels of antibodies indicate an immune reaction to an antigen which threatens the homeostasis of the body and may result in autoimmune reactivity. Cyrex test result comparisons are therefore considered valid only for patients who have tested positive for antibodies. Borderline or "in between" values are not considered trustworthy as those individuals may not develop antibodies. So for the purposes of this study, we have focused exclusively on the participants who were out of range at baseline. And in order to compare the Protease treatment group with the Placebo control group, only analytes where at least one participant from each group tested positive for antibodies at baseline are included here.

The primary immune reaction the body has to an intruder is the production of IgM antibodies in the first days of defense as the immune system is exposed to the antigen. If previous exposures have occurred, a secondary immune response results in IgG production plus IgA, mostly from the musocal lining areas. Higher doses and or extended time in the detox program may have resulted in the IgG+IgA screens being applicable. But for the purposes of this study we have focused on the primary IgM antibody levels. These parameters have yielded seven IgM antigens from various combinations of four participants from Group A (Protease + diet plan) and three from Group B (Placebo + diet plan). The results in this section reflect the averages of the redline participant data points for each group at baseline, then again at midpoint (6 weeks), and at end of study (12 weeks). In general we observed the following trends:

- All of the selected participant groups had a significant drop in all analyte scores at 6 weeks (see ***Figs. 11A-11G***). In fact, with only one exception for each group (see ***Figs. 11B, 11F***) all out of range averages were within range by week 6.
- The Protease group performed better and registered larger numerical improvements compared to the control group participants who were only following the diet plan. The only exception was a virtually identical improvement (see ***Fig. 11A***).
- Two of the analytes under consideration continued to decline until week 12 for one or both groups (see ***Figs. 11C, 11D***).

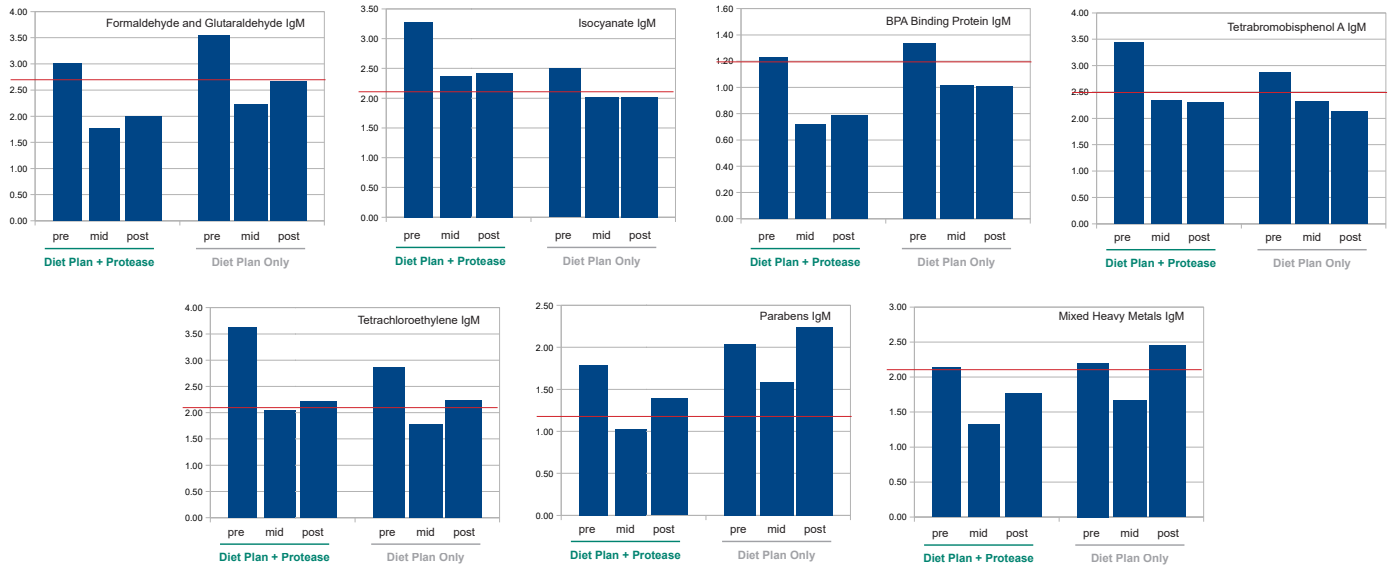

Figure 11 (A,B,C,D,E,F,G). Selected IgM antigen tests

In all other instances in this selected data set, the scores increased from week 6 to week 12, even if only slightly. In fact, in three instances the week 12 scores returned above redline for one or both groups (see ***Figs. 11E-11G***). And in two instances the control group's week 12 scores were actually greater than they were at baseline (see ***Figs. 11F, 11G***). The Cyrex™ Array 11 immune reactivity is concentrated more on its effect on autoimmunity. The urinary analytes were measuring the point when the chemical was entering the body and not attacked by the immune system. But once the body has identified and tagged it as an intruder, the body starts developing antibodies to it and it becomes tracked by the Cyrex™ test. When the antigen is gone from the system, most immunocompetent people return to a base level memory of circulating antibodies. The body tends to hold onto these antibody-producing memory cells. It is feasible that with these trends we are seeing more of antigen/antibody reaction and more complexes are being released by the Protease group versus the control group.

Symptom Surveys

The participants were asked to fill out a symptom questionnaire on the first day and again at the final day of the program. From a list of symptoms (see ***Appendix 2***) participants were instructed to mark each as 'Always', 'Sometimes', or 'Never'. In this study we observed physiological improvements in the release of urinary toxins. These improvements ultimately correlate with improved symptom self-reporting on the questionnaire.

The reduction in the overall amount of symptoms in both groups speaks to the overall success of the diet and lifestyle program (see ***Fig. 12***). However, the more prominent reduction in the amount of 'Always' symptoms in the Protease group shows the advantages of combining a healthy diet and lifestyle with Protease enzyme supplementation.

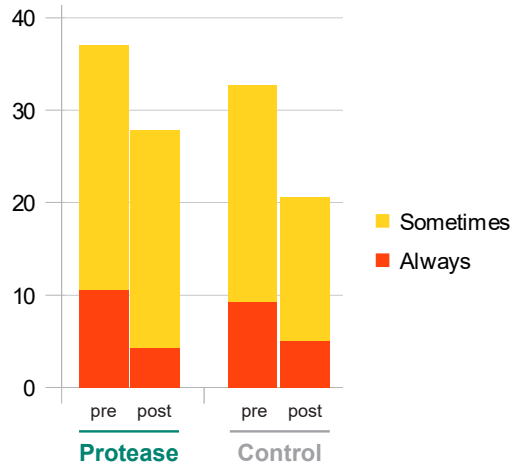

Figure 12. Symptom survey totals

EDCs & PROTEASE | GENERAL RATIONALE

The compounds tested here are all environmental toxins—some are EDCs, while some are not EDCs but still cause an immune reaction when they are identified as a toxin. However, for these purposes the mechanisms of action of EDCs can be grouped into three classes:

- EDCs that act directly or indirectly to stimulate the action of a hormone. Hormones are very powerful molecules, and their secretion and action are tightly regulated: the need of their production or action depends on specific need of specific cells or tissues. Any uncontrolled action by external substances such EDCs taken in the body through foods, cosmetics, medications, etc. may impair or accentuate the homeostatic action of endogenous hormones. As a result of this uncontrolled fact, there could be many metabolic disorders and health challenges.
- EDCs that, in opposite of the ones cited above, inhibit the action of endogenous hormones. So, when these EDCs are in the body they prevent the necessary action of a specific hormone. For instance, the presence of an inhibitory EDC may prevent the action of testosterone in a male and thus leading to infertility and negation of other male secondary characteristics. There are reports that in certain areas this phenomenon is leading to feminization of males.
- EDCs that impair the normal production mechanisms and/or transport of hormones. As hormones are very specific and produced under tightly regulated mechanisms, EDCs could interfere and lead to the absence of the hormone to perform its biological function in the body.

How are the EDCs and other environmental toxins transported in the blood, and what is their normal detoxification? Under normal conditions, an EDC such as BPA, which is one of the most current in the population, enters the blood circulation and is quickly taken to the liver, assuming there is a good blood rheology for transport to the liver where its detoxification is to take place. However, if blood circulation is inadequate, the EDC may linger in tissues and its detoxification will be reduced.

In simple and general terms, bisphenol A is converted in the gut and liver to bisphenol glucuronide for detoxification. Other EDCs may follow the same path. But ultimately toxins and harmful molecules, including EDCs, must go through the liver for chemical modifications by specific enzymes.

Liver detoxification has two phases. The first phase involves the cytochrome P450 enzymes to modify the EDC. At this stage, the EDC, although in the detoxification process, has increased toxicity. Thus, the process must go to the second phase called "phase of conjugation" that involves many other enzymes. These conjugation enzymes will transform the products of the first phase by adding specific chemical groups that will render the toxic EDC into a non-harmful compound that is water soluble and could be eliminated via the stool or urine.

However, bisphenol as well as other EDCs also damage the liver through their oxidative stress. Studies have reported increased liver enzyme levels in blood circulation following exposure to BPA and other EDCs. This is usually an indication of liver injury or damage. Thus, a damaged liver cannot detoxify the body. In fact, a condition of liver damage or fatty liver will intensify the toxicity of bisphenol and other EDCs in the body.

In the present study, there is a general observation of reduced concentration of the various EDCs over a 12-week period in most participants. The level of detoxification varies from participant to participant. As stated in the Materials & Methods, the participants were volunteers. Although pretest samples were taken before participants were separated in groups of "Protease and Diet Plan" and "Placebo and Diet Plan" we did not assess the length of exposure of the participants to the various EDCs tested. Thus, the participants may have different lifestyles and length exposure to the various EDCs.

Our initial hypothesis was to determine if the administration of the Protease may have a beneficial effect by reducing the EDC metabolites in the body. This hypothesis appears to be cautiously verified as most participants showed a reduced level of the compounds and/or their metabolites over the study period. Based on the pharmacological and pharmacokinetics of the proteolytic enzymes used, it could be concluded that the results observed could be due to:

- **Enhanced blood circulation:** The enzymes enhanced blood rheology, thus, improved the delivery of the EDCs to the liver for effective detoxification
- **Bio-presentation of the EDCs for detoxification:** In the body, the EDCs may be bound to various carrier proteins or receptors (free or fixed receptors). The proteases could help release the compounds from the carrier molecules, thus presenting them to the immune system and/or the liver for detoxification
- **Release from tissues:** Many of these EDCs are lipid soluble and thus could be associated with body fat and/or lipoproteins. Through the mechanism of hormone sensitive lipase action, some may be freed and exposed for transport to the liver for detoxification.
- **Improved liver function** by the enzymes as the liver may be damaged over time by oxidative stress. It is possible that the proteases may remove the oxidized proteins thus repairing the free radical damage to the liver and improving its detoxification function.
- **Improved elimination** of the toxins after conjugation in the liver via the bile. Toxins taken by the bile to the digestive tract could be taken to the colon for elimination via the feces or follow the enterohepatic recirculation which may ultimately help their elimination via the urine.
- **Improved kidney function:** Proteolytic enzymes can help cleanse the kidneys by removing oxidized proteins, persistent immune complexes, and other molecular debris that could impair renal function. This action could also indirectly help remove toxins and prevent their bioaccumulation in the body.
- **Enhanced immune action:** as the toxins are released from tissues and carrier proteins, their antigenicity is heightened leading to immune interaction and ultimate removal.

These possible mechanisms, including some associated with the components (antioxidants and other phytonutrients) of the diet plan may all play a role in the improvements observed from the lab test results.

CONCLUSION

This limited short study indicated that proteases as used here have some effect in detoxifying the body. The Protease group showed a trend or reduced toxicity characterized by the urine results of the various analytes tested. For most of the analytes we observed an increased amount of the toxins eliminated in the urine as a function of time, when compared to the control group. We also speculated that there could be more toxins eliminated via other routes, such as the feces, as a result of some action of the proteases.

In terms of antigenicity and immune reaction of the toxins studied, we observed that the Protease group had more immune reaction about the toxins compared to the control group. It is thought that improved immune reaction in the Protease group could be due to the action of the proteolytic enzymes in releasing the toxins from the tissues and/or carriers, thus making these toxins more "visible" to the immune cells and molecules.

This first study of its kind gives some indication of the role of proteases in the body's detoxification process. As the EDCs and the other toxins considered in this study are prevalent in everyone's day-to-day life, simply avoiding them may not be enough. Including proteases as well as other phytonutrients in the diet could be, along with lifestyle change, a very important action in cleansing the body of these human-made chemicals that are prevalent in today's society.

Despite the limitations of the study in terms of the sample size and variability within the participants, the results are very promising as to the benefits of proteolytic enzymes. Further studies are needed to understand some of the mechanisms involved as well as the length of time for toxin removal based on specific individual conditions.

The inflammatory markers indicated that the Protease-treated group had more controlled and efficient inflammatory response as compared to the control group. And the symptom survey results can be correlated with trends of elimination of toxins as evidenced in the urinary excretion of toxins and the immune system recognizing and removing the antigen complexes more efficiently. Clinicians should be aware of treating the patient, not the lab. Symptoms are always the most important factors to pursue.

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References

Chmelíková E, et al. Endocrine Disruptors: General Characteristics, Chemical Nature, and Mechanisms of Action. A review. Med Jour Cell Bio. 2018 Dec;6(4):135-139.

Gore AC, et al. Executive Summary to EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. Endocr Rev. 2015 Dec;36(6):593-602.

Gore AC, La Merrill MA, et al. Endocrine Disrupting Chemicals: Threats to Human Health. The Endocrine Society and IPEN. Feb 2024.

Haggerty DK, et al. Reproductive Toxicology: Pregnancy Exposure to Endocrine Disrupting Chemicals: Implications for Women's Health. Reproduction. 2021 Oct 7;162(5):F169-F180.

Hiller-Sturmhöfel S, Bartke A. The Endocrine System: An Overview. Alcohol Health Res World. 1998;22(3):153-64.

La Merrill MA, et al. Consensus On The Key Characteristics Of Endocrine-Disrupting Chemicals As A Basis For Hazard Identification. Nat Rev Endocrinol. 2020 Jan;16(1):45-57.

Mallozzi M, et al. Endocrine Disrupting Chemicals and Endometrial Cancer: An Overview of Recent Laboratory Evidence and Epidemiological Studies. Int J Environ Res Public Health. 2017 Mar 22;14(3):334.

Vojdani A, Vojdani E. The Role of Exposomes in the Pathophysiology of Autoimmune Diseases I: Toxic Chemicals and Food. Pathophysiology. 2021 Dec 18;28(4):513-543.

APPENDIX 1 | ANTI-INFLAMMATORY DIET

Participants were instructed to choose as often as possible:

- Beef, poultry, seafood, and pork as wild caught seafood and game, cage-free poultry, and beef that has been grass fed, without the use of growth hormones or antibiotics
- Fresh fruits and vegetables, locally grown or organic
- Healthy fats from nuts, seeds, olive, avocado, and coconut; oils that are minimally processed
- Locally grown, organic, and non-GMO foods
- Foods eaten fresh, raw, or close to their natural state
- Water, fresh juice, green tea

All participants were instructed to AVOID the following:

- Alcohol, sodas, caffeine
- All commercial dairy (milk, cheese, yogurt, ice cream, sour cream, etc)
- All grains (wheat, barley, rye, oats, rice, quinoa, amaranth, buckwheat, etc)
- All sugar, high fructose corn syrup, artificial sweeteners
- Legumes (peanuts, kidney beans, black beans, red beans, navy beans, garbanzo beans, lentils, lima beans, peas)
- Processed foods (includes most foods that are pre-packaged, boxed, bagged, microwavable, etc)
- Other (corn)

APPENDIX 2 | SYMPTOM SURVEY

The following are prompts from the symptom survey completed at the beginning and end of the 12-week study period.

- Afternoon fatigue
- Decrease in physical stamina
- General fatigue, tired, sluggish most of day
- Wake up tired even after 6+ hours of sleep
- Poor memory, forgetful, mental sluggishness
- Muscle soreness, stiffness, achy joints
- Increase in fat distribution, abdomen and hips
- Feeling that bowels do not empty completely
- Sense of fullness during and after meals
- Increased thirst and appetite
- Crave sweets during the day
- Crave salt
- Heartburn
- Night sweats
- Nervousness or anxious
- Cannot fall asleep, insomnia
- Cannot stay asleep
- Inability to concentrate or stay focused
- Excessive belching, burping, or bloating
- Pass large amount of foul-smelling gas
- Stools are foul smelling
- Increased sex drive
- Difficulty gaining weight
- Difficulty losing weight
- Headaches
- Migraines
- Fatigue after meal
- Some foods cause sinus congestion/ headaches
- Eating sweets does not relieve sugar cravings
- Irritable, lightheaded, or shaky if meals missed
- Leg nervousness at night, restless leg
- Pain inside of legs or heels
- Stomach pain, burning or aching 1-4 hours after eating
- Mucous-like, greasy, or poorly formed stools
- Undigested foods found in stools
- Unexplained itchy skin
- Use of antacids

APPENDIX 3 | FOLLOW-UP QUESTIONNAIRE

Participants were asked to submit weekly responses to a set of prompts designed to obtain information on their GI comfort, regularity, sleep patterns, energy, challenges with the diet, difficulty with the protocol, and overall experience of health:

- What day in your journal are you on?
- Are you more or less comfortable in your gut during the day and/or at bedtime?
- Have your bowel movements increased, decreased or stayed the same? Explain.
- Has your sleep pattern changed? Sleeping more or less?
- How is your energy throughout the day?
- Are you having difficulty with the diet? Any challenges?

- Are you having difficulty with the protocol?
- What changes in your health are you experiencing?

The study period spanned a hot Texas summer which decreased the opportunities for outdoor exercise for some, and a hurricane made landfall during the midpoint of the study which resulted in power outages that caused some stress and disruption in routines for several participants. Otherwise typical challenges for compliance were reported, such as remembering to take pills between meals, eating right when travelling or in social settings, dealing with occasional illness like ear infection, cold, sinus, PMS, etc.

Giving up alcohol was a challenge for some, but giving up coffee was in many ways the most significant change for participants, affecting those in the Protease group and the control group alike. Some rediscovered wellness as a lasting source of natural energy rather than a temporary boost derived from stimulants and/or the emotional satisfaction of indulgance in food cravings. Here is a selection of the comments submitted each week:

WEEK 1

- "I love caffeine, not having it has been difficult. I miss some processed foods. Somewhat hard, but I can start seeing some good differences. I breathe a lot better, I am not using one of my inhalers, and I have lost about 4 lbs."
- "Cutting out bread, cheese and specifically rice has been very difficult."
- "The first two days were rough, but now my energy is getting more steady. I used to have one bowel movement per day, now I'm having 2-4. I've had a number of people tell me I have lost weight and look thinner."

WEEK 2

- "Increased energy, less joint pain, slight weight loss."
- "Craving coffee and fries and some sweets. But I am losing weight and looking good. And my face is glowing."
- "The fact that my energy is good and I feel pretty good with all I have going on is pretty amazing."

WEEK 3

- "I've lost some inches and my tummy and face look slimmer, that is motivating me to keep on track even more."
- "Clearer skin, flatter tummy."
- "I have lost 5-8 pounds so far."
- "Just feeling good."
- "Energy is better throughout the day."

WEEK 4

- "Afternoon energy seems to be improving. "
- "I've lost over 9 pounds. Even though I haven't lost a ton of weight, people are commenting that it looks like a lot. My knee pain has greatly reduced and I no longer feel buildup on my back teeth like I did before the study when even after brushing my teeth, it always felt filmy."
- "Yesterday I noticed I think my skin did look pretty good and I got two compliments today which was random."

WEEK 5

- "Feeling lighter."
- "No joint pain, more energy, better sleep, better focus."
- "I sleep well and dream almost every night."

WEEK 6

- "More energy, less upset stomach."
- "My face is clear with no pimples, my clothes are big on me, and my tummy is slimming down."
- "My skin looks better, I'm thinner, my eyebrows are returning on the ends which makes me think my thyroid is functioning better. People keep telling me I look younger which is nice!"

WEEK 7

- "Loss of 8 lbs to date."
- "More comfortable during the day, bowel movements increased, sleeping better, energy is good."
- "My gut is now more comfortable throughout the day. When I am home and in my element I am eating clean and healthy. I am feeling good, no pain in my joints, my waist is slimming down."

WEEK 8

- "Feeling better overall, off my antidepressant, started thyroid, won't be returning to my old ways."
- "My face is clear and my skin is glowing. My clothes are fitting me well."
- "Skin looks healthier, hair is healthier. Not too awkward to turn away foods like cookies, coffee, etc. People expect me to eat healthy now which I like!"

WEEK 9

- "Bowel movements are about 2x per day now, sometimes up to 4x if I have a lot of green smoothies."

WEEK 10

- "Very comfortable gut, good sleep, good energy throughout the day, also my joints (knees) are better, not as stiff and can run longer."

ABOUT THE AUTHORS

Dr. Milton Bastidas was born in Colombia and is a graduate of Texas Chiropractic College with additional certification in Functional Medicine. He is Founder of True Lifelong Wellness Center and has been in practice since 1998 treating skeletal conditions, sports injuries, and systemic disorders. Dr. Bastidas is also Vice President of the College of Integrative Medicine (CIM) as well as Director of Research and Development at Transformation Enzyme Corporation. Dr. Bastidas has a passion for soccer which put him through college at Texas Lutheran University and is very sought after in the soccer community for rehabilitation and nutritional counselling. He is also an expert in the use of laboratory analysis and enzyme nutrition as part of a functional and natural approach to wellbeing.

As a lead researcher in Transformation's research and development team, Dr. Bastidas has co-directed two clinical studies showing the benefits of enzyme supplementation on systemic inflammation and gastrointestinal dysfunction as well as a most recent pilot study on the effects of enzyme therapy on Glyphosate detoxification. Dr. Bastidas lives in Houston, TX, with his family. He is currently involved in developing specific treatment protocols depending on the biochemical individuality of the patient.

"My mission is to help patients achieve and maintain optimal health through a holistic and integrative approach. I strive to help my patients regain their health by educating them on how to obtain true lifelong wellness through natural means with the aid of nutrition and enzymes."

Dr. Mahamane Mamadou holds a B.Sc. (University of Wisconsin-Madison), M.Sc. (University of Kentucky, Lexington), and Ph.D. (University of Cincinnati). Dr. Mamadou's post-doctoral fellowship was in the Department of Pharmacology and Cell Biophysics (University of Cincinnati) working on developmental gene expression. Dr. Mamadou's teaching and research activities have been in the areas of protein chemistry, enzymology, food sciences and technology, cell and molecular biology, environmental health, biomedical engineering, and biotechnology. He has taught and conducted research at several universities and has provided consulting and research services for various industries in product development, environmental toxicology, and functional foods as dietary supplements.

Dr. Mamadou continues to be actively involved in health prevention research dealing with various nutritional disorders, degenerative diseases, and the identification of health risk biomarkers. Dr. Mamadou is currently President and CSO of Phytomedic Labs.