Support for Clinical Outcomes Based on Ixcela's Self-Collected Capillary Blood Test

Ixcela is a biotechnology company founded in 2012 by MIT biochemists Erika Angle, PhD and Wayne Matson, PhD, and is based in Beverly, Massachusetts. Ixcela has developed a comprehensive blood-based Functional Gut Assessment (FGA) that provides results for the Gut/Gut Connection, Gut/Body Connection, and Gut/Brain Connection. Ixcela has been used by many world-renowned institutions like MGH, Mayo Clinic, Duke, UCLA, Roper St. Francis Healthcare, the ALS Institute for research, and NASA. Ixcela testing has also been used by professional athletes, Olympians, and thousands of other people who are simply trying to feel their best.

Overview and methodology

This document describes the validation methodology for Ixcela's Functional Gut Assessment (FGA) test. It is divided into three sections, each focusing on one aspect of the FGA test. The link between clinical outcome and root cause is based on accepted clinical practice. The links between root cause and measured metabolites are supported by published literature. The core elements of our therapeutic recommendations are then stated, supported by published literature, or based on accepted clinical practice.

The FGA test

The FGA test is a capillary blood test that has been CLIA certified for repeatability, accuracy, and stability as long as samples are received within 1 week of collection.

Ixcela runs and maintains a certified CLIA lab authorized to analyze these samples. We use an LCECA (liquid chromatography coupled with an electrochemical array detector) analytical platform to analyze our blood samples. This platform has been used for decades by universities, pharma, and the CDC as a reliable analytical platform. Please see the attached research articles for reference about this technology (Kaddurah-Daouk et al., 2010)., (Reavis et al., 2021)., (Cudkowicz et al., 2009)., (Steen et al., 2020)., & (Rosas et al., 2015).

Blood samples are collected on Neoterix Mitra® tips (a dried blood spot equivalent q-tip-like sponge). Collection is performed in the office professionally or at home as kits are designed for simplicity and ease of collection. FedEx 2-day domestic shipping envelopes are provided to ensure samples return to the lab on time. International shipping can be arranged separately if necessary, though at increased cost.

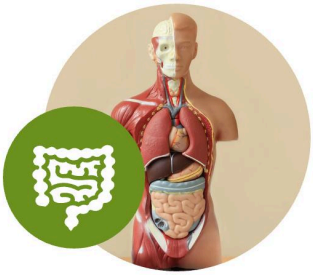
The current FGA test measures:

- Indole-3-Propionic Acid (IPA)
- Indole-3-Lactic Acid (ILA)
- Indole-3-Acetic Acid (IAA)
- 3-Methylxanthine (3MX)
- Total Indoxyl Sulfate (TIS)
- Kynurenine (KYN)
- Xanthine (XAN)
- Tryptophan (TRP)
- Tyrosine (TYR)
- Serotonin (SER)
- Uric Acid (UA)

The 3 areas of assessment

Gut/Gut Connection

Intestinal permeability and gut microbiome diversity



Gut/Body Connection

Underlying drivers of inflammation and visceral adiposity

Gut/Brain Connection

Neurotransmitter availability and blood-brain barrier integrity

Due to the complex and novel nature of many Ixcela metabolites, we have found it best to consider them along metabolic pathways at three points of connection: the gut-gut connection, the gut-body connection and the gut-brain connection. There are certainly points of overlap, but in this primer, considering them in this fashion will be the most efficient way to rapidly digest many novel biomarkers and their important implications for health.

Gut/Gut Connection

Intestinal permeability and gut microbiome diversity

The gut has two domains with immense interactive complexity. The first is the microbiome, comprised of the commensal bacteria, mucus layer and microbe byproducts. The second domain is the human luminal cells which are critical to maintaining a barrier while also facilitating nutrient absorption. Two of the primary drivers in the gut/gut connection portion of the FGA test are intestinal permeability and gut diversity.

Intestinal permeability

Intrinsic to our fundamental understanding of gastrointestinal physiology is that the intestinal epithelium lining the large and small intestines is made of a single layer of simple columnar cells. The luminal surface of these cells directly interacts with the microbiome and all oral intake, e.g. food, supplements and medication. These cells are critical for absorption of nutrients in a highly ordered and regulated manner. The tight junctions attaching these cells to one another are responsible for maintaining the integrity of the barrier between the intestinal lumen and the bloodstream. When the proteins, such as claudins and occludins, required to maintain these tight junctions become compromised, paracellular transport becomes unregulated and luminal contents can flow into the bloodstream with less restriction (Graziani et al., 2019). These inappropriately absorbed substances lead to inflammatory cascade activation with wide ranging clinical and subclinical implications. Proper maintenance of the intestinal epithelium therefore becomes foundational to optimal health and modulated inflammatory response. These cells are renewed every 4-5 days which necessitates the luminal surface be continuously enriched with diverse bacteria, postbiotics and other nourishing substances that support the establishment of tight junctions with high integrity. Antibiotics, inflammatory substances, malicious bacteria and their toxins can damage these tight junctions allowing paracellular transport of unchecked material, thus initiating an inflammatory cascade which further facilitates ongoing loss of intestinal epithelium integrity.

IPA as a measure of intestinal permeability

Low levels of indole-propionic-acid (IPA) have been shown to be directly associated with increased gut permeability (Z.H. Zhao et al., 2019). A positive association is established by the upregulation of tight junction proteins, ZO-1 and occludin, by IPA when at optimal levels. The absence or suboptimal levels of IPA will disrupt intestinal epithelium homeostasis and increase the production of endotoxins in the gut and therefore weaken the intestinal barrier (Li et al., 2021). On the other hand, when IPA is at optimal levels, it strengthens the mucus barrier by increasing mucins (MUC2 and MUC4) and goblet cell secretion products (TFF3 and RELMβ).

Below is the biochemical pathway showing the conversion of tryptophan by various bacterial species in the microbiome into IPA (Roager & Litch, 2018).

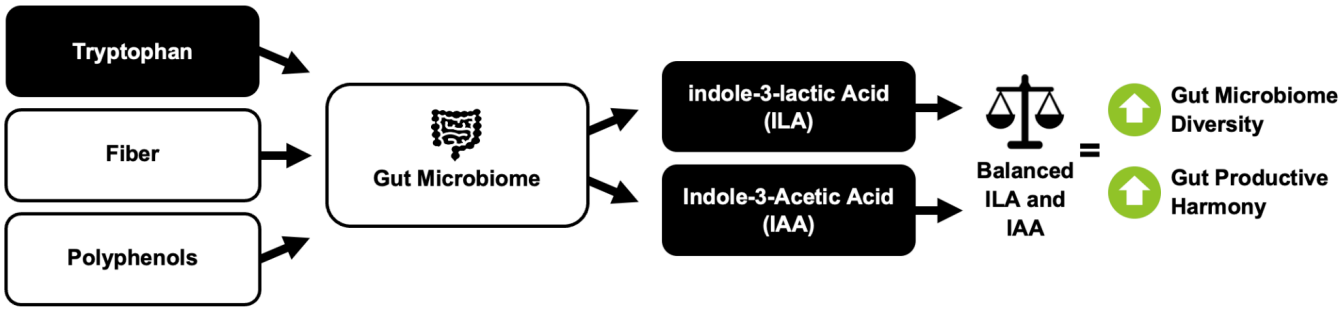
The link between tryptophan and indole propionic acid beyond intestinal permeability

As shown above, IPA is created in the gut through a process involving the conversion of tryptophan by certain bacteria in the gut. Either a deficiency of tryptophan in the diet or a lack of capable gut flora can cause low levels of IPA. While the effects on intestinal permeability are vital, it is important to also recognize the effects beyond the gut. Emerging evidence is demonstrating the role of optimal tryptophan and IPA levels for improving cardiovascular health due to anti-inflammatory properties and reduction in oxidative stress (Li et al., 2022). With the goal of optimizing internal fitness, Ixcela provides unique data with a gut-focus but wide ranging health implications for both general and special populations.

Therapeutic recommendations

When IPA is found to be low in an individual, the next step is to look at the tryptophan level. If the tryptophan level is suboptimal, it must be repleted as the supply of tryptophan is rate-limiting in the microbiome mediated production of IPA. Intrinsic to understanding this pathway is the understanding that no amount of beneficial bacteria or other nutrients can carry out their necessary function without sufficient tryptophan as substrate for the production of IPA. When tryptophan levels are optimal and IPA is low, it becomes clear that skilled labor is the issue rather than substrate supply. In this case, the skilled labor is provided by a sufficient active population of gut bacteria with molecular mechanisms to process tryptophan into IPA. More information about the gut bacteria population cannot be determined by further analysis of IPA and tryptophan so we move on to assessing IAA and ILA covered in the next section.

Gut microbiome diversity



Why gut microbiome diversity matters

The gut microbiome consists of a population of approximately 100 trillion commensal flora that carry out essential functions for human health. A cornerstone of this ecosystem is the need for a high level of diversity. The environment of the gut is ever-changing and ever-moving, thus requiring flora to be resilient in the face of fluctuating nutrient levels, toxic substance exposure (e.g. antibiotics), and competition from pathogen strains. Bacterial species can vary greatly in their functional ability to breakdown nutrients and produce active metabolites. A high level of microbiome diversity helps ensure that nutrients are maximally utilized which leads to greater overall efficiency of dietary intake (Valdes et al., 2018). Higher diversity also leads to improved immune system modulation. When the diversity is high, there is also a reduced chance for a certain genus or species to become dominant, which can happen in many disease states.

Gut microbiome diversity and balanced levels of indole-3-lactic acid (ILA) and indole acetic acid (IAA)

Indole-3-lactic acid (ILA) is a key molecule produced by common gut bacteria, predominantly *Lactobacillus* and *Bifidobacterium*. Importantly, changes in abundance or activity of these bacteria will affect the amount of ILA produced and absorbed into the body. Adequate tryptophan and fiber are important sources of substrate for these bacteria to produce ILA. Therefore, low tryptophan intake can shift the relative abundance and productivity of these strains. Antibiotics and other noxious, inflammatory substances in the gut can negatively impact ILA production. Higher microbiome diversity helps support optimal ILA production even during adverse conditions within the gut because different subpopulations will have heterogeneous responses to any given stressor. To further support meaningful diversity, ILA increases the relative abundance of other tryptophan producing bacteria like those of the Clostridium genus. In this way, ILA has been shown to protect against intestinal inflammation and correct microbial dysbiosis (Wang et al., 2024).

The positive effects of ILA and IAA appear to be from direct action on gut flora. For example, some animal studies have demonstrated that higher IAA can increase *Bacteroides* and decrease *Proteobacteria* and *Firmicutes* (Shen et al., 2022). This is notable because higher relative abundance of *Firmicutes* compared to *Bacteroidetes* has been associated with obesity (Koliada et al., 2017). This finding suggests that optimal ILA and IAA levels don't support gut flora diversity blindly, but enhance the diversity by supporting health-promoting bacteria to achieve greater relative abundance.

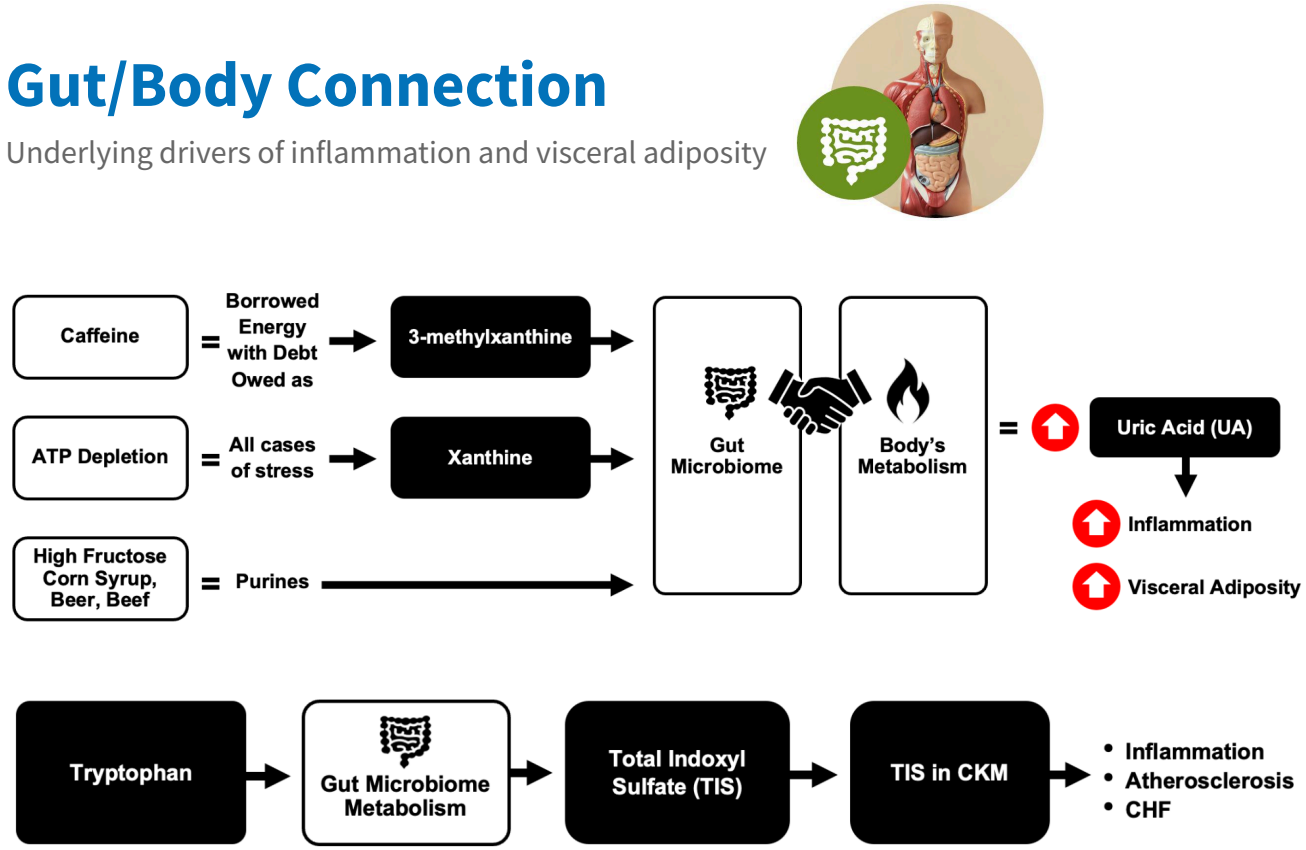
How managing tryptophan in the diet improves production of ILA and IAA

Tryptophan, an essential amino acid in the diet, is metabolized by gut microbes to a variety of tryptophan metabolites, including indole, indolic acid, skatole, and tryptamine, all of which have profound effects on the gut microbial composition, microbial metabolism, the host's immune system, the host-microbiome interface, and host immune system–intestinal microbiota interactions (Gao et al., 2018). Tryptophan appears to be among the first movers in the complex diet-induced host-microbial cross-talk (Roager & Litch, 2018). It is unfortunately quite difficult to estimate tryptophan intake from diet. Numerous factors, such as timing and frequency of protein intake, plant vs animal sourced protein and intestinal surface area differences due to disease states, can all make approximating tryptophan inaccurate based on history. Directly measuring levels in blood correlates well with intake due to the essential nature of this amino

acid. This insight helps provide a mechanism for targeted dietary intervention with a tool for measuring the response to the intervention in the form of retesting.

Therapeutic recommendations

Indole -3- lactic acid (ILA) and indole acetic acid (IAA) levels that are high or low can demonstrate dysfunction in the metabolism of the gut microbiome. The therapeutic goal is to achieve a balance of these. ILA and IAA are both tryptophan-derived bacterial metabolites, thus adequate supply of tryptophan is essential for production. If ILA and IAA are low, tryptophan levels should be reviewed for the next step in the analysis. Repletion of tryptophan from dietary sources can be recommended if low. Alongside increasing tryptophan through dietary protein sources rich in this essential amino acid, diverse sources of fiber and polyphenols should also be recommended to help enrich the gut environment and further support overall flora diversity (Rodríguez-Daza et al., 2021). High levels of IAA and ILA can be due to recent antibiotic use, which causes massive shifts in absolute and relative abundance of gut bacterial strains. Single or low-strain probiotics can also create an inappropriately high relative abundance of certain bacterial genus or species. Additionally, rapid transit chronic diarrhea of any cause, such as chronic anxiety, and a bland, colorless, low-fiber diet can limit bacterial diversity and support dysbiosis in the gut microbiome that is reflected by suboptimal ILA and IAA. Repeat testing in 2-3 months is an excellent way of objectively determining if interventions are effective along with subjective clinical response.



Inflammation

How uric acid (US) and total indoxyl sulfate (TIS) impact Inflammation

Most acute and chronic disease states are driven by inflammation. This includes conditions such as diabetes, coronary artery disease, NAFLD, obesity, neurodegenerative disease, and cancer. Uric acid is well recognized for its role in acute and intercritical gout (Hainer et al., 2014). Uric acid also plays a role in generating chronic inflammation in other disease states. Emerging research has implicated the role of uric acid in inflammatory cascade activation and in blocking anti-inflammatory hormones (detailed below) (Spiga et al., 2017). Total Indoxyl Sulfate (TIS) is well established as a uremic toxin with potent proinflammatory potential through the upregulation of inflammatory cytokines. Emerging evidence has shown the role of indoxyl sulfate in generating adipose tissue inflammation (Tanaka et al., 2020) and vascular inflammation (Nakano et al., 2019). Indoxyl sulfate appears to also interfere with vascular endothelial function by inhibiting nitric oxide production (Hung et al., 2017). Initially, indoxyl sulfate was mainly implicated only in chronic kidney disease (CKD). Fortunately, the newly defined Cardiovascular-Kidney-Metabolic (CKM) Syndrome has demonstrated the interconnectivity and inseparability of cardiovascular diseases, CKD, obesity, and diabetes, which share the common origin of chronic inflammation.

Visceral adiposity

How uric acid (UA) impacts visceral adiposity

Elevated uric acid (UA) levels have been shown to increase inflammation and oxidative stress in adipose tissue, which leads to adipogenesis and inhibition of adiponectin. Adiponectin is a hormone produced by the adipocytes with anti-inflammatory and insulin-sensitizing properties. Clinical evidence has shown that hyperuricemia is associated with increased visceral adiposity and hepatic fat storage independent of anthropomorphic measures of total body fat and other potential confounding factors (Rospleszcz et al., 2020). Hyperuricemia has also been shown to predict weight gain five years prior, demonstrating its robust clinical value in the preventive health setting (Masuo et al., 2003). Furthermore, over a multiyear period, uric acid has been shown to be a "significant predictor of less favorable BMI, triglycerides, HDL, glucose, insulin, and HOMA, independent of age, sex, baseline weight, baseline level of the outcome variable, and weight gain prevention intervention" (Corso et al., 2020).

How 3-methylxanthine (3MX) affects uric acid (UA) levels

3-methylxanthine (3MX) is a byproduct of theophylline and caffeine which is meaningful with the high intake of caffeine among the general population today. Ultimately, 3MX is metabolized into uric acid through various pathways in the microbiome and body. When caffeine is ingested at high amounts, it is helpful to consider the price the body is paying to access the energy this provides. 3MX acts as a ledger to account for what is "owed" for borrowing this energy. The debt is paid through the downstream consequences of higher uric acid levels. While the issues of caffeine intake are apparent at the extremes in terms of increased premature ventricular contractions, anxiety and headaches, the subclinical impact is often missed and the harms smolder under the surface. Compounding the effect on uric acid, increased caffeine intake with

subsequent increased 3MX is associated with poorer sleep and poor sleep is associated with further increases in uric acid (Papandreou et at., 2019).

How xanthine (XAN) affects uric acid (UA) levels

Xanthine (XAN) is a purine base that is a downstream product from ATP utilization and degradation which is ultimately further metabolized into uric acid. Stress of all causes nearly universally leads to increased inflammation and oxidation due to increased ATP utilization. Xanthine oxidoreductase (XOR) is an enzyme that catalyzes the oxidation of hypoxanthine to xanthine to uric acid with ROS production (Chen et al., 2016). Hyperuricemia is caused by overproduction or under-excretion of uric acid with overproduction being driven by increased xanthine as a substrate with XOR as a rate-limiting enzyme.

How total indoxyl sulfate (TIS) affects uric acid (UA) levels

Cell culture experiments found that high levels of uric acid (UA) can reduce endothelial dysfunction (Lin & Tarng, 2017). This protective effect of uric acid may be due to its ability to decrease oxidative stress caused by indoxyl sulfate and to help maintain the production of nitric oxide (NO). Uric acid mitigates the oxidative stress and endothelial dysfunction induced by indoxyl sulfate, suggesting a protective role of uric acid in the context of hemodialysis patients. Uric acid's antioxidant properties at optimal levels may counteract the harmful effects of TIS on vascular health, thereby improving patient outcomes.

How tryptophan (TRP) affects total indoxyl sulfate (TIS) levels

Dietary tryptophan is metabolized by gut bacteria into total indoxyl sulfate (Gryp et al., 2020). This process involves bacterial enzymes like tryptophanase, which convert tryptophan into indole, subsequently converted into indoxyl sulfate in the liver. Elevated levels of indoxyl sulfate are linked to nephrotoxic effects, including oxidative stress, inflammation, and fibrosis, contributing to the progression of Chronic Kidney Disease. A small fraction of tryptophan (~5%) in the gut is converted into indole, while the other fraction is used in the kynurenine (~95%) and serotonin (~1-2%) pathway (Gryp et al., 2020).

Therapeutic recommendations

Historically, uric acid (UA) is only treated to a target when a patient has a history of gout flares. Total indoxyl sulfate (TIS) is rarely measured in clinical practice despite its emergence as an important health marker yielding actionable information independent of other measured factors. Our approach offers a mechanism to develop targeted plans for health optimization. By testing multiple metabolites along the metabolic pathway, we show actionable areas that reveal the underlying root driving the suboptimal endpoint. For example, caffeine intake is often difficult to quantify due to a lack of consistency among many infused drinks and foods. It is possible, and often likely, for patients to significantly underestimate their caffeine intake. By checking 3-methylxanthine (3MX), a direct caffeine metabolite, we can identify whether this is contributing to the suboptimal clinical endpoint. Similarly, stress is difficult for most people to quantify. Stress can come from so many sources in the form of emotional stress and physical stress. Some of this stress is voluntary, such as during intense exercise, but more often, it is involuntary. Dietary choices can also be major drivers of

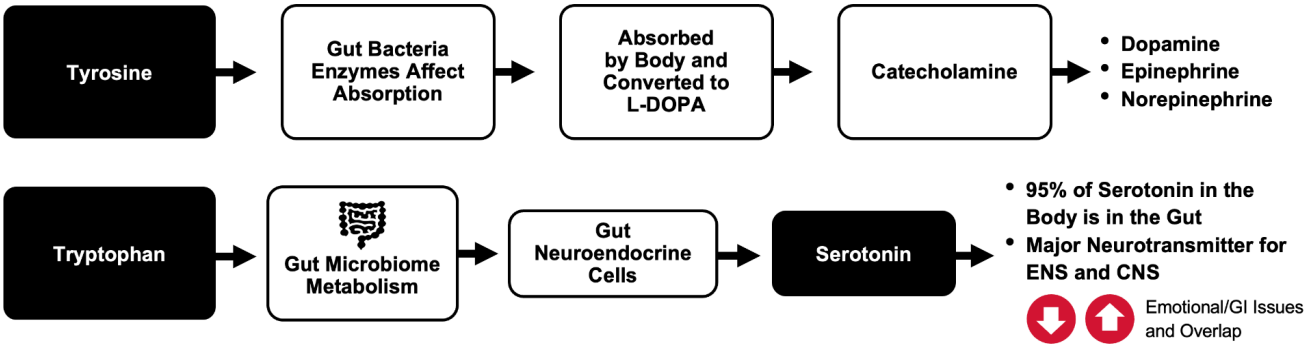
difficult-to-identify physical stress. Having xanthine (XAN) as a tested metabolite gives insight into the level of non-specific sources of stress that cause ATP depletion. Ultimately this xanthine will be metabolized into uric acid with all of its downstream consequences. Clinically, this is an opportunity to delve deeper into the sources of stress and identify lifestyle, dietary, medication, or psychological interventions based on a patient's unique history.

For total indoxyl sulfate (TIS), the primary means of lowering it is by causing changes in the gut where it originates. Based on other individual factors, there may be an opportunity to increase the amount of fiber compared to the amount of protein in the diet. In the setting of high TIS, it is also possible to lower the level by manipulating the relative abundance of gut flora strains with prebiotics and polyphenols. As a uremic toxin, TIS is cleared by the kidney, therefore increasing hydration is encouraged to ensure maximal excretion. Given the emerging recognition of CKM Syndrome, it is also important to focus on the other contributing factors in this syndrome that are modifiable as there are many potential levers to pull when adopting a healthy lifestyle. Routine monitoring of TIS after realizing diet, exercise and other lifestyle modifications is the best way to ensure exposure to this potential toxin does not reach harmful levels.

Gut/Brain Connection

Neurotransmitter availability and blood-brain barrier integrity

Neurotransmitter availability



Root cause and clinical outcome

It is important to be specific as to which "brain" when we discuss the gut/brain connection. As far as the body is concerned, there are two independent though interconnected nervous systems, or "brains", within the body. The Central Nervous System (CNS) is the dominant controller of the body's function. The Enteric Nervous System (ENS) controls the gastrointestinal tract autonomously with input from the CNS. There is bidirectional communication between the CNS and ENS, largely through the vagus nerve. As a consequence, many neurological disorders have neurological and

gastrointestinal clinical signs and symptoms (Rao & Gershon, 2016). Up to 95% of the serotonin in the body is found in the gut, making it the major neurotransmitter of the ENS. This dominant source of serotonin also makes it evident that serotonin measured in the blood is essentially entirely gut-derived.

We understand the extreme clinical effects of this through paraneoplastic conditions like carcinoid tumors, where copious amounts of serotonin are released into the gut with extreme adverse clinical effects of hypermotility and diarrhea. The majority of serotonin is produced by enterochromaffin cells, which are functional gut neuroendocrine cells. The gut microbiome regulates the production and metabolism of serotonin as a tryptophan-derived metabolite. Serotonin plays a significant role in Irritable Bowel Syndrome where research suggests a decrease in serotonin transporter (SERT) leads to higher bioavailable serotonin. Patients with celiac disease and IBS-Diarrhea have been found to have higher blood levels of serotonin, making it a non-specific marker of hypermotility and a tendency to diarrhea (Camilleri, 2009). While serotonin measured in blood is mostly from the gut, it is essential to consider that gut production of serotonin may be stimulated by the CNS and therefore be related to a neurological or psychological disorder.

Tyrosine is a nonessential amino acid that is mostly derived from the diet directly or through the hydroxylation of phenylalanine in the liver. In phenylketonuria, individuals lack the enzyme to convert phenylalanine to tyrosine and must take supplement forms of tyrosine. The neuropsychiatric consequences of severe tyrosine deficiency have been well documented in this condition. Certain gut bacteria have the functional capacity to metabolize dietary tyrosine in the gut prior to absorption in the bloodstream. Tyrosine present in the blood can be directly absorbed or converted in the liver from phenylalanine. Ultimately, it is vital to have sufficient tyrosine which gets converted to L-DOPA in the body. L-DOPA is the catecholamine precursor which ultimately becomes dopamine, norepinephrine, and epinephrine. Tyrosine is also necessary for the production of thyroid hormones (Nussey & Whitehead, 2001).

How tyrosine (TYR) levels correlate with neurotransmitter availability

The amino acid tyrosine is the precursor to dopamine (DA) and norepinephrine, which are neurotransmitters in the brain. Adequate consumption of tyrosine in the diet can help counteract deficits in neurotransmitter levels and cognitive function (Jongkees et al., 2015). Tyrosine consumption is particularly helpful in short-term stressful situations or cognitively demanding situations and supports neurotransmitter production and function.

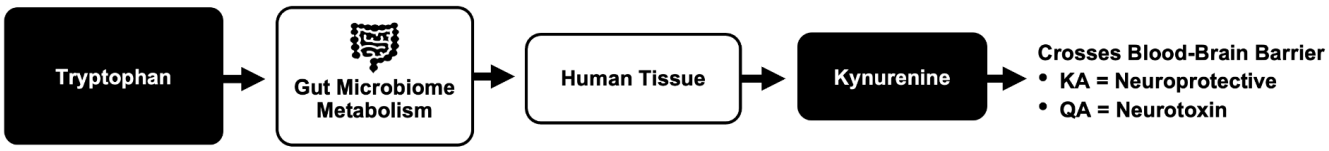
How serotonin (SER) levels impact blood-brain barrier integrity

Intestinal serotonin plays a crucial role in controlling the permeability of membranes in the intestine, brain, and other organs (Szőke et al., 2020). It acts as a hormone, sending continuous regulatory signals throughout the body, including the brain. This signaling function relies on platelets and is largely dependent on gut health. This idea may help explain why an imbalance in gut bacteria (gut dysbiosis) is connected to various health issues and disorders affecting brain development and mental health.

Therapeutic recommendations

Therapeutic planning based on neurotransmitter availability in the form of serotonin and tyrosine levels requires tertiary synthesis of several health parameters. While the gut plays a central role, modifiable factors like stress, sleep quantity and quality, diet, and medications are also involved. Due to this, an individual's unique health issues, such as sleep apnea and anxiety, must be included alongside the objective metabolite laboratory data. The metabolite data serves as an orienting point for this discussion and analysis, accelerating a directional approach. Low levels of serotonin or tyrosine can reveal inadequate protein intake or absorption, which can be seen in vegan diets and malabsorptive states like celiac disease. There has been a relatively recent implication of Small Intestinal Bowel Overgrowth (SIBO) in affecting these levels and can be considered with further directed testing in this setting with other elements driving clinical suspicion (Chojnacki et al., 2022). Also, when lifestyle factors are considered optimized clinically, this testing serves as a reference to the gut and body's chemical perspective. Sometimes, high tyrosine can reveal excessive protein supplementation, especially in regards to excessive protein relative to fiber intake. Sometimes, low serotonin can bring a conversation on insomnia out of obscurity. Ultimately, collective consideration of serotonin and tyrosine with the other Ixcela metabolites in the context of life's situational complexities yields the fullest spectrum view of how to prioritize actions and track improvements.

Blood-brain barrier integrity



Root cause and clinical outcome

With the emerging evidence regarding the importance of blood-brain barrier (BBB) integrity for mental health and the prevention of neurodegenerative diseases, we provide a foundation to ensure supportive elements are optimized. Much of our understanding of the link between the gut and the brain has come from rodent studies due to the need for tissue samples unobtainable in living human subjects. This has yielded some vitally important information regarding our symbiotic relationship with the gut microbiome. Studies on sterile mice have shown decreased tight junctions causing increased permeability of the BBB. Additionally, antibiotic-induced gut dysbiosis in mice has been shown to cause increased permeability of the BBB (Fröhlich et al., 2016). The gut's influence on the BBB has many proposed pathways through gut-derived metabolites such as butyrate and propionate (short-chain fatty acids). Referring back to the studies on sterile mice, when these mice are enriched with butyrate-producing probiotics, their BBB permeability decreases (Parker & Carding, 2020). While we do not measure butyrate directly, we do measure IPA, which is mostly derived from the *Clostridium* genus, which is also largely responsible for the production of butyrate. Similarly, the same fiber-rich foods that support optimal levels of IAA and ILA are also the substrate for gut-derived butyrate. Emerging evidence is also showing IPA's direct role in protecting the BBB (Q Zhao et al., 2022). Notably, IPA also demonstrated neuroprotective properties in a human randomized double-blind placebo-controlled trial where it was shown that higher IPA levels correlated with

increased Brain-Derived Neurotrophic Factor (BDNF), which is known to be neuroprotective and anti-inflammatory within the brain (Kim et al., 2023).

We also measure kynurenine which originates from tryptophan absorbed in the gut and then metabolized by the liver, after which it readily crosses the blood-brain barrier. At optimal levels within the brain, it is further metabolized into kynurenic acid, which is neuroprotective through its role of antagonizing glutamate and alpha-7 nicotinic acetylcholine receptors, thus reducing excitotoxicity. Excessive kynurenine can lead to shunting the metabolic pathway towards producing quinolinic acid, which has neurotoxic properties (Schwarcz et al., 2012).

How serotonin levels impact blood-brain barrier integrity

Intestinal serotonin plays a crucial role in controlling the permeability of membranes in the intestine, brain, and other organs (Szőke et al., 2020). It acts as a hormone, sending continuous regulatory signals throughout the body, including the brain. This signaling function relies on platelets and is largely dependent on gut health. This idea may help explain why an imbalance in gut bacteria (gut dysbiosis) is connected to various health issues and disorders affecting brain development and mental health. We are just starting to understand the complexities of these associations and for the moment are limited to recognizing the importance of balance in this system. We do have emerging evidence suggesting how the protein klotho which does not cross the blood-brain barrier affects brain function through platelet factors (Park et al., 2023). Further connecting the gut and brain, buyrate, a short chain fatty acid (SCFA), can modulate serotonin production for intestinal enterochromaffin cells (Everett et al., 2022). The effects of butyrate on the blood-brain barrier have already been detailed above.

How tryptophan levels impact serotonin and kynurenine levels

Tryptophan (TRP) is an essential amino acid that must be obtained from diet. It is important for building proteins and serves as a starting material for producing several important bioactive compounds. The levels of TRP in our body depend on what we eat and the activity of several metabolic pathways that process TRP. Three main pathways metabolize TRP: the kynurenine (KYN) pathway, the 5-hydroxytryptamine (5-HT) pathway, and the indole pathway (Xue et al., 2023). The most significant of these is the KYN pathway, where over 95% of TRP is broken down into multiple bioactive compounds. Key enzymes in this pathway include TRP-2,3-dioxygenase (TDO), indoleamine-2,3-dioxygenase 1 (IDO1), and IDO2. This pathway plays roles in inflammation, immune responses, and neurotransmission, and it is linked to various diseases. The 5-HT pathway, also known as the serotonin pathway, is involved in several vital bodily functions. TRP can be converted into 5-hydroxytryptophan (5-HTP) and then into serotonin (5-HT) in the brain and gut cells. Serotonin is a crucial neurotransmitter that helps regulate sleep, cognition, and eating behavior. The enzymes IDO, TDO, kynurenine-3-monooxygenase (KMO), and TRP hydroxylase (TPH) are important for controlling TRP metabolism in these pathways.

Therapeutic recommendations

Supporting brain health requires a multimodal approach and comprehensive review of Ixcela metabolites. Dietary strategies that increase IPA and balance IAA and ILA are likely to translate into higher levels of butyrate production which nourishes cells at the blood-brain barrier. Having optimal IPA levels also helps prevent bacterial toxins and other substances from entering the bloodstream and potentially interacting with the BBB. Uric acid should also be considered through a neuroactive lens as studies suggest it can pass through a compromised BBB and cause damage within the brain (Topiwala et al., 2023). Generally, the approach can be lifestyle-driven with improvements in diet, exercise, sleep, and stress management though more recently, probiotics have emerged with butyrate-producing strains.

Optimizing kynurenine requires further clinical investigation once a suboptimal level is identified. Typically, high kynurenine is caused by chronic inflammation or stress secondary to non-specific sources. This could be secondary to a medication like chemotherapy, autoimmune disease, or a chronic infection like gingivitis. Excessive protein intake or vitamin B deficiency can also contribute to high levels. On the other hand, low levels can be caused by inadequate protein (tryptophan) intake and low niacin (vitamin B3) levels. Routine monitoring of kynurenine helps ensure controllable factors are optimized to provide long-term neuroprotective support.

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