FLUIDS iQ®

RESULTS: DRIED BLOOD SPOT TEST

Accession #: 100035536 • Patient: JONATHAN Smith

Patient: JONATHAN Smith
Sex: Male **Age:** 34 yr **Date of Birth:** 1989-01-10
Health Care Professional: Jane Smith

Accession #: 100035536
Sample received: 2023-07-20
Report issued: 2023-08-01
Sample collection: 2023-07-15

ZONULIN FAMILY PEPTIDES (ZONULIN FP) + HISTAMINE : DIAMINE OXIDASE (DAO)

Zonulin FP

Result: 6.8 ng/ml — Optimal

Histamine

Result: 0.6 ng/ml

DAO

Result: 4.1 ng/ml

Histamine : DAO Ratio (x1000)

146.34

* The reference range represents a quintile distribution, each quintile being 20 % of a population determined from archival data.
** Very low Histamine levels may cause signs and symptoms of histapenia (see commentary).
† Very high DAO levels may be linked to early signs and symptoms of abnormal barrier function (see commentary).
†† Very low ratio could be a sign of the breakdown in barrier integrity (see commentary).

Analysis performed by GB Diagnostics, Kingman AZ, USA - CLIA # 03D2182284

GENERAL COMMENTARY Zonulin Family Peptides (Zonulin FP) + Histamine : Diamine Oxidase (DAO)

The comments provided here are for educational purposes only. The results in this report should not be interpreted as diagnostic, nor should they be viewed as treatment recommendations. Those decisions are the responsibility of the health care professional. Moreover, the reference range represents a quintile distribution, each quintile being 20 % of a population determined from archival data (see below).

The FLUIDS iQ Zonulin FP test, which uses the most advanced, polyclonal antibody immunoassay developed to date, is our test for Permeability. By its design, this test measures pre- haptoglobin 2 (see below), known as Zonulin, as well as other very closely related peptides implicated in intestinal permeability.

Zonulin

Zonulin is a protein found in intestinal cells, with production and release mimicking the effect of certain bacterial toxins on the tight junctions of the small intestine. Zonulin, and a small family of closely related peptides, bind to specific receptors only on the apical (luminal) surface of the intestinal epithelia and trigger a cascade of biochemical processes that induces tight junction (TJ) disassembly and a subsequent permeability increase of the intestinal epithelia.[1] This is often referred to as “leaky gut”.

Intestinal permeability changes due to Zonulin have been implicated in many diseases and dysfunction, notably in Celiac Disease and Type I Diabetes, but also in others including the brain, respiratory system and skin.

The gluten glycoprotein, α-gliadin, can activate Zonulin signaling, irrespective of the genetic expression of autoimmunity. This generates a two-way response: Not only can fluid exit, but intestinal contents are able to gain entry in the opposite direction, into the bloodstream. This gliadin-Zonulin ‘leakage’ effect is longer and more pronounced (up to 5-fold greater) in the enterocytes, or intestinal cells, of people with Celiac Disease (CD).[2]

When intestinal tissue is taken from CDs in remission and from non-CD controls with digestive complaints, results show that CDs may produce up to 30 times as much Zonulin as non-CDs and have a three-fold greater intestinal permeability, even though the non-CDs are eating diets containing gluten, while the CDs have been gluten free for over two years.[3]

This strongly suggests that something besides gluten may be contributing to permeability changes in people with celiac disease. It may be that certain types of intestinal dysbiosis (improper balance of bacteria and yeasts in the intestines) prime genetically susceptible individuals to develop CD in response to gluten. In addition, many people who suffer from CD also suffer from other autoimmune disorders. Increased levels of Zonulin are implicated in corticosteroid use, as well as the pathogenesis of insulin dependent diabetes (type 1) and juvenile nonalcoholic fatty liver disease. There is also evidence of its implication in multiple sclerosis, rheumatoid arthritis, skin diseases, as well as inflammatory bowel disease and obesity.[4, 5]

Zonulin also plays an important role in permeability changes in the brain, working as a gatekeeper, not only in the intestine, but also at the blood brain barrier (BBB). This is clearly in evidence when there is an intake of foods containing the α-gliadin or similar proteins. The resulting high Zonulin levels leads to disassembly of the TJs in the vascular epithelium, permitting many molecules, including toxins, to slip through the BBB and resulting in activation of a cerebral inflammatory response.

The resulting symptoms may include anxiety, depression, brain fog, slow mental processing, and emotional disturbances. Over time, this chronic inflammation may progress to neurodegenerative conditions such as dementia, Alzheimer's, and Parkinson's disease.[6]

Zonulin is also involved in the regulation of airway and lung permeability through its action on the TJs of the respiratory epithelial and/or endothelial barriers. This can be seen in Asthma, a complex clinical syndrome characterized by airflow obstruction, airway hyper responsiveness, and inflammation. Increased intestinal permeability in asthmatics may play a role in their susceptibility to environmental allergens. Serum Zonulin levels are high in a subset of subjects affected by Asthma, with 40% of asthmatic patients exhibiting increased intestinal permeability. This suggests that, besides inhalation, an alternative route for the presentation of specific antigens or irritants may occur through the gastrointestinal mucosal immune system, following their intercellular passage through the TJs.[3]

The effect of Zonulin is also evident in lung infections, including Acute Lung Injury (ALI). The role of Zonulin in ALI links the regulation of permeability with the inflammatory response through direct activation of the complement system.[7]

Why Test for Zonulin FP?

Zonulin plays a pivotal role in the control of the tight junctions of the small intestine. As mentioned above, increased Zonulin levels are seen in many conditions and diseases associated with increased intestinal inflammation, with changes in permeability preceding clinical manifestations by up to a year.[8,9] For that reason Zonulin is gaining acceptance as a non-invasive marker of intestinal wall integrity and developing disorders.

The Zonulin FP range in this report is from 0 to 30 ng/ml. It should NOT be interpreted as meaning that this entire range is the optimal range for Zonulin FP. Rather, it represents the range for 95% of randomly selected individuals in a population and includes individuals with no disorders or disease, through to those with diagnosed inflammatory and/or autoimmune disorders.

Values between 0 and 7.8 are considered as Optimal (green). If there are gut issues, they are not sufficient to have an effect on gut permeability.

Values between 7.8 and 10.8 are Borderline (yellow-orange). The effects of gut inflammation, often caused by a combination of dysbiosis and enzyme imbalances, are beginning to have an effect on permeability.

Values from 10.8 to 30 are considered as Elevated (red). Within this red portion of the range one may find individuals showing signs and symptoms of enzyme deficiencies, dysbiosis, acute or chronic inflammatory disease and those with established autoimmune disorders. A small percentage of individuals with acute disorders may show Zonulin FP levels much greater than 30 ng/ml and are noted as Above Range.

Histamine and Diamine Oxidase

Histamine is a nitrogenous compound known to be involved in 23 different physiological functions which include local immune responses, neurotransmission, as well as regulation of physiological function in the gastrointestinal tract.

Histamine is either stored, or rapidly metabolized and inactivated, by either of two degradative enzymes, histamine-N-methyltransferase or diamine oxidase (DAO); the latter being the principal enzyme observed in the digestive tract.

The testing of histamine, together with DAO levels, provides important information that is not revealed with standard food sensitivity tests. The latter is often thought to be the underlying cause of gut inflammation and dysfunction, when in fact the reason may actually be an imbalance of accumulated histamine and the capacity for its degradation by enzymes such as DAO. This imbalance may result in a condition known as Histamine Intolerance (HIT).[11] HIT is an acquired problem and is seen in approximately 3% of the population. Symptoms may occur in up to 20% of the population when histamine-rich food is consumed together with DAO inhibitors, such as alcohol. Women represent about 80% of those affected, with most of them aged 40 or over. It is important to note that the risk of developing this intolerance is increased in those individuals who suffer from inflammatory intestinal diseases or cross-sensitivities.[11]

Why Test for Histamine?

Histamine is involved in many inflammatory and allergic processes, including both immediate and delayed hypersensitivity reactions. Histamine excess can be triggered by its release in the body as a result of a variety of environmental triggers, from the ingestion of foods with high histamine content, a deficiency in DAO, or both.

The Histamine reference range is a quintile distribution, extending from the lowest quintile, represented by values of less than 0.7 ng/ml, to the highest quintile, represented by values greater than 1.5 ng/ml. As histamine values approach the upper limit of the quintile range, there is an increasing probability of HIT.[10]

High levels of histamine (or elevated Histamine : DAO ratio)
- Runny nose, sneezing, congestion
- Itching, hives, skin flushing
- Dizziness or vertigo
- Headache, migraine
- Nausea, vomiting
- Intestinal cramps, gas
- Diarrhea
- Abnormal menstrual cycle
- Shortness of breath
- Abnormal heart rate
- High blood pressure
- Severe allergic reactions (anaphylaxis)

• Abnormalities may also arise in the following:
  - Memory
  - Body temperature
  - Circadian rhythm
  - Locomotion
  - Learning

What causes high histamine levels?

• Allergies (IgE reactions)
• Gluten Intolerance
• Small Intestinal Bacterial Overgrowth (SIBO)
• Intestinal Permeability (“Leaky Gut”)
• Gastrointestinal bleeding
• Histamine-rich foods
• DAO deficiency or DAO-blocking foods: alcohol, energy drinks, and tea
• Genetic mutations (common in people of Asian descent)
• Inflammatory bowel diseases: Crohn’s, ulcerative colitis
• Medications:
  - Non-steroidal anti-inflammatory drugs (eg: Ibuprofen-Motrin, ASA-Aspirin)
  - Antidepressants (eg: Effexor, Zoloft, Prozac, Cymbalta)
  - Immune modulators (eg: Enbrel, Humira)
  - Anti-arrhythmics (eg: Propanolol, Norvasc, Cardizem)
  - H2 blockers (eg: Zantac, Pepcid, Tagamet)

Low levels of histamine (Histapenia):

• Fatigue
• Sleep-wake disorders
• Depression and anxiety in older adults; paranoia in younger people
• Convulsions

What causes low histamine levels?

Excess copper can create low levels by decreasing histamine in the brain. In turn, the lowered levels of histamine allow more copper to accumulate. High copper in the brain may lead to a state of restlessness, insomnia, violence, depression, irritability, paranoia, and high blood pressure.

Why Test for DAO?

The ingestion of histamine rich food, alcohol or drugs that release histamine or block DAO, may provoke an imbalance of accumulated histamine and the capacity for its degradation, already referred to above as Histamine Intolerance (HIT).

An impaired histamine degradation, based on reduced DAO activity and the resulting histamine excess, may cause numerous symptoms mimicking an allergic reaction.[11]

DAO activity does not depend on the DAO alone, but also on cofactors such as vitamin C, vitamin B6, copper or manganese ions. Copper is a central component of DAO. A deficiency in copper can result in insufficient DAO being produced. Vitamin B6 is a cofactor of DAO. If vitamin B6 is missing, DAO is unable to degrade histamine.

Therefore, in assessing HIT via the DAO activity test, one should also consider determining the levels of these cofactors. The symptoms of HIT can be caused by low DAO activity because the above-mentioned cofactors are not sufficiently available.

If the DAO levels are in the normal range but the histamine levels are high, it may indicate that the issue is not insufficient DAO, but rather an overproduction of histamine, due to factors such as gut dysbiosis.

However, if the histamine levels are normal, but the DAO levels are very low, it suggests a possible genetic deficiency of diamine oxidase.

The DAO reference range is a quintile distribution, extending from the lowest quintile, represented by values of less than 8.9 ng/ml, to the highest quintile, represented by values greater than 15.2 ng/ml. The DAO quintile reference range has no significant daily variations or gender differences. As levels go from > 15.2 to < 8.9 ng/ml, it indicates an increasing probability of HIT.[11]

Low levels of DAO:

- Skin rash and pruritis (itching), urticaria (hives), eczema, psoriasis
- Nasal congestion, asthma
- Headache, migraine
- Chronic fatigue
- Anxiety, depression
- Inflammation, irritable bowel syndrome (IBS)
- Estrogen dominance, dysmenorrhea, Premenstrual Syndrome (PMS)
- Muscular pain, fibromyalgia
- Rheumatoid arthritis
- Hypertension, hypotension, arrhythmia,

• Multiple sclerosis and other neurological conditions

Determination of DAO activity, together with a detailed history, helps to differentiate food allergy and histamine intolerance. It should be performed in suspected patients who have headaches, urticaria, pruritus, diarrhea and hypotension, where food allergy has been excluded.[12]

Individuals who are unable to metabolize histamine will often improve with a variety of antihistamines. Because DAO formation occurs in the gastrointestinal system, lower than normal levels are suggestive of poor digestive dysfunction, as well as problems in the intestinal barrier.[13]

The Importance of the Histamine : DAO Ratio

The Histamine : DAO Ratio is helpful in highlighting the imbalances in histamine and DAO levels.

High Ratio: There is a relatively high level of free histamine in the system from both endogenous and exogenous sources, and/or there is an insufficient amount of DAO enzyme available for histamine degradation. It should be noted that even if DAO levels are normal, symptoms may still occur if the histamine levels are very high. As levels reach into the elevated range, histamine intolerance should be considered.

Low Ratio: The amount of free histamine is relatively low, and/or there is sufficient DAO enzyme available to degrade the histamine that is present. An adequate intake of healthy fats and other nutrients, like phosphorus, zinc, magnesium, iron, and vitamin B12, are known to play a role in enhancing DAO activity.[14] Eating mainly low-histamine foods may reduce exposure to histamine and lower its accumulation in the body. This combination of factors will lead to a decrease in the Histamine : DAO ratio.

DAO: A Biomarker of Intestinal Barrier Integrity

The degradative enzyme, Diamine Oxidase (DAO), is the principal enzyme observed in the digestive tract which scavenges extracellular histamine. Recent research has begun to shed light on another important aspect of DAO activity, which is unique among intestinal mucosal enzymes: Circulating blood levels of DAO represent a reliable marker of mucosal maturation & integrity[15]. Indeed, serum DAO activity has been shown to correlate with intestinal permeability of the small intestine.[16]

Intestinal Barrier Changes

Intestinal barrier damage is due to a number of potential causes, amongst them mechanical, microbial and/or enzymatic action. These vectors of damage can be due to:

• Genetic predisposition
• Dysbiosis
• Infections
• Loss of luminal mucous barrier, with a concomitant decrease in levels of SIgA and antimicrobial peptides (AMPs).

• High luminal histamine levels from endogenous or exogenous sources, or both. Increased histamine can occur due to gut microbial action, a diet rich in histamine or histidine, an increased mast cell response as part of an immune reaction and acute or chronic stress.

DAO is synthesized by mucosal cells of intestinal villi crypts. The damage caused by one or more of the factors above can lead to a loss of barrier integrity, which in turn can lead to further inflammatory reactions. All of this will often lead to increased intestinal permeability, which will then enter into a vicious cycle of more inflammation and barrier damage. During the period of initial damage, the mucosal cells release increased amounts of DAO which increases its serum concentration. Therefore, a change in blood DAO concentration is an indication of damage to the intestinal cavity. High DAO in blood is tightly linked to abnormal intestinal barrier function in an acute stage[17].

With more chronic damage, as often seen in cases of inflammatory bowel diseases (IBD), such as Chron's disease or Ulcerative Colitis, one begins to encounter apoptosis of the crypt cells within the villi. This decrease or loss of DAO levels in the gut lumen & blood is a sign of chronic or late stage mucosal damage to the barrier, and is a sign of breakdown in cell architecture. More specifically, it is a decrease in DAO production capacity due to the destroyed or dysfunctional specialized mucosal cells[16]. This makes DAO a sensitive & accurate marker for monitoring Crohn's Disease activity and other inflammatory bowel conditions.

The Histamine : DAO Ratio and Intestinal Permeability

As stated previously, the Histamine : DAO Ratio is helpful in highlighting the imbalances in histamine and DAO levels as well as highlighting changes in intestinal barrier integrity and permeability. Abnormal ratios have been found to highly correlate with many common gut-related issues and other complaints.

High Ratio: A high ratio is an indication that there is a relatively high level of free histamine in the system, often combined with insufficient DAO enzyme available for histamine degradation. When histamine levels are 'normal', and yet the ratio to DAO is still high, it is often a sign of abnormally low production of DAO, due to a breakdown in cell architecture, or even cell destruction (crypt hyperplasia), as seen in cases of IBD and increased intestinal permeability. DAO co-factors may also need to be considered

Low Ratio: When high levels of the DAO enzyme are available to degrade normal or low amounts of free Histamine, it could be a sign of early breakdown in barrier integrity, as DAO is often elevated in the initial stages of mucosal barrier dysfunction.

References

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