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# New Research Report Has Identified New Metabolic Markers for Exocrine Pancreatic Insufficiency in Dogs

Epi4Dogs Foundation Inc.'s purpose is to **advance science and education of EPI Exocrine Pancreatic Insufficiency (EPI) in dogs** by raising funds for EPI research that will yield useful insights or positive outcomes to improve the diagnosis and treatment of the disorder. Epi4Dogs Foundation Inc. has assisted and collaborated with various veterinary investigators to advance studies of the genetics, gastrointestinal, and nutritional aspects of EPI.

**We are thrilled to announce the results of a recent investigation of metabolism in dogs with EPI.** This research was led by a team of investigators at the University of Illinois, including Drs. Patrick Barko and David Williams, both board-certified specialists in small animal internal medicine. **This research utilized a cutting-edge method to measure hundreds of biochemicals involved in metabolic reactions in the blood of dogs with EPI** and compare them to healthy dogs. This approach is called "untargeted metabolomics" and has the potential to reveal new facets of complex disorders like EPI. The results of this study have generated novel insights into the impacts of EPI on metabolism in affected dogs, and has revealed new markers that may aid in the assessment of its impact on gastrointestinal bacteria and resulting intestinal dysbiosis.

The study was conducted in collaboration with Epi4Dogs Foundation Inc. and several of our community members participated in this study by donating samples from their own dogs. This investigation could not have been possible without the assistance and participation of the Epi4dogs community. In particular, **a generous donation from Paula and John Gatens was vital** to its success.

Untargeted metabolomics investigations are complex, and I have attached to this announcement a summary of the study provided from the investigators which is intended to communicate their findings to an audience of non-scientists.

Sincerely,

Olesia Kennedy

Olesia Kennedy
Founder, Epi4Dogs Foundation Inc.

Research Summary:

# Untargeted Analysis of Serum Metabolomes in Dogs with Exocrine Pancreatic Insufficiency

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This research was recently published in the journal “Animals” in a special issue titled “Frontiers in Canine and Feline Gastrointestinal Disease.”

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# Introduction

Exocrine pancreatic insufficiency (EPI) is a digestive disorder caused by a failure of the pancreas to produce digestive enzymes and deliver them to the small intestines. These digestive enzymes are critical to processing dietary proteins, fats, and carbohydrates. In dogs with EPI, lack of these digestive enzymes results in an inability to digest and absorb nutrient from the diet. This leads to clinical signs including weight loss and diarrhea. EPI is diagnosed by the canine trypsin-like immunoreactivity (cTLI) test. Pancreatic enzyme replacement therapy (PERT) is the treatment necessary to reverse the digestive deficit and help affected dogs gain weight and improve stool quality. However, persistent clinical signs, especially diarrhea, are common in dogs with EPI receiving PERT. The reasons for persistent diarrhea in dogs with EPI are most likely related to imbalances in gut bacteria but may also be related to other intestinal disorders.

Our goal in the present study was to detect metabolic fingerprints of persistent gastrointestinal dysfunction in dogs with EPI using untargeted metabolomics. Metabolomics is a field of investigation focused on measuring small chemicals that are present in biologic samples. These small molecules are involved in metabolic interactions that can provide new information that can be used to study complex diseases like EPI.

# Methods

## Recruitment of Dogs with EPI

From the Epi4Dogs patient registry, we recruited dogs that were previously diagnosed with EPI. Samples of blood and feces were collected by the dogs' primary care veterinarians and owners and mailed to the investigators. We confirmed the diagnosis of EPI by measuring cTLI and excluded dogs with cTLI values that were not consistent with a diagnosis of EPI. We also recruited a group of healthy dogs to serve as a control group. These healthy dogs had no clinical signs of gastrointestinal disease and had normal cTLI values. Information about dogs included in this study are present in Table 1.

| | **Healthy** | **EPI** |
|---|---|---|
| **Age (years)** | | |
| Mean (SD) | 5.0 (± 1.74) | 4.3 (± 2.0) |
| **Breed** | | |
| Akita | 0 (0%) | 1 (5%) |
| Australian Shepherd | 0 (0%) | 2 (10%) |
| Border Collie | 0 (0%) | 1 (5%) |
| Cavalier King Charles Spaniels | 0 (0%) | 1 (5%) |
| German Shepherd | 4 (40%) | 9 (45%) |
| Labrador Retriever | 1 (10%) | 1 (5%) |
| Mixed Breed | 4 (40%) | 4 (20%) |
| Pit Bull Terrier | 1 (10%) | 0 (0%) |
| West Highland White Terrier | 0 (0%) | 1 (5%) |
| **Sex** | | |
| Spayed Female | 3 (30%) | 11 (55%) |
| Intact Male | 0 (0%) | 2 (10%) |
| Neutered Male | 7 (70%) | 7 (35%) |

**Table 1:** Information about dogs with EPI included in this investigation.

### Untargeted Metabolomics Analysis

To characterize metabolic differences between dogs with EPI and healthy controls, we measured and analyzed 759 biochemicals in blood samples. We compared the concentrations of these metabolites between dogs with EPI and healthy dogs using various statistical methods. Analysis of untargeted metabolomics data containing hundreds of different chemicals is a complicated undertaking. Owing to their complexity, we will not discuss these methods in detail. However, we provide general explanations that we hope will help non-scientist readers to understand our approach and the meaning of the results.

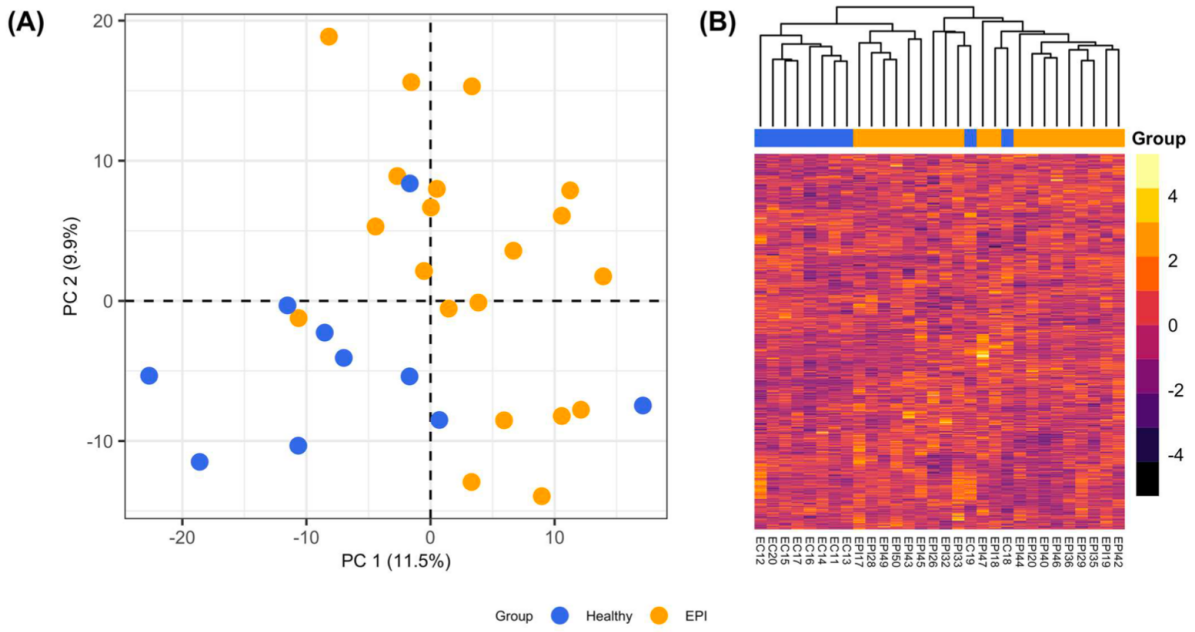
## Results and Discussion

First, we analyzed the data using two methods in which the underlying mathematics do not know about which samples came from dogs with EPI and which came from healthy dogs. These approaches are called “unsupervised” because they do not know the group to which the samples belong. The unsupervised methods included principal component analysis (PCA) and hierarchical clustering. The value in these approaches is that they can tell us whether there are differences in the underlying patterns within the data that distinguish the two groups.

Principal Component Analysis (PCA): This method takes all 759 variables (metabolic chemicals) and looks for correlations among them that explain variation in the data. In the plot below (panel A) each dot represents a mathematical summary of all 759 variables in a single sample from a single dog. Dots that are close to each other

represent samples where the concentrations of the 759 biochemicals are similar to each other. Conversely, dots that are far apart represent samples that are very different from each other. If there are major differences in biochemical profiles between the healthy dogs and dogs with EPI, we expect samples from the two groups to form two clusters – one for EPI samples and one for healthy dog samples. For the most part, this is the pattern we observed. Samples from healthy dogs (blue) cluster in the lower left quadrant, with two exceptions. Samples from dogs with EPI cluster away from the healthy dogs. This means that the biochemical profiles are different between dogs with EPI and healthy dogs.

Hierarchical clustering: This method clusters samples based on similarities in the biochemical profiles generated from measurement of the 759 biochemicals. In the plot below (panel B), the colored bar along the top shows how samples from dogs with EPI (orange) are different from samples from healthy dogs (blue). The tree-like figure at the top of the plot shows how the samples were clustered. The red-orange grid shows the relative concentrations of those 759 biochemicals. The important part is the bar showing how **samples from dogs with EPI and healthy controls form separate clusters**. This indicates that samples from dogs with EPI are very different than those from healthy dogs There are two samples from healthy dogs which cluster with the EPI samples. The reason for this is unknown, but we suspect there are some healthy dogs that have subclinical gastrointestinal dysfunction that may make them similar to dogs with EPI.




Next, we compared the concentrations of each individual metabolite (all 759 of them) between dogs with EPI and healthy dogs to identify statistically significant differences. Out of all 759 metabolites, concentrations of 114 serum metabolites varied significantly between the groups, with 76 being increased and 38 being decreased in dogs with EPI compared with the healthy controls. Table 2 contains a selection of metabolites that varied significantly. In Table 2, each biochemical is organized into a biologic pathway (sub pathway). The q-value represents level of statistical significance (lower p-values are more significant) and the q-value is a statistical measure of whether a given biochemical is likely to be a false-positive (lower q-value is better). The log2FC represents the magnitude of the difference between the EPI and healthy dogs. Negative log2FC values indicate that a biochemical is decreased in EPI dogs, whereas a positive value indicates that a biochemical is increased in dogs with EPI.

Table 2: Significantly variable metabolites

| Biochemical | Subpathway | *p*-Value | q-Value | log2FC |
|---|---|---|---|---|
| **Amino Acids** | | | | |
| Alpha-ketoglutaramate | Glutamate Metabolism | 0.001 | 0.019 | 0.99 |
| Cysteinylglycine Disulfide | Glutathione Metabolism | 0.001 | 0.023 | −1.31 |
| Cysteine-glutathione Disulfide | | 0.003 | 0.043 | −0.93 |
| 4-guanidinobutanoate | Guanidino and Acetamido Metabolism | 0.002 | 0.034 | 1.65 |
| Homocarnosine | Histidine Metabolism | 0.001 | 0.017 | 1.24 |
| 5-aminovalerate | Lysine Metabolism | 0.002 | 0.034 | 0.99 |
| N-acetyl-cadaverine | | 0.001 | 0.024 | 1.19 |
| Cystine | Methionine, Cysteine, s-Adenosylmethionine, and Taurine Metabolism | 0.000 | <0.001 | −2.29 |
| Phenyllactate | Phenylalanine Metabolism | <0.001 | 0.010 | 0.93 |
| Phenylpyruvate | | <0.001 | 0.011 | 1.31 |
| 4-hydroxyphenylacetate | | <0.001 | 0.000 | 2.09 |
| Phenol Sulfate | Tyrosine Metabolism | 0.003 | 0.042 | −1.93 |
| 4-hydroxyphenylpyruvate | | 0.001 | 0.023 | 0.83 |
| 4-hydroxyphenylacetatoylcarnitine | | <0.001 | 0.001 | 3.61 |
| Pro-hydroxy-pro | Urea cycle; Arginine and Proline Metabolism | 0.001 | 0.023 | −1.57 |
| Argininate | | 0.001 | 0.014 | 0.66 |
| 2-oxoarginine | | <0.001 | 0.001 | 2.44 |

**Table 2.** *Cont.*

| Biochemical | Subpathway | *p*-Value | q-Value | log2FC |
|---|---|---|---|---|
| Carbohydrates | | | | |
| 1,5-anhydroglucitol | Glycolysis, Gluconeogenesis, and Pyruvate Metabolism | <0.001 | 0.006 | −0.73 |
| Glycerate | | <0.001 | 0.001 | 1.03 |
| Ribose | Pentose Metabolism | <0.001 | <0.001 | 1.97 |
| **Cofactors and Vitamins** | | | | |
| Threonate | Ascorbate and Aldarate Metabolism | <0.001 | 0.002 | −0.98 |
| Alpha-CEHC Sulfate | Tocopherol Metabolism | <0.001 | 0.010 | −2.03 |
| Pyridoxal | Vitamin B6 Metabolism | 0.002 | 0.028 | −0.79 |
| **Lipids** | | | | |
| 2-hydroxydecanoate | Fatty Acid, Monohydroxy | 0.001 | 0.023 | 1.48 |
| 1-arachidonoyl-GPA (20:4) | Lysophospholipid | <0.001 | 0.008 | 1.02 |
| 1-oleoyl-GPA (18:1) | | <0.001 | <0.001 | 2.27 |
| 1-palmitoyl-GPA (16:0) | | <0.001 | <0.001 | 2.38 |
| Heptanoate (7:0) | Medium Chain Fatty Acid | 0.003 | 0.046 | 0.80 |
| Choline | Phospholipid Metabolism | <0.001 | 0.001 | 0.62 |
| 1-(1-enyl-palmitoyl)-2-oleoyl-GPE (P-16:0/18:1) | Plasmalogen | <0.001 | 0.006 | 0.81 |
| 1-(1-enyl-stearoyl)-2-oleoyl-GPE (P-18:0/18:1) | | <0.001 | 0.011 | 0.92 |
| Sphingomyelin (d18:1/25:0, d19:0/24:1, d20:1/23:0, d19:1/24:0) | Sphingolipid Metabolism | 0.001 | 0.020 | −1.02 |
| Sphingomyelin (d18:1/14:0, d16:1/16:0) | | 0.001 | 0.025 | 0.65 |
| Nucleotides | | | | |
| Xanthine | Purine Metabolism, (Hypo)Xanthine/Inosine-containing | <0.001 | 0.001 | 1.53 |
| Adenosine 5′-monophosphate | Purine Metabolism, Adenine-containing | <0.001 | 0.001 | −2.89 |
| Adenosine | | <0.001 | 0.004 | −2.45 |
| Dihydroorotate | Pyrimidine Metabolism, Orotate-containing | <0.001 | 0.001 | −1.17 |
| Uracil | Pyrimidine Metabolism, Uracil-containing | <0.001 | 0.008 | 1.33 |
| **Peptides** | | | | |
| 4-hydroxyphenylacetylglutamine | Acetylated Peptides | <0.001 | 0.001 | 1.93 |
| Phenylacetylthreonine | | 0.002 | 0.028 | 1.96 |
| 4-hydroxyphenylacetylglycine | | 0.000 | 0.001 | 2.10 |
| Leucylglutamine | Dipeptide | 0.001 | 0.024 | 1.90 |
| **Xenobiotics** | | | | |
| Tartronate (hydroxymalonate) | Bacterial/Fungal | <0.001 | 0.008 | 0.94 |
| Perfluorooctanesulfonic Acid | Chemical | 0.002 | 0.034 | −2.11 |
| S-(3-hydroxypropyl)mercapturic Acid | | 0.001 | 0.026 | 0.76 |
| Hydroquinone Sulfate | Drug–Topical Agents | 0.002 | 0.028 | −1.86 |
| Quinate | Food Component/Plant | <0.001 | 0.006 | 3.16 |

From the results presented in table 2, we identified many biochemicals which are known to be made by intestinal bacteria, including species of *Lactobacillus*, including biochemicals in the phenylalanine and tyrosine metabolism sub pathways. Previous studies have identified increased abundance of *Lactobacillus* in dogs with EPI due to small intestinal bacterial overgrowth (SIBO) and our findings have identified novel blood markers that could be used to assess whether a dog with EPI is affected by SIBO. We also identified significant elevations in a chemical called kynurenine which may be related to intestinal inflammation. These markers could be used to identify causes of persistent diarrhea in dogs with EPI.

# Conclusions

This investigation identified significant differences in 114 blood metabolites between dogs with EPI and healthy dogs. Differences in certain amino acids were probably related to a persistent failure of the intestines to absorb important nutrients from the diet. Changes in but bacterial metabolites were consistent with small intestinal bacterial overgrowth and increased kynurenine suggests the presence of intestinal inflammation in dogs with EPI. The importance of these findings is unknown and our group is actively involved in follow-up investigations to understand whether these insights can be used to improve the diagnosis and treatment of dogs with EPI.