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Can canines function as a model organism for human metabolism?

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Targeted Metabolomics analysis using LC-MS for determining metabolic changes after two different diets

Introduction

The health benefits of eating a more natural, less processed diet has been an area of intense study for decades. The different effects of feeding companion dogs more natural diets has come into focus as a result, although the field is still quite new[1].

Anecdotal evidence, now supported with emerging experimental findings has indicated that a raw, high-fat, low-carbohydrate diet lowers the overall risk of common diseases in canines[1]. The same has been shown in humans[2].

Our primary objective has been to provide quantitative evidence for how dietary choice is linked to metabolic changes in canines. We have focused specifically on determining metabolic differences between raw food diets (high-fat, low carbohydrate*) and the most commonplace diet, kibble (low-fat, high carbohydrate**).

Another objective has been to determine whether canines can function as a model organism for certain human metabolic responses to dietary intake, as many vital metabolic pathways are shared between the two species[3] . The metabolome of an organism is primarily influenced by diet, and hence so is their overall health.

Materials & Methods

To achieve this, a total of 54 Staffordshire bull terriers were divided into two dietary groups, and were fed a diet of commercially available raw and dry diets, respectively, over a period of approx. 4-5 months. Owners fed the dogs a freely varying diet prior to baseline. Blood serum samples from only 8 dogs were included in our first analysis due to high running costs, taken at baseline and at the end of the dietary intervention. The concentrations of 102 key metabolites were measured using targeted Liquid-Chromatography Mass-Spectrometry (LC-MS). Of these metabolites, 13 have been shown to be tightly associated with sequential steps in the *methylation pathway*[4], and we turned our focus to this subset for subsequent analysis.

**Raw food diet = Fat 41,7-45,4% Protein 44.8-46.6% Carbohydrate 0%*
*** Dry food diet = Fat 16,0%, Protein 25,3%, Carbohydrate 44.5%*
The percentages are per unit weight.
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Data collection & processing

Statistical analysis, including principle component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) were used to measure significant changes in the targeted metabolite concentrations between the two groups after dietary intervention using $p \leq 0.05$. Heatmaps are a common method for quickly visualizing significant changes between groups. Initial analysis of the Staffordshire bull terriers ($n=8$), were illustrated using omics-type data processing software, such as metaboanalyst[4]. The samples were clustered pairwise using Ward's method, which determines the combination of pairs that yields the smallest squared distance between samples. This hence generates a relative scale between (-2 ,2), which determines the intensity of up- and downregulation of the metabolites between groups.

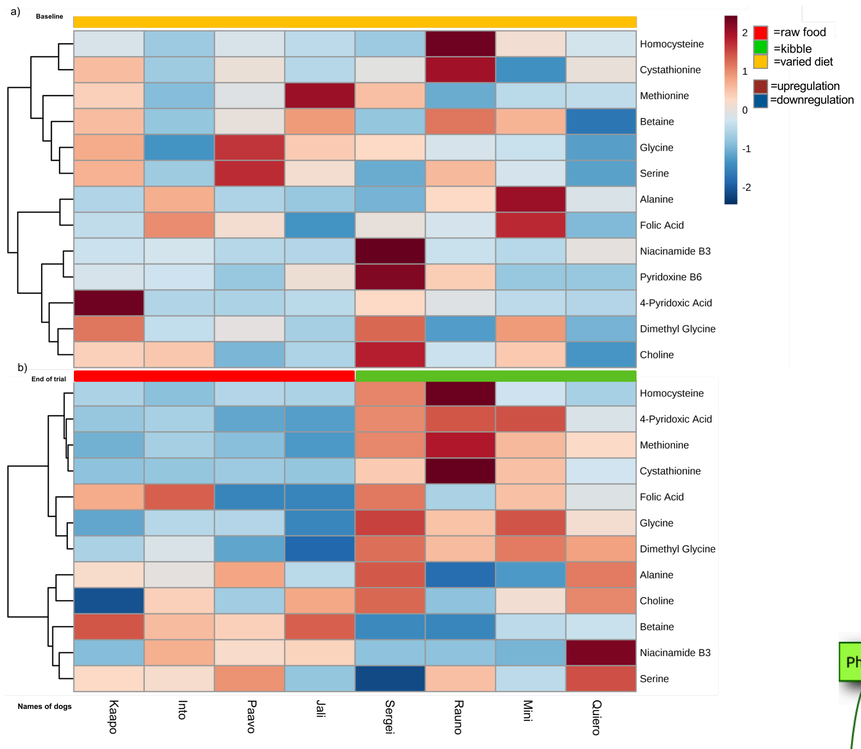

Figure 1- two heatmaps (a&b) showing metabolite changes between baseline (a) and post-dietary intervention (b) of Staffordshire bull terriers (n=8) as colour intensity, blue for downregulation, red for upregulation. Clustering, shown to the left, performed with Ward's method.

Results & Discussion

Using metabolic concentration data from 8 dog's serum samples, we have determined that key metabolites of the methylation pathway, such as homocysteine and dimethyl glycine can be found in differing concentrations at the end of the two dietary interventions. This is illustrated as varying colour intensities, red for upregulation, and blue for downregulation, for each metabolite between the raw and kibble food groups *(Fig.1b)*. The metabolites presented in *Fig. 1a&b* are closely tied to the methylation pathway *(Fig.2)*, which has vital roles for detoxification and immune function in both canines and humans[5].

Previous studies have indicated that the changes observed in these metabolites, especially the increased build up of homocysteine in the kibble diet cohort, may be caused by abnormal liver and kidney function, as well as insufficient uptake of different B vitamins[6].

In conclusion, based on the metabolites studied, we hypothesise that Staffordshire bull terriers eating a raw, high-fat, low-carbohydrate diet may be less prone to suffer from metabolic responses associated with abnormal liver and kidney function, as well as insufficient B-vitamin uptake[5]. However, the underlying biochemical causes for this need to be further studied. We chose to perform this dietary intervention on canines as they offer total compliance to the dietary intervention given, something which is an obstacle in human trials. For this reason, and due to many numerous shared metabolic pathways, we suggest canines may be surprisingly useful as a model organism for dietary intervention experiments.

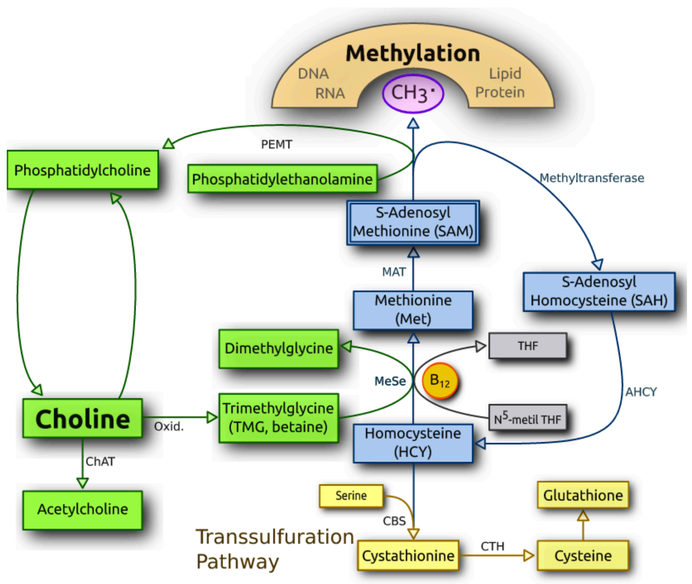

*Fig 2. a simplified illustration*** of a vital portion of the methylation pathway, which may have severe consequences if perturbed.*

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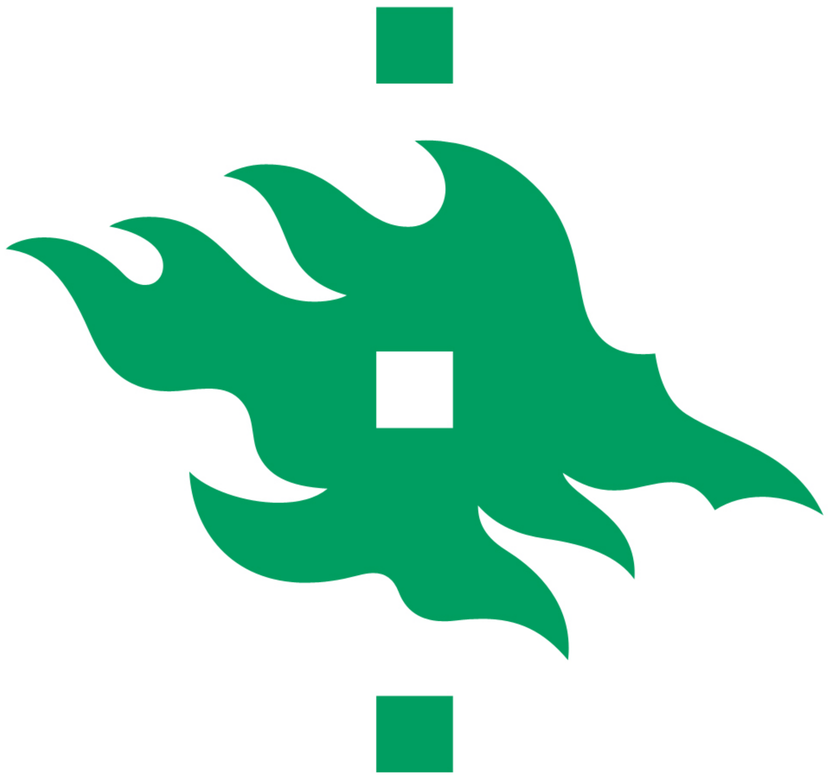

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