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Short Communication

Effect of kibble and raw meat diets on peripheral blood mononuclear cell gene expression profile in dogs

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ABSTRACT

Peripheral blood mononuclear cell (PBMC) gene expression microarray profiling is a minimally invasive tool used in human diet intervention studies. In this study, PBMC gene expression was determined in dogs fed kibble or raw red meat diets for 9 weeks to test the hypothesis that diet influences canine immune cell gene expression profiles. The two diets were associated with differences in PBMC gene expression profiles, which corresponded with changes in plasma IgA concentrations. Analysis of PBMC gene expression profiles might provide useful insights into the long term effects of diet on health outcomes in dogs.

The links between diet, chronic inflammation and disease risk are well-established in human beings (Ahluwalia et al., 2013; Nasef et al., 2017), but there are few studies investigating the role of diet in health outcomes in dogs. Diet-related changes in gene expression might provide an early indication of long term health effects. In human diet intervention studies, peripheral blood mononuclear cells (PBMCs) have been used as a proxy for changes in gene expression in other cell types, such as hepatocytes and adipocytes (de Mello et al., 2012). Although PBMC gene expression analysis has been used to investigate disease states in dogs (Hulanicka et al., 2014; Garncarz et al., 2016), the effect of diet on gene expression in canine PBMCs has not been studied.

We hypothesised that diet alters immune cell gene expression profiles in dogs. To test this hypothesis, we used blood samples from a previously published study (Bermingham et al., 2017), in which adult dogs (Harrier hounds; mean age 5.81 years, standard error 0.72 years; range of weights 21.8–31.9 kg) were fed either a premium kibble diet (kibble; $n = 8$) or a raw red meat diet (meat; $n = 7$) for 9 weeks (Massey University Animal Ethics Committee approval number 14/37; date of approval 17 April 2014). The dogs were maintained on a base diet (mixture of kibble and canned food) for at least 4 weeks prior to the start of the experiment, when they were changed to either a 100% kibble diet or a raw red meat diet (Table 1).

The daily dietary energy intake offered was adjusted weekly to maintain a consistent body weight using the formula: Metabolisable energy for maintenance (ME_m kcal) = 132 × bodyweight (BW kg)$^{0.75}$. Blood samples were collected at baseline (time 0), and after 3, 6 and 9 weeks to compare the PBMC gene expression for each diet over time, and between diets at each time-point. Plasma immunoglobulin A (IgA) and IgG concentrations were measured as biomarkers of immune status.

PBMCs were isolated from blood samples using density centrifugation media (Lympholyte-Mammal; Cedarlane Laboratories; CL5110) and total RNA was extracted using the RNeasy Protect Cell Mini Kit (Qiagen; 74624). All samples passed the technical threshold requirements for amplification and labelling, i.e. 28s:18 s peak ratio > 1.5 and RNA integrity number (RIN) > 8 (2100 Bioanalyzer; Agilent Technologies). Global gene expression profiles were analysed using canine v2 4x44k two colour arrays (G2519F-021193; Agilent Technologies). Samples were hybridised to the microarrays using a randomised design, ensuring that all samples compared on each array had comparable RINs, to prevent any bias during the competitive hybridisation step. Microarray slides were scanned using a DNA Microarray Scanner G2565CA (Agilent Technologies). The raw data have been deposited in the NCBI Gene Expression Omnibus[1] (GEO accession number GSE103529).

Genes differentially expressed between diets and over time were identified using an Empirical Bayes modified *t* statistic in the

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[1] https://www.ncbi.nlm.nih.gov/geo/ (accessed 5 September 2017).

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Table 1
Summary of diet composition (Bermingham et al., 2017).

	Kibble diet	Meat diet[a]
DM %	91.5	24.7
Crude protein % DM	29.9	76.3
Crude fat % DM	27.1	17.9
Crude fibre % DM	2.4	0.6
Crude ash % DM	6.0	4.6
NFE % DM	34.6	0.6
Metabolisable energy content (kcal/kg diet)	3748	4543

DM, dry matter; NFE, nitrogen free extract, calculated by difference: 100 – (crude protein + crude fat + crude fibre + ash).
[a] 73% beef muscle, 10% beef liver, 5% bone chip, 5% beef tripe, 3.5% beef heart, 3.5% beef kidney, 0.2% mineral pre-mix.

limma package (version 3.18.13) of R (version 3.2.1; R Foundation for Statistical Computing). Differentially expressed genes were clustered into functional groups and pathways using ingenuity pathway analysis (IPA version 36601845; Qiagen Bioinformatics). The data were filtered with a fold-change cut-off ± 1.5 and adjusted P value < 0.05. The tissue type was limited to 'immune cells' to ensure that results were biologically relevant to PBMCs.

Canonical pathway analysis (Fig. 1a) showed that, prior to feeding the experimental diets (0 weeks), no pathways had differentially expressed genes between the kibble-fed and meat-fed dogs. Gene expression profiles differed between kibble-fed and meat-fed dogs at 3 and 6 weeks, with numerous pathways enriched with differentially expressed genes (11 pathways at 3 weeks, 15 pathways at 6 weeks). However, this between-diet difference disappeared by 9 weeks. This is supported by hierarchical clustering of the groups (Fig. 1b). Canonical pathway analysis of differences within a diet group over time (Fig. 2) showed that the kibble diet caused more differences (75 pathways) compared to the meat diet (50 pathways). This suggests that the effects of changing the pre-experiment diet to the experimental diets were greater than the difference between the two experimental diets.

IPA upstream regulator analysis was used to identify regulators that may be responsible for the observed differences in gene expression. Compared to time 0, after 3 weeks the kibble diet induced expression of pro-inflammatory cytokine genes, including CD40LG, interleukin (IL) 2 and IL1β, as well as other factors associated with increased immune responses, such as STAT6, TREM1, ITK, DOCK8 and MTD88. In contrast, the meat diet inhibited expression of pro-inflammatory cytokines, including IL2, interferon γ, CCL5 and IL15, and reduced the expression of immune-associated transmembrane receptors, such as FOXO1, CD40 and Toll-like receptor 4. These results are consistent with the kibble diet having a pro-inflammatory effect and the meat diet having an anti-inflammatory effect. This is highlighted in the regulatory effects network, which shows numerous inflammatory processes activated in kibble-fed vs. meat-fed dogs at 3 weeks (Fig. 3).

Plasma IgA and IgG concentrations were quantified by ELISA (Bethyl Laboratories) and normalised to the initial concentration for each dog. Diets were compared in GenStat v18.1 using residual maximum likelihood (REML) analysis with an auto-regression order 1 (AR-1) covariance model to take account of the repeated measures. For IgA the 'treatment x time' effect was significant ($P < 0.05$). Plasma IgA concentrations were lower in the meat-fed dogs than the kibble-fed dogs after 3 weeks ($P < 0.05$), but not at 6 and 9 weeks ($0.05 < P < 0.1$; Fig. 4a). Diet did not affect plasma IgG concentrations (Fig. 4b).

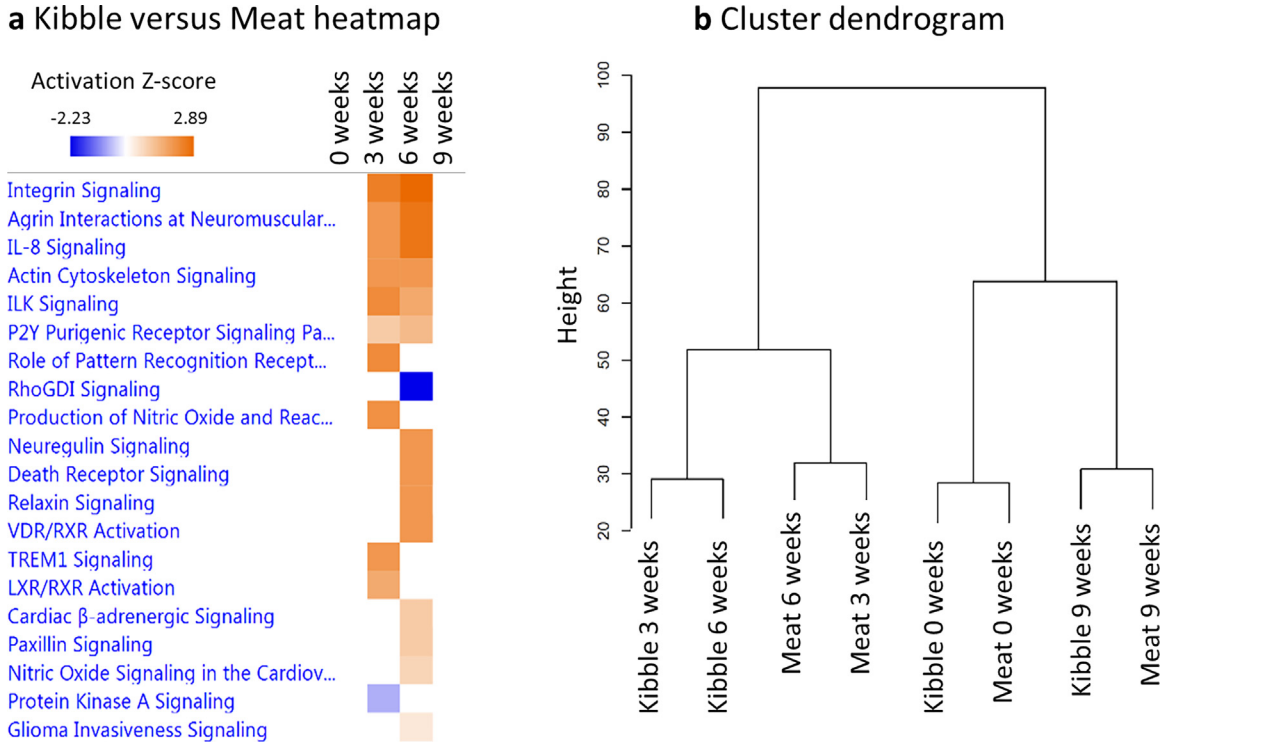

Fig. 1. Comparison of peripheral blood mononuclear cell gene expression in dogs fed kibble or meat diets for 9 weeks. (a) Heatmap of the canonical pathways enriched with differently expressed genes at each time point. (b) Cluster dendrogram illustrating the hierarchical clustering of the sample groups (diet × time).