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The effect of a kibble diet versus a raw meat-based diet on energy metabolism biomarkers in dogs

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ABSTRACT

Considering that dogs originate from carnivorous wolves and do not have a requirement for carbohydrates, it may be questioned whether high-carbohydrate diets are beneficial to their health. It is unknown how low-carbohydrate raw meat-based diets (RMBDs) affect dogs' energy metabolism in comparison to traditional high-carbohydrate kibble diets. In this diet intervention study, 46 client-owned Staffordshire Bull Terriers ate either a kibble or a RMBD for a median of 4.5 months. Before and after the trial, fasting blood samples were analyzed for glucose, glycosylated hemoglobin (HbA1c), insulin, glucagon, triglycerides, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), β-hydroxybutyrate (BHB), the homeostatic model assessment of insulin resistance (HOMA-IR), and triglyceride-glucose (TyG) index. Bodyweight was also evaluated. Comparisons were made between the two diet groups at baseline and end, and between baseline and end within both diet groups. The kibble diet significantly increased HbA1c, total cholesterol, BHB, and bodyweight, whereas the RMBD significantly increased BHB, and decreased glucose, glucagon, cholesterol, and TyG index. At the end of the trial, the kibble fed dogs had significantly higher concentrations of triglycerides, total cholesterol, HDL, LDL, and VLDL compared to the RMBD fed dogs, and the RMBD fed dogs had significantly lower glucagon and TyG index, and higher BHB compared to the kibble fed dogs. No changes were found in insulin and HOMA-IR. In conclusion, kibble and RMBDs have different effects on energy metabolism in dogs. More research is needed on how these forms of feeding may affect dogs' long-term health.

1. Introduction

Dry food, or kibble diets, have long been the most common feeding style for dogs, but raw meat-based diets (RMBDs) have recently gained increasing popularity among dog owners (Empert-Gallegos et al., 2020). Kibble and RMBDs differ greatly in their macronutrient content, especially regarding carbohydrates. Raw meat-based diets, which typically consist of raw meat, offal, bones, fish, eggs, vegetables, fruits, berries, and various supplements, contain little or no carbohydrates, mostly in the form of fiber (Dillitzer et al., 2011; Freeman et al., 2013; Morgan et al., 2022). Kibble diets typically contain 30–60 % refined carbohydrates from potatoes or cereal grains such as wheat, corn, or rice (Carciofi et al., 2008; Spears and Fahey, 2004). For people, overconsumption of refined carbohydrates is widely associated with negative health effects such as high glucose and insulin concentrations, insulin resistance (IR), obesity, systemic inflammation, and metabolic dysfunction, as well as an increased risk for chronic diseases such as type 2 diabetes, cardiovascular disease, and cancer (Clemente-Suárez et al., 2022). Although dogs originate from carnivorous wolves, the domestication process has led to increased copy numbers of AMY2B, the gene coding for pancreatic amylase, which has improved dogs' ability to digest starch (Axelsson et al., 2013). However, dogs do not have a requirement for carbohydrates in their diet (Kronfeld et al., 1977), and there also seems to be a great variation in the AMY2B copy numbers in different dog breeds, like in different human populations (Arendt et al., 2014). Furthermore, it has been shown that dogs prioritize fat and

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protein over carbohydrates (Roberts et al., 2018) and there is evidence of low-carbohydrate, high-fat diets improving the performance in working dogs (Gal et al., 2021; Kronfeld et al., 1977). In comparison to dogs fed kibble diets, dogs fed RMBDs have been reported to have better dental, ear, and skin health (Hiney et al., 2021) as well as lower alkaline phosphatase (ALP) activity (Algya et al., 2018; Anturaniemi et al., 2020a; Hiney et al., 2021) and higher fecal anti-inflammatory markers, suggesting improved gastrointestinal homeostasis and immune function as well as increased feed digestibility (Hiney et al., 2024). In addition, low-carbohydrate diets were recently reported to have an anti-inflammatory effect in dogs, implied by the downregulation of several proinflammatory cytokines/chemokines and their receptors (Tavener et al., 2024). It can therefore be questioned whether it is beneficial for dogs to eat high-carbohydrate diets. As in humans, carbohydrates are the major macronutrient affecting postprandial glucose concentrations in dogs (Nguyen et al., 1994). High-carbohydrate diets have been found to increase postprandial glucose and insulin concentrations in dogs, whereas diets with lower carbohydrate content lower glucose and insulin concentrations and have been suggested to increase insulin sensitivity and improve glycemic control (André et al., 2017; Elliott et al., 2012). However, there are no studies on how a RMBD affects dogs' energy metabolism in comparison to a traditional kibble diet.

Although the RMBD might have less fat and more protein than the classic ketogenic diet, which is very high in fat, moderate in protein, and very low in carbohydrates, recent studies have shown that dogs fed high-fat or RMBDs have higher concentrations of the ketone body β-hydroxybutyrate (BHB) compared to dogs fed high-carbohydrate or kibble diets (Hiney et al., 2024; Jackson, 2022; Tavener et al., 2024). This suggests that they enter a metabolic state called nutritional ketosis, where blood glucose concentrations are low and ketone bodies, such as BHB, are elevated and used for energy. The physiological process of ketogenesis is comparable in dogs and humans, although it takes longer for dogs to reach ketosis, and they also do not reach the same intensity of ketosis as humans (Crandall, 1941). In humans, the ketogenic diet has been associated with anti-inflammatory (Youm et al., 2015) and anti-oxidative effects (Knowles et al., 2018), as well as improvement of mitochondrial function (Zhou et al., 2021), and it has also been considered a promising nutritional approach for improving IR (Paoli et al., 2023; Skow and Jha, 2022), obesity and type 2 diabetes (Yuan et al., 2020), epilepsy (Han et al., 2021), and cancer (Zhu et al., 2022).

Various biomarkers can be used to assess energy metabolism. Glycated hemoglobin or HbA1c is formed when glucose molecules are irreversibly bound to hemoglobin during the lifetime of the erythrocytes, and it reflects the average blood glucose concentration over the preceding 2–3 months (Higgins et al., 1982). This marker has previously been used to assess glycemic status in diabetic dogs (Norris and Schermerhorn, 2022), but to our knowledge, has never been used in a diet intervention study. Insulin and glucagon are peptide hormones that are secreted from the pancreas to maintain blood glucose homeostasis, with insulin lowering glucose concentrations and glucagon elevating them (Idowu and Heading, 2018). Triglycerides and cholesterol are blood lipids that can either originate from the diet or be endogenously synthesized during high availability of glucose (Hellerstein et al., 1996; Luna-Castillo et al., 2022). Due to being insoluble in water, these blood lipids are transported through plasma in particles called lipoproteins, including high density lipoproteins (HDL), low density lipoproteins (LDL), and very low-density lipoproteins (VLDL) (Watson and Barrie, 1993). The homeostatic model assessment of insulin resistance (HOMA-IR) is a clinical marker for IR that has been used in both humans (Ferrannini and Mari, 1998) and dogs (Xenoulis et al., 2011). The triglyceride-glucose (TyG) index is another IR marker that has been used in humans (Lopez-Jaramillo et al., 2023) and rats (Sousa et al., 2018), but to date not in dogs.

We have previously reported changes in hematology and biochemistry analytes between dogs fed kibble versus RMBDs (Anturaniemi et al., 2020a) and we now wanted to continue by studying the effect of these two diet types on dogs' energy metabolism. To do this, we analyzed several energy metabolism biomarkers, including glucose, HbA1c, insulin, glucagon, triglycerides, total cholesterol, HDL, LDL, VLDL, TyG index, HOMA-IR, and BHB before and after a diet intervention where dogs were fed either a high-carbohydrate kibble diet or a low-carbohydrate RMBD. We also measured bodyweight before and after the trial. Our hypothesis was that these two diet types would have vastly different effects on dogs' energy metabolism, which would be reflected in the studied biomarkers and bodyweight.

2. MATERIALS AND METHODS

2.1. Animals and study design

This study was part of a larger diet intervention study on the associations between diet and canine atopic dermatitis (CAD) in privately owned Staffordshire Bull Terrier dogs (Anturaniemi et al., 2020a, 2020b), and therefore both atopic and healthy dogs participated in the study. The diagnosing of CAD is explained further by Anturaniemi et al. (2020b). However, CAD status was not included as a variable in this study as the focus was on comparing the energy metabolism biomarkers between diet groups, and therefore the dogs' health status will not be addressed further in this study. The dogs were recruited into the trial using a Breed Club newsletter, Facebook, and by contacting respondents of the DogRisk questionnaire (a Finnish version can be found at http://bit.ly/427aGBa). The clinical study was conducted in a veterinary teaching hospital at the University of Helsinki during 2013–2014. All dog owners signed a written consent form. The study protocol was approved by the Animal Experiment Board in Finland (ELLA) (permit number: ESAVI/3244/04.10.07/2013).

The study included two or three visits. The baseline (BL) and end (E) visits, which took place before and after the diet intervention, were compulsory for all dogs, and only dogs that came to both visits were used in the analysis. Part of the dogs also came to an extra, pre-study visit, here referred to as before baseline (BBL), to start an elimination diet to help make the CAD diagnosis. At BL and E visits, dogs underwent a thorough physical examination, and blood samples were collected.

Sixty-eight dogs were recruited for the study. The owners were first contacted by phone, and after the phone interview, 58 dogs were considered eligible to participate in the study. Inclusion criteria for healthy dogs were those over 3 years of age with no skin diseases, and inclusion criteria for atopic dogs were those over 1 year of age with no other skin conditions. Four dogs either did not show up or were not eligible to participate in the study, and these dogs were excluded. Thus, the total number of animals included in the diet intervention trial was 54. These dogs were randomly divided into either a kibble or RMBD group and stratified for previous diet, health status, and disease severity using a computerized randomization list. At first there were 26 dogs assigned to the kibble group and 28 dogs to the RMBD group. Three dogs, all from the kibble group, refused to eat their kibble diet and were allowed to change into the RMBD group. This led to the kibble group having 23 dogs and the RMBD group having 31 dogs. Eight dogs discontinued the trial: five owners chose not to continue the trial because the diet was unsuitable for their dog (kibble group $n = 3$, RMBD group $n = 2$), one was diagnosed with immune mediated hemolytic anemia (kibble group), one was euthanized (RMBD group), and one owner was unreachable at the time of the E visit (RMBD group). The final dataset comprised 46 dogs that completed the trial, and this number of dogs still provided adequate statistical power. As this study was part of a larger diet intervention study, and the energy metabolism biomarkers were among the last analyses performed, we ran out of samples for some of the dogs. To solve this, we used either BBL or BL samples, referred to as combined baseline (cBL), when we did not have enough BL samples. This was done for the analysis of HbA1c, where we used 12 BBL samples. For analysis of HDL, LDL, and VLDL, only E samples were used. Some dogs were also excluded from the analyses. In the analysis of HbA1c, one

dog was excluded due to a lack of sample volume collected at the E visit. In the analysis of glucose, eight dogs were excluded: one due to lack of samples and seven due to not having fasted prior to sampling. In the analysis of insulin, 19 dogs were excluded due to a lack of samples. In the analysis of glucagon, 12 dogs were excluded due to lack of samples. In the analysis of bodyweight, nine dogs were excluded due to lack of information of bodyweight at either the BL or E visit. In the analysis of total cholesterol, two dogs were excluded due to lack of samples and seven due to not having fasted. In the analysis of HDL, LDL, and VLDL, six dogs were excluded due to lack of samples and five due to not having fasted. In the analysis of triglycerides and BHB, seven dogs were excluded due to not having fasted. For the statistical analyses, this left us with 45 dogs for HbA1c, 37 for glucose, 27 for insulin, 34 for glucagon, 37 for total cholesterol, 34 for HDL, VDL, and VLDL, 37 for triglycerides and BHB, and 38 for bodyweight. The above is summarized in the flow chart in Fig. 1.

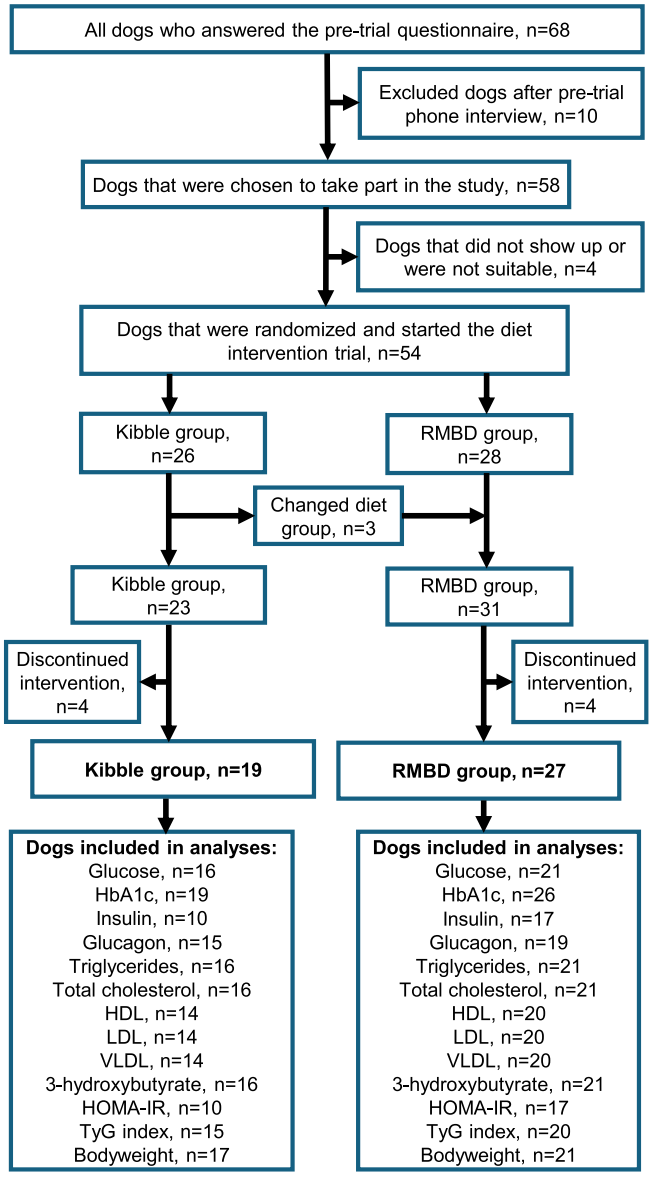

Fig. 1. Flow chart of the included and excluded dogs. HbA1c, glycated hemoglobin; HOMA-IR, the homeostatic model assessment of insulin resistance; HDL, high-density lipoprotein; LDL, low-density lipoprotein; RMBD, raw meat-based diet; TyG, triglyceride-glucose; VLDL, very low-density lipoprotein.

2.2. Experimental diets

Three different diets were used in this study: one commercial kibble diet and two different commercial RMBDs. The kibble diet was Hill's Science PlanTM Canine Adult Sensitive Skin with Chicken, and the two RMBDs were MUSH Vaisto® Pork-Chicken-Lamb and MUSH Vaisto® Beef-Turkey-Salmon. The owners could choose either one of the RMBDs or use both, since many were concerned that their dogs were sensitive to certain animal proteins. However, the RMBDs were very similar regarding macronutrient profiles and processing. All three diets have been stated as complete and balanced by the manufacturers, and detailed nutrient compositions according to the manufacturers are shown in Tables 1a and 1b.

The macronutrient proportions as a percentage of metabolizable energy (% ME) for the kibble and the RMBD is presented in Fig. 2. Dog owners were advised to feed the dogs the proper amount according to bodyweight (at BL), as recommended by the manufacturer. They were instructed to transition their dogs to the study diet over one week by gradually reducing the dogs' previous food while increasing the new food. Water was allowed ad libitum. All dogs were fed different diets before the diet intervention trial started.

2.3. Measurement of bodyweight and body condition score

The dogs were weighed prior to all sampling using an electronic veterinary use platform balance scale (Model Kern EOS 150K100NXL, Kern & Sohn Gmbh, Germany), which uses a measurement accuracy of 0.1 kg over a measurement range from 3 to 150 kg. The body condition score (BCS), which is a subjective, semiquantitative method of evaluating body composition, was assessed during the BL visit using the 1–5 scale with 1 meaning very thin and 5 meaning obese (German, 2006).

Table 1a
Composition and analytical constituents of the kibble diet (Hill's Science Diet Canine Adult Sensitive Stomach & Skin with Chicken[a] dog food, Hill's Pet Nutrition, Inc.) fed to adult Staffordshire Bull Terriers in the diet trial.

Analytical Constituent	In food	In dry matter
Protein (%)	25.3	27.5
Fat (%)	16	17.4
Carbohydrate (NFE) (%)	44.5	48.4
Fiber (crude) (%)	1.3	1.4
Ash (%)	4.9	5.3
Moisture (%)	8	-
Calcium (%)	0.66	0.72
Phosphorus (%)	0.58	0.63
Calcium: Phosphorus	1.1	1.1
Sodium (%)	0.35	0.38
Potassium (%)	0.64	0.7
Magnesium (%)	0.07	0.08
Omega−3 fatty acids (%)	1.2	1.3
Omega−6 fatty acids (%)	4.8	5.2
Additives per kg		
Vitamin A (IU)	9600	10435
Vitamin D (IU)	480	522
Vitamin E (mg)	600	652
Vitamin C (mg)	70	76
Iron (mg)	53.7	58.4
Iodine (mg)	0.9	1.0
Copper (mg)	5.3	5.8
Manganese (mg)	5.6	6.1
Zinc (mg)	111	121
Selenium (mg)	0.15	0.16
Beta-carotene (mg)	1.5	1.6

NFE, nitrogen-free extract. [a]Composition (2013): Rice, maize, poultry meat meal (min. chicken 23 %), maize gluten meal, dried whole egg, vegetable oil, flaxseed, digest, animal fat, potassium chloride, salt. The diet is stated as a complete diet by the manufacturer.

Table 1b
Composition and analytical constituents of the two RMBDs (Vaisto dog foods, Mush Ltd.) fed to adult Staffordshire Bull Terriers in the diet trial.

Analytical Constituent	In food	In dry matter
Pork-chicken-lamb[a]		
Protein (%)	15.2	38
Fat (%)	20	50
Carbohydrate (NFE) (%)	0.0	0.0
Ash (crude) (%)	4.20	10.5
Fiber (crude) (%)	0.60	1.5
Moisture (%)	60.0	0.0
Phosphorus (%)	0.65	1.6
Calcium (%)	1.09	2.7
Calcium: Phosphorus	1.7	1.7
Analyzed ingredients per kg (different batch)[c]		
Omega−3 fatty acids (%)		0.4
Omega−6 fatty acids (%)		3.8
Vitamin A (IU)		143050
Vitamin D (IU)		698
Vitamin E (mg)		46.6
Iron (mg)		123
Iodine (mg)		1.86
Copper (mg)		24.2
Manganese (mg)		8.8
Zinc (mg)		119
Selenium (mg)		0.62
Beef-turkey-salmon[b]		
Protein (%)	15.0	42.5
Fat (%)	15.8	44.8
Carbohydrate (NFE) (%)	0.0	0.0
Ash (crude) (%)	3.70	10.5
Fiber (crude) (%)	0.80	2.3
Moisture (%)	64.7	0.0
Phosphorus (%)	0.34	1.0
Calcium (%)	0.45	1.3
Calcium: Phosphorus	1.3	1.3
Analyzed ingredients per kg (different batch)[c]		
Omega−3 fatty acids (%)		1.1
Omega−6 fatty acids (%)		2.7
Vitamin A (IU)		80890
Vitamin D (IU)		2130
Vitamin E (mg)		54.4
Iron (mg)		82.1
Iodine (mg)		1.64
Copper (mg)		31.5
Manganese (mg)		7.4
Zinc (mg)		79.6
Selenium (mg)		0.73

[a]Composition (2013): (pork-chicken-lamb): Finnish pork 46 % (meat, bone, lung, cartilage, heart, liver), Finnish chicken 29 % (meat, bone, gizzard, skin, heart, cartilage, liver), Finnish lamb 20 % (bone, meat, lung, cartilage, liver), vegetables 5 % (spinach, broccoli, lettuce, cold-pressed sunflower oil), egg < 1 %. [b]Composition (2013): (beef-turkey-salmon): Finnish beef 47 % (rumen, meat, lung, heart, cartilage, liver), Finnish turkey 38 % (meat, bone, cartilage), Norwegian salmon 10 % (salmon including bones), vegetables. 5 % (broccoli, lettuce, apple, carrot, cold-pressed sunflower oil, camelina oil). The foods have been stated as complete and balanced by the manufacturer. [c]Ingredients were analyzed by the manufacturer from different food batches and provided to the researchers by MUSH Ltd.

2.4. Energy metabolism biomarkers

Blood samples were collected at BBL, BL and E of the trial. All blood samples were collected from the jugular vein into Vacuette® 6–10 mL plain serum tubes by a closed method (Vacutainer® Safety-Lok™ Blood collection sets, Becton Dickinson, Meylan, France). For serum samples, plastic vials without coagulants were used. The collected blood was allowed to clot for a minimum of 30 min and then centrifuged (2100 ×g, 15 min). For plasma samples, vials with lithium heparin (Li-hep) were used, and for whole blood samples, both evacuated collection tubes containing potassium ethylene diamine tetra-acetic acid (EDTA) and Li-hep tubes were used. All samples were supposed to be fasting samples, but as mentioned, some owners forgot this, and these dogs' samples were excluded from further analysis. All samples were stored at −80°C until analysis.

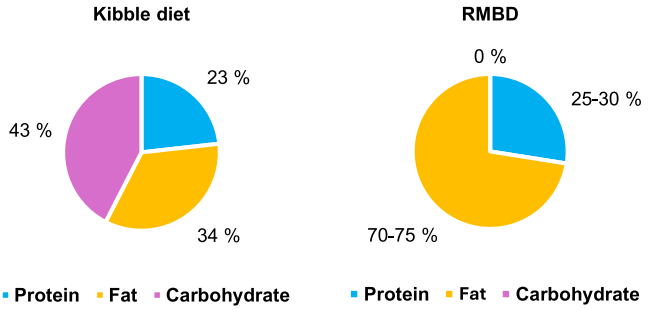

Fig. 2. The macronutrient proportions as a percentage of metabolizable energy (% ME) for the kibble and the raw meat-based diet (RMBD). This does not include fiber.

2.4.1. Glucose

Glucose concentrations were analyzed from serum using a photometric method (Konelab 30i, ThermoFisher Scientific, Vantaa, Finland). The method employs glucose oxidase and a modified Trinder color reaction, catalyzed by the enzyme peroxidase. Glucose is oxidized to D-gluconate by glucose oxidase with the formation of an equimolar amount of hydrogen peroxide. In the presence of peroxidase, 4-aminoantipyrine and phenol are oxidatively coupled by hydrogen peroxide to form a quinonimine dye, colored in red. The intensity of color in the reaction is measured at 510 nm and it is proportional to the glucose concentration in the sample. Analyses were performed immediately or one day after a visit.

2.4.2. Glycosylated hemoglobin (HbA1c)

Glycosylated hemoglobin was analyzed using the Baycom Diagnostic's A1CARE Assay, which uses a dried blood spot (DBS) mail-in test manufactured at an FDA approved facility designed for use on canine and feline glycosylated hemoglobin. The A1CARE Assay has an average inter-assay CV of 9.6 % and the average intra-assay CV is 2.6 %. The DBS methodology employed solves the need for a stable sample and the time it takes for domestic and international shipping to the lab when blood drops are applied to the DBS membrane supplied by Ahlstrom-Munksjo Grade 226. Frozen blood samples were thawed in the refrigerator overnight and then transferred by pipette to the DBS test forms after a short vortex treatment. The DBS samples were allowed to completely dry overnight and then placed in a biologically approved envelope for mailing to Baycom Diagnostics and then transported to and tested by Baycom Diagnostics using a Molecular Devices SpectraMax iD5 Multi-Mode Microplate Reader (Baycom Diagnostics, Florida, USA). During testing, species specific Baycom manufactured controls were run on each assay plate for 2 % glycation, 6 % glycation, 8 % glycation, 12 % glycation and 30 % glycation. In addition, the Lyphochek Hemoglobin A1C Linearity Set Human A1C controls were purchased from Bio-Rad USA for assay testing and validation of the A1CARe assay for each lot of the DBS collection test request forms and species-specific reagents. A more detailed description of the methodology can be found in the publication by Norris and Schermerhorn (2022).

2.4.3. Insulin

Insulin was analyzed from serum at the animal diagnostic laboratory Movet Oy (Kuopio, Finland). The analysis was first made using an immunoluminometric method (Siemens Immulite 2000, Insulin REF L2KIN2, Siemens Healthcare GmbH, Erlangen, Germany) but this proved unsuitable for analyzing insulin in dogs. Therefore, a solid phase two-site bovine-specific enzyme immunoassay method (Bovine Insulin ELISA, Mercodia AB, Uppsala, Sweden) was used instead. This had an intra-assay CV of 8.2 % and an inter-assay CV of 9.5 % and 7.7 % for low

and medium concentration, respectively.

2.4.4. Glucagon

The samples were collected into evacuated collection tubes containing potassium EDTA and placed on ice. Blood samples were centrifuged at 2100 ×g for 15 min to separate plasma, which was then stored at −80°C until analysis. Glucagon was analyzed at the Department of Agricultural Sciences of Helsinki University (Helsinki, Finland). Measurements were performed using a Millipore's Glucagon Radioimmunoassay (RIA) Kit, GL32K (Millipore, St. Charles, MO, United States).

2.4.5. Triglycerides and β-hydroxybutyrate

Analysis of triglycerides and BHB was performed from Li-hep plasma as described by Pohjanvirta et al. (2012) using a clinical chemistry analyzer (Konelab 30i, ThermoFisher Scientific, Vantaa, Finland).

2.4.6. Total cholesterol

The collected blood was allowed to clot and then centrifuged at 2100 ×g for 15 min. Serum total cholesterol was measured using a Konelab 30i chemistry analyzer (ThermoFisher Scientific). Analyses were performed immediately or one day after a visit.

2.4.7. HDL, LDL, and VLDL

Analyses of HDL, LDL, and VLDL were performed later than the rest of the analyses from serum E samples that had been stored at −80°C for approximately ten years. Concentrations of HDL and LDL were measured using test kits (HDL-Cholesterol Plus, LDL-Cholesterol) and an Indiko Plus Clinical Chemistry Analyzer (Thermo Fisher Scientific) at MILA laboratories, Helsinki, Finland. VLDL was calculated using the Friedewald formula [VLDL (mmol/l) = triglycerides (mmol/l) / 2.2] (Friedewald et al., 1972), and for this, triglyceride concentrations were analyzed a second time from the same serum samples (R^2 linear correlation with previous triglyceride samples was 0.959).

2.4.8. HOMA-IR

The HOMA-IR was calculated as previously described in dogs (Xenoulis et al., 2011) according to the formula: [Basal serum insulin concentration (mU/L) × basal serum glucose concentration (mmol/L)] / 22.5.

2.4.9. Triglyceride glucose (TyG) index

The TyG index was calculated as previously described in human studies (Guerrero-Romero et al., 2010) according to the formula: Ln [fasting triglycerides (mg/dl) × fasting glucose (mg/dl) / 2]. For this, the triglyceride and glucose concentrations were converted from mmol/l to mg/dl.

2.5. Statistical analysis

All statistical analyses were performed using SPSS software (version 28, IBM Corp, Armonk, NY, USA). Normality was assessed using Kolmogorov-Smirnov and Shapiro-Wilk tests. To compare energy metabolism biomarkers and bodyweight at BL and E between the kibble and RMBD groups, the independent samples T test was used if normality assumption held. Otherwise, differences were tested using the Mann-Whitney *U* test. Group differences at study E were evaluated using analysis of covariance (ANCOVA) with diet as fixed factor, adjusting for baseline concentrations and intervention duration as covariates. To compare changes in energy metabolism biomarkers and bodyweight between BL and E within the diet groups, the paired-samples T test or the Wilcoxon Signed-rank test were used, depending on the normality. Normality assumption held in the analysis of glucose, insulin, glucagon, triglycerides, total cholesterol, HDL, LDL, VLDL, BHB, HOMA-IR, TyG index, and bodyweight measurements, whereas HbA1c measurements were not normally distributed. In all tests, the statistical significance was set at $p < 0.05$.

3. Results

3.1. Clinical characterization of the study dog population

A total of 46 dogs completed the diet intervention, of which 19 belonged to the kibble group and 27 to the RMBD group. The dietary intervention lasted for 50–188 days (median 136 days) in the dogs included in this study with no statistical difference between groups. Also, no statistical differences in background characteristics were found between the two groups (Table 2). Twenty-five of the dogs underwent an elimination diet (duration 46–213, median 80 days) before starting the diet intervention.

3.2. Energy metabolism biomarker results

The comparisons of energy metabolism biomarkers between the kibble and RMBD groups at BL and E and between BL and E within each diet group are shown in Fig. 3. More detailed data (mean±SD, min-max) can be found in Supplementary Table 1. There were no differences in any of the parameters between the diet groups at BL or cBL.

The mean glucose concentration decreased significantly between BL and E within the RMBD group ($p = 0.026$), while there was no significant change in the kibble group. At the end of the trial, there was no significant difference in mean glucose concentrations between the two diet groups.

The mean HbA1c percentage increased significantly between cBL and E within the kibble group ($p = 0.026$), whereas no change was seen within the RMBD group. At the end of the trial, there was no significant difference in mean HbA1c percentage between the two diet groups.

No changes in insulin were found between BL and E within the two diet groups, and there was no significant difference in mean insulin concentrations between the two diet groups at the end of the trial.

The mean glucagon concentration decreased significantly within the RMBD diet group ($p = 0.004$), while there was no change in glucagon concentrations within the kibble group. At the end of the trial, the mean glucagon concentration was significantly lower in the RMBD group compared to the kibble group ($p = 0.004$).

During the trial, the mean triglyceride concentration decreased in the RMBD group, although not reaching statistical significance ($p = 0.053$), while there was no change in triglycerides within the kibble group. At the end of the trial, the RMBD fed dogs had a significantly lower mean triglyceride concentration compared to the kibble fed dogs ($p = 0.006$).

The mean total cholesterol concentration increased significantly

Table 2
Background characteristics of the study dogs that completed the diet intervention (n = 46).

	Kibble group		RMBD group		P value
	n	*Mean ± SD*	*n*	*Mean ± SD*	
Age at BL (years)[a]	19	5.61 ± 2.73	27	4.76 ± 2.58	0.213
Sex (male/female)[b]	19	9/10	27	15/12	0.765
Bodyweight at BL (kg)[a]	17	18.04 ± 3.42	25	17.70 ± 3.26	0.750
Body condition score at BL (scale 1–5)[a]	18	3.06 ± 0.24	27	2.89 ± 0.51	0.145
Health status (CAD/healthy)[b]	19	15/4	27	20/7	0.742
Previous diet (kibble/RMBD/mixed)[b,c]	19	7/6/6	27	8/13/6	0.594
Duration of diet intervention trial (days)[a]	19	135.68 ± 36.26	27	131.41 ± 30.50	0.667

BL, baseline; CAD, canine atopic dermatitis; SD, standard deviation. [a]Independent samples *t*-test. [b]Chi-Square test
[c]Previous diet of either ≥ 80 % kibble, ≥ 40 % RMBD, or a mix of kibble and/or RMBD and/or home cooked food.

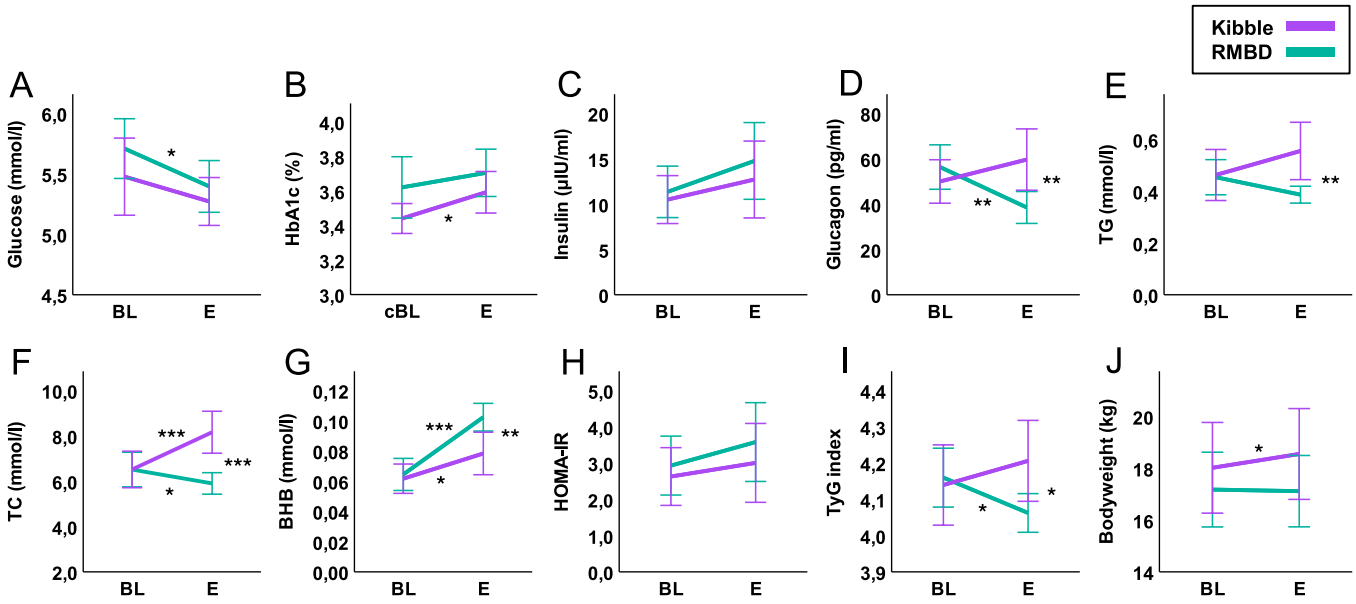

Fig. 3. Line graphs showing baseline (BL) or combined baseline (cBL) and end (E) mean values for glucose (A); glycated hemoglobin, HbA1c (B); insulin (C); glucagon (D); triglycerides, TG (E); total cholesterol, TC (F); β-hydroxybutyrate, BHB (G); the homeostatic model assessment of insulin resistance, HOMA-IR (H); triglyceride-glucose, TyG, index (I); and bodyweight (J) in the kibble (purple line) and the raw meat-based diet (RMBD) (turquoise line) groups. The error bars represent 95 % confidence intervals. Asterisks (*P < 0.05, **P < 0.01, ***P < 0.001) indicate statistically significant differences between BL and E within diet groups (asterisk above or below lines) or between diet groups at E (asterisk on the right side of the lines). There were no differences in any of the parameters between diet groups at BL or cBL.

between BL and E within the kibble group ($p < 0.001$) and decreased significantly within the RMBD group ($p = 0.027$). At the end of the trial, the total cholesterol concentration was significantly higher in the kibble fed dogs compared to the RMBD fed dogs ($p < 0.001$). When we looked at the dogs' previous diets, we found that the dogs that had been eating a kibble diet (>70 % of the total diet) before entering the study had significantly higher baseline total cholesterol concentrations compared to the dogs that had been eating a RMBD ($p = 0.004$). The kibble fed dogs also had significantly higher concentrations of HDL ($p < 0.001$), LDL ($p = 0.014$), and VLDL ($p = 0.007$) compared to the RMBD fed dogs at the end of the trial (these are not included in Fig. 3 since only E data was used, but mean values can be found in Supplementary Table 1). In one of the kibble-fed dogs, the VLDL could not be calculated, due to the dog having a triglyceride concentration higher than what is allowed in the Friedewald formula.

The mean BHB concentration increased significantly within both the kibble group ($p = 0.018$) and the RMBD group ($p < 0.001$), but at the end of the trial, BHB concentrations were significantly higher in the RMBD group compared to the kibble group ($p = 0.003$). Also, the dogs that had been eating a RMBD diet before entering the study had significantly higher baseline BHB concentrations compared to the dogs that had been eating a kibble diet ($p = 0.004$).

Bodyweight increased significantly (+0.53 kg) between BL and E within the kibble group ($p = 0.02$), whereas no significant change was seen between BL and E within the RMBD group. However, there was no significant difference in bodyweight between the two diet groups at the end of the trial.

Intervention duration was tested as a covariate using ANCOVA for all biomarkers, but it did not significantly affect any outcome (data not shown).

4. Discussion

This study showed that feeding a high-carbohydrate kibble diet versus a low-carbohydrate RMBD to pet dogs for a median of 4.5 months had significantly different effects on energy metabolism.

The decrease in glucose in the RMBD fed dogs is in line with findings by Elliott et al. (2012) and André et al. (2017) showing that a lower amount of carbohydrates in dogs' diet resulted in lower postprandial glucose concentrations, although our study presented fasted and not postprandial concentrations, so the results might not be directly comparable. However, in a study by Ober et al. (2016) there was no difference in fasting glucose concentrations in detection dogs when they were fed a low-fat kibble diet with 41 % of ME carbohydrate versus a high-fat kibble diet with 14 % ME carbohydrate for 12 weeks. Likewise, Jackson (2022) did not find any difference in fasting glucose concentrations between dogs fed a high-carbohydrate diet versus a high-fat, low-carbohydrate diet. However, our results are not directly comparable with studies that have used only processed foods and/or had at least some carbohydrates in their low-carbohydrate diets. In humans, it is well-known that consumption of low-carbohydrate diets lead to a reduction in fasting blood glucose (Yuan et al., 2020). During a two-month diet intervention, fasting glucose concentrations decreased significantly in bodybuilders eating a ketogenic diet versus a western diet (Paoli et al., 2021). A recent meta-analysis also concluded that carbohydrate restricted diets, especially ketogenic diets, were effective in lowering fasting glucose concentrations in humans with type 2 diabetes (Jing et al., 2023).

The percentage of HbA1c increased in the kibble fed dogs in our study, while it was not affected in the RMBD fed dogs. The kibble diet contained considerable amounts of rice and maize, which are fast-digesting starch sources that readily elevate blood glucose concentrations. According to Teixeira et al. (2018), a maize-based diet, when compared to a diet with peas and barley, led to higher blood glucose concentrations and poorer glycemic control in diabetic dogs. We were surprised to see that the percentage of HbA1c did not decrease in the RMBD fed dogs, considering that carbohydrate restricted diets, especially ketogenic diets, are known to lower HbA1c percentage in humans (Jing et al., 2023). However, falsely elevated HbA1C concentrations can occur in any condition where the lifespan of the erythrocyte is increased or the erythrocyte turnover is decreased, such as during deficiencies of iron, vitamin B12, or folate (Radin, 2014). The dogs in our study were from the same cohort of dogs as in our previous study where we reported that blood concentrations of iron, vitamin B12, and folate decreased

significantly in the RMBD fed dogs. This could reflect an increased need for these nutrients due to increased erythropoiesis, indicated by increased erythrocyte counts and hemoglobin concentrations, when eating a high-protein diet (Anturaniemi et al., 2020a). The duration of the dietary intervention varied between 50 and 188 days. This could have had an impact on the HbA1c results, considering that the average lifespan of the dog's erythrocyte is 86–106 days (Cline and Berlin, 1963) and HbA1c accumulates throughout the lifespan of the erythrocyte (Higgins et al., 1982). However, only seven out of the 45 dogs that were included in the analysis of HbA1c had a diet intervention that lasted below 86 days, and these were evenly distributed between diet groups (three in the kibble group and four in the RMBD group).

Glucagon concentrations decreased significantly in the RMBD group during the trial and were significantly lower in the RMBD fed dogs compared to the kibble fed dogs at the end of the trial. Glucagon concentrations typically rise when blood glucose concentrations are low, such as during fasting or when eating low-carbohydrate diets (Gannon and Nuttall, 2004; Hoover et al., 2021). In humans, glucagon concentrations increase after ingesting pure fat (Radulescu et al., 2010) and decrease after ingesting pure glucose (Carr et al., 2010). Söder et al. (2016) also reported that postprandial glucagon concentrations increased in dogs after they were fed a high-fat diet (51 % fat, 26 % carbohydrate, and 23 % protein of ME). However, it is difficult to compare their results with ours since the macronutrient composition of the diets differed and since postprandial and fasting glucagon concentrations serve different physiological functions and are not directly comparable. All these studies contradict our findings of decreased glucagon in dogs fed a low-carbohydrate, high-fat RMBD. One explanation could be that glucagon decreased due to a reduced need for glucagon-stimulated gluconeogenesis, as the dogs were using fat, or ketones, for energy instead of glucose. According to a study on rats, ketone bodies such as BHB had a direct inhibitory effect on glucagon secretion (Ikeda et al., 1987), and further research is required to confirm whether a similar mechanism operates in dogs. In addition, diets with high protein content can stimulate insulin, which has a suppressive effect on glucagon release (Nuttall et al., 1984). This hypothesis is supported by the fact that insulin increased more in the RMBD fed dogs than in the kibble fed dogs during the current trial, although the increase was not significant. It has also been shown that fasting in dogs, unlike in humans, does not greatly affect their glucose concentrations and their glucagon concentrations remain unchanged (de Bruijne et al., 1981). However, the discrepancy between our glucagon results and previous findings requires further study.

Consuming refined carbohydrates (e.g., rice and maize) leads to high availability of glucose and endogenous synthesis of triglycerides by the liver and adipose tissue (Luna-Castillo et al., 2022). Our results of higher triglycerides in kibble fed dogs are in line with the study by Algya et al. (2018) showing that dogs fed a kibble diet had significantly higher serum triglyceride concentrations compared to dogs fed raw or mildly cooked diets, despite the kibble having a much lower fat content (13 % versus 28 %–34 %). Also in line with our finding is a recent metabolomics study including 2068 dogs' serum samples where dogs eating a RMBD were found to have lower triglycerides compared to dogs eating a diet consisting of either kibble, both kibble and raw food, or other food types (Puurunen et al., 2022). Carbohydrate-induced hypertriglyceridemia has also been seen in mice fed fat-free, high-starch diets (Sheorain et al., 1980). Similarly to our finding, human research has also shown an association between higher carbohydrate-intake and elevated triglyceride concentrations (Jung and Choi, 2017; Schwingshackl and Hoffmann, 2013). Furthermore, the ketogenic diet has been shown to decrease blood triglycerides when compared to the western diet (Paoli et al., 2021). Elevated triglyceride concentrations in dogs have been associated with IR, inflammation, obesity, diabetes mellitus, pancreatitis, hypothyroidism, hyperadrenocorticism, and hepatobiliary disease (Xenoulis et al., 2022; Xenoulis and Steiner, 2010). Miniature Schnauzers with hypertriglyceridemia were reported to be 192 times more likely to have high serum ALP activity compared to dogs with normal triglyceride concentrations (Xenoulis et al., 2008). We as well as others have previously reported higher ALP activity in kibble fed dogs compared to RMBD fed dogs (Algya et al., 2018; Anturaniemi et al., 2020a; Hiney et al., 2021). We also found a moderate positive correlation between ALP activity and triglyceride concentrations at the end of the trial in the current study (data not shown). In contrast, both Xenoulis et al. (2020) and Miceli et al. (2021) reported that low-fat kibble diets reduced triglyceride concentrations in dogs with severe hypertriglyceridemia, although their results are not directly comparable to ours since their study diets contained significant amounts of carbohydrates. Although all dogs in our study had triglyceride concentrations that were within the RIs, future studies should elucidate the mechanisms behind lower triglyceride concentrations in RMBD fed dogs.

Despite the high content of saturated animal fat in the RMBD, the dogs fed this diet had decreased cholesterol concentrations, whereas the dogs fed the high-carbohydrate kibble diet instead had increased concentrations of total cholesterol, HDL, LDL, and VLDL. Three dogs in the kibble group even surpassed the upper RIs (3.7–9.8 mmol/l) for total cholesterol. Consuming diets with high amounts of refined carbohydrates raises glucose and insulin concentrations in humans, which in turn stimulates the liver to produce more cholesterol through a process called de novo lipogenesis (Hellerstein et al., 1996). In line with our results, Boretti et al., 2020 reported that dogs fed a kibble diet had higher total cholesterol compared to dogs fed a home-made, balanced, RMBD. Likewise, Puurunen et al. (2022) reported in their metabolomics study that dogs fed a RMBD had lower concentrations of total cholesterol, HDL, and VLDL compared to dogs fed a kibble diet, although LDL was not affected by diet in their study. Phungviwatnikul et al. (2020) reported that dogs fed a higher carbohydrate diet had higher total cholesterol compared to dogs fed a diet with less carbohydrates and more protein and fiber. High-carbohydrate, low-fat diets have also been found to increase the cholesterol content in livers of mice (Zhang et al., 2023). In addition, our results are in line with recent evidence from human studies suggesting that a ketogenic diet decreases the concentrations of total cholesterol and LDL (Yuan et al., 2020) and that carbohydrate-restricted diets are superior to low-fat diets in improving lipid markers (Gjuladin-Hellon et al., 2019). However, there are also conflicting results. Algya et al. (2018) found no difference in total cholesterol concentrations between dogs fed a RMBD versus a kibble diet. However, the RMBD in their study contained 27 % carbohydrates (on a dry matter basis) from sweet potatoes, making it difficult to compare their findings with ours. Likewise, Chiofalo et al. (2019) reported that dogs fed a high-carbohydrate, low-protein, low-fat diet had lower cholesterol concentrations compared to dogs fed a low-carbohydrate, high-protein, high-fat diet, although the low-carbohydrate diet in their study also contained around 30 % carbohydrates, making it difficult to compare results. Although increased LDL is a known risk factor for atherosclerosis in humans (Wadhera et al., 2016), dogs seem to be resistant to the development of atherosclerosis and their predominant plasma lipoprotein is HDL, and not LDL as in humans (Zhao et al., 2023). However, further studies are needed to clarify the cause of higher cholesterol in kibble versus RMBD fed dogs and whether there is a negative effect on dogs' health.

The RMBD fed dogs had significantly higher concentrations of BHB compared to the kibble fed dogs at the end of the trial, suggesting that they were using ketone bodies for energy in the absence of carbohydrates. This supports a recent study by Hiney et al. (2024) where dogs fed a RMBD for 28 days had higher serum BHB concentrations compared to dogs that were fed kibble. While our study's intervention lasted longer (median 136 days) compared to 28 days in the study by Hiney et al., the findings are consistent. In another study, by Jackson (2022), beagle dogs fed a high-fat, low-carbohydrate diet, with similar macronutrient content as our RMBD, had higher BHB concentrations compared to dogs fed either high-carbohydrate or high-protein, low-carbohydrate diets. The mean BHB concentration of the RMBD fed dogs

in our study was similar to that of the high-fat diet fed dogs in Jacksons study. In another study, dogs fed an ultra-low carbohydrate, high-fat diet had elevated serum BHB and lower glucose concentrations during the week before whelping, when there is a negative energy balance, compared to dogs fed a high-carbohydrate, low-fat diet (Romsos et al., 1981). Fasting for two weeks can induce higher BHB concentrations (0.35 mmol/l) in dogs (de Bruijne et al., 1981), while diet-induced ketosis in our study resulted in lower BHB concentrations, reflecting a mild state of ketosis compared to that seen during fasting. However, fasting in dogs results in lower ketone concentrations compared to those seen in humans after fasting for the same period (Grundler et al., 2024). This shows that the concentration of ketones rises less and more slowly in dogs than in humans, which was reported already in 1941 (Crandall, 1941). The reason for the increase in BHB in the kibble group remains unclear but could be due to higher fat content in the skin diet formulation used in the current study compared to the dogs' diets prior to the diet intervention, although further research is required to understand this finding.

The TyG index decreased significantly in the RMBD fed dogs during the trial. At the end of the trial, these dogs also had significantly lower TyG index values compared to the kibble fed dogs, suggesting improved insulin sensitivity, similarly to what has been found in humans eating low-carbohydrate or ketogenic diets (Paoli et al., 2023; Skow and Jha, 2022). A 12-week diet intervention with a very-low carbohydrate, high-fat diet significantly decreased the TyG index compared to baseline values in a human study (Cipryan et al., 2022). To our knowledge, this is the first time the TyG index is included as a biomarker in a canine study, and results should therefore be interpreted with caution due to lack of established reference values or similar studies. Furthermore, we did not find any significant changes in the other marker for insulin resistance, HOMA-IR. Nevertheless, future studies should aim to clarify whether a RMBD increases insulin sensitivity in dogs.

Bodyweight increased significantly in the kibble fed dogs during the trial. The two main ingredients in our study kibble were rice and maize, which are rapidly digestible carbohydrates. The consumption of these carbohydrates causes a rapid absorption of glucose, which through insulin and other hormones stimulate lipogenesis in the liver and adipose tissue, causing an increased fat deposition (Ludwig et al., 2021). According to one study, dogs had significantly increased plasma insulin concentrations for 2–8 h after consuming a high-starch meal, whereas no changes in insulin concentrations were found after consumption of a low-starch meal (Hewson-Hughes et al., 2011). Another study found that when dogs were overfed to develop obesity and insulin resistance, an increase in plasma insulin concentrations was associated with development of obesity, suggesting that weight gain increase insulin concentrations or vice versa (Gayet et al., 2004). We found no significant difference in fasting insulin concentrations in our study. However, it is possible that increased postprandial insulin concentrations were contributing to the observed weight gain in the kibble fed dogs. Comparable with our findings, Jackson (2022) showed that dogs fed a low-carbohydrate, high-fat diet had, despite an increased provision of calories, a lower mean bodyweight compared to dogs fed a high-carbohydrate diet or a low-carbohydrate, high-protein diet. The author found that the reduction of dietary carbohydrates by replacement with either protein or fat increased the energy required to maintain bodyweight, with fat having a greater effect. Gal et al. (2021) also reported that dogs fed a high-carbohydrate kibble diet for one month maintained their bodyweight, whereas dogs fed an ultra-low-carbohydrate, high-fat diet lost approximately 1.2 kg. In any case, the difference in bodyweight in the kibble fed dogs in our study was only 0.5 kg, which represents a 3 % change in average bodyweight across all dogs in the kibble group, and this probably did not have any physiological relevance. In addition, all dogs included had a median of 3 in BCS (on a scale of 1–5), and thus no obese dogs participated in this study. The number of dogs included in this study is too small to make strong conclusions about the dietary effects on changes in bodyweight. Also, as the weight of lean muscle and fat are different, it is impossible to say if more fat was lost and more lean mass was gained or vice versa in either of the two groups, since body composition was not assessed in this study.

This study had some limitations. First, the limited sample size could have affected the results. The results might also have been affected by the fact that dogs consumed a variety of diet types before the intervention. Furthermore, the dogs did not live in a controlled environment during the trial and could therefore have been exposed to other foodstuffs, although we tried to control this by using a food diary and saw that the owners had been very stringent with the diets. It is also important to consider that both healthy and atopic dogs participated in this study, and the dogs' health and treatment status may have affected the results, although the number of atopic and healthy dogs was evenly distributed between the two diet groups. A small number of dogs (two at BL and six at END) were on cortisone (glucocorticoid) treatment, which can affect energy metabolism biomarkers; however, because these dogs were evenly distributed between the two diet groups, the overall impact on group comparisons is likely minimal. In addition, one dog was treated with cyclosporine, but given the small number, this is unlikely to have influenced the overall results. Considering that age alters energy metabolism (Fahey et al., 2008), the results may have been affected by the dogs' age, which varied between 1 and 13 years. However, we found no significant age difference between the two diet groups. Sex and neuter status may also influence energy metabolism, but subgroup analyses in our study were limited by small sample sizes and incomplete data on neutering. However, the male/female distribution was similar between the diet groups. In terms of breed characteristics, the Staffordshire Bull Terriers is a heavily muscled breed and considering that skeletal muscle is the primary site of postprandial glucose uptake and a major determinant of whole-body energy metabolism (Merz and Thurmond, 2020), this may limit the generalizability of our findings to other breeds, although it should not bias the comparisons between the two diet groups. Since energy metabolism is known to vary among dog breeds (Gomez-Fernandez-Blanco et al., 2018), we rather consider it a strength that our study included a single breed. Another factor that could have altered the glycemic control is stress (Velasco et al., 2022), which is difficult to measure. The blood samples used in this study were collected during the years 2013–2014, and analyzed during 2014–2020 (HDL, LDL, and VLDL were analyzed in 2024), and had therefore been frozen for a long time, which could have altered some of the biomarkers. Lipid biomarkers (Muzakova et al., 2020), HbA1c (Selvin et al., 2005), insulin (Retnakaran et al., 2019), and BHB (Fritzsche et al., 2001) have all been considered stable during long-term storage in −80 °C. Glucagon however is considered less stable, being prone to degradation, and therefore its measured concentrations may be somewhat underestimated. However, even if the quantities might have decreased, they would have decreased similarly within both groups, so the fact that there were significant differences would not have changed.

In conclusion, the high-carbohydrate kibble diet was associated with changes often linked to adverse metabolic health, such as increased long-term blood sugar, blood lipids, and bodyweight, while the low-carbohydrate RMBD promoted metabolic responses generally considered more favorable, such as decreased blood sugar and blood lipids. In addition, RMBD-fed dogs showed a greater reliance on ketones. Further studies are warranted to clarify the long-term health implications of these two feeding strategies.

CRediT authorship contribution statement

Sarah Holm: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Anna Hielm-Björkman:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Robin Moore:** Writing – review & editing, Conceptualization. **Kristiina**

A. Vuori: Writing – review & editing, Visualization, Conceptualization. **Siru Salin:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Manal Hemida:** Writing – review & editing, Conceptualization. **Johanna Anturaniemi:** Writing – review & editing, Supervision, Resources, Investigation, Conceptualization. **Emilia Baarman:** Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The RMBDs (MUSH Ltd.) and part of the kibble diets (Hills via Berner Ltd.) were provided for free, however, these companies had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tvjl.2025.106462.

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