Understanding age-related metabolic changes is a critical step in the identification of targets for therapies intended to extend life span and health span. Studying the metabolic changes associated with natural aging can be difficult due to the long duration of this process. Therefore, experimental models that mirror some aspects of the metabolic deterioration associated with aging are necessary to facilitate the practical discovery and testing of clinical interventions.

One potential model already widely used in laboratory species, including dogs, is the high-fat diet (HFD). This involves feeding animals a diet typically composed of 40% to 60% fat, mainly sourced from lard, and can be either for short (1 to 2 weeks) or long-term (months) administration. The HFD model has been shown to rapidly induce several key metabolic and clinical features that occur with natural aging.

**OBJECTIVE**

The goal of this study was to characterize changes induced by a high-fat diet in body composition, insulin levels and sensitivity, blood lipids, and other key biomarkers also associated with the metabolic dysfunction that occurs with natural aging.

**ANIMALS**

24 male Beagle dogs, 3 to 7 years of age, of mixed castration status.

**METHODS**

Dogs were randomly assigned to continue twice daily feeding of the commercial adult maintenance diet (n = 12, including 2 intact) that they were previously fed or to a high-fat diet (12, including 2 intact) for 17 weeks between December 1, 2021, and April 28, 2022. Assessments included body composition (weight, body condition score, and adipose mass determined by deuterium enrichment), clinical chemistries, plasma fatty acid quantification, oral glucose tolerance test, and histology of subcutaneous and visceral adipose biopsy samples.

**RESULTS**

The high-fat diet led to increased body weight, body condition score, fat mass and adipocyte size, hyperinsulinemia and peripheral insulin resistance, and elevations in serum lipids, including cholesterol, triglycerides, and several species of free fatty acids. Leptin levels increased in dogs fed a high-fat diet but not in control dogs. There were no significant changes in routine clinical chemistry values in either group.

**CLINICAL RELEVANCE**

Feeding a high-fat diet for 17 weeks led to potentially deleterious changes in metabolism similar to those seen in natural aging in dogs, including hyperinsulinemia, insulin resistance, and dyslipidemia. A high-fat diet model may provide insights into the similar metabolic dysfunction that occurs during natural aging.

**Keywords:** high-fat diet, fatty acids, insulin, aging, dogs
has also been associated with shorter life spans in dogs, as in humans and rodents. A

Aging dogs also experience insulin resistance and increased fasting insulin levels, metabolic changes associated with multiple age-related pathologies in humans and rodents. Age-associated changes in adipose mass and distribution contribute to this insulin-resistant phenotype.

To date, few studies have explicitly connected these changes to clinical outcomes. A lifetime caloric restriction study did identify insulin resistance as a predictor of life span and chronic disease in control-fed dogs. Given the significance of insulin resistance in the pathogenesis of age-associated diseases in humans and other species, identifying and mitigating insulin resistance and hyperinsulinemia in aging dogs are likely to be important elements in extending life span and health span.

Dyslipidemia is also a common feature of aging in dogs, although it may be underappreciated due to the species’ resistance to the atherosclerotic vascular disease most readily associated with these changes in humans. Older dogs show increased levels of cholesterol, triglycerides, and free fatty acids (FFAs) compared with young dogs.

In dogs, HFD induces obesity, increased visceral fat mass, insulin resistance, and changes in lipid metabolism and in adipose tissue structure and function. These changes are similar to those seen with natural aging in this species and occur in a few months rather than over several years.

The purpose of this study was to further characterize the metabolic and physiologic changes associated with the HFD diet model and how those changes may correspond to the metabolic aging phenotype in dogs. Specifically, changes in body composition, insulin levels, insulin resistance, and blood lipid profiles were compared between dogs on a standard diet and HFD.

Methods

Animals

All animal studies were submitted to and approved by the ClinVet Institutional Animal Care and Use Committee. Certificates of approval were issued. The study was designed to allow the use of the study animals in compliance with the ClinVet policy on the ethical use of animals, using the most recent version of South African National Standard SANS 10386: the Care and Use of Animals for Scientific Purposes, as a reference. All animals were returned to the ClinVet colony upon completion.

Study design

The study was conducted between December 1, 2021, and April 28, 2022, in a phased approach. All adult dogs were fed ad libitum normal, control diet consisting of 55% metabolizable energy (ME) from fat (VetsBrands Premium adult maintenance dog food, manufactured by VetsBrands; Reg No. V24369) for a minimum of 2 months before the week 0 measurements. Afterward, dogs were randomly assigned to either a control group or a HFD group for 17 weeks. The HFD consisted of a 74% ME from a fat mixture of dry food (VetsBrands Premium adult maintenance dog food, manufactured by VetsBrands; Reg No. V24369), wet food (Husky Adult [Chunky], manufactured by Purina; Reg No. V13339), and pork lard. Dietary macronutrient composition is described (Supplementary Table S1). Dogs in the control group were continued on dry food only through the conclusion of the study. Study assessments by week are outlined (Figure 1).

Nutrition and anthropometric measurements

Dogs were fed twice daily, (4 hours apart) except on days of oral glucose tolerance testing (OGTT), where dogs received their full daily ration once, after the last blood collection. During each meal, food was weighed before offering and dogs were given approximately 2 hours to consume the food before any remaining food was removed and weighed again. Food intake was calculated by subtracting the remaining food from the initial offering. Daily food intake was used to determine a weekly mean intake for analysis. Caloric consumption was calculated based on caloric density by gram of the 2 diets offered (control or HFD) multiplied by grams consumed. Dogs were weighed weekly in a fasted state at the same time of day. A 9-point body condition score (BCS) was determined weekly by the staff veterinarian.

Oral glucose tolerance test

Oral glucose tolerance tests were performed at week 5 and week 15 as previously described by Coate et al. Briefly, dogs had access to water (access was maintained posttesting) but were fasted for at least 12 hours overnight before conducting the procedure. Blood was sampled at 20 and 0 minutes before glucose bolus to establish fasting glucose and insulin values. Blood was collected utilizing an 18-gauge IV catheter to minimize discomfort from repeated venipuncture. Approximately 2 mL of blood was collected in black-capped coagulation tubes with beads for insulin, and 1.3 mL of blood was collected for glucose in yellow-capped sodium fluoride tubes. At t = 0, an oral glucose bolus (0.9 g/kg) was administered using a syringe. Blood was collected at 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 120, 180, and 240 minutes postglucose bolus. Blood samples settled at room temperature for 30 to 120 minutes before being centrifuged at 3,750 X g for at least 10 minutes at room temperature (20°C to 30°C). Samples were then frozen at −20°C until analyzed. Serum and plasma were analyzed for glucose and insulin levels, respectively, for each time point, by IDEXX Bioanalytics.

Clinical chemistry

Measurements for clinical chemistry were performed on samples collected the week before the dietary intervention (week 0) and on weeks 9 and 17. As previously described, fasting glucose and insulin were averaged from fasting measurements pre-OGTT. Glucose and insulin were quantified using
a bench-top clinical analyzer (AU480; Beckman Coulter, Inc) and an immunoassay system (Advia Centaur XP; Siemens), respectively, and were analyzed by IDEXX Bioanalytics. Blood samples were centrifuged at 3,750 \( \times g \) for at least 10 minutes at room temperature (20 °C to 30 °C) and analyzed on the day of collection.

Measures of serum leptin, nonesterified fatty acids (NEFAs), triglycerides, cholesterol, AST, ALT, ALP, and creatine kinase were derived from fasting blood samples. Serum leptin concentrations were measured by single-analyte ELISA (Canine Leptin ELISA; Millipore EZCL-31K). Serum NEFA concentrations were measured using the Cobas 6000 Chemistry Analyzer (Charles River Laboratories). Serum clinical chemistry and triglyceride assessment were performed and quantified using bench-top chemistry analyzers (AU840 [Beckman Coulter] and Alinity [Abbott Laboratories]) by PathCare.

Fatty acid quantification

Fasting canine plasma samples were analyzed on weeks 9 and 17 by gas chromatography-mass spectrometry (GC/MS) at Metabolon Inc using the fatty acid metabolism targeted panel for the determination of the total content of saturated fatty acids (SFAs), monounsaturated fatty acids (MUfAs), polyunsaturated fatty acids (PUfAs), and FFAs, after conversion into their corresponding fatty acid methyl esters. A complete list of the individual fatty acid species in each aggregated group of SFAs, MUfAs, PUfAs, and FFAs is provided (Supplementary Table S2). Blood samples were centrifuged at 2,500 \( \times g \) for 15 minutes at approximately 4 °C. Aliquots of plasma were pipetted into tubes and lyophilized. An internal standard solution was added to the lyophilized plasma samples. The solvent was removed by evaporation under a stream of nitrogen. The dried sample was subjected to methylation/transmethylation with methanol/sulfuric acid, resulting in the formation of the corresponding fatty acid methyl esters of FFAs and conjugated fatty acids. The reaction mixture was neutralized and extracted with hexanes. An aliquot of the hexanes layer was injected into a GC/MS system (7890A/5975C; Agilent Technologies). Mass spectrometric analysis was performed in the single ion monitoring positive mode with electron ionization. Quantitation was performed using both linear and quadratic regression analysis generated from fortified calibration standards prepared immediately before each run. Raw data were collected and processed using standard software (MassHunter GC/MS Acquisition B.07.04.2260 and MassHunter Workstation Software Quantitative Analysis for GC/MS B.09.00/Build 9.0.647.0; Agilent Technologies). Data reduction was performed using Office 365 (ProPlus Excel; Microsoft Corp).

Body composition

At week 16, canine plasma samples were collected and analyzed for body composition assessment through Metabolic Solutions for deuterium and oxygen-18 enrichment by cavity ring-down spectroscopy using a Liquid Water Isotope Analyzer with
automated injection system, (version 2 upgrade; Los Gatos Research). In brief, animals were fasted overnight for 12 hours and body weight was recorded before dosing. A solution of deuterium (D$_2$O 50 mg/kg; Cambridge Isotope Laboratories 99%; No. DLM-4) and oxygen-18 enrichment (H$_2$O; 150 mg/kg; Sigma-Aldrich 97%; No. 329878) was orally administered to the dogs, and blood samples were collected in EDTA tubes 2 hours postdose. Blood samples were centrifuged at 3,750 g for at least 10 minutes at approximately 4°C. Plasma proteins were removed by adding approximately 5 mg zinc sulfate monohydrate to 25 to 50 μL plasma in a microcentrifuge tube.

Samples were vortexed and spun at 8,000 rpm to precipitate proteins. The plasma protein-free supernatant was injected 8 times, and the average of the last 3 measurements was used for data analysis. A standard curve was run before and after samples for calculation of deuterium and oxygen-18 enrichment as parts per million (parts per thousand) relative to Vienna Standard Mean Ocean Water. Data analysis was performed with standard software (LWIA Post Analysis Software v2.1.0; Los Gatos Research). The unprocessed mass spectrometric data is expressed as a fraction of the initial dose given as suggested by the consensus report by Prentice.\(^{35}\)

The δ-deuterium and oxygen-18 values for the predose (δ$_{\text{pre}}$) and postdose (δ$_{\text{dose}}$; 2 hours postdose) samples were quantified, diluted with tap water, and recorded. The deuterium and oxygen-18 content of the tap water (δ$_{\text{tap}}$) and diluted dose (δ$_{\text{dose}}$) were contributed to the calculation of energy expenditure based on the Technical Recommendations for Use inHumans,\(^{25}\) which has shown to apply to dogs as well.\(^{26}\) This was quantified with the following equation:

$$X = \frac{(\delta_{\text{dose}} - \delta_{\text{pre}})}{(\delta_{\text{dose}} - \delta_{\text{tap}})} \times \frac{18.02a}{\text{WA}}$$

where W is the amount of water (grams) used to dilute the dose, A is the amount of dose (grams) administered to subject, and a is the amount of dose (grams) diluted for analysis.

Total body water to assess body composition was calculated by Metabolic Solutions. This measurement was derived from the reciprocal of the intercept of the decay line for each isotope. Deuterium values were adjusted by 1.04, and the oxygen-18 values were adjusted by 1.01 to account for nonexchangeable hydrogen/oxygen in the body.\(^{25}\) Lean mass was derived by dividing total body water by 0.71, the anticipated percentage of water in fat-free wet weight in canines.\(^{27}\) The remaining mass was subtracted from body weight to quantify fat mass.

**Adipocyte histology**

Adipose tissue biopsies (approx 200 mg) were collected at week 17 from visceral and SC adipose tissue in the thoracic and abdominal regions, respectively. Before biopsy collection, dogs were anesthetized with an intramuscular injection of 0.06 to 0.10 mg/kg of medetomidine and 3.0 to 4.0 mg/kg of ketamine. Tissue samples were collected 10 to 30 minutes after administration of the anesthetics. Tissue samples were stored in 70% ethanol at room temperature for 24 hours and transferred to 10% formalin for storage. All samples were trimmed, processed, and embedded as formalin-fixed paraffin-embedded blocks. Blocks were sectioned at 4 μm onto positively charged SuperFrost slides for maximal tissue adherence. One slide for each SC and visceral adipose sample was cut and stained with wheat germ agglutinin (WGA), which binds to glycoproteins of the cell membrane to allow for measurements of cross-sectional cell area. An additional slide of each adipose sample was cut to serve as a negative control (without WGA staining of the cell membrane). Lipid regions within WGA-stained tissue sections were identified and quantitated as a percent of the total image area. Adipocyte diameters were measured using an automated analysis (Pathology Intelligence; Reveal Biosciences) of whole slide images.

**Statistical analysis**

Means and SD are reported for all analytes measured for each group assignment and time point. All serum biochemistry, body weight, food intake, caloric consumption, and BCS were analyzed using repeated-measures ANOVA (RM-ANOVA), as they were all measured longitudinally with at least 3 observations. For each of these longitudinal outcomes, RM-ANOVA model included group, time, and a group-by-time interaction as fixed effects. Post hoc comparisons of estimated marginal means generated from this RM-ANOVA model were used to test differences between groups within each time point. Post hoc marginal mean difference scores, SEs, and 95% CIs are reported (Supplementary Table S3). Fatty acids, body composition, and adipocyte histology were each ascertained at 2 time points. Two-sample t tests were used to assess group differences in these measures at each time point. Mean differences, SEs, and 95% CIs are reported. Simple correlations between continuous measures were assessed using Pearson correlations. Simple linear regression was used to estimate and visualize linear fits and variance explained. All data were visually inspected for outliers and normality. If outliers were identified, relevant sensitivity analyses were performed. Statistical significance was set to $\alpha = 0.05$. Statistics were performed using standard software (R version 4.3.2; The R Foundation).

**Results**

**Chronic HFD feeding increases food intake and weight gain**

Male Beagle dogs (n = 24) ages 3 to 7 years old of mixed castration status were supplied by ClinVet of ClinGlobal (South Africa). Each group (control, n = 12; HFD, 12) contained 2 intact dogs, with the remaining dogs having been castrated. At week 0, the average BCS was 5.0 ± 0.6 (mean ± SD) and body weight was 16.7 ± 2.6 kg. At baseline (week 0), there was no significant difference in weekly food intake between dogs assigned to the HFD group (250.6 ±
66.1 g) compared to the dogs assigned to the control group (237.7 ± 36.3 g, *P* = .0600; Figure 2). After 1 week of changing diets, dogs fed a HFD consumed significantly more food per week compared to the dogs in the control group for the remainder of the study when assessed by mean time point comparisons (486.0 ± 118.8 g and 242.4 ± 32.2 g, respectively, *P* < .001). Similarly, no differences were seen at week 0 in caloric consumption between the HFD group and control group (571.3 ± 150.7 calories and 542.0 ± 82.7 calories, respectively, *P* = .567), but after 1 week of the dietary intervention, dogs fed

![Figure 2](image)

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**Table 1**—Comparison (2-sample *t* test) of plasma fatty acid profiles for 24 healthy male Beagles after having been fed either a high-fat diet (HFD) (n = 12) versus a control maintenance diet (12) for 17 weeks between December 1, 2021, and April 28, 2022, in a randomized, controlled trial to characterize changes in body composition, insulin levels and sensitivity, blood lipids, and other key biomarkers associated with the metabolic dysfunction that occurs with natural aging.

| Fatty acid (μg/mL)          | Week 9                  | Week 17                 | *P* value
|-----------------------------|-------------------------|-------------------------|----------
|                             | Control1                | HFD2                    |          |
| Linoleic acid               | 1,199.8 ± 153.4         | 1,441.8 ± 440.4         | .086     |
| Oleic acid                  | 487.7 ± 76.9            | 552.1 ± 199.9           | .309     |
| Palmitic acid               | 549.7 ± 68.0            | 737.9 ± 190.0           | .004**   |
| Σ Free fatty acids          | 2,502.5 ± 314.5         | 2,931.0 ± 897.4         | .133     |
| Σ Monounsaturated fatty acids| 671.2 ± 118.9           | 703.4 ± 277.4           | .715     |
| Σ Polyunsaturated fatty acids| 326.3 ± 65.0            | 410.0 ± 254.7           | .277     |
| Σ Saturated fatty acids     | 1,273.2 ± 143.9         | 1,602.0 ± 418.2         | .017*    |

1Control diet contained 55% metabolizable energy from fat (VetsBrands Premium adult maintenance dog food, manufactured by VetsBrands; Reg No. V24369, reformulated since the present study) and 100% dry food by weight.

2HFD contained 74% metabolizable energy from fat mixture of dry food (VetsBrands Premium adult maintenance dog food, manufactured by VetsBrands; Reg No. V24369, reformulated since the present study), wet food (Husky Adult [Chunky], manufactured by Purina; Reg No. V13339, discontinued), and pork lard and 62.5% dry food, 25% wet food, and 12.5% pork lard by weight.

* *P* < .05; ** *P* < .01; and *** *P* < .001.
a HFD consumed significantly more calories than the control group (1,031.7 ± 252.2 calories and 552.7 ± 73.5 calories, respectively, \( P < .001 \)), maintaining this pattern throughout the remaining weeks.

At week 0, there was no significant difference in body weight between dogs assigned to the HFD group (16.4 ± 2.7 kg) compared to the dogs assigned to the control group (17.1 ± 2.5 kg, \( P = .576 \); Figure 2). After 7 weeks, dogs fed a HFD gained significantly more body weight compared to dogs in the control group (18.9 ± 3.1 kg and 16.3 ± 2.3 kg, \( P = .046 \)). Finally, at week 0, there were no significant differences between BCS in the HFD group (4.8 ± 0.5) compared to controls (5.3 ± 0.6, \( P = 0.116 \)). After 5 weeks, dogs in the HFD group displayed a significantly greater BCS compared to controls (6.2 ± 1.0 and 5.6 ± 0.5, respectively, \( P = 0.040 \)), which continued through all remaining weeks, with the exception of week 10.

**Chronic HFD feeding leads to changes in fasting clinical chemistry**

By week 9, chronic HFD feeding compared to the control group resulted in significantly higher cholesterol (HFD, 8.7 ± 6.3 mmol/L; control, 5.1 ± 0.9 mmol/L; \( P = .018 \)) and leptin (HFD, 9.5 ± 8.7 ng/mL; control, 1.2 ± 0.6 ng/mL; \( P = .002 \)). After 17 weeks, HFD feeding compared to controls resulted in significantly higher triglyceride (HFD, 1.0 ± 0.7 mmol/L; control, 0.5 ± 0.1 mmol/L; \( P = .003 \)), cholesterol (HFD, 8.2 ± 4.2 mmol/L; control, 5.3 ± 0.7 mmol/L; (trending))
$P = .051$) and leptin levels (HFD, 12.8 ± 10.4 ng/mL; control, 1.7 ± 0.6 ng/mL; $P < .001$). There were no HFD-associated changes in ALP, ALT, AST, creatine kinase, or NEFA at week 9 or week 17. Full results are provided (Supplementary Table S4).

**Fatty acids associated with metabolic dysfunction increase from chronic HFD feeding**

Significant elevations in plasma palmitic acid and SFAs were observed in the HFD group compared to the control group at week 9 (Table 1). Furthermore, these values continued to rise through the continuation of the study, remaining significantly different between groups by week 17. Additionally, at week 17, significant rises were seen in linoleic acid, oleic acid, and FFAs in the HFD-fed group compared to the control group. After 17 weeks, the only fatty acids that did not significantly rise as a result of the HFD were the more metabolically favorable MUFAs and PUFAs.

**Chronic HFD feeding results in elevated fat mass and expansion of adipocytes**

Chronic HFD feeding for 16 weeks led to significantly greater fat mass in the HFD group compared

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**Figure 4**—Schematic of the blood sampling protocol of the oral glucose tolerance test (OGTT) (A). Vertical bar graphs of the mean ± SD depicting comparisons (2-sample $t$ test) of fasting glucose (B), insulin (C), glucose area under the curve (AUC; D), and insulin AUC (E) for the dogs described, grouped by diet (control [gray] vs high-fat diet [HFD; blue]). The horizontal bars above the plots indicate results that differed significantly between groups. *$P < .05$. 

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to the control group (5.0 ± 2.7 kg and 1.1 ± 1.8 kg, respectively, P < .001; Figure 3). However, no significant differences were seen in fat-free mass. After 17 weeks of the HFD, visceral (2,663.1 ± 895.6 µm², P = .014) and SC adipocyte size (3,037.9 ± 815.4 µm², P < .001) was significantly greater in the HFD group compared to the control group.

Indicators of insulin sensitivity are significantly impacted by the HFD

Fasting insulin was significantly higher in the HFD group dogs by week 5 (20.0 ± 12.9 mIU/L vs 11.2 ± 2.9 mIU/L, P = .031) and week 15 (23.4 ± 14.4 mIU/L vs 12.2 ± 3.7 mIU/L, P = .016), (Figure 4). By 15 weeks, HFD group dogs experienced significant elevations in
Lipid species correlate with insulin action, regardless of diet

Pearson correlations demonstrated that aggregated FFA levels are positively associated with increasing fasting insulin (ρ = 0.78, R² = 0.61, P < .001), insulin AUC (ρ = 0.83, R² = 0.68, P < .001), triglycerides (ρ = 0.90, R² = 0.81, P < .001), and cholesterol (ρ = 0.47, R² = 0.22, P = .002) in all dogs (Figure 5). Additionally, clinically measured triglycerides and cholesterol were positively associated with fasting insulin (ρ = 0.66, R² = 0.44, P < .001; ρ = 0.75, R² = 0.56, P < .001) and insulin AUC (ρ = 0.71, R² = 0.50, P < .001; ρ = 0.77, R² = 0.60, P < .001; Figure 5). Two dogs from the HFD group displayed higher yet physiologically acceptable values for several of these outcomes, compared to the other dogs in the study. An identical analysis with these 2 dogs removed, produced similar results indicating aggregated free fatty acid levels are positively associated with increasing insulin AUC (ρ = 0.54, R² = 0.29, P = .010), triglycerides (ρ = 0.52, R² = 0.27, P = .014), and cholesterol (ρ = 0.87, R² = 0.75, P < .001; Supplementary Figure S2 and Table S5). Furthermore, cholesterol was positively associated with fasting insulin (ρ = 0.44, R² = 0.19, P = .040) and insulin AUC (ρ = 0.66, R² = 0.43, P < .001). Trending relationships were observed between FFAs and fasting insulin (ρ = 0.41, R² = 0.16, P = .061), as well as triglycerides and insulin AUC (ρ = 0.40, R² = 0.16, P = .068).

Discussion

The results of this study support the utility of HFD in dogs as a model of metabolic changes similar to those seen with natural aging. By definition, an experimental model is not identical to the phenomenon it is used to investigate. However, such models can recapitulate key features of the natural process being studied and inform understanding and manipulation of this process. While HFD does not replicate all aspects of natural aging in dogs, it does produce a sufficiently similar metabolic profile to provide genuine insight into the mechanisms of canine aging and how these might be measured and manipulated clinically.

As in naturally aging dogs, the dogs fed HFD experienced increases in fasting insulin and peripheral insulin resistance. In other species, such as rodents and humans, these changes are often accompanied by an increase in fasting blood glucose and an increased risk of diabetes mellitus. Dogs, however, do not consistently show elevated fasting glucose levels in response to HFD, and they appear to have an exceptional capacity for increasing insulin secretion and avoiding hyperglycemia even in the face of significant peripheral insulin resistance. As a result, diabetes mellitus is relatively less common in dogs than in other species, and veterinarians often do not test for hyperinsulinemia and changes in insulin sensitivity in normoglycemic individuals. However, insulin resistance has been implicated in other species as a risk factor for several important age-associated diseases that do occur commonly in dogs. For example, neoplasia has been associated with insulin resistance, as has all-cause mortality. These results show that a HFD model can be useful for characterizing changes in insulin sensitivity and for testing the potential impact of therapies targeting these changes. This is an important step in developing therapies to mitigate the negative health consequences of age-associated changes in insulin levels and the effects this may have on specific tissues.

The present study demonstrates that HFD induces increases in fasting lipid levels similar to those seen in natural aging. Cholesterol, triglycerides, and fatty acids increased with HFD, and the increases in cholesterol and triglycerides were correlated with hyperinsulinemia, further supporting the case that dyslipidemia accompanies, and may contribute to, the insulin resistance associated with both HFD and natural aging.

Dogs are not as susceptible to atherosclerotic cardiovascular disease as humans and rodents fed HFDs, so the clinical significance of dyslipidemia is not always appreciated. This phenotype may contribute to sarcopenia, chronic inflammation, and other clinically significant consequences of both obesity and natural aging. Hyperlipidemia independent of diet has been associated with pancreatitis, hepatopathy, and ocular disease in dogs. Further investigation may show other negative health outcomes associated with the changes in lipid levels and metabolism associated with aging and modeled by HFD.

The HFD used in this study also induced significant increases in leptin concentrations. Leptin is a hormone produced by adipose tissue. In metabolically healthy individuals, leptin serves to modulate satiety and regulate fat storage and energy metabolism. However, in both aging and obesity, leptin resistance can develop, which leads to a dysregulation of energy balance. One of many possible contributors to this resistance is elevated triglycerides, raising the possibility that dyslipidemia can impair leptin function.

Elevated leptin levels have also been implicated as a contributing factor in chronic inflammation and the pathogenesis of some age-associated diseases, such as osteoarthritis. High-leptin levels associated with obesity in dogs are suspected to be associated with chronic inflammation and potentially pancreatitis and cardiac disease. Leptin may also increase with age in nonobese dogs, though the evidence for this is limited. The pattern of leptin increase seen in the dogs fed HFD in this study supports the value of this model for studying the metabolic dysfunction associated with obesity and possibly with natural aging.

While body condition scores were significantly greater in the HFD group than in the control group,
the differences were small and unlikely to be seen as meaningful on routine clinical examination (6.3 ± 1.0 on HFD and 5.6 ± 0.5 on a 9-point scale). This illustrates that significant metabolic dysfunction can occur before the onset of clinical obesity. Other research has also shown that body condition scoring may not be the most sensitive tool for detecting changes in body weight and composition associated with age-related diseases. Monitoring markers of metabolic dysfunction in aging dogs, such as blood lipids and insulin levels, could potentially be a useful adjunct to body condition scoring as a means of predicting the risks of negative clinical outcomes associated with aging.

There were several limitations to this study. The use of HFD to study the types of metabolic dysfunction seen with aging is relatively new in dogs, and there is no accepted standard for the specific diet or feeding strategy to be used. In this study, the control diet was relatively high in fat (55% ME from fat) compared with typical adult maintenance diets (20% to 40% ME from fat). The differences in metabolic health and body composition between the groups might have been even greater if the controls had been fed a diet with a more typical proportion of ME from fat.

The primary source of fat in the HFD was pork lard. The fat in the control diet consisted predominantly of ostrich and salmon oil. Some research suggests that different fat sources have different impacts on metabolic health in dogs. For example, a HFD based on added salmon oil may be less likely to induce insulin resistance than one based on pork lard. The fat in the control diet consisted predominantly of salmon oil. Some research suggests that different fat sources have different impacts on metabolic health in dogs. For example, a HFD based on added salmon oil may be less likely to induce insulin resistance than one based on pork lard. This diet was clearly not representative of a diet with a more typical proportion of ME from fat. The fat in the control diet consisted predominantly of ostrich and salmon oil. Some research suggests that different fat sources have different impacts on metabolic health in dogs. For example, a HFD based on added salmon oil may be less likely to induce insulin resistance than one based on pork lard.

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This preliminary evaluation of a HFD hopefully paves the way for future research to further clarify the potential of this model to recapitulate changes associated with aging in dogs. Future research with this model should give insights into these changes, their potential clinical consequences, and possible future targets for interventions to address age-associated metabolic dysfunction.

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Finally, the study was conducted over a relatively short period, and negative clinical outcomes were not expected nor assessed. In future work, it would be useful to conduct longer studies to examine the clinical impact of HFD-associated metabolic derangements, including the development of disease states that may also be seen in dogs with natural aging.

This preliminary evaluation of a HFD hopefully paves the way for future research to further clarify the potential of this model to recapitulate changes associated with aging in dogs. Future research with this model should give insights into these changes, their potential clinical consequences, and possible future targets for interventions to address age-associated metabolic dysfunction.

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None reported.

**Supplementary Materials**

Supplementary materials are posted online at the journal website: avmajournals.avma.org