Effect of increased dietary protein and decreased dietary carbohydrate on performance and body composition in racing Greyhounds

Richard C. Hill, VetMB, PhD; Daniel D. Lewis, DVM; Karen C. Scott, PhD; Mayuko Omori, DVM; Melissa Jackson, DVM; Deborah A. Sundstrom, BS; Galin L. Jones, MStat; John R. Speakman, PhD; Celeste A. Doyle, BS; Richard F. Butterwick, PhD

Objective—To determine effects of increased dietary protein and decreased dietary carbohydrate on hematologic variables, body composition, and racing performance in Greyhounds.

Animals—8 adult Greyhounds.

Procedure—Dogs were fed a high-protein (HP; 37% metabolizable-energy [ME] protein, 33% ME fat, 30% ME carbohydrate) or moderate-protein (MP; 24% ME protein, 33% ME fat, 43% ME carbohydrate) extruded diet for 11 weeks. Dogs subsequently were fed the other diet for 11 weeks (crossover design). Dogs raced a distance of 500 m twice weekly. Rectal temperature, hematologic variables before and after racing, plasma volume, total body water, body weight, average weekly food intake, and race times were measured at the end of each diet period.

Results—When dogs were fed the MP diet, compared with the HP diet, values (mean ± SD) differed significantly for race time (32.43 ± 0.48 vs 32.61 ± 0.50 seconds), body weight (32.8 ± 2.5 vs 32.2 ± 2.9 kg), Hct before (56 ± 4 vs 54 ± 6%) and after (67 ± 3 vs 64 ± 8%) racing, and glucose (131 ± 16 vs 151 ± 27 mg/dl) and triglyceride (128 ± 17 vs 104 ± 28 mg/dl) concentrations after racing.

Conclusions and Clinical Relevance—Greyhounds were 0.18 seconds slower (equivalent to 0.08 m/s or 2.6 m) over a distance of 500 m when fed a diet with increased protein and decreased carbohydrate. Improved performance attributed to feeding meat to racing Greyhounds apparently is not attributable to increased dietary protein and decreased dietary carbohydrate. (*Am J Vet Res* 2001;62:440–447)

Greyhound racing is a major industry in many countries, but few studies have examined the relationship between nutrient composition of diets and performance in racing Greyhounds. Toll et al[1] reported that Greyhounds ran slower when dietary fat increased from 31 to 75% **metabolizable energy (ME)**. Our laboratory group reported[2] that Greyhounds ran faster when dietary fat increased from 25 to 32% ME and dietary protein increased from 21 to 25% ME. It was not clear, however, whether dietary protein or dietary fat was responsible for this difference in performance. Dogs fed the diet higher in protein and fat, however, had an increased Hct.[2] In racing sled dogs, a diet very high in protein (> 40% ME) increased plasma volume,[3] and increasing dietary protein to more than 32% ME protein prevented a decline in Hct observed when dogs were fed a 28% ME protein diet.[4] Therefore, dietary protein may affect Hct and performance in Greyhounds.

Most trainers feed a mixture of meat (beef or chicken) and a proprietary extruded food to their Greyhounds, because they believe that meat must be included in the diet for a dog to perform optimally.[5] Most commercial extruded diets contain 20 to 28% ME protein and 25 to 45% ME fat, whereas beef contains 25 to 40% ME protein and 60 to 75% ME fat.[6] Adding meat to an extruded diet increases dietary protein to more than 30% ME and dietary fat to more than 50% ME. Thus, protein or fat could be responsible for the perceived improvement in performance of Greyhounds when meat is added to an extruded diet.[5] The purpose of the study reported here was to measure the effect of an increase in dietary protein on performance, hematologic variables, and body composition in racing Greyhounds.

Materials and Methods

Animals—Ten Greyhounds trained to chase a lure on a racetrack were donated by Greyhound breeding kennels for use in the study. Two dogs incurred muscle injuries during the study and were withdrawn from racing. Data from the 8 remaining dogs (4 females, 4 males; body weight [mean ± SD], 31.7 ± 2.7 kg; 2.5 to 4.5 years old) were analyzed.

Dogs were judged to be clinically normal and cared for as described elsewhere,[2] except that dogs were treated topically with a parasitacide[a] (2.68 ml of a 0.29% solution of fipronil) on arrival and once per month throughout the study to remove fleas and ticks. Dogs were cared for in accordance with principles outlined in the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*.[7] The study was approved by the University of Florida Institutional Animal Care and Use Committee.

All dogs were fed once daily in the morning after an exercise period. Each dog was offered food in excess of its

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From the Department of Small Animal Clinical Sciences, College of Veterinary Medicine (Hill, Lewis, Scott, Omori, Jackson, Sundstrom), and the Department of Statistics, Institute of Food and Agricultural Sciences (Jones), University of Florida, Gainesville, FL 32610–0126; the Department of Zoology, University of Aberdeen, Aberdeen AB24 3TZ, Scotland (Speakman); Kal Kan Pet Foods Inc, PO Box 58853, 3250 E 44th St, Vernon, CA 90058 (Doyle); and the Waltham Centre for Pet Nutrition, Freeby Ln, Waltham-on-the-Wolds, Leics LE14 4RT, England (Butterwick).
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estimated requirement[2] and allowed to eat for 30 to 40 minutes. Excess food was removed once each dog had voluntarily stopped eating. The amount of food offered and any residual food were weighed to determine food intake. Each dog was weighed after it had urinated but immediately before exercise each week. Dogs were photographed to document body condition during the final week of each diet period.

Diets—Diets fed consisted of a **high-protein (HP)** extruded diet or a **moderate-protein (MP)** extruded diet. As we increased dietary protein in the HP diet, dietary carbohydrate was designed to decrease proportionately, with dietary fat remaining the same in each diet. This was achieved by increasing the amount of soy protein isolate, poultry meal, and corn gluten meal by 5.9% each, increasing the amount of dried egg by 1.3%, and decreasing the amount of brewer's rice by 5.2% and whole corn by 13.8% in the final HP diet, compared with amounts for the MP diet. Diets were formulated to contain similar concentrations of vitamins and minerals and to conform to **American Association of Feed Control Officials (AAFCO)** recommendations for adult dogs.[8] Each diet was manufactured as a single batch by a commercial company.[b]

Procedure—All dogs were fed a commercial extruded dog food[c] for 5 weeks and then a mixture of the 2 experimental diets (50% HP diet:50% MP diet) for 2 weeks during a 7-week acclimatization period. Dogs then were randomly assigned to 2 groups (4 dogs/group; 2 males and 2 females/group). One group of dogs was fed the HP diet for weeks 1 to 11 and then the MP diet for weeks 12 to 22. The other group was fed the diets in the reverse order.

Apparent digestibilities were measured during weeks 10 and 21. A representative sample (1 kg) of each extruded diet was stored at –20 C for subsequent analysis. Feces were collected on 4 consecutive days, including 1 race day, by direct catch in plastic bags with wire-twist closures.[d] Feces were weighed, frozen at –20 C, lyophilized, and weighed again to determine dry weight. Samples from each week for each dog were pooled and homogenized by being ground in a blender[e] to the consistency of powder.

Food and feces were analyzed at the laboratory of a commercial company,[f] as described elsewhere.[2] Protein, fat, ash, insoluble fiber, and moisture were subtracted from the total to obtain **nitrogen-free extract (NFE)**. Amino acid composition was measured at the laboratory of another commercial company,[g] using a procedure advocated by the Association of Official Analytic Chemists.[9] Digestibilities of nutrients were calculated as 100 minus the percentage of each nutrient consumed that was collected in the feces. The ME density and percentage ME provided by each major nutrient were calculated by multiplying these average digestibilities by the gross energy values of 4.4 (5.65 – 1.25, to account for nitrogen energy loss in the urine), 9.4, and 4.15 kcal/g for protein, fat, and NFE, respectively.[2,10]

All dogs exercised for 15 minutes twice daily in a 30 × 30-m grass paddock and raced in pairs once or twice per week. Each race was a distance of 500 m on a 400-m oval track (soft sand and clay) with 10° banking on the corners. During racing, dogs chased a mechanical lure. Dogs were randomly assigned to race and starting position. During the 7-week acclimatization period, dogs raced once per week for 3 weeks and then twice weekly for 4 weeks. During each 11-week diet period, dogs rested for the first 2 weeks, raced once per week during the third week, and raced twice weekly for the remaining 8 weeks. Race times were measured with the aid of a camera[h] positioned at the finish line during the final 4 weeks of each diet period (equipment failure prevented measurements during earlier weeks). Ambient temperature and humidity were measured at the track on each race day.

Rectal temperature was measured and blood samples were obtained in the kennel before racing and at the track 5 minutes after racing during week 11 of each diet period. Blood samples were obtained by jugular venipuncture and immediately transported on ice to the laboratory for analysis; CBC counts, serum biochemical analyses, and blood gas analyses were performed, as described elsewhere.[2]

Lean body mass and plasma volume were measured 10 hours after the morning meal (2 hours after the afternoon exercise period) on the day after a race during week 11 of each diet period. Dogs were allowed access to water for 30 minutes after exercise, after which dogs were not allowed access to water until blood samples had been obtained.

Total body water and plasma volume were measured, using dilution of deuterium oxide and Evan's Blue dye,[11] respectively. A known weight (0.1 g/kg of body weight) of deuterium oxide and Evan's Blue dye[i] (0.5% solution) were injected IV through a butterfly catheter inserted in the left cephalic vein. Syringes and the catheter were flushed with 10 ml of saline (0.9% NaCl) solution via a 3-way stopcock. Blood samples were obtained from the right cephalic vein 0, 15, 30, and 60 minutes after infusion to measure dilution of Evan's Blue dye and 0, 2, 4, and 6 hours after infusion to measure deuterium dilution. Blood samples were transferred to a 3-ml tube containing lithium heparin as an anticoagulant.[j] Blood samples for determination of Evan's Blue dye content were centrifuged (500 × g), and plasma was stored at –70 C until analysis. Concentration of Evan's Blue dye in plasma was measured spectrophotometrically.[11] A regression of the logarithm of the concentrations of Evan's Blue dye plotted against time interval from injection was extrapolated to time 0 to provide an estimate of concentration of Evan's Blue dye at the time of infusion. Plasma volume was calculated by dividing the administered dose of Evan's Blue dye by the estimated concentration of Evan's Blue dye at the time of infusion. Blood volume was calculated with 0.96 as a correction factor for trapped plasma by dividing the plasma volume by ([1 – 0.96] × PCV)/100.[12]

Samples for deuterium analysis were sealed in 100-µl capillary tubes and refrigerated until analysis. Deuterium enrichment of plasma was measured by use of mass spectrometry at a university laboratory.[k] Water was extracted from encapsulated blood samples, using the pipette-distillation method of Nagy.[13] Aliquots (10 µl) of the resulting distillate were reduced to hydrogen, using lithium aluminum hydride[l] as the reducing reagent.[14] The resulting hydrogen was cryogenically purified and injected into the inlet of a gas-source isotope-ratio mass spectrometer.[m] Enrichment was calculated relative to a standard gas[n] injected into the mass spectrometer. The absolute ratio of the reference gas was established by assaying it against international standards (ie, **standard mean ocean water [SMOW]**, **standard light Arctic precipitate [SLAP]**, and International Atomic Energy Agency 304a and b). Three characterized standards were assayed in conjunction with the samples each day, and their values were used to correct the data for day-to-day variation in machine performance. Enrichments, relative to the standard, were adjusted to the SMOW-SLAP scale and then were converted to **parts per million (ppm)**, using 0.00015555 as the absolute ratio for SMOW. All samples were assayed in quadruplicate.

Enrichment of deuterium in the injection solution was 98.6%, which was achieved by making a dilution of a known weight of injection material in a known weight of characterized background water and measuring isotope enrichment in the resulting mixture.[15] The $^{18}O_2$ enrichment in the same injection solution was estimated to be 3,493 ppm, using analyses of the same materials, by the small-sample equilibration method.[16] Another 4 dilutions, using isotopically characterized background water, revealed that the amount estimated by use of the isotope dilution method did not dif-

fer significantly from the known quantity of water in the vessels and was accurate within 1%.

Deuterium enrichment in plasma appeared to have reached a plateau by 4 hours in most dogs but declined slightly at 6 hours in 1 dog. Therefore, the dilution space of deuterium was calculated for all dogs at 6 hours after injection, using the derived injection enrichments for deuterium and $^{18}O_2$ and the measured background and equilibrium enrichments of deuterium in the blood samples determined by use of an equation reported by Speakman.[17] It was assumed that the enrichment of $^{18}O_2$ in the injection solution was 3,493 ppm (measured) and in body water was 1,980 ppm, which is realistic for animals at this latitude.[18] Varying this latter value by 20 ppm did not have an impact on the final calculated dilution space. Body water was calculated by dividing the dilution space by 1.04, because the hydrogen dilution space exceeds the desiccation spaces by approximately 4%.[17] Fat-free mass was calculated by assuming that total body water constituted 73% of the fat-free mass.[19] Fat mass was calculated as the difference.

Statistical analysis—Results were reported as mean ± SD. Statistical analyses were performed, using a standard software package.° A normal probability plot of the data was inspected visually, and the Shapiro-Wilk test was performed to assess whether data were normally distributed. Variables that appearing not to be normally distributed at all time points or showing evidence of unequal variances were logarithmically transformed prior to analysis. Average daily food intake (weight of food as fed), average ME intake relative to metabolic body weight (ie, body weight$^{0.75}$), and average race time were calculated for each week of the study.

Data were analyzed as a 2-period crossover design with a within-period repeated-measures factor, using generalized least squares estimation.[20] Measurements over time within each period (ie, weekly measurements [average food and energy intake, body weight, and race times]) and measurements before and after racing were treated as within-subject repeated measures. An autoregressive heterogeneous covariance structure was used to explicitly model the correlation between measurements obtained at these time points. Moreover, a random effect for each subject was included. Whereas diet was used as a within-subject factor, period and sequence were used as between-subject factors. Diet-by-time interactions also were tested. When sequence effects were found, differences attributable to diet were tested, using data from the first period only. This essentially was the method of analysis suggested by Hills and Armitage.[21] Concentrations determined after racing were adjusted for postrace hemoconcentration, using the following transformation:

$$C_{adj} = C_{obs} \times (P_1/P_2),$$

where C_{adj} was the adjusted postrace concentration, C_{obs} was the observed postrace concentration, P_1 was the total protein concentration before racing, and P_2 was the total protein concentration after racing. Adjusted and unadjusted concentrations after racing were compared separately with the concentration before racing. Body weights were compared between the start (week 1) and end of the study (week 22), and body compositions were compared between diets, using a paired *t*-test. Ambient temperature and humidity were regressed against race time, using data from all dogs throughout the duration of the study.

Type-I and -II errors were set at 0.05 and 0.2, respectively, for use in detecting differences of 2 kg in body weight, 30 g/d in food intake, 10 kcal/kg$^{0.75}$ per d in energy intake, 0.5 seconds in race time, 200 ml in plasma volume, 5% in total body water, 0.03 in pH, 0.3 mg/dl in protein, albumin, globulin, or creatinine concentrations, 1 mEq/L in potassium, calcium, or phosphorus concentration, 2 mEq/L in sodium, chloride, or bicarbonate concentration, 2 mg/dl in BUN concentration, 10 mg/dl in glucose and triglyceride concentrations, 10 U/L in **alanine transaminase (ALT)**, **aspartate transaminase (AST)**, and **alkaline phosphatase (ALP)** activities, 300 U/L in creatine kinase, lipase, and amylase activities, 3% in Hct, and 2 fl in mean corpuscular volume.[22]

Results

The HP diet contained 37.3% ME protein, 33.0% ME fat, and 29.7% ME carbohydrate. The MP diet con-

Table 1—Effect of diet on average body weight, food intake, metabolizable energy (ME) intake, and race time in 8 racing Greyhounds

Variable	High-protein diet	Moderate-protein diet
Race time (s)*	32.61 ± 0.50	32.43 ± 0.48
Average speed (m/s)*	15.34 ± 0.24	15.42 ± 0.23
Food intake (g/d)	499 ± 111	514 ± 66
ME intake (kcal/kg$^{0.75}$ • d)	150 ± 29	154 ± 17
Body weight (kg)*	32.2 ± 2.9	32.8 ± 2.5

Values are reported as mean ± SD.
*Values differ significantly ($P \leq 0.05$) between diets.

Table 2—Effect of diet on body composition in 8 racing Greyhounds

Variable	High-protein diet	Moderate-protein diet
Total body water (%)	71 ± 7	68 ± 6
Lean body mass (%)	96 ± 9	93 ± 8
Fat mass (%)	3 ± 9	7 ± 8
Plasma volume (ml/kg)	50 ± 10	50 ± 10
Blood volume (ml/kg)	110 ± 15	114 ± 16

Values are reported as mean ± SD; none were significantly different between diets.

Table 3—Effect of diet on rectal temperature and results of venous blood gas analysis in 8 racing Greyhounds

Variable	Diet	Before racing	After racing
Temperature (C)*	High protein	38.8 ± 0.4	41.4 ± 0.5
	Moderate protein	38.8 ± 0.4	41.6 ± 0.4
pH*	High protein	7.40 ± 0.02	7.06 ± 0.06
	Moderate protein	7.41 ± 0.02	7.06 ± 0.08
Bicarbonate (mEq/L)*	High protein	25.3 ± 1.5	7.5 ± 2.0
	Moderate protein	25.6 ± 2.0	7.2 ± 1.8
PCO_2 (mm Hg)*	High protein	41 ± 3	28 ± 7
	Moderate protein	41 ± 2	26 ± 5
PO_2 (mm Hg)*	High protein	52 ± 6	76 ± 8
	Moderate protein	54 ± 4	80 ± 9

Values reported are mean ± SD.
*Values obtained after racing are significantly different ($P \leq 0.001$) from values obtained before racing.

Table 4—Effect of diet on hematologic variables in racing Greyhounds*

Variable	Diet	Before racing	After racing
Hct (%)†‡	High protein	54 ± 6	64 ± 8
	Moderate protein	56 ± 4	67 ± 3
RBC count ($\times 10^6$ cells/μl)‡	High protein	8.1 ± 0.8	9.5 ± 1.1
	Moderate protein	8.4 ± 0.4	9.9 ± 0.3
Hemoglobin (g/dl)†‡	High protein	19.4 ± 1.4	23.0 ± 1.4
	Moderate protein	20.4 ± 0.7	24.1 ± 0.5
Mean corpuscular volume (fl)‡	High protein	66 ± 2	68 ± 2
	Moderate protein	66 ± 4	67 ± 4
WBC count ($\times 10^3$ cells/μl)‡	High protein	5.7 ± 1.2	6.2 ± 1.4
	Moderate protein	5.9 ± 0.8	6.4 ± 0.9
Segmented neutrophils ($\times 10^3$ cells/μl)	High protein	4.0 ± 0.7	4.2 ± 0.8
	Moderate protein	4.2 ± 0.8	4.6 ± 0.8
Lymphocytes ($\times 10^3$ cells/μl)	High protein	1.1 ± 0.2	1.2 ± 0.6
	Moderate protein	1.0 ± 0.4	1.1 ± 0.5

Values reported are mean ± SD.
*Values reported are for only 7 dogs, because blood samples from 1 dog clotted and were not available for analysis. †Values are significantly different ($P \leq 0.05$) between diets. ‡Values obtained after racing are significantly different ($P \leq 0.05$) from values obtained before racing.

Table 5—Effect of diet on results of serum biochemical analyses in 8 racing Greyhounds

Variable	Diet	Before racing	After racing	Adjusted for after racing*
Lactate (mmol/L)‡§	High protein	0.4 ± 0.2	24.8 ± 2.8	20.2 ± 1.8
	Moderate protein	0.5 ± 0.3	24.0 ± 4.1	19.3 ± 3.1
Sodium (mEq/L)‡§	High protein	144 ± 2	155 ± 2	127 ± 5
	Moderate protein	145 ± 1	155 ± 2	125 ± 4
Potassium (mEq/L)§	High protein	4.2 ± 0.3	4.1 ± 0.3	3.4 ± 0.3
	Moderate protein	4.3 ± 0.2	4.3 ± 0.3	3.5 ± 0.2
Chloride (mEq/L)†‡§	High protein	116 ± 2	114 ± 3	93 ± 4
	Moderate protein	116 ± 2	113 ± 3	91 ± 3
Calcium (mg/dl)‡§	High protein	9.5 ± 0.2	9.9 ± 0.4	8.1 ± 0.4
	Moderate protein	9.4 ± 0.2	10.0 ± 0.3	8.0 ± 0.3
Ionized calcium (mg/dl)‡§	High protein	5.4 ± 0.3	5.2 ± 0.1	4.2 ± 0.2
	Moderate protein	5.3 ± 0.2	4.9 ± 0.2	4.1 ± 0.1
Phosphorus (mg/dl)‡§	High protein	2.5 ± 0.4	1.8 ± 0.4	1.5 ± 0.4
	Moderate protein	2.5 ± 0.3	1.8 ± 0.6	1.5 ± 0.5
Total protein (g/dl)‡	High protein	5.9 ± 0.3	7.2 ± 0.3	5.9 ± 0.3
	Moderate protein	5.9 ± 0.3	7.3 ± 0.3	5.9 ± 0.3
Albumin (g/dl)‡§	High protein	3.0 ± 0.2	3.8 ± 0.1	3.1 ± 0.2
	Moderate protein	3.0 ± 0.2	3.9 ± 0.3	3.1 ± 0.2
Globulin (g/dl)‡	High protein	2.8 ± 0.2	3.4 ± 0.3	2.8 ± 0.2
	Moderate protein	2.9 ± 0.2	3.4 ± 0.2	2.8 ± 0.1
Creatinine (mg/dl)‡§	High protein	1.2 ± 0.2	1.8 ± 0.2	1.5 ± 0.2
	Moderate protein	1.3 ± 0.2	1.7 ± 0.3	1.4 ± 0.3
BUN (mg/dl)†‡§	High protein	20 ± 4	21 ± 4	17 ± 3
	Moderate protein	16 ± 1	17 ± 1	14 ± 1
Glucose (mg/dl)†‡§ ‖	High protein	104 ± 10	151 ± 27	124 ± 25
	Moderate protein	106 ± 5	131 ± 16	105 ± 13
Triglyceride (mg/dl)†‡§ ‖	High protein	32 ± 11	104 ± 24	85 ± 18
	Moderate protein	34 ± 8	128 ± 17	103 ± 12
Cholesterol (mg/dl)‡§	High protein	158 ± 38	187 ± 39	154 ± 36
	Moderate protein	152 ± 22	182 ± 28	146 ± 21
ALT (U/L)†‡§	High protein	39 ± 10	65 ± 13	53 ± 11
	Moderate protein	45 ± 10	72 ± 22	58 ± 17
AST (U/L)‡§	High protein	32 ± 7	74 ± 11	60 ± 9
	Moderate protein	36 ± 12	81 ± 15	65 ± 11
ALP (U/L)‡	High protein	67 ± 15	80 ± 18	66 ± 15
	Moderate protein	75 ± 18	90 ± 24	72 ± 19
CK (U/L)	High protein	200 ± 164	274 ± 136	222 ± 104
	Moderate protein	218 ± 226	274 ± 177	218 ± 135
Total bilirubin (mg/dl)‡§	High protein	0.20 ± 0.05	0.12 ± 0.05	0.10 ± 0.04
	Moderate protein	0.22 ± 0.07	0.12 ± 0.05	0.10 ± 0.04

Values reported are mean ± SD.
§Values obtained after racing and adjusted for hemoconcentration were significantly different ($P \leq 0.05$) from values obtained before racing. ‖ A significant ($P \leq 0.05$) diet × time of sample collection (before vs after racing) interaction was detected for this variable.
See Table 4 for remainder of key.
ALT = Alanine transaminase. AST = Aspartate transaminase. ALP = Alkaline phosphatase. CK = Creatine kinase.

tained 24.2% ME protein, 33.1% ME fat, and 42.7% ME carbohydrate. Thus, dietary fat was similar in the 2 diets, but there was 50% more dietary protein and 30% less NFE in the HP diet, compared with the MP diet (**Appendix 1**). Amino acid distribution was similar for the 2 diets, but there was approximately 50% more of each amino acid in the HP diet than in the MP diet (**Appendix 2**). Essential nutrient composition was similar for the 2 diets, and both diets conformed to AAFCO recommendations for adult dogs[8]; however, the HP diet contained slightly more of most essential nutrients (**Appendix 3**). The digestibility of major nutrients was similar for both diets (**Appendix 4**).

Diet composition varied little during the study. Analysis of major and minor nutrient composition of a sample of each diet obtained at the time of manufacture yielded almost identical results to those reported for samples obtained during the digestibility trials. Similarly, amino acid analysis yielded similar results to those reported here.

Body weight was unchanged between week 1 (32.3 ± 2.8 kg) and week 22 (32.1 ± 3.1 kg), and mean ME intake throughout the study was 156 ± 26 kcal/kg$^{0.75}$ per d. Photographs of the dogs taken during the last week of each diet period suggested that body condition was unaffected by diet. Daily food and ME intake were similar when dogs were fed each of the 2 diets, but mean body weight was slightly but significantly more when dogs were fed the MP diet than when fed the HP diet (32.8 vs 32.2 kg). Mean race times were significantly shorter (32.43 vs 32.61 seconds) and dogs were significantly faster (15.42 vs 15.34 m/s) for the 500-m race when fed the MP diet than when fed the HP diet (**Table 1**). Race times varied significantly ($P = 0.04$) from week to week. Ambient temperature and humidity increased from –4 C and 15%, respectively, during week 1 to 28 C and 55%, respectively, by week 22, but neither factor appeared to affect race times (correlation coefficient was not significantly different from 0 and $r^2 \leq 0.01$). Diet did not have an effect on body composition (**Table 2**).

The amount of dietary protein did not have an effect on body temperature and most hematologic variables (**Tables 3-5**). Some hematologic results represented data from only 7 dogs, because 1 blood sample collected after racing clotted before analysis; all other results represented data from 8 dogs. The Hct, hemoglobin, BUN, glucose, triglyceride, and chloride concentrations, and ALT activity had small but significant differences or interactions attributable to diet, and diet had a small but not significant($P = 0.1$) effect on RBC count. Rectal temperature, Hct, RBC count, hemoglobin concentration, mean corpuscular volume, WBC count, venous P_{O_2}, lactate, sodium, total calcium, total protein, albumin, globulin, creatinine, BUN, glucose, triglyceride, and cholesterol concentrations, and ALT, AST, and ALP activities all increased significantly and venous pH, P_{CO_2}, bicarbonate, chloride, ionized calcium, phosphorus, and total bilirubin concentrations all decreased significantly ($P < 0.001$) after racing. When concentrations after racing were adjusted on the basis of hemoconcentration, only lactate, triglyceride, and glucose concentrations and ALT and AST activities increased markedly with racing; other variables decreased with racing or were unchanged. We did not detect sequence effects.

Discussion

The study reported here revealed that racing Greyhounds ran slower (0.18 seconds or 0.08 m/s) for a 500-m distance when dietary protein was increased from 24 to 36% ME and dietary carbohydrate was decreased from 43 to 30% ME. This is equivalent to a difference of 2.5 m for a 500-m race and may represent the difference between winning and losing. Speeds (15.4 m/s) reported here were slower than those observed at commercial race tracks with hard surfaces, because a soft sand training track was used to minimize the risk of injury; however, speeds of our dogs were comparable to those for other scientific studies at this facility and elsewhere.[2,23] Furthermore, dogs used in this study were donated for our use, potentially because they were too slow for commercial racing.

The increase in dietary protein or decrease in dietary carbohydrate or a change in minor nutrient content between diets could have been responsible for the observed detrimental effect on performance. It also is possible that changes in 2 or more nutrients acted synergistically. Nevertheless, it is not possible in a single experiment to separate these effects. Therefore, explanations as to why dogs ran slower must be speculative until additional experiments have been performed.

Deficiencies of protein, amino acids, or other essential nutrients could not explain the difference in performance. The 2 diets contained more protein, amino acids, and other nutrients than are recommended by the **National Research Council (NRC)** and AAFCO for adult dogs, and there were more of most essential nutrients in the HP diet than the MP diet. The biological value and relative proportions of protein sources varied between the 2 diets, but the distribution of essential amino acids and protein digestibility was similar between the 2 diets. The NRC recommends that adult dogs should be fed at least 8% ME protein when fed purified diets,[10] and AAFCO recommends that adult dogs should be fed at least 18% ME protein when fed commercial pet foods.[8] The NRC and AAFCO do not suggest a maximum amount of intake. However, analysis of the results reported here suggests that Greyhounds in training may have a maximum protein requirement above which excess protein has a detrimental effect. It also suggests that increased dietary protein is not responsible for beneficial effects on performance when meat is added to the diet of racing Greyhounds. It remains to be determined, however, whether Greyhounds will perform optimally when fed diets containing < 24% ME protein.

In another study performed by our laboratory group,[2] we found that dogs ran faster when dietary protein increased from 21 to 25% ME and dietary fat increased from 25 to 32% ME with a concurrent decrease in dietary carbohydrate from 54 to 43% ME. However, Toll et al[1] reported that Greyhounds ran slower when dietary fat increased from 31 to 75% ME and dietary carbohydrate decreased to low amounts. Together, results of those studies suggest that excess

amounts of protein and fat and inadequate amounts of fat and carbohydrate may be detrimental to performance in sprinting dogs, but the optimum proportion of protein, fat, and carbohydrate and the manner in which they interact with each other to affect performance needs further evaluation.

Glucose oxidation is the principle source of energy at high rates of energy expenditure (80% of energy source at a rate of 85% maximal oxygen consumption), and consumption of high-carbohydrate diets increases resting muscle glycogen in endurance racing dogs.[24,25] However, diets that are higher in fat and lower in carbohydrate increase stamina in dogs undertaking endurance exercise.[25] Furthermore, the amount of energy from fat oxidation in dogs is twice that in less aerobic species such as humans and goats.[26,27] Finally, most muscle fibers in the limbs of Greyhounds and crossbred dogs have high oxidative activity.[28,29] Thus, it seems most likely that there may be an optimum percentage of each of these nutrients, and excess protein may limit other nutrients.

Analysis of results of studies with racing sled dogs has suggested that dogs require increased dietary protein when undertaking endurance exercise. Plasma volume increased in racing sled dogs when dietary protein exceeded 40% ME,[3] and Hct declined during training when dogs were fed a diet containing 28% ME protein but not when dogs were fed diets containing ≥ 32% ME protein.[4] In the study reported here, we did not detect evidence for an effect of dietary protein on plasma volume and total body water in these racing Greyhounds, but Hct and hemoglobin concentration were slightly higher in dogs fed less protein and more carbohydrate. Therefore, an increase in Hct may explain the improved performance of dogs fed the MP diet, but increased amounts of dietary protein did not appear to affect body composition in sprinting dogs in a manner similar to that in endurance racing dogs.

Plasma volume (50 ± 10 ml/kg) was similar in these Greyhounds to that reported for adult dogs of other breeds (mean values of 47 to 55 ml/kg),[12,30-32] but blood volume (112 ± 15 ml/kg) for these Greyhounds was greater than that reported for adult dogs of other breeds (mean values of 78 to 98 ml/kg)[12,30-32] because PCV was higher in these Greyhounds. In 1 study,[33] mean plasma volume and blood volume of trained Greyhounds (77 and 158 ml/kg, respectively) was much higher than that of untrained Greyhounds (59 and 128 ml/kg, respectively). Those values are much higher than values for the trained Greyhounds of the study reported here, but differences in methods probably are responsible.

Total body water in these racing Greyhounds (69 ± 6% of body weight) was greater than that reported for adult dogs of other breeds (mean values of 50 to 66%),[17,30,34] but fat mass was only 5 ± 9%, compared with up to 35% fat mass in adult dogs of other breeds.[17,30,34] It is difficult to make direct comparisons among studies, because methods vary, but Greyhounds probably have more muscle mass and less fat than dogs of most other breeds.[35] The fat mass estimate also may have been inaccurate if Greyhound fat-free mass contains appreciably more or less than 73% water.

Daily energy consumption (156 kcal/kg$^{0.75}$ per d) was similar to that reported for Greyhounds trained at this facility.[2] Dogs were slightly (0.6 kg) heavier when fed the MP diet, but we did not detect differences in estimated ME consumption or digestibility between diets. The excess protein in the HP diet may have been used less efficiently, however, than the carbohydrate in the MP diet, because the heat increment of dietary protein is greater than that of carbohydrate.[36] Rectal temperature was measured before feeding, which may explain the reason that the change in heat increment did not affect body temperature.

Changes in hematologic variables observed after racing were similar to those reported for Greyhounds that raced a distance of 500 m.[23,37-40] A 50-fold increase in plasma lactate concentration caused severe postrace acidosis (pH 7.06), which was partly compensated by a slight decrease in serum phosphorus concentration and venous P_{CO_2}. Total protein concentrations increased by 22%, probably as a result of fluid shifts and dehydration (Table 2). Many hematologic variables also increased a small amount, but when adjusted for dehydration, concentrations of most hematologic variables decreased after racing. Adjusted serum triglyceride and glucose concentrations increased markedly after racing, but triglyceride concentrations increased more and glucose concentrations increased less in dogs when fed the MP diet, compared with when dogs were fed the HP diet. This increased fat availability may explain the improved performance in dogs fed the MP diet. Both diets contained a similar amount of dietary fat, but it is possible that increased dietary carbohydrate in the MP diet increased insulin release, inhibiting lipolysis and stimulating lipogenesis. Concentration of BUN was slightly higher when dogs were fed the HP diet, reflecting increased protein turnover, but the increase was nominal and of questionable physiologic importance.

Resting serum phosphorus concentrations were low (2.5 ± 0.4 mg/dl) in all dogs and decreased after racing to 1.8 ± 0.5 mg/dl (equivalent to 1.5 ± 0.4 mg/dl when adjusted for hemoconcentration), whereas other investigators found that serum phosphorus concentrations increase after a race.[37,40] Two chemical assays yielded similar phosphorus concentrations. Concentrations reported here are those obtained with the assay that yielded slightly higher concentrations. Phosphorus is required for glycolytic intermediates and ATP. Moderate hypophosphatemia (1 to 2.5 mg/dl) usually does not cause clinical signs, whereas severe hypophosphatemia (< 1 mg/dl) may result in lysis of RBC.[41] Adjusted phosphorus concentrations decreased to < 1 mg/dl in only 3 dogs, but there was a significant (P = 0.006) decrease in resting PCV from 58 ± 3% during week 8 to 52 ± 3% during week 16. Number of RBC and hemoglobin concentration decreased proportionately. Nevertheless, blood volume did not change significantly from week 11 to 22 (104 ± 16 vs 109 ± 13 ml/kg), because plasma volume increased significantly (P = 0.002) from 45 ± 6 ml/kg during week 11 to 55 ± 9 ml/kg during week 22. Therefore, it is possible but questionable that hypophosphatemia caused this decrease in PCV. The MP and HP diets contained 0.85

and 1.0% phosphorous, respectively (dry-matter basis). This is well above the minimum NRC recommendation for adult and growing dogs and is similar to the AAFCO recommendation for growth.[8,10] However, phosphorus requirements of Greyhounds in training may be greater than those for inactive dogs. Unless there was a severe difference in bioavailability of phosphorus between the diets, dietary phosphorus probably was not responsible for the difference in performance, because there was more phosphorus in the HP diet than the MP diet.

The study reported here provides additional evidence that diet can affect the performance of Greyhounds involved in sprint exercise, and the nutrient requirements of these dogs may differ from those of sled dogs involved in endurance exercise. Performance of Greyhounds appeared to decrease when dietary protein increased from 24 to 37% ME protein, whereas endurance racing dogs appear to require increased dietary protein. This has important implications for the formulation of diets for Greyhounds. The traditional practice of adding meat to a commercial extruded food formulated for dogs usually increases dietary protein and reduces dietary carbohydrate. The purported increase in performance when meat is added to the diet probably cannot be attributed to this change in major nutrient composition.

[a]Frontline Topspot, Rhone-Merieux, Athens, Ga.
[b]Kal Kan Foods Inc, Vernon, Calif.
[c]Advance, Kal Kan Foods Inc, Vernon, Calif.
[d]18-oz Whirl-Pak bags, Fisher Scientific, Pittsburgh, Pa.
[e]Osterizer Galaxie, Oster, Laurel, Miss.
[f]Waltham Centre for Pet Nutrition, Leics, England.
[g]Covance Laboratories Inc, Madison, Wis.
[h]Nikkor 43-86 mm zoom, Nikon, Melville, NY.
[i]Evans Blue E2129, Sigma Chemical Co, St Louis, Mo.
[j]Vacutainer tubes, Becton Dickinson, Rutherford, NJ.
[k]Department of Zoology, University of Aberdeen, Aberdeen, Scotland.
[l]Lithium Aluminium Hydride, Poole, Dorset, UK.
[m]VG Optima, Micromass Ltd, Manchester, England.
[n]CP grade hydrogen, BOC, Aberdeen, Scotland.
[o]SAS/STAT, version 6.12, SAS Institute Inc, Cary, NC.

References

1. Toll PW, Pieschl RL, Hand MS. The effect of dietary fat and carbohydrate on sprint performance in racing Greyhound dogs, in *Proceedings*. 8th Int Racing Greyhound Symp, 1992;1–3.

2. Hill RC, Bloomberg MS, Legrand-Defretin V, et al. Maintenance energy requirements and the effect of diet on performance of racing Greyhounds. *Am J Vet Res* 2000;61:1566–1573.

3. Reynolds AJ. The effect of diet on performance, in *Proceedings*. Performance Dog Nutr Symp, 1995;10–20.

4. Kronfeld DS, Hammel EP, Ramberg CF, et al. Hematological and metabolic responses to training in racing sled dogs fed diets containing medium, low, or zero carbohydrate. *Am J Clin Nutr* 1977;30: 419–430.

5. Kohnke JR. Nutrition for the racing Greyhound. In: Bloomberg MS, Dee JF, Taylor RA, eds. *Canine sports medicine and surgery*. Philadelphia: WB Saunders Co, 1998;328–335.

6. Beltsville Human Nutrition Research Center. *USDA nutrient database for standard reference*. Riverdale, Md: Nutrient Data Laboratory, Agricultural Research Service, Beltsville Human Nutrition Research Center, 1997.

7. National Research Council. *Guide for the care and use of laboratory animals*. Publication No. 85-23 (rev). Bethesda, Md: National Institutes of Health, 1985.

8. American Association of Feed Control Officials Inc. *Official publication 1994*. Atlanta, Ga: American Association of Feed Control Officials Inc, 1994;110–116.

9. Association of Official Analytical Chemists. Method #982.30. In: *Official methods of analysis*. 15th ed. Gaithersburg, Md: Association of Official Analytical Chemists International, 1990;1096–1097.

10. National Research Council. *Nutrient requirements of dogs*. Washington, DC: National Academy Press, 1985.

11. Zweens J, Frankena H. An improved method for the determination of the plasma volume with Evans Blue. *J Clin Chem Clin Biochem* 1981;19:919–924.

12. Jain NC. Blood volume and water balance. In: *Schalms veterinary hematology*. 4th ed. Philadelphia: Lea & Febiger, 1986;87–102.

13. Nagy KA. *The doubly labeled water method: a guide to its use*. Los Angeles: University of California, 1983.

14. Bocek P, Deml M, Tesarik K. The determination of ^2H_2O in water and biological fluids by gas chromatography. *J Chromatogr* 1973;87:246–249.

15. Halliday D, Miller AG. Precise measurement of total body water using trace quantities of deuterium oxide. *Biomed Mass Spectrom* 1977;4:82–87.

16. Speakman JR, Nagy KA, Masman D, et al. Interlaboratory comparison of different analytical techniques to determination of oxygen-18 abundance. *Anal Chem* 1990;62:703–708.

17. Speakman JR. Equation 17.11. In: *Doubly labeled water: theory and practice*. 1st ed. London: Chapman & Hall, 1997;306.

18. Taylor HP. The application of oxygen and hydrogen isotope studies to problems of hydrothermal alteration and ore deposition. *Econ Geol* 1974;69:843–883.

19. Rathbun EN, Pace N. Studies on body composition. I. The determination of total body fat by means of the body specific gravity. *J Biol Chem* 1945;158:667–676.

20. Littell RC, Milliken GA, Stroup WW, et al. *SAS system for mixed models*. Cary, NC: SAS Institute Inc, 1996;633.

21. Hills M, Armitage P. The two period cross-over clinical trial. *Br J Pharmacol* 1979;8:7–20.

22. Marks RG. Determining the proper sample size. In: *Designing a research project*. Belmont, Calif: Wadsworth Inc, 1982;129–171.

23. Ilkiw JE, Davis PE, Church DB. Hematologic, biochemical, blood-gas, and acid-base values in Greyhounds before and after exercise. *Am J Vet Res* 1989;50:583–586.

24. Weibel EW, Taylor CR, Weber J-M, et al. Design of the oxygen and substrate pathways. VII. Different structural limits for oxygen supply to muscle mitochondria. *J Exp Biol* 1996;199:1699–1709.

25. Kronfeld DS. Diet and the performance of racing sled dogs. *J Am Vet Med Assoc* 1973;162:470–473.

26. de Bruijne JJ, Ketone body metabolism in fasting dogs.Utrecht:Drukkerij Elinkwijk, BV, 1982;93.

27. McLelland G, Zwingelstein G, Taylor CR, et al. Increased capacity for circulatory fatty acid transport in a highly aerobic mammal. *Am J Physiol* 1994;266:R1280–R1286.

28. Gunn HM. Differences in the histochemical properties of skeletal muscles of different breeds of horses and dogs. *J Anat* 1978;127:615–634.

29. Guy PS, Snow DH. Skeletal muscle fiber composition in the dog and its relationship to athletic ability. *Res Vet Sci* 1981;31: 244–248.

30. Lammerant J, Haxhe J-J. Relationships between cardiac output, blood volume and body composition in chloralose anesthetized normal dogs. *Arch Int Physiol Biochim* 1964;72:585–598.

31. Koh H, Ishihara H, Miyahara I, et al. Does the initial distribution volume of glucose reflect plasma volume after haemorrhage in dogs? *Can J Anaesth* 1995;42:163–167.

32. Vollmar AM, Montag C, Preusser U, et al. Atrial naturetic peptide and plasma volume of dogs suffering from heart failure or dehydration. *Zentralbl Veterinarmed* [A] 1994;41:548–557.

33. McKeever KH, Schurg WA, Convertino VA. Exercise training-induced hypervolemia in Greyhounds: role of water intake and renal mechanisms. *Am J Physiol* 1985;248:R422–R425

34. Ballevre O, Anantharaman-Barr G, Gicquello P, et al. Use of doubly labelled water method to assess energy expenditure in free living cats and dogs. *J Nutr* 1994;124:2594S–2600S.

35. Gunn HM. The proportions of muscle, bone and fat in two different types of dog. *Res Vet Sci* 1978;24:277–282.

36. Blaxter K. The utilization of the energy of food. In: *Energy metabolism in animals and man*. Cambridge, UK: Cambridge University Press, 1989;254–289.

37. Rose RJ, Bloomberg MS. Responses to sprint exercise in the Greyhound: effects on haematology, serum biochemistry and muscle metabolites. *Res Vet Sci* 1989;47:212–218.

38. Snow DH, Harris RC, Stuttard E. Changes in haematology and plasma biochemistry during maximal exercise in Greyhounds. *Vet Rec* 1988;123:487–489.

39. Nold JL, Peterson LJ, Fedde MR. Physiological changes in the running Greyhound (*Canis domesticus*): influence of race length. *Comp Biochem Physiol* 1991;100A:623–627.

40. Pieschl RL, Toll PW, Leith DE, et al. Acid-base changes in the running Greyhound: contributing variables. *J Appl Physiol* 1992; 73:2297–2304.

41. Yawata Y, Hebbel RP, Silvis S, et al. Blood cell abnormalities complicating the hypophosphatemia of hyperalimentation: erythrocyte and platelet ATP deficiency associated with hemolytic anemia and bleeding in hyperalimented dogs. *J Lab Clin Med* 1974;84: 643–653.

Appendix 1

Proximate analysis, fatty acid, fiber, and estimated metabolizable energy (ME) content of 2 diets fed to racing Greyhounds

Nutrient*	High-protein diet	Moderate-protein diet
Protein (%)	38.9	26.0
Fat (%)	14.8	14.9
Linoleic acid (%)	2.01	2.06
Linolenic acid (%)	0.09	0.09
Arachidonic acid (%)	0.06	0.04
NFE (%)	30.4	43.5
Ash (%)	7.0	6.0
Crude Fiber (%)	0.4	0.6
Soluble fiber (%)	0.8	0.1
Insoluble fiber (%)	2.2	3.1
Moisture (%)	6.7	6.5
ME density (kcal/g)	4.07	4.11

*Values are reported on an as-fed basis.
NFE = Nitrogen-free extract.
Ingredients for the 2 diets were brewer's rice, whole yellow corn, soy protein isolate, low-ash poultry meal, corn gluten meal, brewer's yeast, dried egg, iodized salt, digest, beef tallow, vegetable oil, and a vitamin-mineral premix.

Appendix 2

Amino acid composition of 2 diets fed to racing Greyhounds

Amino acid*	High-protein diet	Moderate-protein diet
Arginine	2.25	1.43
Histidine	0.83	0.52
Isoleucine	1.44	0.96
Leucine	3.59	2.38
Lysine	1.84	1.11
Cystine	0.55	0.37
Methionine	0.67	0.45
Tyrosine	1.38	0.90
Phenylalanine	1.82	1.17
Threonine	1.41	0.89
Tryptophan	0.36	0.21
Valine	1.64	1.16
Alanine	2.22	1.57
Aspartate	3.10	1.90
Glutamate	5.63	3.51
Glycine	2.13	1.47
Proline	2.30	1.50
Serine	1.75	1.06

*Values are reported on an as-fed basis.

Appendix 3

Mineral and vitamin analysis of 2 diets fed to racing Greyhounds

Nutrient	High-protein diet	Moderate-protein diet
Minerals		
Calcium (g/kg)	12.2	9.6
Phosphorus (g/kg)	10.3	8.5
Sodium (g/kg)	6.5	5.4
Potassium (g/kg)	5.9	5.3
Magnesium (g/kg)	0.7	0.7
Iron (mg/kg)	350	295
Copper (mg/kg)	23	20
Zinc (mg/kg)	196	193
Manganese (mg/kg)	16	15
Selenium (mg/kg)	0.5	0.4
Iodine (mg/kg)	1.2	1.6
Vitamins		
A (IU/g)	11.8	8.4
E (IU/kg)	88	86
D_3 (IU/kg)	1,470	1,320
B_1 (mg/kg)	1.0	1.2
B_2 (mg/kg)	7.1	5.9
B_3 (mg/kg)	100	47
B_6 (mg/kg)	3.1	2.5
B_{12} (mg/kg)	0.1	0.1
Pantothenic acid (mg/kg)	21	19
Folic acid (mg/kg)	1.3	1.0
Choline chloride (mg/kg)	2.4	2.1
Biotin (g/kg)	0.1	0.1

Appendix 4

Digestibility of major nutrients in 2 diets fed to 8 racing Greyhounds

Nutrient*	High-protein diet	Moderate-protein diet
Organic matter	92 ± 4	93 ± 2
Dry matter	88 ± 5	90 ± 2
Protein	89 ± 5	87 ± 3
Fat	97 ± 1	97 ± 1
NFE	95 ± 2	97 ± 1

*Values reported are percentages.
Values are reported as mean ± SD.