Effect of Diet on Vitreous Humor and Serum in Black-tailed Jackrabbits

Scott E. Henke[1]
Stephen Demarais[2]
Department of Range and Wildlife Management, Texas Tech University, Lubbock, Texas 79409

ABSTRACT

The responses of serum and vitreous humor constituents to two levels of nutrition were compared in 23 adult, male black-tailed jackrabbits (*Lepus californicus*). The vitreous humor constituents were similar or linearly correlated to serum constituents for urea nitrogen, triglycerides, and glutamic-oxaloacetic transaminase. The remaining vitreous humor constituents were neither similar nor predictive of serum constituents. Vitreous humor glucose, cholesterol, albumin, total protein, and gamma glutamyltransferase, and serum urea nitrogen, triglycerides, cholesterol, total protein, albumin, and gamma glutamyltransferase were affected by 25% feed restriction. Vitreous humor has potential as a source for biochemical indicators of nutritional intake only if additional baseline vitreous humor constituent values are determined.

KEY WORDS: condition, jackrabbit, *Lepus californicus*, nutrition, serum, stress, vitreous humor

Knowledge of nutrition is essential to gain insight on the interaction of wildlife and their habitat. Blood characteristics used to assess the nutritional status of wildlife (LeResche et al. 1974, Warren and Kirkpatrick 1978) may be influenced by collection stress (Jacobson et al. 1978). In addition, in cases of sudden death, hematologic evaluation is limited because of coagulation (Coe 1972). Although obtaining a vitreous humor sample typically involves euthanasia, the vitreous humor can be an alternative source for measuring certain metabolites such as urea nitrogen and creatinine (Lane and Lincoln 1985, DeLiberto et al. 1988). Vitreous humor is easily accessible and less subject to chemical changes than serum (Coe 1972), may retain its quantifiable constituent levels longer than serum following death (Schoning and Strafuss 1981, Henke and Demarais 1992), and has been shown in humans to be less susceptible than serum to acute physical changes (Jaffe 1962). Urea nitrogen in vitreous humor and serum were correlated in cattle (Lane and Lincoln 1985), domestic rabbits (Henke and Demarais 1992), and white-tailed deer (*Odocoileus virginianus*) (DeLiberto et al. 1988). The effect of specific condition levels on vitreous humor must be determined (LeResche et al. 1974) before the vitreous humor can be accepted as a reliable alternative to serum as a source of biochemical nutritional indices. If successful, then vitreous humor would offer a postmortem diagnostic source of animal condition. For example, eyes could be obtained at hunter check stations from recently-hunted animals to assess their nutritional condition rather than having to be immediately present at the kill site to obtain blood samples before coagulation occurs.

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Therefore, we evaluated the potential use of vitreous humor as a source for biochemical indicators of nutritional intake. Black-tailed jackrabbits (*Lepus californicus*) were selected as an animal model for other wild animal applications because they had large eyes that contained an adequate sample of vitreous humor, were numerous in the surrounding area, and were easily captured. Specific objectives included to 1) compare serum and vitreous humor values between rabbits placed on *ad libitum* diets and 75% of their *ad libitum* intake, 2) determine the effect of nutritional intake on serum and vitreous humor values, 3) estimate predictability of serum values using vitreous humor values, and 4) establish baseline physiological values for black-tailed jackrabbits in northwest Texas.

METHODS

Twenty-three adult, male black-tailed jackrabbits were caught by the drive corral method (Henke and Demarais 1990*a*) within the city limits of Lubbock, Texas during 30 April-8 May, 1988. Animals were individually housed indoors in stainless steel rabbit cages. A grid rack allowed urine and feces to fall out. Jackrabbits were provided *ad libitum* water and Purina Rabbit Chow Complete blend (Ralston Purina Co., St. Louis, MO) and allowed to adapt to confinement and feed. The amount of feed that each jackrabbit consumed was determined by the daily difference of the amount of feed given and amount of feed remaining. Jackrabbits were considered adapted when their daily feed consumption stabilized. Consumption was considered stable when feed consumption remained within 10% of the 7-day running mean for a 7-day period. After the adaption period, jackrabbits were assigned randomly to 1 of 2 diets, either *ad libitum* or 75% of their *ad libitum* intake, for a 2-week period. Feed intake reduced by 25% resulted in jackrabbits with lower post-trial body weight and kidney fat indices, enlarged spleens and adrenal cortex widths, increased bilirubin and cortisol concentrations, and depressed immune function (Henke and Demarais 1990*b*).

Euthanasia was accomplished with a 1.5cc intraperitoneal injection of T61 euthanasia solution (Taylor Pharmaceuticals, Decatur, IL). The time period between removal from the cage and death was < 3 minutes. A blood sample was taken via a heart puncture with a 12cc syringe and a 18 gauge needle and centrifuged at 1,400 rpm for 15 minutes. Serum was collected and used for chemistry analysis.

Both eyes of each jackrabbit were removed from their sockets and washed free of blood. The vitreous humor was collected, centrifuged at 1,400 rpm for 15 minutes; the gelatinous portion was discarded, and the liquid phase was collected and used for chemistry analysis. Animals were aged using eye lens weights to verify adult status (Rongstad 1966).

Vitreous humor and serum samples were analyzed for urea nitrogen (UN), glucose, triglycerides, cholesterol, total protein, albumin, lactic dehydrogenase (LDH), creatine phosphokinase (CPK), glutamic-oxaloacetic transaminase (GOT), and gamma glutamyltransferase (GGT) using the MultiStat III-plus fluorescence light scattering microcentrifugal analyzer (Instrumentation Laboratory, Lexington, MA). Reagents and standards were obtained from the manufacturer. Controls analyzed with each run consisted of two commercially-produced human samples (Fisher Scientific, Inc., Springfield, NJ) and the appropriate pooled animal samples. Concentrations of standards had to be within 3% of their known concentration for the chemical analysis to be accepted.

Statistical analyses were performed on log-transformed data due to non-normality of distributions. Distribution of residuals were retested using the Shapiro-Wilk test at $P < 0.05$ to ensure normality of log-transformed data (SAS Institute 1989). Because relationships between serum constituents and animal condition are known, mean serum and vitreous humor constituents within treatments were compared using similarly as serum. Each variable's association between serum and vitreous humor paired Student's t-test (SAS Institute 1989) to determine if vitreous humor reacted wasdetermined using Pearson's correlation coefficients (SAS Institute 1989). Equations predicting concentrations of se-

rum constituents from concentrations of vitreous humor constituents were developed by use of least-squares linear regression (SAS Institute 1989). The effect of diet was tested separately within the serum and vitreous humor samples with Student's t-test (SAS Institute 1989). Statistical analyses were considered to have potential biological significance at $P \leq 0.10$ (Tacha et al. 1982).

RESULTS

The time period for death to occur did not differ ($P = 0.89$) between treatment groups. Corresponding right and left vitreous humor samples were similar ($P > 0.23$) for all constituents in both treatment groups so vitreous humor data were averaged within animals for supplemental analyses.

Urea Nitrogen (UN)

Urea nitrogen was lower ($P < 0.004$) in vitreous humor than in serum for both treatment groups (Table 1). Feed restriction increased UN in serum ($P = 0.001$) but did not affect vitreous humor ($P = 0.19$) (Table 1). A linear relationship ($r = 0.63$, $P = 0.03$) was observed between vitreous humor UN and serum UN in the control group (Table 2). The predictive equation of log-transformed UN using vitreous humor is: Serum UN = 1.40 + 0.59 (Vitreous humor UN).

Glucose

Glucose was lower ($P < 0.0001$) in vitreous humor than in serum for both treatment groups (Table 1). Feed restriction did not affect glucose levels in the serum ($P = 0.26$), but did affect vitreous humor levels ($P = 0.05$) (Table 1). There were no linear relationships between serum and vitreous humor glucose for either treatment group (Table 2).

Triglycerides

Triglyceride levels were similar between serum and vitreous humor ($P > 0.29$) for both treatment groups (Table 1). Feed restriction increased triglyceride levels in serum ($P = 0.002$) but did not affect vitreous humor levels ($P = 0.24$) (Table 1). A linear relationship ($r = -0.64$, $P = 0.03$) was observed between vitreous humor and serum triglycerides in the stressed group (Table 2). The predictive equation of log-transformed triglycerides using vitreous humor of nutritionally stressed jackrabbits is: Serum Triglycerides = 5.65 - 0.16 (Vitreous humor Triglycerides). However, for this equation to be meaningful, the nutritional condition of jackrabbits must be assessed first (see Henke and Demarais 1990*b*).

Cholesterol

Cholesterol was lower ($P < 0.0001$) in vitreous humor than in serum for both treatment groups (Table 1). Feed restriction increased cholesterol in serum and vitreous humor ($P < 0.0001$) (Table 1) . There were no linear relationships between serum and vitreous humor cholesterol for either treatment group (Table 2).

Total Protein

Total protein was greater ($P < 0.0001$) in vitreous humor than in serum for both treatment groups (Table 1). Feed restriction decreased total protein levels in the serum and vitreous humor ($P < 0.0001$) (Table 1). There were no linear relationships between serum and vitreous humor total protein for either treatment group (Table 2).

Albumin

Albumin was lower ($P < 0.0001$) in vitreous humor than in serum for both treatment groups (Table 1). Feed restriction decreased albumin levels in the vitreous humor ($P = 0.04$) and serum ($P = 0.0002$) (Table 1). There were no linear relationships between serum and vitreous humor albumin for either treatment group (Table 2).

Table 1. The effect of source (serum vs. vitreous humor) and treatment (control vs. nutritional stress) on blood chemistry values for adult male black-tailed jackrabbits.

	Serum				Vitreous Humor				P-Values			
	Control (N=12)		Stress (N=11)		Control (N=12)		Stress (N=11)		Stress Effect		Source Effect	
Constituents	0	SE	0	SE	0	SE	0	SE	Serum	Vit. Humor	Control	Stress.
Urea Nitrogen (mg/dl)	23.1	1.0	29.5	1.2	18.8	0.9	21.4	1.7	0.001	0.19	0.004	0.001
Glucose (mg/dl)	215.3	15.0	193.9	6.6	98.2	8.6	76.6	6.2	0.26	0.05	0.0001	0.0001
0Triglyceride (mg/dl)	84.9	8.8	140.6	12.6	85.7	23.0	235.7	114.5	0.002	0.24	0.29	0.48
Cholesterol (mg/dl)	41.9	1.5	67.4	3.7	15.4	1.0	22.0	0.9	0.0001	0.0001	0.0001	0.0001
Total Protein (g/dl)	24.9	0.4	16.5	0.7	58.0	0.4	46.7	2.3	0.0001	0.0001	0.0001	0.0001
Albumin (g/dl)	4.3	0.1	3.6	0.1	1.4	0.2	0.9	0.1	0.0002	0.04	0.0001	0.0001
GGT (IU/L)[a]	4.2	0.6	6.0	0.7	0.7	0.1	1.7	0.4	0.05	0.001	0.0001	0.0001
LDH (IU/L)[a]	228.5	24.9	193.4	29.6	73.0	35.0	90.5	42.5	0.22	0.52	0.0001	0.002
CPK (IU/L)[a]	289.0	43.1	240.1	42.5	145.0	47.6	166.8	43.2	0.38	0.60	0.004	0.09
GOT (IU/L)[a]	82.4	20.2	59.6	7.4	58.3	17.4	48.7	11.3	0.38	0.99	0.10	0.17

[a]GGT, gamma glutamyltransferase; LDH, lactic dehydrogenase;CPK, creatinephosphokinase; GOT, glutamic-oxaloacetic transaminase.

Table 2. Pearson's correlation coefficients on log-transformed data for serum and vitreous humor in 23 adult, male black-tailed jackrabbits on two levels of nutrition.

	Treatments			
	Non-stress		Stress	
Constituents	rho	P	rho	P
Urea nitrogen	0.63	0.03	0.03	0.92
Glucose (mg/dl)	0.18	0.57	0.49	0.13
Triglyceride (mg/dl)	0.49	0.10	- 0.64	0.03
Cholesterol (mg/dl)	-0.15	0.64	0.29	0.39
Total Protein (g/dl)	-0.22	0.48	- 0.18	0.59
Albumin (g/dl)	0.24	0.45	-0.31	0.35
GGTa (IU/L)	-0.06	0.84	-0.18	0.61
LDHa (IU/L)	-0.20	0.54	0.50	0.12
CPKa (IU/L)	0.20	0.53	-0.29	0.38
GOTa (IU/L)	0.76	0.004	-0.47	0.14

[a]GGT, gamma glutamyltransferase; LDH, lactic dehydrogenase; CPK, creatine phosphokinase; GOT, glutamic-oxaloacetic transaminase.

Gamma Glutamyltransferase (GGT)

Gamma glutamyltransferase was lower ($P < 0.0001$) in vitreous humor than in serum for both treatment groups (Table 1). Feed restriction increased GGT in vitreous humor ($P = 0.001$) and in serum ($P = 0.05$) (Table 1). There were no linear relationshipsbetween serum and vitreous humor GGT for either treatment group (Table 2).

Lactic Dehydrogenase (LDH)

Lactic dehydrogenase was lower ($P < 0.002$) in vitreous humor than in serum for both treatment groups (Table 1). Feed restriction did not affect LDH levels in the serum ($P = 0.22$) or vitreous humor ($P = 0.52$) (Table 1). There were no linear relationships between serum and vitreous humor LDH for either treatment group (Table 2).

Creatine Phosphokinase (CPK)

Creatine phosphokinase levels were lower ($P < 0.09$) in vitreous humor than in serum for both treatment groups (Table 1). Feed restriction did not affect CPK levels in the serum ($P = 0.37$) or vitreous humor ($P = 0.60$) (Table 1). There were no linear relationships between serum and vitreous humor CPK for either treatment group (Table 2).

Glutamic-oxaloacetic Transaminase (GOT)

Glutamic-oxaloacetic transaminase levels were similar between vitreous humor and serum ($P > 0.10$) for both treatment groups (Table 1). Feed restriction did not affect GOT levels in the serum ($P = 0.38$) or vitreous humor ($P = 0.99$) (Table 1). A linear relationship

($r = 0.76$, $P = 0.004$) was observed between vitreous humor GOT and serum GOT in the control group (Table 2). The predictive equation of log-transformed GOT using vitreous humor is: Serum GOT = 2.51 + 0.47 (Vitreous humor GOT).

DISCUSSION

Vitreous humor constituents were poor predictors of serum values. Because biochemical indicators of nutritional condition in blood are sensitive to antemortem stress, hemolysis, and postmortem interval (Mautz et al. 1980, Henke and Demarais 1992), vitreous humor has been suggested as a potential alternative medium for certain metabolites due to the vitreous barrier, which may nullify the problems associated with hematological analysis (Coe 1972). However, the vitreous barrier caused the vitreous humor to be insensitive to short-term dietary differences, which explains the dissimilarity between serum and vitreous humor values for all variables except triglycerides and GOT. Differences in penetration of chemically-related substances into the vitreous body of New Zealand white rabbits (*Oryctolagus cuniculus*) were attributed to a vitreous barrier, which was selective to lipoid solubility and to the electrical charge of molecules (Bleeker et al. 1968). In addition, because the vitreous humor and serum reacted differently to feed restriction, the predictive equations offered for UN, triglycerides, and GOT are only meaningful if the nutritional condition of the animal is assessed first. However, such an assessment is not always possible, which would render the predictive equations without merit.

Glucose, UN, CPK, and GOT in the vitreous humor and serum were correlated in winter-captured black-tailed jackrabbits (Henke 1989). The differential response of several constituents in the vitreous humor between the present study and previous research with black-tailed jackrabbits (Henke 1989) suggested that either the vitreous humor can react erratically for the same biochemistry within the same animal model, or a seasonal variation within the sources exist. A similar phenomenon was observed in white-tailed deer where only winter serum and vitreous humor glucose were correlated in summer and winter-captured deer (DeLiberto 1987).

Serum and vitreous humor UN were not affected by feed restriction. The increased serum UN with feed restriction was most likely caused by tissue catabolism because the feed-restricted jackrabbits also lost weight (Henke and Demarais 1990*b*). Warren and Kirkpatrick (1978) attributed elevated serum UN levels in feed-restricted cottontail rabbits (*Sylvilagus floridanus*) to a similar process. Vitreous humor UN was not indicative of recent nutritional intake by jackrabbits.

Serum glucose was not affected by the 25% feed restriction, which suggested that the feed restriction either was not severe enough or long enough for the jackrabbits to use up their glucose reserves (Collins 1982). Vitreous humor was sensitive of recent carbohydrate intake by jackrabbits though.

Increased triglycerides suggest that fats were being mobilized from peripheral lipid deposits due to low caloric intake (Collins 1982). Because vitreous humor triglycerides were unaffected by the feed restriction, the vitreous humor would not be an adequate source to assess triglycerides in jackrabbits.

Increased levels of cholesterol suggest that dietary protein was insufficient for liver synthesis of lipoproteins (Collins 1982). Decreased protein intake can result in a decreased utilization of total protein and albumin (Watson and Sodeman 1985).

Increased levels of vitreous humor GGT in the feed restricted group suggested that vitreous humor GGT may be a sensitive indicator of recent nutritional intake. Liver damage due to caloric and protein malnutrition can increase GGT (Iber and Latham 1985)

Increased levels of LDH, CPK, and GOT would indicate severe and/or long-term liver damage (Iber and Latham 1985). However, because the feed restriction was only for two weeks, hepatiosis was not expected and did not occur.

The use of vitreous humor will not replace serum as the standard medium for biochemical analysis. However, vitreous humor may have potential as a source for some biochemical indicators of nutritional intake. Vitreous humor glucose, cholesterol, total protein, albumin, and GGT were sensitive indicators of feed restriction. Vitreous humor total protein, albumin, and GGT of black-tailed jackrabbits also were not affected by acute handling stress (Henke 1989). The range of Anormal values for these vitreous humor constituents needs to be addressed prior to the vitreous humor having practical application. The serum and vitreous humor constituent levels presented can be used as baseline values for *ad libitum*-fed and feed-restricted black-tailed jackrabbits.

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