Scientific Report

TISSUE MINERAL ANALYSIS PATTERNS IN 564 DOGS

Ava L. Frick, DVM, FAIS*
1825 Denmark Road, Union, MO 63084,
dravafrick@avafrick.net | Cell: 314-808-6309; Fax: 636-590-0424
*Corresponding author

Arlene H. Tolen, MPH, NC
6207 N. Cattle Track Road, Scottsdale, AZ 85250

ABBREVIATIONS
ARL – Analytical Research Laboratory
CLIA – Clinical Laboratory Improvement Amendments
TMA - Tissue Mineral Analysis
Note: Standard elemental abbreviations are used throughout, for example Mg for Magnesium

Abstract

Tissue Mineral Analysis (TMA) is a technique using soft tissue hair or fur biopsy that provides a reading of the mineral deposition in the cells and interstitial spaces of the hair over a 2 to 3 month period. TMA can be used to understand metabolism. It is another scientific measure that can expand our understanding of health and processes that impact illness in dogs. Mineral excess or deficiency is known to produce certain physical and psychological symptoms. The correlation of TMA results with clinical signs seen in patients is discussed in this paper.

Tissue mineral levels and electrolyte patterns of calcium (Ca), magnesium (Mg), sodium (Na), and potassium (K) were analyzed in 564 dogs (300 male, 264 female; 99% neutered or spayed) of variable breeds. Their ages ranged from 1 to 15 years. Cases included all dogs presented to the authors within a 12-year period.

Ninety-four percent (94%) of the 564 dogs were found to have high Na to K ratios with low Ca and Mg. Ca, Mg, Na, and K are prevalent minerals in the body, regulate osmotic balance, and are involved in most body functions. According to mineral analysis research, both results (levels and ratios) are indicative of inflammation.

TMA is an accurate and scientifically valid approach to assessing metabolic function and balance. TMA is responsive not only to the trace mineral levels in the diet, but also to all other factors which influence their metabolism including stress, exercise, and endocrine and gastro-intestinal function.

INTRODUCTION

Hair mineral analysis is a tissue mineral biopsy blueprint of one's biochemistry and the nutritional metabolic activity that occurred during the period of hair formation. Hair and fur are chemically indistinguishable, having the same chemical composition, and are made of keratin. The primary difference between hair and fur is the word usage. The hair of non-human mammals is referred to as "fur," while humans are said to have hair. For the purposes of this paper, "hair" refers to both hair and fur.

Hair is formed from clusters of matrix cells that make up the follicles. During the growth phase, the hair is exposed to the internal metabolic environment such as the circulating blood, lymph, and extracellular fluids. As hair continues to grow and reaches the surface of the skin, its outer layers harden, locking in the metabolic products accumulated during the period of formation. Hair analysis reveals individual metabolic states in addition to the mineral status of the individual animals (1, 2, 3).

Hair is 10-15% porous, and washing agents used in the laboratory process can remove not only the exogenous elements but also penetrate inside the shaft and wash out some of the loosely bound minerals. Washing a

sample during test preparation removes quantities of water-soluble macrominerals. When hair samples are not washed by the laboratory prior to assessment, the analysis can accurately measure macrominerals. Therefore, it is important to select a laboratory that does not wash the submitted specimen prior to the mass spectrometer analysis. The laboratory will instruct the practitioner on the protocol for collecting the sample from the animal (4-7).

For animals treated with topical insecticides and pesticides, it is recommended to collect the sample during the last week of the treatment cycle (i.e., 1 week before the next application for most products). The animal can be bathed and clipped 4 hours later. Alternately, the ventrum, where the sample will be collected, can be lightly sprayed with alcohol, wiped clean, and then clipped, avoiding the topical application zone.

TMA is a non-invasive and a cost-efficient screening test (8). It has been used throughout the world to assess individual or herd nutritional status, providing a sensitive indicator of the long-term metabolic trends from the effects of diet, stress, and toxic metal exposure (9-14). Most deficiencies in animals are brought about by altered relationships of minerals within the body. It has become evident that both the retention and loss of minerals by the animal are equally as important as the nutrients consumed from the feed itself. This is valuable in determining dietary needs as well as for recommending supplementation (15-17). Mineral ratios and levels can be changed by the presence of toxic metals, nutritional deficiencies, infections, illness, or stress from a myriad of sources (18, 19).

Assessment of an individual's stage of stress and oxidation rate (how fast the body metabolizes the food in exchange for energy) can be determined by TMA (20). Measured stress can come from external or internal sources. Helping or treating stress with specific minerals can improve the health status, resulting in better physical and emotional responses. Many factors can influence the stress patterns of a TMA report. Trends for common psychological conditions such as depression, hyperactivity, anxiety, and mood swings may be identified through TMA utilizing the levels and ratios of calcium (Ca), magnesium (Mg), sodium (Na), and potassium (K). Research confirms the intimate connection between the body's biochemistry and many emotional states (21-24).

Changes in body chemistry are reflected in the cellular metabolism as measured by the hair tissue mineral levels and inter-relationships found via TMA. These can indicate metabolic dysfunction before clinical signs occur. While blood values reflect what is in the blood, hair analysis provides a record of how the body stores and disposes of elements. The choice of hair as a testing medium is based on the fact that blood chemistries change dynamically from day to day, while hair values give a more stable view of the overall mineral nutrition (25).

The purpose of this retrospective study was to show how TMA has been used on a large dog population to establish typical mineral patterns; to present why TMA is a valid test to aid in nutritional assessment; to ascertain how these patterns affect function, health and behavior; and to help determine reasons why this can be occurring in dogs across the U.S.

MATERIALS AND METHODS

Tissue mineral levels and electrolyte patterns of Ca, Mg, Na and K were analyzed in 564 dogs (300 male, 264 female; 99% neutered or spayed) of variable breeds. Their ages ranged from 1 to 15 years and included all dogs presented to the authors within a 12- year period. The health conditions or clinical signs of the dogs ranged from dermatitis, anxiety, nervousness, aggression, gastrointestinal conditions, metabolic conditions, neuromuscular, pain, and degenerative joint disease. Some samples from apparently healthy dogs were run for a baseline assessment by their owners (**Table 1**).

Table 1: Presenting Clinical Signs or Conditions

Presenting Clinical Signs or Conditions	Number of Dogs Included in Group
Dermatological	370
Anxiety, Aggression, Nervousness	302
Gastrointestinal	252
Metabolic Conditions (diabetes, heart disease, liver disease, kidney disease, cancer)	218
Pain	201
Neuromuscular	151
Degenerative Joint Disease	117
Apparently Normal	47

Seven groups of conditions or clinical signs that were seen in 517 of the total group are listed here. Many of the dogs had multiple problems. Another 47 dogs were apparently healthy without any complaints from the owners. The TMA in those 47 was run as a baseline. Their data is also included in the total statistics reported.

The fur was collected via clipping or cutting from the ventrum, not longer than 1.25 cm in length and taken close to the skin. Samples were weighed on a small scale provided by the laboratory to at least 100 mg (less than 100 mg would produce insufficient sampling), placed in a paper envelope, and submitted to the Analytical Research Laboratory (ARL) in Phoenix, Arizona.

Computer-controlled mass spectrometers and induction-coupled plasma (ICP) instruments are used today at hair testing laboratories in the U.S. All commercial hair testing laboratories in the U.S. are licensed and inspected annually by the federal government as part of the Clinical Laboratory Improvement Amendments (CLIA) act. In this study, testing was performed on a Perkin Elmer Elan 9000 ICP Mass Spectrometer. Results are reported as mg % (parts per million times 10). The source of standards for TMA used by the ARL is provided from original dog research by Parmae Laboratory (Texas) along with 40 years of research data collected by Dr. Paul Eck of ARL. A completed report includes the optimal reference range, the patient's level, toxic metals, and mineral ratios (**Table 2**).

Table 2: Example of TMA report

PATIENT NAME	Dog		Pet Clinic	
SEX: M	AGE: 9	DATE: 3/24/2017	LAB NO. 606832	CLIENT ACCT. NO. 91199

NUTRIENT MINERAL LEVELS

Calcium (Ca)	Magnesium (Mg)	Sodium (Na)	Potassium (K)	Iron (Fe)	Copper (Cu)	Manganese (Mn)	Zinc (Zn)	Chromium (Cr)	Phosphorus (P)
610	192	94	191	26.8	3.7	3.8	45.8	.19	89.3
578	182	89	181	25.4	3.5	3.6	43.4	.18	84.6
546	172	84	171	24.0	3.3	3.4	41.0	.17	79.9
514	162	79	161	22.6	3.1	3.2	38.6	.16	75.2
482	152	74	151	21.2	2.9	3.0	36.2	.15	70.5
450	142	69	141	19.8	2.7	2.8	33.8	.14	65.8
418	132	64	131	18.4	2.5	2.6	31.4	.13	61.1
386	122	59	121	17.0	2.3	2.4	29.0	.12	56.4
354	112	54	111	15.6	2.1	2.2	26.6	.11	51.7
322	102	49	101	14.2	1.9	2.0	24.2	.10	47.0
290	92	44	91	12.8	1.7	1.8	21.8	.09	42.3
258	82	39	81	11.4	1.5	1.6	19.4	.08	37.6
226	72	34	71	10.0	1.3	1.4	17.0	.07	32.9
194	62	29	61	8.6	1.1	1.2	14.6	.06	28.2
162	52	24	51	7.2	0.9	1.0	12.2	.05	23.5
130	42	19	41	5.8	0.7	0.8	9.8	.04	18.8
98	32	14	31	4.4	0.5	0.6	7.4	.03	14.1
66	22	9	21	3.0	0.3	0.4	5.0	.02	9.4
34	12	4	11	1.6	0.1	0.2	2.6	.01	4.7
2	0	0	1	0.2	0	0	0.2	0	0
116.0	22.0	254.0	46.0	18.4	1.0	1.144	12.0	0.146	22.0

Toxic Metals

Lead (Pb)	Mercury (Hg)	Cadmium (Cd)	Aluminum (Al)	Arsenic (As)
3.6	.04	.12	.12	.12
2.7	.03	.09	.09	.09
1.8	.02	.06.	.06	.06
0.9	.01	.03	.05	.05
0.036	0.008	0.006	41.78	0.028

Selenium (Se)	0.164	Nickel (Ni)	0.036

Ratios	Mean Ratios	Pt Ratio
Ca/Mg	3.13	5.27
Ca/K	3.18	2.52
Ca/P	6.88	5.27
Na/Mg	0.47	11.55
Na/K	0.48	5.52
Zn/Cu	13.28	12.00

Testing by:
Accutrace Lab
2225 W. Alice Ave
Phoenix, AZ 85021

CLIA # 03D0641886

Omega Statistics (Murrieta, CA) performed all statistical analyses described on a fee-for-service basis. A series of Pearson's Product Moment correlations were performed to investigate bivariate relationships between the variables of Na, Ca, and Mg. Following the correlational analysis, a series of one-sample t-tests were performed to compare mean levels of the 4 minerals of Ca, Mg, Na, and K with the laboratory normative values. A total of 4 1-sample t-tests were performed, 1 for each of the mineral types.

A series of 1-sample t-tests were then performed to compare mean levels of the 3 mineral ratios of Ca/Mg, Na/K, and Ca/K with normative values. A total of 3 1-sample t-tests were performed, 1 for each of the mineral ratios.

A repeated measures analysis of variance (ANOVA) was performed to compare the values for each dog across the minerals of Ca, Mg, Na, and K. Prior to analysis, the values of the 4 minerals were normalized to rank the values for each mineral on a scale between 0 and 1. The normalization was performed because each of the 4 mineral types was assumed to vary in its individual ranges of mg%. Normalization of the mineral values allowed all 4 variables to be compared on the same metric (values between 0 and 1).

ANOVA was then performed to compare the normalized values for each dog across the minerals ratios of Ca/Mg, Na/K, and Ca/K. Prior to analysis, the values of the 3 mineral ratios were also normalized to rank the values for each mineral ratio on a scale between 0 and 1 (**Table 3**).

Table 3: Pearson's Product Moment Correlation Coefficients for Mineral Concentration in Study of Canines (N=564)

Mineral	1	2	3
1. Calcium (Ca)			
2. Magnesium (Mg)	0.819**		
3. Sodium (Na)	0.116**	0.180**	
4. Potassium (K)	0.229**	0.171**	0.434**

***p < .01*

Table 4 presents measures of central tendency for the 4 minerals studied and the 3 minerals ratios of the collected data, as well as the normative mean values for each of the 4 minerals.

Table 4: Measures of Central Tendency of Mineral Levels and Mineral Ratios in the Canines of Study (N=564)

Mineral/Ratio	M	SD	Mdn	Range	M-Norm
Calcium (Ca) – mg%	96.05	87.65	68.00	4 - 902	227.00
Magnesium (Mg) – mg%	19.91	18.78	14.00	2 - 211	72.00
Potassium (K) – mg%	50.62	59.88	34.00	2 - 760	71.00
Sodium (Na) – mg%	198.44	183.92	140.00	10 - 1162	34.00
Ca/Mg	5.24	1.95	5.00	0.50 - 18.00	3.153
Na/K	5.39	4.65	4.00	0.17 - 34.00	0.479
Ca/K	3.59	6.19	2.13	0.10 -112.75	3.197

Results

Ca, Mg, K, and Na were analyzed. Statistically significant differences were found between the sample means and the normative means of these 4 minerals.

A statistically significant difference was noted between the mean value of Ca from the sample (M = 96.05, SD = 3.98; N = 564) and the normative mean of Ca [M = 227; t (563) = -35.48; p < .0005]. The mean value of Ca from the sample was significantly less than the normative mean. A statistically significant difference was noted between the mean value of Mg from the sample (M = 19.91, SD = 18.78; N = 564) and the normative mean of Mg [M = 72; t (563) = -65.89; p < .0005]. The mean value of Mg from the sample was significantly less than the normative mean. A statistically significant difference was noted between the mean value of K from the sample (M = 50.62, SD = 59.88; N = 564) and the normative mean of K [M = 71; t (563) = -8.08; p < .0005]. The mean value of K from the sample was significantly less than the normative mean. A statistically significant difference was noted between the mean value of Na from the sample (M = 198.44, SD = 183.92; N = 564) and the normative mean of Na [M = 34; t (563) = 21.23; p < .0005]. The mean value of Na from the sample was significantly greater than the normative mean.

A statistically significant difference was noted between the mean value of Ca/Mg from the sample (M = 5.25, SD = 1.95; N = 564) and the normative mean of Ca [M = 3.15; t (563) = 25.52; p < .0005]. The mean value of Ca/Mg from the sample was significantly greater than the normative mean. A statistically significant difference was noted between the mean value of Na/K from the sample (M = 5.39, SD = 4.65; N = 564) and the normative mean of Na/K [M = 0.48; t

(563) = 25.09; p < .0005]. The mean value of Na/K from the sample was significantly greater than the normative mean. A statistically significant difference was not found between the mean value of Ca/K from the sample (M = 3.59, SD = 6.19; N = 564) and the normative mean of Ca/K [M = 3.197; t (563) = 1.50; p = 0.134].

Discussion

There are thousands of biochemical reactions that ultimately control metabolism, digestion, and the regeneration of body tissues. The vast majority of these reactions depend on minute amounts of trace minerals for their activity. If these essential minerals are not present to fuel the processes, then the body's ability to regenerate, metabolize, or break down noxious substances is compromised.

Because hair in the growth phase is exposed to the internal metabolic environment (circulating blood, lymph, and extracellular fluids) and retains the metabolic products presented to it as it hardens, it becomes a perfect tissue sample for testing body function, metabolic trends, and toxic metals. TMA is a technique using soft tissue mineral biopsy that provides a reading of the mineral deposition in the cells and interstitial spaces of the hair over a 2 to 3 month period. This form of testing allows for measurement capabilities of nutritional alterations and improved recommendations for vitamin and mineral supplementation or nutrient augmentation.

Ninety-four percent (94%) of the 564 dogs in the study population had low Ca, Mg, and K, and high Na. This correlates to a metabolic stress pattern, and the consistency of these mineral patterns should not be ignored. Other studies have reported similar findings; they linked this mineral pattern to inflammation influencing T cells, cytokines, and anti-inflammatory plasma proteins (26, 27). Another set of parallel data confirmed that the deficiency of essential mineral elements and Na overload can directly cause lipid peroxidation and eventually hepatic damage (28).

Hair analysis offers a perspective for studying both "disease" trends and for studying "stress" and physiological coping mechanisms (29, 30). Disease trends and metabolic changes can be documented and supported by using hair TMA (31-33).

Ca and Mg levels have been measured in patients with fibromyalgia (34). Hair Ca concentration also correlates to coronary heart disease (35). Levels of Ca and Mg have been studied as part of the metabolic syndrome (36). Both a deficiency and excess of these electrolytes have been shown to be of pathogenic value in the development of endocrine disorders and thyroid disease (37-40).

Nutrients can be unavailable for many reasons. Malabsorption or bio-unavailability of specific minerals can occur due to gut interference; improper form; lack of other needed vitamins, minerals, or amino acids; and ligand interruption, to name a few. Low taurine, low K, and high Na all lead to low tissue Mg levels. Studies of both animals and humans link minerals to healthy functioning of the nervous system and behavior (41, 42). Nutrient minerals must be available to support positive behavior.

Ca plays a fundamental role in metabolic processes, which can be altered by small changes in extracellular ionized Ca concentrations (43-46). A tissue Ca deficiency can exhibit as a sympathetic dominant state (alarm or fright-flight reactions), anxiety, bruising, high blood pressure, "fast" oxidation/metabolic state, insomnia, irritability, muscle cramps and spasms, nervousness, hyperactivity, osteoporosis, weakened ligaments and tendons, and tooth decay (47,48). When the extracellular fluid level of Ca ions drops below normal, the nervous system becomes progressively more excitable because of increased permeability of the neuronal membrane to Na (49).

A deficiency of tissue Mg affects the autonomic nervous system, behavior, muscle tone and function (50-53). Clinical signs include poor appetite, irritability, weakness, muscle tremors, tetany, twitching, numbness, tingling, confusion, disorientation, personality changes, apathy, memory loss, skin lesions, tissue calcification, elevated cholesterol, cardiovascular changes, tachycardia, elevated parathyroid hormone, pancreatitis, and stress (42).

Magnesium deficiency increases catecholamine secretion and sensitivity to stress, and may promote aggressive behavior (54). Overall, Mg can act as a neuron protector against aggression. Magnesium helps to protect the nervous system locally and globally, is a psycho-stabilizer, and plays an important role in aging (55, 57). Increased catecholamine-induced intracellular Mg loss is a causal factor in urinary loss of Mg (56).

When tissue K is deficient relative to Na, the body will tend to develop hypertension, arrhythmias, and fatigue (57). Excess Na will be able to enter the cell, which interferes with cellular metabolism, especially protein synthesis. When this happens, the cellular response is expressed as loss of energy and manifests in the individual as exhaustion, submissive behavior, and heightened anxiety or panic (58).

High tissue Na levels are indicative of excessive adrenal gland activity and excitability. Excess Na combined with too much phosphorus, low tissue levels of Ca, Mg, zinc

(Zn), and K result in adrenal insufficiency (59). Na, when elevated in the tissue, is a stress that is associated with anger and fear, the emotions of the "fight or flight" response, and an indication of body inflammation (60).

With the activation of the stress response, the adrenal glands increase their secretion of the hormone aldosterone, which increases the retention of Na in the cells and tissues. As Na is retained, Mg and Ca are lost from the cells and tissues. The loss of Zn and Mg under stress appears to facilitate the stress response by allowing for greater retention of Na (61, 62).

The primary mechanism by which psychological factors predispose one towards a disease process is by means of the stress response and its effect on nutrient minerals at the cellular level. The stress response involves general systems responses in which psychological, neurological, endocrine, and immune system phenomena occur. Cellular energy production and neuroendocrine interference ensues. Mineral imbalance is a factor in physiological stress and antisocial behavior (63, 64).

Stress has the capacity to impact minerals even more than other nutrients. Zn and Mg are lost from the body's tissue reservoir in the "Acute Stage" of stress. Ca and copper are lost in the "Resistance Stage" of stress. Loss of Na and K are associated with the "Exhaustion Stage" of stress. Both internal and external stressors create an increased need for vitamins and minerals to recover and to prevent depletion and breakdown.

Dogs are carnivorous biased, which means they derive optimal health and well-being by eating meat. Even though they have omnivorous capacity and many manage to survive on grain and carbohydrate-rich foods, a solely grain-based diet is not the best choice for maximizing digestion, absorption, health, and disease-free longevity.

Grains have been shown to interfere with Zn and Ca assimilation (46). Eating carbohydrates lowers Ca and Mg, and raises Na. Ca and Mg are sedative elements, or known as the calming minerals. Lowering their levels therefore increases the sensitivity of the sympathetic division of the autonomic nervous system and prepares the dog to respond to either fight or flight.

Mineral composition of the body is dependent not only on food intake but on the efficiency or inefficiency of neuroendocrine function (65). Hormones are known to influence nutrients at several levels including absorption, excretion, transportation, and storage. Nutrients in turn can exert an influence on hormones (39, 66, 67). The generations of dogs who were fed the dry grain-based foods may have an impact on today's canine

offspring that are now showing physiological imbalance. Pesticides, herbicides, and insecticides are known to act as endocrine disruptors in humans and animals. Herbicides and fungicides affect Ca transport in plant mitochondria. Herbicides and insecticides have been shown to interfere with Ca metabolism in birds. Further, 354 drugs have been reported to interact negatively with Ca, Mg, and Zn. Toxic metals affect mineral metabolism and availability. Tannins and polyphenols from tea, for example, reduce Mg in tissue. These are all stressors that affect minerals (68, 69).

Conclusion

The majority (94%) of the 564 dogs studied were found to have a high Na/K ratio with low Ca and Mg. According to mineral analysis research, both results (the levels and the ratio) are indicative of inflammatory processes and stress. This correlates well with the clinical signs (general, physiological, and psychological) reported in the cases studied.

Macrominerals are essential in metabolism in that they activate catalytic and enzyme functions. Hair analysis is used not only for measuring those minerals but also to monitor the nutritional state of the dog until treatment benefits are achieved and the effects of the treatment protocol have been stabilized. The combination of feed ratio and hair analysis is an invaluable screening tool to determine the correct program of diet and supplementation for each dog's specific needs. TMA is responsive not only to trace mineral levels in the diet but to all other factors which influence their metabolism including stress, exercise, and endocrine and gastro-intestinal function.

When performed to standards and correctly interpreted, a TMA can be used as a screening tool in canine wellness programs; and for those in suboptimal health, for monitoring mineral deficiencies, mineral excesses, biochemical characteristics, system imbalances, supporting behavior, and endocrine indices (70-75). The use of hair TMA offers a sophisticated and cost-effective approach (less than a typical chemistry/CBC panel) that is very affordable for any practitioner to incorporate into their nutritional assessment evaluations. There is currently nothing else available in the way of a simple laboratory test that gives the veterinarian or nutritional consultant a perspective of the internal nutritional status of an animal. It can be a method to comply with AVMA and AAHA recommendations regarding the importance of offering annual or semi-annual nutritional evaluations. This enables the veterinarian to present a nutritional consultation that has significant value and direction toward resolving health issues using individualized nutrition.

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