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ORIGINAL PAPER

HAIR MANGANESE LEVELS IN DOGS FROM WARSAW IN RELATION TO BREED, SEX, AGE AND BODY WEIGHT*

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Abstract

Pet animals live in relatively similar conditions to those of man. Therefore, they are considered to be available for monitoring the influence of various environmental factors on the organism, including trace elements. Manganese is an element essential for the metabolism and growth of organisms as a major constituent of several metalloenzymes, hormones and proteins. Tissues of mammals usually contain manganese localized in parenchymal organs, melanin-containing cells, in the bones and in skin appendages including hair, which is a stable matrix that presents numerous advantages for biomonitoring and provides information about short- and long-term exposure and the temporal exposure pattern by segmental analysis. The aim of the study was to test the use of canine coat hair as a biomarker of body manganese content in relation to factors such as breed, sex, age and body weight of animals.We measured manganese concentration in 120 hair samples collected from dogs of the following breeds: Yorkshire Terrier (YT), West Highland White Terrier (WHWT) and Schnauzer Dog (SCHD). The data were analyzed in relation to sex, age and body weight of dogs. Manganese levels were determined using flame atomic absorption spectrometry (FAAS). The hair of the studied dogs was found to be low in manganese compared to data on other carnivorous species. The highest level (5.66 mg kg^{-1} dry weight) was found in the coat hair of YT dogs. In the other two breeds, the values measured were 3.4 and 2.87 mg kg^{-1} dry weight in WHWT and SCHD, respectively. There was a decrease in hair manganese content in the coat of older WHWT and SCHD dogs. A reverse relationship was found in YT dogs. Sex was a factor determining hair manganese level in WHWT. The results indicate low environmental exposure of the dogs from Warsaw agglomeration to the manganese. The results of our comparisons between breeds do not confirm other authors' claims about great variation in hair manganese content.

Keywords: dogs, manganese, animal hair, indicator.

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INTRODUCTION

Manganese is a common metal in nature and like other metals, such as zinc, copper or iron, is essential for the functioning of the body of both humans and animals (Soetan et al. 2010, Zheng et al. 2011). Tissues of terrestrial mammals usually contain 0.2 to 10 mg kg^{-1} of manganese localized mainly in parenchymal organs and melanin-containing cells, but also in the bone and integumentary system appendages, such as hair. The element is a part of a number of biochemical processes as a component of metalloenzymes, which regulate metabolism of carbohydrates, lipids and proteins (Arnhold et al. 2002, Aschner, Aschner 2005, Soetan et al. 2010). Its role in metabolic processes implies a particular importance of the metal for the normal growth of the body, immunological response processes, regulation of energy production and preventing cell oxidative damage (Bjørklund et al. 2017). Manganese is also essential for the reproductive processes in humans and animals, hence its deficiencies may lead to fertility conditions in individuals of either sex (Avila et al. 2013). It has been demonstrated that the element is actively involved in each stage of the female sexual cycle. Manganese is essential for the normal development of ovulation, occurrence of estrous symptoms, semen transport, fertilization, embryonic implantation, fetal development and labor (Anke et al. 1994, Arnhold et al. 2002, Avila et al. 2013). Experiments on farm animals fed a diet low in manganese resulted in reproductive disorders in the form of poor heat signs, low conception rates and frequent miscarriages. Other manganese deficiency symptoms include bone of limbs and scull deformities resulting from disturbed endochondral ossification and intramembranous ossification processes, limb joint deformities, neurological disorders, and growth retardation (Anke et al. 1994).

Although manganese is an essential element, its excessive exposure leads to a variety of symptoms generally referred to as manganism. Manganese poisoning symptoms in humans resemble those observed in Parkinson's disease. They mainly result from oxidation of catecholamines by trivalent manganese complexes and from production of reactive oxygen species, which are toxic to the structures of the central nervous system associated with the extrapyramidal system function (Olanow 2004, Aschner et al. 2007, Aschner et al. 2009, Zheng et al. 2011).

Due to possible toxicity of manganese, it is crucial to find a proper biomarker of its level in the body, especially one that would be easy to collect and allow avoiding application of any invasive procedures. Wang et al. (2008) propose saliva as a non-invasive manganese testing bioindicator in humans. Namely, the authors found that manganese content in saliva reflects its blood serum level. Analysis of manganese contents in the integumentary system appendages, such as hair, claws or nails, provide interesting data as well. Results of studies that have been carried out since the 1970s substantiate the conclusion that hair manganese levels most adequately reflect its supply

in the body of animals and humans, better than other analyzed tissues (Hayashi et al. 1981, Yoshinaga et al. 1990, Bader et al. 1999, Kośla, Skibniewska 2010, Soetan et al. 2010, Filistowicz et al. 2011). Thus far, no extensive studies have been conducted on the content of manganese in hairs of carnivorous pet animals in the urban environment. Dogs may represent an indicator of exposure to selected metals, as they share the habitat with their caretakers, drink the same water and sometimes eat the same food. The aim of the study was to test the use of canine coat hair as a biomarker of body manganese content in relation to factors such as sex, age and body weight of the animals.

MATERIAL AND METHODS

The material in the form of hair samples was collected from the back of various breeds of dogs held as pets in homes across the city of Warsaw. Samples were collected from 120 dogs, including 74 Yorkshire Terriers (YT, 36 females and 38 males), 12 West Highland White Terriers (WHWT, 3 females and 9 males) and 34 Schnauzer Dogs (SCHD, 15 females and 19 males). Preparation of the samples was carried out according to methods described by Kośla et al. 2004, Kośla, Skibniewska 2010). Hair samples were washed in distilled water, then washed again three times in redistilled water and degreased in a Soxleth apparatus using 70% ethyl alcohol. Pre-prepared material was dried at 105°C until constant weight, measured for dry weight (d.w.) and incinerated in a muffle furnace at 450°C. The ash was transferred to 10-ml flasks with double-distilled water, and 2.5% HCl was added for acidification. Manganese was measured in the filtrate by flame atomic absorption spectrometry (FAAS) in a reference laboratory. Statistical analysis was carried out using a Statistica 12.0™ package (StatSoft Poland). Normality of distribution was tested with the *W* Shapiro-Wilk test. Due to the non-parametric distribution of data, significance of differenced between groups was tested using the Kruskal-Wallis test at the significance level $p \leq 0.05$.

RESULTS AND DISCUSSION

Hair manganese levels in the analyzed dogs are presented in Table 1. A significantly higher Mn content was found in YT females in relation to WHWT females. There were no significant differences in hair manganese levels between WHWT males and the other groups. Mean values in SCHD (both males and females) differed significantly from those in YT males and females (Table 2).

Table 1

Manganese content depending on breed and sex of dogs (mg kg^{-1})

Breed	Sex	AM	*n*	SD	Min	Max	IQR	Median
WHWT	f	2.01	3	0.41	1.64	2.46	0.82	1.94
WHWT	m	4.79	9	3.33	1.56	10.30	4.50	3.52
SCHD	f	3.01	15	1.84	1.04	6.53	3.51	2.37
SCHD	m	2.72	19	2.51	0.82	11.94	2.25	2.18
YT	f	5.93	36	3.58	0.86	14.80	4.47	5.19
YT	m	5.38	38	3.26	1.22	12.50	2.96	4.27
Total		4.70	120	3.31	0.82	14.80	2.00	3.91

f – females, m – males, AM – arithmetic mean, SD – standard deviation, Min – minimum, Max – maximum, IQR – range between Q^{25} and Q^{75}

Table 2

Statistical differences between individuals of either sex representing each breed

Breed	Sex	f (1)	m (2)	f (3)	m (4)	f (5)	m (6)
WHWT	f (1)					*	
WHWT	m (2)						
SCHD	f (3)					*	*
SCHD	m (4)					*	*
YT	f (5)	*		*	*		
YT	m (6)			*	*		

* $p \leq 0.05$

Hair metal concentration analysis is the domain of mainly human toxicology (Flache et al. 2015). This is due to the fact that human hair represents good analytical material which has many advantages, such as matrix stability, ease and low cost of sampling and storage, as well as applicability for evaluation of effects of both short- and long-term exposure to given metals (Chyla, Żyrnicki 2000, Rashed Soltan 2005, Zhang et al. 2007, Gil et al. 2011, Martín-Cameána et al. 2014). Despite the numerous advantages, hair as an analytical material has some drawbacks, for example the difficulty of distinguishing blood-transported metals embedded in hair structure by the skin cells from the impurities coming directly from the external environment; there is also a large variation in the manganese content associated with hair color as well as the age and sex of the subjects (Barbosa et al. 2005, Gil et al. 2011, Martín-Cameána et al. 2014). In spite of these drawbacks, hair is used for manganese level analysis, since its content in the hair reflects the overall manganese burden of other tissues of the organism (Huang, Cao 2003). Cowan et al. (2009) claim that people with high occupational exposure to manganese may exhibit its hair content exceeding 2000%

in relation to unexposed subjects; however, this can be the result of external contamination of the analyzed material. In terms of data interpretation, a lack of reference databases of hair manganese levels in animals and humans represents another problem. Manganese concentration in hair depends on many factors such as age, color, breed and animal gender. Stanek et al. (2016), who analyzed Polish Konik horses, both housed in stables and free-ranging in a nature reserve, found no differences in hair manganese levels between mares and stallions. As a result of Mn dietary supplementation, its content in the coat of all studied horses increased significantly, yet no differences were found between horses of either sex. In horses without Mn supplementation, the content of this element was 22.75 mg kg^{-1} in stable horses, and 34.41 mg kg^{-1} in free-ranging horses. In a paper on hair manganese levels in the European bison from Białowieża, Skibniewski et al. (2010) found an average Mn content of 15.2 ± 4.3 mg kg^{-1} fresh tissue. Mn content in females was 16.0 ±4.4, while in males it equalled 14.0 ± 4.2 mg kg^{-1}. These results suggest that the level of manganese in the hair cover is rather due to environmental exposure, and the sex does not seem to be a significant factor.

Maeoca and Prada (1987) measured hair manganese levels in healthy dogs ranging from 17.0 to 97.5 mg kg^{-1}, on average 57.4 mg kg^{-1}. If we compare these data with our results, manganese levels in canine hair in Brazil are several times higher than in Poland. Cholewa et al. (2014*a*), who studied farmed polar foxes, found on average 52.62 to 67.56 mg kg^{-1} of manganese depending on a farm. The same authors report in another paper that hair manganese levels in the red fox, depending on the color variant, ranged from 37.56 to 63.27 mg kg^{-1} in females and from 43.01 to 59.83 mg kg^{-1} in males (Cholewa et al. 2014*b*). These levels are also much higher compared to what we found in the hair of Warsaw dogs. Surprisingly, the values measured in our study are more similar to data of farm animals representing other taxa, such as ruminants, rather than what might be expected from carnivores. Manganese in sheep wool remains in the range 0.35 to 20 mg kg^{-1}, depending on the state of the environment and the origin of animals (Patkowska-Sokoła et al. 2009). Wool of Polish, Greek and Syrian sheep contained manganese, according to the same authors, at the level of 3.37 ± 1.65, 4.43 ± 1.8 and 22.93 ± 13.93 mg kg^{-1} dry weight, respectively. Normal manganese levels in European sheep's wool range from 0.35 to 20 mg kg^{-1}. Demonstrably higher wool content of Mn was found in samples coming from Syria (Patkowska-Sokoła et al. 2009).

Manganese levels in appendages of the integumentary system may show seasonal variations as well. Anke et al. (1975) found that the manganese concentration in pig bristles changed significantly depending on the season of collection. The highest levels were measured in June, 12.5 mg kg^{-1}, whereas the lowest were in November, 2.5 mg kg^{-1} ($p < 0.001$). The authors claim that Mn in porcine hairs change in an annual cycle.

Manganese concentration in the hair of red deer at various sites in Germany (Anke et al. 1980) ranged from 1.9 ± 0.9 to 16 ± 7.2 mg kg^{-1}, the diffe-

rence between the extreme values being highly significant at $p < 0.001$. Such manganese levels in roe deer inhabiting the same sites in Germany (Anke et al. 1980) ranged from 1.2 ± 0.8 to 2.5 ± 1.3 mg kg^{-1}. In this case, the authors also found significant differences between the sites.

Average hair manganese levels in wild and domesticated even-toed ungulates are as follows: red deer, 7.0, fallow deer, 3.1, raw deer, 2.4, muffon, 41.0, sheep, 9.5, goat, 2.0 and cattle, 11.0 mg kg^{-1} (Anke et al. 1980). According to the authors, the manganese content in animal hair is more species--specific than it is that in other tissues. The color of the coat seems to have an impact. Chyla and Żyrnicki (2000) found that the average manganese content in the dog's melange, black and grey hair was: 2.29, 1.88 and 1.00 mg kg^{-1} d.w. respectively. Considering the previously mentioned relationship between the color of hair and its manganese concentration, which has been observed by numerous authors (Hayashi et al. 1981, Sturaro et al. 1994, Chyla, Żyrnicki 2000, Barbosa et al. 2005, Gil et al. 2011), our data reveal this association only for WHWT females, whereas the mean Mn level in white hair of males was nearly 2.4-fold higher. This result was higher than observed in SCHD dogs of either sex. The mean value in females was 3.01 mg kg^{-1} d.w., whereas in males the mean level was 2.72 mg kg^{-1} d.w. The highest concentration of manganese was found in the "golden" hair of YT. A similar pattern in dogs was found by Hayashi et al. (1981), who found the highest Mn content in colored dogs' hair. These observations are consistent with those carried out on humans (Sturaro et al. 1994), as the hair of redheaded people contain higher manganese concentrations.

Considering the age of the studied animals (Tables 3 and 4), WHWT age groups up to 3.5 years and 4-5 years differed significantly from the oldest group of YT (more than 5 years). Schnauzer dogs (SCHD) up to 3.5 years of age did not differ from other groups. In the case of Schnauzers, a similar pattern was observed in the groups of older individuals (4 to 5 years of age and older that 5 years), which differed significantly from all YT age groups.

Table 3

Manganese content in hair in relation to breed and age of dogs

Breed	Age (years)	AM	n	SD	Min	Max	IQR	Median
WHWT	up to 3.5	4.40	10	3.31	1.64	10.3	4.53	2.33
WHWT	4–5	2.54	2	1.39	1.56	3.52	1.96	2.54
SCHD	up to 3.5	4.53	8	3.25	1.00	11.94	1.63	4.08
SCHD	4–5	2.63	12	1.95	0.93	6.53	2.81	2.08
SCHD	more than 5	2.07	14	1.05	0.82	4.25	1.60	1.77
YT	up to 3.5	5.06	42	3.12	0.86	13.54	3.10	4.27
YT	4–5	6.02	23	3.11	1.80	12.78	3.72	5.06
YT	more than 5	7.40	9	4.86	1.60	14.80	8.88	5.40

AM – arithmetic mean, SD – standard deviation, Min – minimum, Max – maximum, IQR – range between Q^{25} and Q^{75}

Table 4

Statistically significant differences in hair Mn levels in relation to breed and age of dogs

Breed	Age (years)	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
WHWT	up to 3.5 (1)								*
WHWT	4 to 5 (2)								*
SCHD	up to 3.5 (3)								
SCHD	4 to 5 (4)						*	*	*
SCHD	more than 5 (5)						*	*	*
YT	up to 3.5 (6)				*	*			*
YT	4 to 5 (7)				*	*			
YT	more than 5 (8)	*	*		*	*	*		

* $p \leq 0.05$

The effect of age on the content of manganese in hair has been confirmed in the studies conducted by Hayashi et al. (1981), who analyzed Mn content in the hair collected from the dogs. The authors found 1.39 mg kg^{-1} d.w. of manganese in younger individuals (up to one years old) and 1.96 mg kg^{-1} d.w. in older dogs (over 5 years old).

Tables 5 and 6 present the hair manganese levels in dogs in relation to their breed and body constitution type expressed as body weight. In the SCHD, there were differences between individuals weighing up to 5 kg and those of higher body weights (Table 6). Among dogs of various breeds of

Table 5

Manganese content in canine hair relation to breed and body weight

Breed	Body weight	AM	n	SD	Min	Max	IQR	Median
SCHD	up to 5 kg	2.35	6	1.81	0.82	4.74	3.55	1.55
SCHD	6-10 kg	3.35	17	2.77	1.00	11.94	2.99	2.37
SCHD	more than 10 kg	2.35	11	1.17	1.09	4.25	2.25	2.23
YT	up to 5 kg	5.65	74	3.41	0.86	14.8	4.45	4.70

AM – arithmetic mean, SD – standard deviation, Min – minimum, Max – maximum, IQR – range between Q^{25} and Q^{75}

Table 6

Statistically significant differences in canine hair Mn content in relation to breed and body weight

Breed	Body weight	(1)	(2)	(3)	(4)
SCHD	up to 5 kg (1)		*	*	*
SCHD	6-10 kg (2)	*			
SCHD	more than 10 kg (3)	*			
YT	up to 5 kg (4)	*			

* $p \leq 0.05$

a similar body weight, the breed had a significant effect on the Mn content. Significant differences were found in hair Mn content between SCHD of up to 5 kg and YT group, which had a significantly higher Mn content. The differences in Mn content in relation to body weight could not be compared with other data, as no relevant report have been found in the literature.

In conclusion, manganese concentrations in keratin skin appendages in humans and various animal species living in various habitats and places are characterized by high variability, with the levels being different by manifold.

CONCLUSIONS

The hair of the studied dogs was found to be low in manganese compared to data on other carnivorous species, which may indicate low environmental exposure to the element. The highest manganese content was found in the YT breed. The mean values in the two other breeds were similar, 3.4 and 2.87 mg kg^{-1} d.w., respectively, WHWT and SCHD. In the case of WHWT, there was a clear effect of the sex on the content of Mn in hair. WHWT and SCHD breeds revealed a decline in manganese content in older individuals. A reverse relationship was found in YT. Despite the presence of statistically significant differences between the studied groups, the results of our comparisons between breeds do not confirm other authors' claims about great variation in hair manganese content.

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