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Mercury interferes with endogenous antioxidant levels in Yukon River subsistence-fed sled dogs

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Abstract
Before adopting modern corn-and-grain-based western processed diets, circumpolar people had a high fat and protein subsistence diet and exhibited a low incidence of obesity, diabetes and cardiovascular disease. Some health benefits are attributable to a subsistence diet that is rich in omega-3 fatty acids and antioxidants. Pollution, both global and local, is a threat to wild foods, as it introduces contaminants into the food system. Northern indigenous people and their sled dogs are exposed to a variety of contaminants, including mercury, that accumulate in the fish and game that they consume. The sled dogs in Alaskan villages are maintained on the same subsistence foods as their human counterparts, primarily salmon, and therefore they can be used as a food systems model for researching the impact of changes in dietary components. In this study, the antioxidant status and mercury levels were measured for village sled dogs along the Yukon River. A reference kennel, maintained on a nutritionally balanced commercial diet, was also measured for comparison. Total antioxidant status was inversely correlated with the external stressor mercury.

Keywords: mercury, subsistence food, salmon, antioxidant status, Yukon River, sled dogs

1. Introduction

Rural Alaskan communities typically live a subsistence lifestyle to survive and to uphold traditional, cultural and spiritual values. In the past, traditional foods including native plants, wild game and salmon, blended with the physical endurance needed to live a subsistence lifestyle and provided the nutritional bases for individual and community health (Loring *et al* 2008). Today it is not well understood how contaminants in the food system interact with these nutrient rich foods and how antioxidants and physical endurance reduce the hidden 'risks' associated with environmental toxins like mercury in their food sources (Belanger *et al* 2006, Mozaffarian and Rimm 2006, Tyrrell 2006).

Sled dog mushing, once used only as a means of transportation, has evolved into a modern international sport. Comparative studies, spanning this diversity of climate and diet, provide an ideal opportunity for research in nutrition, exercise, physiology and the immune system. Sled dog research provides a unique model because these dogs can be found all over the world in relatively large numbers that are exercised and housed under similar conditions. Dogs, in general, are a proven model for aging, immune function, toxicology and cognitive disorders (Greeley *et al* 2001, Milgram *et al* 2002, Strasser *et al* 1993).

Sled dogs in northern climates are exposed to the same environmental hazards as their human counterparts (Dunlap *et al* 2007, Hansen and Danscher 1995). In many Alaskan villages, sled dogs are still a fundamental part of a traditional lifestyle, used for trapping, packing and transportation. Most of these villages are small settlements, established on or near rivers to facilitate travel and to gather their food supply. The diet of both the villagers and their sled dogs in Alaska is often comprised of a variety of wild game, fish and marine mammals. In addition to being a fundamental component of the village diet, salmon is the primary food source for sled dogs throughout the year along Alaska's river systems (Andersen 1992). There is an increased use of processed diets in villages for both sled dogs and humans, but economical and cultural factors contribute to continual use of subsistence foods. The diet of the sled dogs can be controlled and monitored more easily than the diet of their human counterparts. When subsistence food is unavailable, for instance in the middle of winter, sled dogs are maintained on commercial food with little variation.

Before western diets, circumpolar people had a low incidence of diabetes, cancer and cardiovascular disease (Bang *et al* 1971, Dyerberg *et al* 1975, Lanier *et al* 2006, Martinsen *et al* 2006). Health benefits have been attributed to this northern, low carbohydrate diet, rich in omega-3 fatty acids and antioxidants offered from indigenous subsistence foods (Adler *et al* 1994, McGrath-Hanna *et al* 2003, Mozaffarian and Rimm 2006, Storlien *et al* 1987). Diets high in antioxidant-rich foods are associated with reduced risk of cardiovascular diseases, cancers and Alzheimer's disease (Block *et al* 2002).

In order to quantify antioxidant status in sled dogs in their natural environment while maintained on a subsistence diet, we surveyed river sled dogs using a reliable index for antioxidant potential (TAP). Since there is an increasing concern in northern communities with the possible health implications surrounding subsistence food and contaminants in the ecosystem, the need to survey the baseline levels of antioxidants in the subsistence diet of dogs and their health in relation to increasing global contaminants such as mercury has been stressed by elders (Hild 1998, Krauss 2004, Tyrrell 2006). The Yukon River system is ideal for such a naturalistic survey because it traverses the state of Alaska and parts of western Canada. We observed a significant inverse relationship between total mercury concentration and antioxidant status in subsistence-fed sled dogs, indicating that mercury can reduce antioxidant levels and potentially cause dietary insufficiency.

2. Material and methods

2.1. Animals

Alaskan huskies, *Canis lupis familiaris* raised in four villages along the Yukon River in Alaska and a reference kennels located in Salsha, Alaska (Latitude, 65°N) were used as test subjects. The Institutional Animal Use and Care Committee at the University of Alaska Fairbanks approved this study (#04–16). There were 12 dogs sampled at each site, including the reference kennel. The village dogs were in or around Russian Mission (62°N), Galena (64°N), Rampart (65°N) and Fort Yukon (66°N) (figure 1). The dogs that were used in this study were typical racing sled dogs with similar lineage, sex and age range. All dogs ranged from 1 to 8 years of age with the majority of the dogs within the peak racing years of 2–5. Each kennel had an even sex distribution, with Galena, Rampart and Salcha having slightly more females. Housing arrangements varied from kennel to kennel, but most of the dogs were tethered with 2 m chains with access to shelter, water and food (Dunlap *et al* 2007).

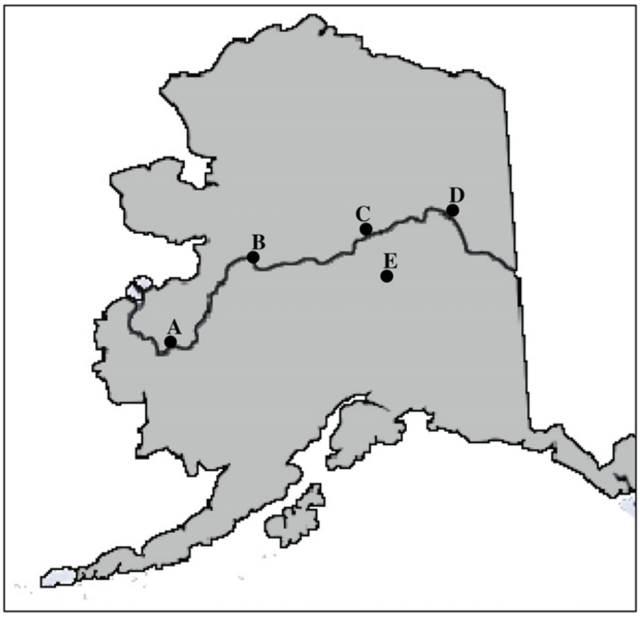

Figure 1. Locations of the sample sites along the Yukon River in the state of Alaska. (A) is Russian Mission, (B) is Galena, (C) is Rampart, (D) is Fort Yukon, and (E) is Salcha, the reference site.

2.2. Diet

A minimum diet adaptation period of 14 days is common in many studies (Kuhnt *et al* 2006, Rondanelli *et al* 2011). A diet recall was done for the 2 months preceding sampling. Village dogs were primarily maintained on subsistence diets, which included black bear, moose, pike and salmon for a minimum of 2 months. Some diets were supplemented with small amounts of donated human food from the community and commercial feed within the past 2 months. However, easily 90% of the food source in all villages in the past 2 months was seasonal cooked salmon from local salmon runs. Dogs in the reference kennel in Salcha, Alaska were maintained on a meat-based commercial dog food (Purina Pro Plan) that was devoid in fish, fish oil or fish by products. This diet for both village dogs and reference dogs is typical for this time of year. The kennel owners determined the amount of food fed to each dog individually but dogs were maintained at a healthy body condition. Healthy body condition is defined as easily palpable ribs and vertebral spinal processes, with a slight depression between the wings of the ileum (Laflamme 1997, Reynolds *et al* 1999). On the sampling day, dogs were fed at least 12 h prior to blood collection to ensure that the dogs were in a post-absorptive state. Sled dogs are fed once a day.

Table 1. TAP mean concentrations in village sled dogs and differences between villages. (Note: This table shows mean concentrations and standard deviations for TAP. Differences in mean concentrations between each village are shown and significant differences of $P < 0.05$ are indicated with an asterisk (*). The largest differences observed were between reference kennel and village sled dogs.)

	Village				
Variable village	Russian mission	Galena	Rampart	Fort Yukon	Salcha
TAP mean (mM)	0.133 ± 0.04	0.122 ± 0.03	0.152 ± 0.03	0.131 ± 0.01	0.208 ± 0.07
Russian mission	0.000				
Galena	0.011	0.000			
Rampart	−0.019	−0.030	0.000		
Fort Yukon	0.002	−0.009	0.021	0.000	
Salcha	−0.075*	−0.086*	−0.056*	−0.077*	0.00

2.3. Blood sampling

Sampling was done between August and October of 2006. All dogs were sampled between 11:00 am and 1:00 pm. The time of the year for sampling was determined based on local salmon runs and to ensure that the diet of village dogs was primarily salmon. Hair samples and blood were drawn from each village at the same time. Blood was drawn by venipuncture from the cephalic into a 12 ml syringe. Blood samples were centrifuged at 2500*g* for 10 min on site, transferred into freezer vials, flash frozen in liquid nitrogen and stored at −70 °C until they were analyzed (Dunlap *et al* 2006).

2.4. Biochemical analysis

The biochemical analysis for total antioxidant power (TAP) was performed at the University of Alaska Fairbanks. A commercial assay from Oxford Biomedical Research Laboratory (#TA 01) was used to determine plasma total antioxidant power, using plasma samples collected in vacutainers containing Na^+ citrate. In this assay, the ability of the sample to reduce Cu^{2+} to Cu^+ was applied as an index of the sample's antioxidant capacity. The antioxidant concentrations of the samples were determined by further extrapolation from a standard curve developed from known concentrations of uric acid (Dunlap *et al* 2006).

2.5. Mercury analysis

Total mercury (THg) concentrations were determined on hair samples (0.2 g) collected with stainless-steel surgical scissors from the front dorsal area of the neck near the skin from the same dogs and at the same time as blood sampling. Hair samples were washed prior to digestion. THg concentration in the hair samples was measured at Frontier Geosciences (Seattle, WA) using the cold vapor atomic fluorescence spectrometry (CVAF) method. The results are reported on a wet weight (w/wt) basis as ng/g (ppb), and previously published (Dunlap *et al* 2007).

2.6. Statistical analysis

Samples were analyzed using SAS statistical software. Analysis of variance was performed on TAP to evaluate the effects of sampling location on the measured index. Significant differences between sampling sites was determined using Tukey's Studentized range test. A linear regression was performed between THg and TAP at the kennel level ($n = 5$). Differences were considered significant at $P \leqslant 0.05$.

3. Results and discussion

Mean total antioxidant power (TAP) in Yukon River sled dogs is summarized in table 1. Total antioxidant power in village sled dogs did not differ significantly between Yukon River villages. However, sled dogs from the control kennel, Salcha, maintained on high quality commercial food, had significantly higher TAP then all villages (table 1).

The effect of mercury in the dogs on TAP in the village sled dogs was compared. When TAP mean level is compared with exposure to mercury (table 1), plasma TAP was inversely correlated ($P = 0.026$) with THg concentrations in the hair of sled dogs. The hair THg concentration in the village dogs along the river ranged from 139 to 15 800 ng/g (Dunlap *et al* 2007).

The current health risk from exposure to chemicals in food and from food-borne pathogens to both humans and their sled dogs through the consumption of fish is not clearly understood in rural Alaska. Some potential risks associated with human health in which a sled dog model would be useful, include cardiovascular disease, impaired immune function, and decreased neural and motor development in children (Belanger *et al* 2006, Tyrrell 2006, Loring *et al* 2010). A sled dog model also provides information on the impacts of changing lifestyles in villages (Belanger *et al* 2006, Loring *et al* 2008).

The body composition of migrating salmon drastically change during their journey from the ocean and are in a starved state using their body reserves to arrive at their destination. In the ocean, they are relatively strong and healthy. Nutrient composition of the salmon, including their polyunsaturated fatty acid profile, probably varies along the river run of 1000 km (Dunlap *et al* 2011). As the salmon migrate, their lipid reserves are mobilized, and the beneficial effects of omega-3 against the toxic affects of stressors like mercury may be dampened. Selected fatty acid levels in these sled dogs is published elsewhere (Dunlap *et al* 2011).

Overall, we observed a significant inverse correlation between mercury exposure and antioxidant status ($P = 0.026$). The production of free radicals and peroxidized lipids by Hg is reported to be one potential mechanism in the development of cardiovascular disease. Mercury is capable of increasing cellular damage through the generation of reactive species that modify sulfhydryl groups in enzymes, lipids, ion channels and receptors, and interfering with endogenous antioxidants systems (Belanger *et al* 2006). The inverse relationship observed in sled dogs suggests that mercury exposure reduces or interferes with endogenous antioxidant levels. It is still extremely difficult to establish a cause and effect relationship between chronic contaminant exposure and overall health because of the myriad of other influences such as infectious diseases (Feingold *et al* 2010). This study adds to the weight of evidence that mercury levels should be kept low in the diet to reduce its contribution to other disease processes.

Salmon are rich in polyphenolic antioxidants, such as tocopherols and carotenoids (Rajasingh *et al* 2006, Yamamoto *et al* 2001). Notably, the carotenoid astaxanthin that is responsible for the bright red color that is characteristic of salmon flesh. During migration, astaxanthin relocates from the flesh to the skin and gonads of the fish and switches from the free form to an esterified form. Additionally, there is a dramatic loss in whole-body carotenoid levels (Rajasingh *et al* 2006). It would be expected that sled dogs living closest to the delta mouth, and eating salmon that are just embarking on the long migration, would have higher antioxidant status. This however, was not the case—suggesting that the elevated mercury levels interfere with antioxidant status.

Indigenous populations are keenly aware of changes in their environment and food supply (Hild 1998, Krauss 2004). Much of the contamination in subsistence foods is derived from pollutants that are not used in Alaska, indicating that exposure is a global concern especially for indigenous people (Krauss 2004, Mozaffarian and Rimm 2006). We measured total mercury in this study. While the species of mercury is important when it comes to toxicological significance, published literature shows that the per cent of methyl mercury in salmon muscle tissue is generally above 90% (Zhang *et al* 2001), and the level of methyl mercury in marine mammals is also very high (Dehn *et al* 2006). Continued monitoring of health effects associated with subsistence diets is necessary, and sled dogs provide a good model system for humans living in the north (Dunlap *et al* 2007, 2006, Reynolds *et al* 1999).

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