Polish Journal of Veterinary Sciences Vol. 18, No. 4 (2015), 677–682

DOI 10.1515/pjvs-2015-0087

Original article

Assessment of iron in uterine and testicular tissues and hair of free-ranging and household cats

P. Rzymski[1], P. Niedzielski[2], P. Dąbrowski[3]

[1] Department of Environmental Medicine, Poznan University of Medical Sciences, Rokietnicka 8, 60-806 Poznań, Poland
[2] Department of Analytical Chemistry, Faculty of Chemistry, Adam Mickiewicz University, Umultowska 89, 61-614 Poznań, Poland
[3] Veterinary Clinic, Zbąszyńska Street 26, Poznań, Poland

Abstract

Iron (Fe) represents a highly essential element for various biological processes. In spite of this very little is known as regards its status in mammalian reproductive tissues and factors that may potentially influence it. At the same time, there is an ongoing debate as to whether analyses of the Fe content in hair can provide reliable information on its tissue burden. Therefore, the aim of the present study was to investigate the content of Fe in the testicular and uterine tissues, and hair of the domestic cat (*Felis catus*) and how this content relates to an animal's age, weight, physical activity, inhabited environment and diet. The median Fe content in the feline reproductive tissues amounted to 50.8 ppm and in hair to 180.2 ppm. As found, free-ranging cats were characterized by a significantly higher Fe content in reproductive tissues, particularly in the uterus. Age, weight and physical activity had no effect on determined Fe levels. The type of commercial diet (wet, dry or combined) given to household cats also had no influence upon Fe status in hair and tissue although males fed exclusively on dry food had a lower Fe_{testis}:Fe_{hair} ratio. Hair Fe level was positively correlated with that found in the reproductive tissues (Rs=0.30). This study extends the body of information on Fe distribution in felines, demonstrates the difference between free-ranging and household cats and provides evidence that Fe hair status may, at least partially, reflect the status of this element in the feline reproductive system.

Key words: *Felis catus*, iron, reproduction, hair analysis, bioaccumulation

Introduction

Iron (Fe) is a highly essential element that participates in various metabolic processes and pathways including oxygen and electron transport, synthesis of DNA, RNA and proteins, cell proliferation and differentiation as well as regulation of gene expression (Pantopoulos et al. 2012). Its deficiency in mammals,

Correspondence to: P. Rzymski, e-mail: rzymskipiotr@ump.edu.pl

which results from decreased Fe intake, has been linked with anemia (Eussen et al. 2015). On the other hand, an excess of Fe can lead to the generation of reactive oxygen species through Fenton and Haber-Weiss reactions and subsequent oxidative stress cellular damage (Galaris and Pantopoulos 2008). Therefore, it is essential to monitor its status so that proper clinical decisions can be arrived at (Bohn 2013).

Various standardized methods to assess Fe status in mammals, including domestic cats, are known and include measurements of serum Fe concentrations, serum ferritin and transferrin levels and total iron binding capacity (which is a transferrin saturated with Fe). As found, endogenous excretion of Fe with urine and feces cannot be regarded as an active route of elimination of its excess (Bohn 2013). Instead, Fe is retained in organs and tissues with a biological half-life estimated at 4-7 years (Green et al. 1968, Heinrich 1970). It is known to accumulate at high levels mainly in the liver and brain (Yehuda and Youdim 1989, Nuttall et al. 2003) but recent studies have demonstrated that it can also be detected in hair in which, due to the continuous contact of hair follicle with bloodstream and the high content of sulfhydryl-containing keratin, Fe can be bound and deposited while the hair grows (Chaturvedi et al. 2014). Consequently, hair can potentially serve as a non-invasive matrix for assessment of long-term nutritional status in investigated subjects (Bissé et al. 1996). However, little is known about the presence of Fe in mammalian hair or exogenous factors that may influence its content. Moreover, there is an ongoing debate as to whether hair analyses can reflect (and to what extent) a general burden on tissue level (Długaszek and Kopczyński 2014, Rzymski et al. 2015). Relatively little is also known regarding the concentrations of Fe in the mammalian reproductive system although it has been shown to play a dualistic role in their procreation. Iron homeostasis protects against anemia, a risk factor for preterm delivery and subsequent low birth weight (Allen 2000) as well as reduced sperm quality (Soliman et al. 2014). On the other hand, an excess of dietary Fe can result in increased levels of an unbound form that reacts readily with water, generates free radicals and leads to oxidative stress. In males, increased Fe level has already been linked to morphological alterations and atrophy in testes, impaired spermatogenesis and impaired reproductive performance (Tvrda et al. 2015) while in females it has been associated with endometriosis (Kobayashi et al. 2009) and preterm birth (Sakata et al. 2008).

As levels of available Fe are highly dependent on lifestyle and diet (Crouter et al. 2012, Sjöberg and Hulthén 2015), the comparison of its content in free-ranging and household animals, particularly of one species, such as the domestic cat (*Felis catus*), can potentially serve as a valuable source of information on the endogenous and exogenous factors that may influence the status of this element due to distinctively different conditions of existence. For example, household cats are usually fed a commercial diet and may vary from free-ranging individuals as regards their level of physical activity (Remillard 2008, Plantinga et al. 2011).

The aim of the present study was to assess and compare the status of Fe in household and free-ranging cats by analyzing reproductive tissues (uterus and testis) obtained during spay and neuter surgery as well as by evaluating the content of this element in feline hair. The relation between cats' age, weight and physical activity and determined Fe levels was also investigated. For household cats, the potential effect of a commercial diet (dry, wet or combined) on Fe profile in the reproductive tissues and hair was studied. Finally, the usefulness of hair Fe analyses in assessment of the tissue burden of this element was assessed. The results of this study are important in understanding how lifestyle, inhabited environment and diet can influence the status of one of the most biologically essential elements in cats.

Materials and Methods

Collection and preparation of samples

Samples of uterine and testicular tissues as well as hair were collected from a total of 44 free-ranging and household individuals of domestic cat (*Felis catus* L. 1758) undergoing spay and neuter surgical procedures at the veterinary clinic. Due to technical difficulties (the need to proceed with routine medical procedures) the ovarian tissues were not collected for further analyses. As cats shed their hair mostly during spring and autumn, sample collection was performed between June and August 2014. Information on animal characteristics was acquired through examination at the veterinary clinic and a questionnaires filled in by pet owners or persons responsible for catching the free-ranging individuals. General information on the studied group is given in Table 1.

In the case of free-ranging animals, surgery was performed in accordance with the National Sterilization Project while in household cats, upon the undersigned will of the pet owner. All procedures were performed as a routine medical treatment using a surgical stainless steel knife and pliers. To avoid metal contamination, samples of uterine and testicular tissues for further analyses were obtained by cutting out the

Table 1. The main characteristics of the studied groups of cats.

Age [months][a]	8 (3-96)
Weight [kg][a]	3.0 (1.5-5.0)
Sex	
Female (n)	28
Male (n)	16
Lifestyle	
Household (n)	34
Free-ranging (n)	10
Physical activity	
Low (n)	10
Moderate (n)	23
High (n)	11
Diet (only household cats fed with commercial food)	
Commercial wet (n)	7
Commercial dry (n)	7
Commercial wet and dry combined (n)	16

[a] median (range)

untouched areas with blades made of zirconium carbonate. The tissue was then profusely flushed (to remove blood), immersed in sterile normal saline solution, placed in cryogenic tubes (Nunc™, ThermoScientific, United States) and stored at -40°C prior to metal determination.

Samples of hair from the lower abdominal area were collected prior to the sterilization procedure using an electric razor, and sealed in plastic bags. To remove external contaminants, the hair samples were washed with acetone (Sigma, ACS Reagent, ≥99.5%) followed by water and acetone again. Washed hair was dried in an oven to a constant weight, placed in sealed plastic tubes and stored in darkness at room temperature prior to metal content analyses.

Analyses of iron content

Collected uterine and testicular tissues, and hair samples were handled using plastic instruments with special care taken to avoid any contamination. Thawed tissues were dried in an oven at 40°C, flushed twice with MilliQ water (Millipore, USA) to ensure the removal of blood remnants and dried again to a constant weight. All samples (hair, uterine and testicular tissues) were subjected to a complete digestion performed with suprapur 14 mol/L HNO_3 (Sigma-Aldrich, Germany) in sealed plastic tubes using an oven (80°C). The concentration of iron in the investigated samples was determined by the fast sequential atomic absorption spectrometer SpectrAA 220 FS (Varian, Australia) equipped with HCL lamps (Varian, Australia) and a Sampling System with an Electronic Control Module SIPS-20 (Varian, Australia). Following optical conditions were applied: wavelength 248.3 nm, slit 0.2 nm, with background correction from a deuterium lamp. The calibration was performed using multi-element standard analytical solutions (Merck, Germany). A control without any tissue but containing HNO_3 was performed in order to exclude the interference of any procedural step on metal content determination – the iron content was below the limit of detection. The final results were given as ppm of Fe (mg Fe kg^{-1} sample).

Calculations and statistical analyses

To assess potential relation with Fe content in hair and tissues, the following ratios were calculated: Fe_{testis}:Fe_{hair} and Fe_{uterus}:Fe_{hair}. The results were analyzed using STATISTICA 10.0 software (StatSoft, U.S.A.). Gaussian distribution was tested with the Shapiro-Wilk test, and because most of the data did not meet this assumption, non-parametric methods were employed. To evaluate the differences between two independent groups, the Mann-Whitney U test was used. If more than two groups were compared, the matrix of the Mann-Whitney U test was applied. Relations between two datasets were determined with Spearman\s rank correlation coefficient (Rs). $p<0.05$ was considered as statistically significant.

Results

Iron status in reproductive tissues

The median Fe content in feline reproductive tissues was 50.8 ppm and revealed no significant differences between the uterine and testicular tissues. In general, free-ranging cats were characterized by significantly ($p<0.05$) higher Fe status in the reproductive tissues than household cats (difference in medians 59.4 ppm). A detailed comparison found that the uterine but not testicular tissue differed between free-range and household cats (Fig. 1). Cats' age and weight were not related to Fe concentrations in the uterus and testis. Moreover, the level of physical activity of animals had no effect on the metal content. Finally, the type of commercial diet used in household cats did not alter Fe content in the reproductive tissues (Fig. 2).

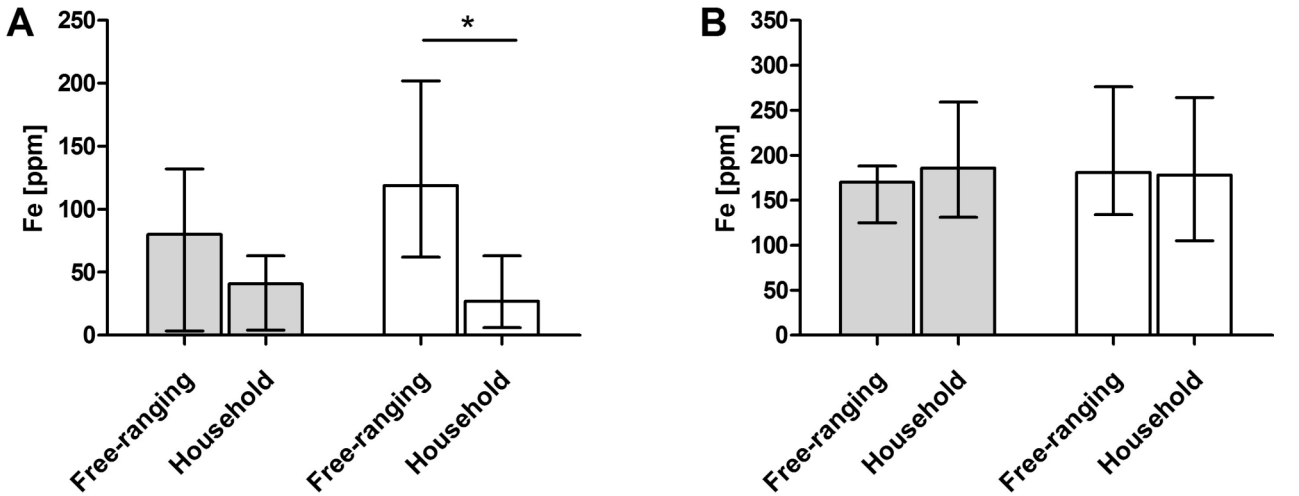

Fig. 1. The status of Fe (median and interquartile range) in reproductive tissues (testis – black; uterus – white) (A) and hair (males – grey; white – females) of free-ranging and household cats. Asterisk indicates statistically significant difference ($p<0.05$).

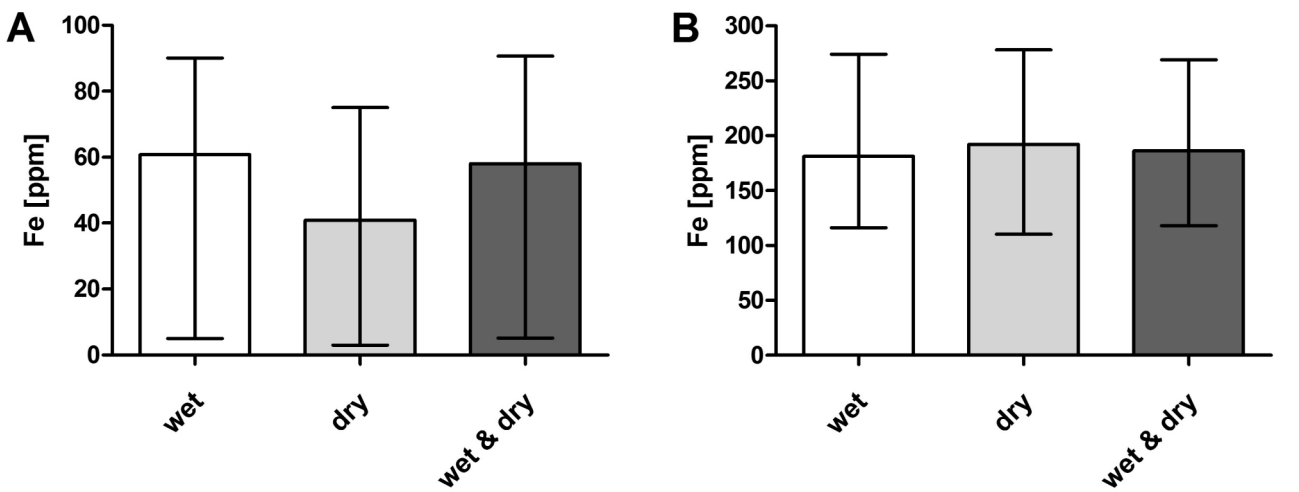

Fig. 2. The status of Fe (median and interquartile range) in reproductive tissues (A) and hair (B) of household cats in relation to commercial diet.

Iron status in hair

The median content of Fe in hair was 180.2 ppm. No difference between the males and females were found. Neither was any difference observed in Fe content between free ranging and household cats (Fig. 1). Age, weight and level of physical activity did not influence the Fe concentrations and no differences were observed between cats fed dry, wet and combined diet (Fig. 2).

Relationship between iron in hair and tissues

A statistically significant positive correlation between Fe level in hair and reproductive tissues was found (Rs=0.30). None of the considered variables (age, weight, level of physical activity, inhabited environment and commercial diet) affected the Fe_{uterus}:Fe_{hair} ratio. In turn, the Fe_{testis}:Fe_{hair} ratio calculated for household cats fed wet food (median value 0.34) or wet and dry food combined (median value 0.36) differed significantly ($p<0.05$) from this calculated for cats given dry food exclusively (median value 0.12).

Discussion

Analyses of the elementary content in mammalian reproductive tissues have so far been performed only rarely (Rzymski et al. 2014a, b, Rzymski et al. 2015). The present study is the first to address the status of Fe in the feline reproductive system and to demonstrate that it can be detected at various levels. It represents a unique, single occasion to study the level

of Fe in the uterus and testis of animals varying in age, weight, physical activity, diet and lifestyle, and to evaluate whether any relationship with the Fe content of hair occurs. It has previously been suggested that hair analysis of Fe may be useful to complement evaluations of body iron status determined with markers most commonly used to diagnose and monitor Fe deficiency, such as serum Fe, ferritin and haemoglobin level (Bissé et al. 1996). However, tissue Fe levels can be quantified using biopsy sections of the liver, spleen and bone marrow (or both) with Prussian blue stain (Schultheiss et al. 2002). To our knowledge no association between tissue and hair Fe has been studied to date. As found in the present study, the concentrations in hair are significantly, positively but weakly associated with levels found in the feline reproductive tissue and therefore may, to some extent, reflect the general body Fe burden but its potential as a useful measure of internal tissue Fe status should be treated very cautiously.

The most important finding is the significantly higher concentrations found in the reproductive tissues, particularly in the uterus, of free-ranging cats than in those collected from household individuals. This observation is likely attributable to the difference in the dietary habits of these two animal groups. The commercial food products consumed by household cats are usually a good source of Fe as they are predominantly based on meat. On the other hand, they may contain ingredients not normally eaten by cats such as algae or plants, which are not a source of heme Fe (Buffington 2008). Moreover, household cats are usually fed within a fixed schedule (e.g. three times a day) while free-rangers may eat more often and thereby deliver higher loads of Fe. Finally, the diet of free-ranging cats may not be only based on prey but also on human waste, nevertheless it may still be more diversified than that of household individuals which are repeatedly fed the same food (e.g. the owner's favourite). In comparison with recommended allowances for household cats, free-rangers were observed to consume larger amounts of Fe (Plantinga et al. 2011). It is likely that these differences arise from other nutritional goals, supporting survival and procreation in free-ranging cats and optimizing health and longevity in household individuals. As shown in monkeys, free-ranging individuals are characterized by higher Fe content than caged individuals, possibly as a result of a higher oral intake of Fe (Marriott et al. 1996). Interestingly, no similar difference in Fe content was found in hair collected from household and free-ranging cats. It should, however, be stressed that feline hair is shed year round, most intensively during the spring and autumn (Grandjean and Butterwick 1999) and therefore, its chemical content may be subject to a dynamic turnover.

There is an ongoing debate on what constitutes a satisfactory diet for household cats (Buffington 2008). As previously demonstrated, some commercially available dry foods for cats and dogs tend to have a lowered Fe content compared to corresponding wet products (Duran 2010). Importantly, the bioavailability of Fe in pet food is often low, particularly in forms of oxide or carbonate (NRC 2006). Interestingly, in the present study diet did not influence the Fe content in hair, uterus or testis alone but had an effect on the Fe_{testis}:Fe_{hair} ratio. Males which were fed a combined diet (wet and dry food) or wet food revealed higher values than those given dry food exclusively. This may indicate that feeding cats with wet or combined food promotes the storage of biologically available Fe in tissues over its accumulation in hair, in which the element is bound with thiol protein groups and immobilized. It should, however, be noted that compared groups were low and therefore, these results should be treated cautiously.

Conclusions

The present study demonstrated that Fe can be detected in feline reproductive tissues and hair at various, significant but weakly correlated levels. As found, the status of Fe in reproductive tissues, particularly in the uterus, has been associated with cats' lifestyles – free-ranging animals were characterized by significantly higher levels of this element. However, the type of commercial diet did not affect the level of Fe in the uterus, testis or hair alone, cats fed wet or combined food (wet and dry) had largely increased Fe_{testis}:Fe_{hair} ratio than those fed exclusively dry food.

Acknowledgments

We would like to thank all the pet owners and cat catchers for their participation in this research.

References

Allen LH (**2000**) Anemia and iron deficiency: effects on pregnancy outcome. Am J Clin Nutr 71(Suppl 5): 1280--1284.

Bissé E, Renner F, Sussmann S, Scholmerich J, Wieland H (**1996**) Hair iron content: possible marker to complement monitoring therapy of iron deficiency in patients with chronic inflammatory bowel diseases? Clin Chem 42: 1270-1274.

Bohn AA (**2013**) Diagnosis of disorders of iron metabolism in dogs and cats. Vet Clin North Am Small Anim Pract 43: 1319-1330.

Buffington CA (**2008**) Dry foods and risk of disease in cats. Can Vet J 49: 561-563.

Chaturvedi R, Banerjee S, Chattopadhyay P, Bhattacharjee CR, Raul P, Borah K (2014) High iron accumulation in hair and nail of people living in iron affected areas of Assam, India. Ecotoxicol Environ Saf 110: 216-220.

Crouter SE, DellaValle DM, Haas JD (**2012**) Relationship between physical activity, physical performance, and iron status in adult women. Appl Physiol Nutr Metab 37: 697-705.

Długaszek M, Kopczyński K (**2014**) Correlations between elements in the fur of wild animals. Bull Environ Contam Toxicol 93: 25-30.

Duran A, Tuzen M, Soylak M (2010) Trace element concentrations of some pet foods commercially available in Turkey. Food Chem Toxicol 48: 2833-2837.

Eussen S, Alles M, Uijterschout L, Brus F, van der Horst-Graat J (**2015**) Iron intake and status of children aged 6-36 months in europe: a systematic review. Ann Nutr Metab 66: 80-92.

Galaris D, Pantopoulos K (**2008**) Oxidative stress and iron homeostasis: mechanistic and health aspects. Crit Rev Clin Lab Sci 45: 1-23.

Grandjean D, Butterwick R (**1999**) Waltham Course on Dog and Cat Nutrition. Waltham Centre for Pet Nutrition, Waltham.

Green R, Charlton R, Seftel H, Bothwell T, Mayet F, Adams B, Finch C, Layrisse M. (**1968**) Body iron excretion in man: a collaborative study. Am J Med 45: 336-353.

Heinrich HC (**1970**) Iron Deficiency. In: Hallberg L, Harwerth HG, Vannotti A (eds) Pathogenesis, Clinical Aspects, Therapy. Academic Press, London, pp 213-296.

Kobayashi H, Yamada Y, Kanayama S, Furukawa N, Noguchi T, Haruta S, Yoshida S, Sakata M, Sado T, Oi H (**2009**) The role of iron in the pathogenesis of endometriosis. Gynecol Endocrinol 25: 39-52.

Marriott BM, Smith JC Jr, Jacobs RM, Jones AO, Altman JD (**1996**) Copper, iron, manganese, and zinc content of hair from two populations of rhesus monkeys. Biol Trace Elem Res 53: 167-183.

NRC (**2006**) Nutrient Requirements of Dogs and Cats. The National Academies Press, Washington DC.

Nuttall KL, Palaty J, Lockitch G (**2003**) Reference limits for copper and iron in liver biopsies. Ann Clin Lab Sci 33: 443-450.

Pantopoulos K, Porwal SK, Tartakoff A, Devireddy L (**2012**) Mechanisms of mammalian iron homeostasis. Biochemistry 51: 5705-5724.

Plantinga EA, Bosch G, Hendriks WH (**2011**) Estimation of the dietary nutrient profile of free-roaming feral cats: possible implications for nutrition of domestic cats. Br J Nutr (Suppl 1): 35-48.

Remillard RL (**2008**) Homemade diets: attributes, pitfalls, and a call for action. Top Companion Anim Med 23: 137-142.

Rzymski P, Rzymski P, Tomczyk K, Niedzielski P, Jakubowski K, Poniedziałek B, Opala T (**2014a**) Metal status in human endometrium: relation to cigarette smoking and histological lesions. Environ Res 132: 328-333.

Rzymski P, Niedzielski P, Rzymski P, Poniedziałek B Tomczyk K, Opala T (**2014b**) On the presence of aluminium in human endometrial tissue and potential factors that may influence it – a pilot study. J Medical Science 83: 326-331.

Rzymski P, Niedzielski P, Poniedziałek B, Rzymski P, Pacyńska J, Kozak L, Dąbrowski P (**2015**) Free-ranging domestic cats are characterized by increased metal content in reproductive tissues. Reprod Toxicol 58: 54-60.

Sakata M, Sado T, Kitanaka T, Naruse K, Noguchi T, Yoshida S, Shigetomi H, Onogi A, Oi H, Kobayashi H (**2008**) Iron-dependent oxidative stress as a pathogenesis for preterm birth. Obstet Gynecol Surv 63: 651-660.

Schultheiss PC, Bedwell CL, Hamar DW, Fettman MJ (**2002**) Canine liver iron, copper and zinc concentrations and association with histologic lesions. J Vet Diagn Invest 14: 396-402

Sjöberg A, Hulthén L (**2015**) Comparison of food habits, iron intake and iron status in adolescents before and after the withdrawal of the general iron fortification in Sweden. Eur J Clin Nutr 69: 494-500.

Soliman A, Yassin M, De Sanctis V (**2014**) Intravenous iron replacement therapy in eugonadal males with iron-deficiency anemia: Effects on pituitary gonadal axis and sperm parameters; A pilot study. Indian J Endocrinol Metab 18: 310-316.

Tvrda E, Peer R, Sikka SC, Agarwal A (**2015**) Iron and copper in male reproduction: a double-edged sword. J Assist Reprod Genet 32: 3-16.

Yehuda S, Youdim MB (**1989**) Brain iron: a lesson from animal models. Am J Clin Nutr 50 (Suppl 3): 618-625.