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Manganese, copper and copper enzymes in blood of Icelandic sheep: Relevance to scrapie

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ABSTRACT

The aim of this study was to investigate whether amounts of manganese or copper in blood, or the activity of copper enzymes (ceruloplasmin oxidative activity in serum and superoxide dismutase [SOD1] activity in erythrocytes) could be related to the sporadic occurrence of scrapie on sheep farms in Iceland. The farms were divided into three Categories: Category 1: scrapie-free (never afflicted by scrapie, or prior to 1960 and then restocked with healthy sheep); Category 2: scrapie-prone (afflicted by scrapie after 1980 and restocked with healthy sheep); and Category 3: scrapie-afflicted (scrapie diagnosed in the respective flocks during the experimental period, autumn 2001 – autumn 2003). Blood samples were collected for analysis of manganese, copper and SOD1 activity from 2-5 year old ewes on 13 farms in all categories in the Vatnsdalur valley area in northern Iceland as well as from ewes on 3 scrapie-afflicted farms but located in other areas of the country. Ceruloplasmin oxidative activity was only analysed in serum from ewes in a preliminary study based on farms in all Categories in four different areas (the Vatnsdalur valley area inclusive). Manganese concentration in blood did not differ significantly between non-pregnant ewes on farms in the three Categories. The manganese concentration was not significantly different in blood of pregnant ewes on farms in the three Categories but it was significantly lower than in blood of the non-pregnant ewes. SOD1 activity did not differ significantly between erythrocytes in non-pregnant ewes on farms in the three Categories. Neither did SOD1 activity differ significantly in erythrocytes from pregnant ewes but it was significantly higher than in the non-pregnant ewes. Copper concentration did not differ significantly in blood of non-pregnant ewes on farms in the three Categories. Copper concentration tended to increase in pregnancy and it reached the level of significance in two out of three Categories. Ceruloplasmin oxidative activity in serum of ewes on farms in all categories appeared to be the same. There are three main conclusions from this research. First, the results do not support the postulate that scrapie in Iceland (and other countries) is associated with low levels of copper and/or high levels of manganese. Second, there is apparently no decrease or derangement in ceruloplasmin oxidative activity or SOD1 activity in sheep on scrapie-afflicted farms. Third, the results emphasize the necessity, as previously noted, to describe explicitly the state of the sheep when they are taken into experiment (non-pregnant, pregnant etc.).

Key words: blood, copper, copper enzymes, manganese, scrapie, sheep

YFIRLIT

Mangan, kopar og koparensím í blóði íslensks sauðfjár: Tengsl við riðu

Markmiðið með rannsókninni var að kanna hvort þéttni mangans eða kopars í blóði sauðfjár eða virkni koparensíma (oxunarvirkni cerúlóplasmíns í sermi og virkni súperoxíðdismútasa [SOD1] í rauðum blóðkornum) gæti tengst staksettri (sporadic) uppkomu riðu á sauðfjárbúum á Íslandi. Bæjum var skipt í þrjá flokka: riðulausir bæir ("scrapie-free"; riða annað hvort aldrei komið upp eða fyrir 1960 og þá skipt um fé), fjárskiptabæir ("scrapie-prone"; riða komið upp eftir 1980, og skipt um fé) og riðubæir ("scrapie-afflicted"; riða greind á rannsóknartímabilinu, frá hausti 2001 til hausts 2003). Blóðsýnum til ákvörðunar á mangan, kopar og SOD1 virkni var safnað úr 2-5 vetra gömlum ám á 13 bæjum í öllum flokkum í tveimur samliggjandi dölum norðanlands (Vatnsdalur – Víðidalur), og einnig úr ám á 3 riðubæjum annars staðar á landinu. Cerúlóplasmínvirkni var eingöngu ákvörðuð í sermi úr ám í forrannsókn sem gerð var á bæjum í öllum flokkum á fjórum svæðum á landinu (þar með talið Vatnsdals-Víðidalssvæðið). Ekki var marktækur munur á manganþéttni í blóði lamblausra áa frá bæjum í flokkunum þremur. Ekki var heldur marktækur munur á manganþéttni í blóði lambfullra áa í flokkunum þrem, en þéttnin var marktækt minni í lambfullum ám en í lamblausum. Það var ekki marktækur munur á SOD1 virkni í rauðum blóðkornum lamblausra áa á bæjum í flokkunum þrem. Marktækur munur á SOD1 virkni var heldur ekki í rauðum blóðkornum úr lambfullum ám í þessum flokkum, en virknin var marktækt meiri en í lamblausu ánum. Ekki var marktækur munur á koparþéttni í blóði lamblausra áa á bæjum í flokkunum þrem. Koparþéttnin hafði tilhneigingu til að aukast á meðgöngu og sást marktæk aukning í tveimur af þremur flokkum. Cerúlóplasmínvirkni í sermi áa virtist vera eins á bæjum í öllum flokkum. Helstu niðurstöður þessarar rannsóknar eru þrjár: 1) Niðurstöðurnar styðja ekki þá fullyrðingu að riða tengist lítilli koparþéttni og/eða mikilli manganþéttni. 2) Virkni koparensímanna cerúlóplasmíns og SOD1 virtist ekki vera afbrigðileg í sauðfé á riðubæjum. 3) Niðurstöðurnar undirstrika nauðsyn þess, eins og áður hefur verið nefnt, að skilgreina nákvæmlega ástand sauðfjár þegar gerðar eru málma- og ensímmælingar á því (lamblausar ær, lambfullar ær o.s.frv.).

INTRODUCTION

Scrapie belongs to the group of diseases called prion diseases (also named transmissible spongiform encephalopathies - TSEs) that are neurodegenerative and uniformly fatal, and some of which are known in humans. Although TSEs have many similarities to other amyloidoses, like Alzheimer´s disease, the unique and distinguishing feature of TSEs is that they alone are transmissible. However, most prion diseases have sporadic occurrence, suggesting that environmental factors, like metals, may influence the progress or recurrance of these diseases (Prusiner 2001, Brown 2002, Jóhannesson et al. 2003, 2005).

Scrapie is still encountered sporadically each year in Iceland, mostly in previously affected areas, in spite of stringent precautionary efforts (including culling of sheep, quarantine of farms for years, disinfection of stables and instalment of new and healthy stock of sheep). Although causes of sporadic recurrence of scrapie on farms in Iceland remain unknown, recent data indicate that high concentration of manganese in forage, or a high manganese/copper ratio in forage, may have a preventive effect on the recurrence of scrapie (Jóhannesson et al. 2004a). Purdey (2000) on the other hand, has postulated that the occurrence of scrapie in Iceland could be related to low amounts of copper and/or high amounts of manganese in herbage. The possible association between differences in concentrations of manganese and copper in brain and blood and the occurrence of experimental scrapie in mice has been studied by Wong et al. (2001) and Thackray et al. (2002) among others as is reviewed by Jóhannesson et al. (2003).

In previous studies decreased or deranged activity of ceruloplasmin and superoxide dismutase (SOD1), both copper containing enzymes controlling oxidative reactions in biological systems and sensitive markers of copper status, were found in serum and erythrocytes of human patients suffering from neurodegenerative diseases of the central nervous system (Snaedal et al. 1998; Tórsdóttir et al. 1999, 2000, 2001). Whether similar changes are found in sheep on scrapie-afflicted farms has not been studied up to the present time.

The purpose of the present study was first to investigate whether manganese and copper concentrations in blood of ewes on scrapie-afflicted farms could be related to the occurrence of clinical scrapie similar to what has been found for forage. Secondly, we intended to study whether changes in ceruloplasmin oxidative activity in serum or the activity of SOD1 in erythrocytes of ewes could be related to occurrence of clinical scrapie in sheep.

Pregnancy may influence enzymic activities as well as the concentrations of metals in the blood of ewes, as has been shown for example for copper and ceruloplasmin (Howell et al. 1968) and SOD1 in erythrocytes (Jóhannesson et al. 2003). The study therefore, whenever possible, was comprised of both non-pregnant and pregnant ewes. Ceruloplasmin oxidative activity was only included in a preliminary study as the results clearly indicated that it was the same in ewes on scrapie-free, scrapie-prone and scrapie-afflicted farms.

MATERIALS AND METHODS

Categories and location of farms

Sheep farms were divided into three categories in accordance with the history of scrapie as previously described by Jóhannesson et al. (2004b). Those farms included in *Category 1* (ten in number), referred to as *scrapie-free*, had either never been afflicted by scrapie (seven farms) or scrapie had been absent for more than 40 years after the farms were restocked with new flocks of sheep from areas free of scrapie (three farms). Three of the farms are located in a county in western Iceland where scrapie has never been recognized. The remaining seven farms are located in the Svarfaðardalur valley or the Vatnsdalur valley area (including the Vatnsdalur and Víðidalur valleys), two scrapie-prone areas in northern Iceland. These two areas and one in the southern part of the country where scrapie has been diagnosed on several farms after 1980 (Jóhannesson et al. 2004a) provided the farms in *Category 2* (twelve farms). Of these farms, referred to as *scrapie-prone*, five are located in Árnes County in southern Iceland and seven in the Svarfaðardalur valley and the Vatnsdalur valley area. Scrapie has seemingly been eradicated on these farms by slaughtering and, after expiration of the statutory quarantine period, replacing sheep with healthy animals. This practice has resulted in the absence of the disease on these farms for at least eight years. On the farms in *Category 3* (seven farms), referred to as *scrapie-afflicted*, scrapie had been diagnosed during the experimental period (2001-2003). These farms are found in the well-known scrapie-prone areas in northern and southern Iceland (Svarfaðardalur valley, Vatnsdalur valley area and in Árnes County). The ewes on the scrapie-afflicted farms were either non-pregnant or in the pregnant state when scrapie was diagnosed. Usually scrapie was diagnosed in one or two animals in the respective flocks and the entire flocks were, in accordance with government regulations, killed shortly afterwards.

Preliminary study of ceruloplasmin oxidative activity and SOD1 activity (autumn 2001-spring 2002)

For each sheep, two blood samples were drawn from the jugular vein by a skilled individual; one tube being used for determination of ceruloplasmin oxidative activity and the other for determination of SOD1 activity. Blood samples were collected in 7.5 ml tubes specially prepared for metal analysis (Sarstedt). Blood samples were collected in two rounds. In the first round in September-October 2001, samples were taken from about ten ewes on each farm a few weeks after the sheep had been rounded up and driven from the highland pastures and before the animals were admitted to stables for winter feeding. From early December through early January 2002 the ewes were ushered to the rams for breeding. A second sampling round took place in March 2002 because pregnant ewes fed in stables for prolonged periods may exhibit altered enzymic activities. This study included 6 farms in Category 1, seven in Category 2, but only one farm in Category 3, this being the only farm diagnosed with scrapie during the period of the preliminary study. The ewes on the scrapie-afflicted farm were pregnant when diagnosis was made and were accordingly only sampled in the pregnant state.

Studies of manganese and copper in blood and SOD1 activity in erythrocytes of ewes

Two- to 5 year-old ewes on nine farms in Categories 1 and 2 in the Vatnsdalur valley area were sampled both as non-pregnant in autumn 2002 and in the pregnant state in spring 2003. The procedure for collecting blood samples was the same as is described above. In both rounds blood was collected from six to nine animals on each farm for analysis of SOD1 activity. In both rounds a second blood sample was also collected (also in tubes specially prepared for metal analysis) from five to six of the same animals on each farm for the determination of manganese and copper concentrations in blood. Scrapie was diagnosed on seven farms from autumn 2001 to autumn 2003. Four of these farms are in the Vatnsdalur valley area but three are located in other areas of the country. When these farms were diagnosed with scrapie (usually 1-2 animals per flock) the ewes were either non-pregnant or pregnant and were culled shortly after being sampled (cf. above). Seven to fifteen ewes were sampled on each scrapie-afflicted farm for determination of SOD1 activity and six to twelve of them were also sampled for analysis of manganese and copper.

Ceruloplasmin oxidative activity in serum was determined at the Department of Pharmacology and Toxicology, University of Iceland, with the manual kinetic assay originally described by Boyett et al. (1976). The blood samples were centrifuged at 4 °C and 3000 rpm for 10 minutes within 48 hours after collection. The serum was then stored in the freezer at -20 °C until the determination of ceruloplasmin oxidative activity, which occurred within 20 days after sample collection. The coefficient of variation (C.V.) for this assay was 6.1%. Results are expressed as units ml^{-1}.

Superoxide dismutase (SOD1) activity in erythrocytes was determined at the Department of Pharmacology and Toxicology, University of Iceland, with a spectrophotometric assay (Biooxytech® SOD-525) as originally described by Nebot et al. (1993). The reagents used in the analyses were obtained from Oxis International, Inc., Portland, Oregon, USA. Within 48 hours after sample collection the blood samples were centrifuged and the erythrocytes separated from the plasma. Superoxide dismutase activity was determined within 140 days (20 weeks) after sample collection. The coefficient of variation (C.V.) for this assay was 7.3%. Results are expressed as SOD-525 units.

Preparation of samples and analyses of manganese and copper

Manganese and copper in blood were determined at the Fisheries Laboratories, Reykjavík, by flame atomic absorption spectrophotometry (Perkin Elmer 1100B) with D2-background correction and external standards in solvents matching the sample solvents. Samples were digested in two different ways. First, samples were digested in duplicate in closed quartz bombs with Teflon lids by nitric acid (Merck, Suprapur). After digestion, the solvent was evaporated to dryness and the residue diluted to an appropriate volume by dilute nitric acid (Merck). Secondly, samples in duplicate were solubilized with nitric acid and hydrochloric acid followed by dry-ashing with magnesium nitrate as an ashing aid (Merck). The residue was dissolved in 5 M hydrochloric acid (Merck) and diluted to an appropriate volume. The respective solutions were then analysed for manganese and copper. Typical detection limits were 0.5 ng ml^{-1} for manganese and 3 ng ml^{-1} for copper.

As a quality control measure, certified reference materials were used (mussel tissue [CRM 278] and cod muscle [CRM 422] from BCR) with satisfactory recoveries (a certified standard for manganese and copper in sheep blood is to our knowledge not available). Additionally, these methods have been applied by the Fisheries Laboratories in proficiency testing programmes for trace elements in various foods (e.g. in programmes managed by the Swedish National Food Administration).

Results of manganese and copper determinations gave comparable results with the two digestion procedures used. In the case of copper determinations both digestion procedures were always applied to the samples. In the case of manganese determinations both digestion procedures were used on many occasions, but on some occasions only one of the two procedures was used. Results of copper determinations are accordingly presented as the means of quadruplicate measurements and results of manganese determinations either as the means of duplicate or quadruplicate measurements. The results are expressed as ng ml^{-1}.

Statistical analysis

Analysis of variance (ANOVA) was used for the comparison of results within each sampling round (non-pregnant and pregnant, respectively). The Student-Newman-Keuls test was used for all pairwise comparisons where significant differences were found in the ANOVA. Linear regression analysis was used to test the relationship between copper concentrations in blood and SOD1 activity in erythrocytes. The level of significance was taken as $P \leq 0.05$.

RESULTS

In a preliminary study the mean ceruloplasmin oxidative activity was in the range of 45-50 units ml^{-1} for non-pregnant ewes on the scrapie-free and scrapie-prone farms. In pregnancy the enzymic activity increased and was on average in the range of 55-60 units ml^{-1} for the same ewes on these farms. In samples from pregnant ewes on the only scrapie-afflicted farm included in this study the mean enzymic activity was also in the same range as found for pregnant ewes on the scrapie-free and scrapie-prone farms. No significant difference was detected in ceruloplasmin oxidative activity between ewes, non-pregnant or pregnant, on farms in the three Categories. It should be noted that very high values occurred in serum from two pregnant ewes in Category 1 (scrapie-free).

Determination of SOD1 activity was also included in the preliminary study. In contrast to ceruloplasmin oxidative activity, SOD1 activity was found to be significantly lower in non-pregnant and pregnant ewes on scrapie-prone farms than in non-pregnant or pregnant ewes on scrapie-free farms (Jóhannesson et al. 2003). Determination of SOD1 activity was therefore extended and was performed in samples from non-pregnant and pregnant ewes on farms in all categories along with analyses of manganese and copper.

Manganese concentration in blood did not

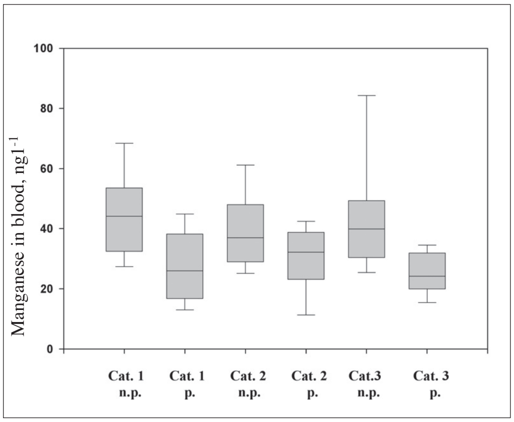

Figure 1. Distribution of manganese concentrations in blood from non-pregnant (n.p.) and pregnant (p.) ewes on scrapie-free farms (Cat.1), scrapie-prone farms (Cat.2) and scrapie-afflicted farms (Cat.3). Ewes on the scrapie-afflicted farms were either pregnant or non-pregnant when scrapie was diagnosed and they were culled shortly after sampling. The boxes represent median and 25th percentiles and the vertical bars the 5th percentiles.

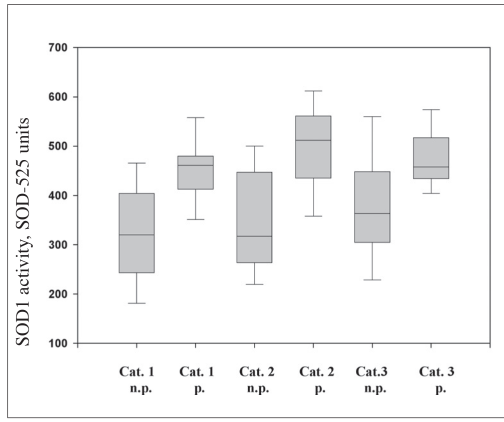

Figure 2. Distribution of SOD1 activity in erythrocytes from non-pregnant (n.p.) and pregnant (p.) ewes on on farms in all categories (see text to Figure 1).

differ significantly between non-pregnant ewes in the three Categories (means: 45 ng ml^{-1} $\pm$ 16 s.d., 40 ng ml^{-1} $\pm$ 13 and 45 ng ml^{-1} $\pm$ 22 for Categories 1, 2 and 3, respectively). In the blood of pregnant ewes manganese concentration was significantly lower on all occasions than in the blood of non-pregnant ewes (means: 31 ng ml^{-1} $\pm$ 14 s.d., 32 ng ml^{-1} $\pm$ 11 and 26 ng ml^{-1} $\pm$ 7, respectively). SOD1 activity in erythrocytes did similarly not differ significantly between non-pregnant ewes in the three Categories (means: 351 SOD-525 units $\pm$ 104 s.d., 348 units $\pm$ 105 and 355 units $\pm$ 110, respectively). In the blood of pregnant ewes SOD1 activity was significantly higher than in the blood of non-pregnant ewes (means: 454 SOD-525 units $\pm$ 70 S.D., 503 units $\pm$ 82 S.D. and 475 units $\pm$ 60 S.D., respectively). Distribution of the manganese concentration in blood and SOD1 activity in erythrocytes in non-pregnant and pregnant ewes on scrapie-free (Cat.1), scrapie-prone (Cat.2) and scrapie-afflicted farms (Cat.3) is shown in Figures 1 and 2. The median values and the 5th and 25th percentiles are indicated in the Figures.

Copper concentration in blood did not differ significantly between non-pregnant ewes in the three Categories (means: 914 ng ml^{-1} $\pm$ 206 s.d., 822 ng ml^{-1} $\pm$ 190 and 876 ng ml^{-1} $\pm$ 156, respectively). The average copper cocentration was significantly higher in the blood of pregnant ewes than in non-pregnant ewes on scrapie-prone farms (Cat.2) and scrapie-afflicted farms (Cat.3) but not on the scrapie-free farms (Cat.1). In the blood of non-pregnant ewes the copper concentration was on six occasions lower than 600 ng ml^{-1}, the lowest concentration being 220 ng ml^{-1}. In the blood of the pregnant ewes the lowest copper concentrations were in the range of 600-800 ng ml^{-1}. The highest copper concentrations were in the range of 1200-1400 ng ml^{-1} for ewes on farms in all Categories.

Copper concentration in the blood of the ewes was, with one exception, not correlated with SOD1 activity in erythrocytes. Only in the case of non-pregnant ewes on scrapie-prone farms did the copper concentration happen to be positively related to the SOD1 activity.

DISCUSSION

Essentially this study is based on analyses of blood samples collected from non-pregnant and pregnant ewes on scrapie-free, scrapie-prone and scrapie-afflicted farms in the Vatnsdalur valley and in an adjacent area in northern Iceland. In addition blood samples were collected from ewes on scrapie-afflicted farms where scrapie was diagnosed during the experimental period but located in other areas in the country. As the number of scrapie-afflicted farms is always likely to be low during any chosen experimental period this fact may hamper studies of this kind.

The Vatnsdalur valley area is one of the areas in Iceland, most affected by scrapie. In this area scrapie has been found sporadically on several farms during the recent decades while other farms, located in the same environment, have remained free of the disease. If environmental factors like manganese or copper, or the activity of copper enzymes, are related to the occurrence of clinical scrapie such connection should be explored in a locality like the Vatnsdalur valley area. As stated above the life span of Icelandic sheep falls each year into two distinct phases. It was therefore deemed necessary to sample the sheep when they were non-pregnant in the autumn as well as pregnant in the spring, whenever possible.

No difference of significance was found between manganese concentration in the blood of non-pregnant ewes on farms in Categories 1,2 and 3. During pregnancy the manganese concentration in the blood decreased and the decrease was statistically significant (Figure 1). In the available literature there are no concise data on the concentration of manganese in the blood of non-pregnant and pregnant ewes. The decrease during pregnancy could possibly indicate that manganese in pregnant sheep is for some reason concentrated in the placenta. This is known to occur in pregnant women as far as selenium is concerned (Osman et al. 2000).

The copper concentration was not statistically different in the blood of non-pregnant ewes on farms in Categories 1, 2 and 3. Copper concentration is known to increase in mammals during pregnancy (Ettinger 1984, Sharma & Sharma 1997). This was also observed in the present study in two out of three Categories where the difference reached the level of significance.

The lowest normal value for copper in the blood of sheep is now thought to be about 600 ng ml^{-1} (Suttle 1993) while previously the so-called normal range was as wide as 250-2000 ng ml^{-1} (Keen & Graham 1989). In our study the average concentration of copper in the blood of non-pregnant ewes in Categories 1, 2 and 3 was in the range 820-920 ng ml^{-1} and it was higher in the blood of the pregnant ewes. Individual copper concentrations in blood of ewes on farms in all three Categories, whether non-pregnant or pregnant, were, with a few exceptions, above 600 ng ml^{-1} and all but one was higher than 250 ng ml^{-1}. The highest individual concentrations for both pregnant and non-pregnant ewes did not exceed 1500 ng ml^{-1}. Copper concentration in the blood of ewes on farms in all Categories can thus in general be assumed to be in the normal range.

In the blood of non-pregnant ewes on farms in all categories the concentration of copper was approximately twenty times the concentration of manganese. In forage we have previously found the reverse, i.e. the concentration of manganese was twenty times the concentration of copper in forage on scrapie-free farms (Jóhannesson et al. 2004a). The difference in concentrations from forage to blood can, in part at least, be explained by the extremely low bioavailability of manganese in the gastrointestinal tract of ruminants, about 1% or less, while the bioavailability of copper is about ten times higher (Søli 1980, Spears 2003). The relatively low bioavailability of manganese in comparison with copper is also apparently borne out by the finding that in the liver of Icelandic lambs the concentration of copper was ten times the concentration of manganese (Reykdal & Thorlacius 2001). The possible protective effect of a high concentration of manganese in the forage of sheep against the occurrence of clinical scrapie would thus logically be confined to the cellular border of the gastrointestinal tract, which is considered the main port of entry for the prion protein in the sheep (Jóhannesson et al. 2004a). In this context it is of interest that the low glutathione peroxidase activity in the blood of sheep on scrapie-prone and scrapie-afflicted farms as compared to scrapie-free farms (Jóhannesson et al. 2003) is more or less matched by low manganese concentration in the forage of sheep on these farms (unpublished results).

In a preliminary study ceruloplasmin oxidative activity did not differ statistically in serum from non-pregnant ewes on scrapie-free and scrapie-prone farms located in four different areas in the country. During pregnancy the activity tended to increase in ewes in all Categories. The results indicate that the availability of copper for synthesis of ceruloplasmin was of similar magnitude in non-pregnant and pregnant ewes on farms in all categories.

SOD1 activity, which is considered a sensitive marker of copper status (Andrewartha & Caple 1980, Harris 1992), did not differ statistically in erythrocytes from non-pregnant ewes on scrapie-free, scrapie-prone and scrapie-afflicted farms. In pregnancy the average SOD1 activity increased significantly in ewes on farms in all Categories (Figure 2). Increased SOD1 activity in pregnancy was only on one occasion found to be significantly correlated with the levels of copper in the blood. This fact may indicate that copper was generally transferred from other compartments to the SOD1 apoenzyme. It is nevertheless evident that SOD1 activity could not have increased significantly in pregnancy unless it was based on access to sufficient amounts of copper to form the complete holoenzyme.

Taken together, our results contradict the postulate of Purdey (2000) that scrapie in Iceland (and other countries) could be associated with low levels of copper and/or high levels of manganese in herbage. Our previous results of forage analyses moreover indicate that high concentration of manganese may, as mentioned, have a protective effect on the occurrence of clinical scrapie (Jóhannesson et al. 2004a).

Jóhannesson and coworkers (Snaedal et al. 1998, Tórsdóttir et al. 1999, 2000, 2001, Jóhannesson et al. 2003) have shown, that the oxidative activity of ceruloplasmin and the SOD1 activity are either decreased or deranged in patients suffering from Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis and in older patiens with Down's syndrome. This phenomenon was described as copper dyshomeostasis as the copper concentration in plasma was in the normal range for humans on all occasions. The results presented here do not indicate that changes of this nature are seen in blood of ewes on scrapie-afflicted farms or on farms in the other two categories.

In summary, the results presented here indicate that the concentration of manganese and copper and the activities of the two copper enzymes studied are not statistically different in blood (serum, erythrocytes) of ewes on scrapie-free, scrapie-prone and scrapie-afflicted farms. The results (Figures 1 and 2) also emphasize the necessity, as previously noted for selenium (Jóhannesson et al. 2004b), to describe explicitly the state of sheep when they are taken into an experiment (non-pregnant, pregnant, housed or kept outside of sheds etc.).

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