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Metabolic changes of cadmium and zinc in kidney and liver tissue during foetal development in Romney and Merino sheep

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ABSTRACT

In adult sheep, cadmium (Cd) accumulates in both liver and kidney tissue in an age dependant manner. To quantify the accumulation of Cd in the developing foetus and investigate the relationship with the Cd status of the maternal parent, liver and kidney samples were collected from both Romney and Merino foetuses, approximately fortnightly, from 60 days after conception up to parturition, and from the suckling lamb, weekly over three weeks. Matching liver and kidney samples were also obtained from the dam. Tissue sub-samples were analysed for Cd by Zeeman electrothermal atomic absorption and for zinc (Zn) by plasma emission spectrometry. The same tissues were also analysed for metallothionein-mRNA by dot blot hybridisation of total RNA extracts to assist in identifying regulatory aspects of Cd accumulation in hepatic foetal tissue and any relationship with Zn. Cadmium concentrations (means ± s.e.; ng Cd/g FW) in pre-parturition and neonate Romney lamb liver (kidney) tissue compared with those in the adult were: 1.1±0.1 (2.5±0.25), 2.4±0.4 (2.8±0.4) and 139±8 (1208±99) respectively. Merino tissue Cd concentrations were similar to those in the Romney. Although Zn concentrations in liver tissue from the developing foetus decreased with increasing gestation time, Cd concentrations remained low and relatively constant over this period, but increased markedly after parturition. There was no correlation between Cd in tissue from the dam and that of the progeny and the data indicate that although placental transfer of Cd is minor, a small influx of Cd from the dam into the foetal liver occurs concomitantly with a much larger flux of Zn into the developing liver between 110 and 130 days after conception. Both Cd and Zn concentrations in foetal and adult liver and kidney tissue were correlated with MT mRNA.

Keywords: Cadmium; zinc; foetal development; placental transfer.

INTRODUCTION

In adult sheep, cadmium (Cd) accumulates in both liver and kidney tissue in an age dependant manner. We have previously shown that accumulation is most rapid over the few months immediately after weaning (Lee *et al.* 1994b). However as turnover of Cd from the kidneys is slow, in older ewes (3-5 yrs) concentrations of Cd may be in excess of 1mg Cd/kg FW of kidney tissue. Liver stores of Cd, which are much more mobile than those in the kidney, and total body Cd are also elevated. Although over 99% of daily Cd intake by a grazing sheep is excreted, and as a result daily net absorption is small, total body Cd content, especially that in the liver and kidney, continues to increase with age. For example we have shown that in an animal with a daily Cd intake of 750 μg/day, 746 μg Cd/day is excreted in the faeces, with small amounts in the urine and milk (about 0.5 and 0.2 μg Cd/day respectively). The liver and kidney accumulates 1.3 and 0.4 μg Cd/day respectively, while the remainder (2.3 μg Cd/day) is distributed in all other body tissues (Grace *et al.* 1993, Lee *et al.* 1994b). Cadmium which may be mobilised from some tissues, especially the liver, may impact on the developing foetus through placental transfer. Cadmium and zinc (Zn) in animals bind with the same metal-regulatory protein - metallothionein (MT) - which is involved in transport and storage of both metals. Cadmium binds to MT more strongly than Zn and it is this strong binding of Cd within the MT complex which provides the basis for Cd detoxification in animals (Petering and Fowler, 1986). In their early stage of development the livers of foetal animals contain elevated concentrations of zinc (Zn) most of which is bound with MT (Paynter *et al.* 1990). The purpose of the present investigation, therefore, was: to quantify the accumulation of Cd in the developing foetus and compare this with Zn movement in the liver and kidney; to establish any relationship with the Cd status of the maternal parent; and to identify factors affecting its accumulation up to, and shortly after, parturition.

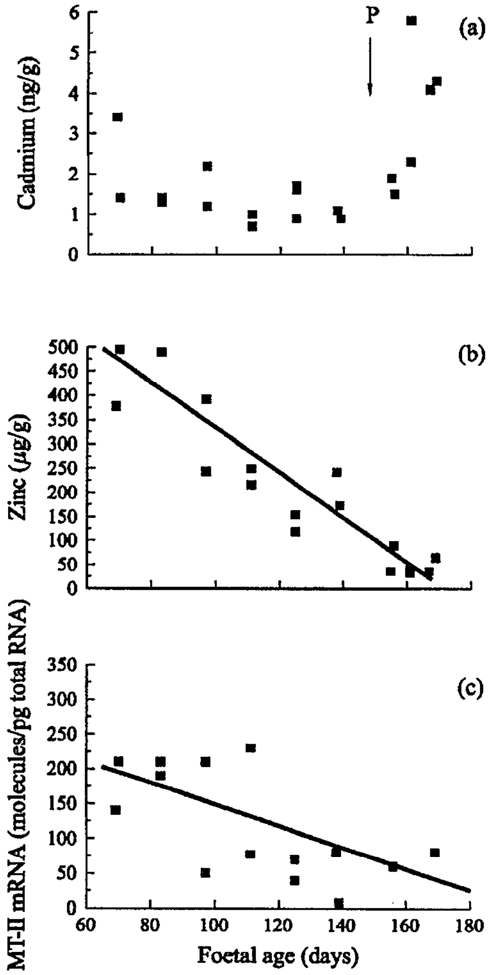
MATERIALS AND METHODS

Liver and kidney samples were collected from both Romney and Merino foetuses, approximately fortnightly, from 60 days after conception up to parturition, and from the suckling lamb, weekly over three weeks (Hocking Edwards *et al.* 1994). Matching liver and kidney samples were also obtained from the dam. Tissue sub-samples were analysed for Cd by Zeeman electrothermal atomic absorption and for Zn by plasma emission spectrometry (Lee, 1983). Metallothionein, a high cysteine-containing protein, binds both Cd and Zn and is important in both regulation and storage of these metals. The same tissues were therefore analysed for MT-mRNA by dot-blot hybridisation of total RNA extracts to assist in identifying regulatory aspects of Cd accumulation in hepatic foetal tissue and any relationship between Cd and Zn. (Lee *et al.* 1994a).

RESULTS AND DISCUSSION

Figure 1 gives the change in Cd, Zn, and MT-II mRNA concentrations in liver tissue from 60 to approximately 150

Figure 1: Concentration of (a) Cd, (b) Zn and (c) MT-II mRNA in liver tissue from the developing foetus and neonate of Merino sheep from 60 days after conception to three weeks post-parturition (P, approximate parturition date).



day old foetus (ie up to parturition) and from neonates up to three weeks of age from Merino dams. Data from Romney animals over the same time period was similar. The concentration of Zn (Fig. 1b) in liver tissue of the early foetus was high. This has been observed in other studies and could be attributed to its role in an especially active period of cell replication and protein synthesis (Paynter *et al.* 1990). Zinc concentration decreased linearly with foetal development ($r^2 = 0.84$, $P < 0.001$) to a level in the three week neonate which was similar to that of the adult sheep. The concentration of MT-II mRNA (indicative of MT protein synthesis) was correlated with Zn ($r^2 = 0.38$, $P = 0.012$) and showed a similar decline with foetal age (Fig. 1c). However the trend for Cd (Fig. 2a) in the developing foetal liver was different to that of Zn (Fig. 2b). The Cd concentration in liver tissue was very low and remained relatively constant up to parturition, after which a marked rise occurred. In the three week old neonate the Cd concentration had more than doubled. There was no significant difference in foetal liver concentrations of Cd between the Merino and Romney. There was also no significant relationship between the Cd concentrations in the foetal liver (or kidney) and those of the maternal parent. That is those maternal parents with a high Cd status did not have offspring with correspondingly high organ Cd levels.

The organ weights in the developing foetus increase rapidly in mass, particularly over the first 100 or so days of gestation, and it is therefore noteworthy to examine the Cd and Zn levels in the liver and kidney in terms of total content or pool size. These data are given in Fig. 2 for Cd and Zn in foetal and neonate liver for the combined data from both Merino and Romney animals and in Fig. 3 for the corresponding kidney data (there was no significant difference between the Romney and Merino data). It should be noted that the amount of Zn in both tissues was over 3 orders of magnitude greater than for Cd. The Cd content in the foetal liver (Fig. 2a) and in the matching kidney (Fig. 3a) remained low indicating little input up to parturition, after which the content increased as a consequence of the neonates independence from the dam and its direct exposure to the environment and exogenous sources of Cd (including that in milk). However, between about 110 and 130 days of gestation a small flux of Cd, into the liver especially, was apparent. Thus, although small, there was some placental transport of Cd into the foetus. This input was concurrent with the movement of Zn into the liver which was markedly much greater (Fig. 2b). The additional Zn was then mobilised from the liver after about 120 days and was presumably utilised for further foetal development. There was some storage of Zn in the kidney, with the Zn content continuing to increase up to and after parturition (Fig. 3b). A similar pattern was also seen for the Cd content in the kidney (Fig. 3a). In both the foetus and adult, Cd and Zn were correlated ($P<0.05$) with MT synthesis. Cadmium accumulates in the kidney bound to MT protein (which binds Cd more tightly than Zn; Petering and Fowler, 1986). Cadmium continues to accumulate rapidly in both liver and kidney tissue of grazing sheep especially over the first six months after parturition and in the kidney, continues to accumulate with age but at a slower rate (Lee *et al.* 1994b).

FIGURE 2: Total content of (a) Cd and (b) Zn in the liver from the developing foetus and neonate of Merino and Romney sheep from 60 days after conception to three weeks post-parturition (P, approximate parturition date).

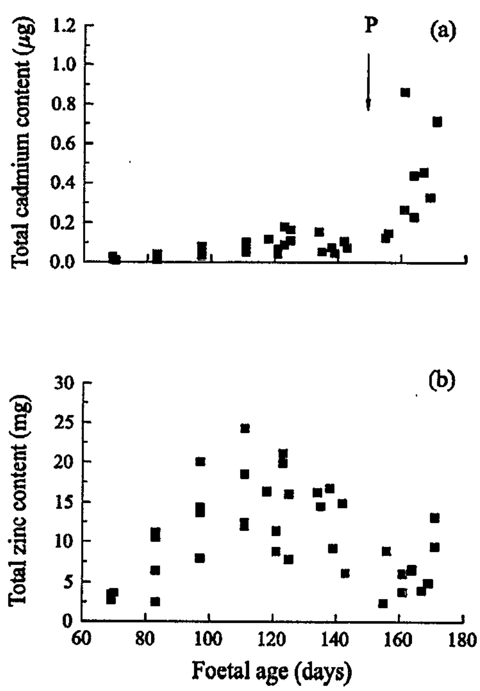


FIGURE 3: Total content of (a) Cd and (b) Zn in the kidney from the developing foetus and neonate of Merino and Romney sheep from 60 days after conception to three weeks post-parturition (P, approximate parturition date).

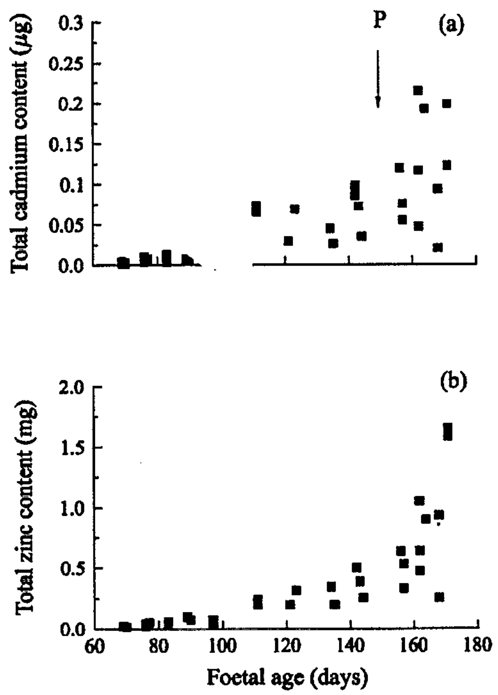


Table 1 gives comparative data for the mean Cd concentrations in liver and kidney tissue from foetus, neonate and their mixed-aged maternal parents. Early in life the Cd concentrations were extremely low in both organs, indicating that from conception to parturition, placental transfer of Cd was minimal. In one particular ewe for which Cd concentrations in the liver and kidney were especially high (>5µg Cd/g fresh tissue), foetal organ concentrations were not significantly different to those of foetus from dams with much lower Cd tissue concentrations. Cadmium concentrations in the dams blood from this study were not determined, however in other work we have found that the concentration of Cd in plasma was typically in the range 0.1-0.3 ng Cd/g plasma (Lee and Rounce, unpublished work). Variation in blood Cd concentrations related to age of animal, total body content of Cd and Cd intake is not known.

TABLE 1: Mean concentrations (ng Cd/g fresh weight) of cadmium in liver and kidney tissue from foetus (average for 60-150d of gestation), neonate (average for 1-3 weeks post-parturition) and their corresponding maternal parents. Results are mean ± s.e .

	Romney		Merino	
	Liver	Kidney	Liver	Kidney
Foetus (60 - 150 d)	1.1±0.1(18)†	2.5±0.25(17)	1.45±0.2(13)	1.5±0.2(12)
Neonate (1 - 3 weeks)	2.4±0.4(6)	2.8±0.4(5)	3.3±0.7(6)	3.4±0.4(6)
Maternal parent (3-5 yrs)	139±8(28)	1208±99(26)	205±68(15)	953±159(15)

† ( ) No. of animals

CONCLUSION

The concentration of Cd in organs of the maternal parent did not appear to influence Cd uptake by the developing foetus. This seems logical as the potential for Cd to replace Zn in key processes would have marked toxic effects. Although there was an indication of some placental transport of Cd analogous to that of Zn during foetal development (about 110-130 days), the Cd content of foetal organs remained low until parturition. After parturition the neonate would absorb Cd directly from its environment and must then develop its own mechanism for detoxification. The relationship between the level of Cd intake by the dam and foetal Cd accumulation is the subject of future work.

REFERENCES

Grace, N.D., Rounce, J.R. and Lee, J. 1993: Intake and excretion of cadmium in sheep fed fresh herbage. *Proceedings of the New Zealand Society of Animal Production*, **53**: 251-253.

Hocking Edwards, J.E., Birtles, M.J., Harris, P.M., Parry, A.L., Paterson, E., Wickham, G.A. and McCutcheon, S.N. 1994: Prenatal wool follicle development in Romney, Merino and Merino-Romney cross sheep. *Proceedings of the New Zealand Society of Animal Production*, **54**: 131-134.

Lee, J. 1983: Multi-element analysis of animal tissue by inductively coupled plasma emission spectrometry. *ICP Information Newsletter*, **8**: 553-561.

Lee, J., Treloar, B.P. and Grace, N.D. 1994a: Metallothionein and trace element metabolism in sheep tissues in response to high and sustained zinc dosages. II. Expression of metallothionein m-RNA. *Australian Journal of Agricultural Research*, **45**: 321-332.

Lee, J., Grace, N.D. and Rounce, J.R. 1994b: Cadmium accumulation in liver and kidney of sheep grazing ryegrass/white clover pastures. *Proceedings of the New Zealand Animal Production Society*, **54**: 31-34.

Paynter, J.A., Camakaris, J. and Mercer, F.B. 1990: Analysis of hepatic copper, zinc, metallothionein and metallothionein-Ia mRNA in developing sheep. *European Journal of Biochemistry*, **190**: 149-154.

Petering, D.H. and Fowler, B.A. 1986: Discussion summary. Roles of metallothionein and related proteins in metabolism and toxicity: Problems and perspective's. *Environmental Health Perspectives*, **65**: 217-224.