Response of trace element concentrations in plasma of sheep to chronic infusion of a recombinant variant of IGF-1

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ABSTRACT

As part of a study measuring the effect of a variant of insulin-like growth factor-1(long-arg^3-IGF-1; LR3IGF-1) on tissue amino acid utilisation in sheep, changes in plasma Cu, Fe and Zn concentrations were observed. LR3IGF-1 was infused continuously over 21 days into the skin of 6 sheep using a bilateral arterio-venous preparation. Another 6 sheep were infused in the same way with saline only (controls). Feed intakes were the same for both groups. Arterial blood samples were obtained prior to the start of treatment and at 1, 7, 14 and 21 days. Plasma samples were prepared and analysed for concentrations of endogenous IGF-1, insulin, ceruloplasmin, macro-elements (Ca, Mg, Na, P and S) and the trace elements, Cu, Fe, Mn and Zn. Plasma concentrations of the macro-elements were unchanged over the treatment period and were not significantly different between the two groups. However there was a significant effect of treatment ($P<0.001$) and treatment x time interaction ($P=0.025$) on the Cu concentration in plasma. After 7 days the Cu concentration in plasma of the LR3IGF-1 group increased from 1.06±0.04 to 1.84±0.11 μg/mL compared with 0.94±0.07 and 1.27±0.08 μg/mL for the control group at 0 and 7 days respectively. This difference continued for the remainder of the treatment period. Zinc concentrations in plasma significantly ($P<0.001$) decreased in response to LR3IGF-1 treatment (overall means: 0.83 and 0.69 mg/mL for the treatment and control groups respectively; LSD, 0.06). Plasma Fe concentrations declined with time, but there was no significant treatment effect. Within the treatment group plasma Cu concentration was significantly ($P<0.05$) correlated with concentration of endogenous IGF-1 and strongly negatively correlated with the concentration of Fe in plasma. Within both groups concentrations of Cu and ceruloplasmin in plasma were strongly correlated ($P<0.001$). These results suggest that chronic endocrine imbalances, such as those imposed from the LR3IGF-1 treatment, may result in changes to tissue distribution of trace elements. Such effects may result in secondary complications. However, an increase in Cu availability for nonhepatic tissue use may be beneficial, particularly in situations of mild, chronic, Cu deficiency.

Keywords: Plasma; Insulin-like growth factor-1; copper; zinc; ceruloplasmin; sheep.

INTRODUCTION

In New Zealand agriculture the status of several trace elements, especially that of copper (Cu), is of special interest. Over recent years several studies have indicated that changes in the transport and metabolism of micronutrient trace elements e.g. Cu, iron (Fe) and zinc (Zn), and their metalloproteins, are regulated by hormones (Cousins, 1985; DiSilvestro, 1988). Recently we have been interested in the effects of one of these, insulin-like growth factor (IGF-1) - an important mediator of animal growth (McGuire *et al.*, 1992) - and its influence on local blood flow and amino acid utilisation in peripheral tissues of sheep (Harris *et al.*, 1993). The interdependency of Zn and IGF-1 in growth retardation has been established and plasma IGF-1 could be a useful indicator for diagnosing Zn deficiency, in monogastrics (Cossack, 1991) and in calves (Kirchgessner and Heindl,. 1993). Further, alterations in Cu and Zn metabolism associated with the diabetic state (Zn is co-released with insulin) are known (Failla, 1983). The effects of both Cu and IGF-1, acting either independently or together, may have important implications for bone growth (DiSilvestro *et al.*, 1988; Cymaluk and Smart, 1993). In a recent investigation we administered a variant of IGF-1 (Long-arg^3-IGF-1; LR3IGF-1) into a skin artery over 21 days and measured a range of metabolic changes including amino acid uptake, wool follicle characteristics and wool production (Hocking Edwards *et al.* 1995). In this paper we report concomitant whole body changes to plasma trace element concentrations in response to the LR3IGF-1 treatment.

MATERIAL AND METHODS

Twelve, 1-year old, castrated male Romney sheep (30-35kg) were surgically modified as described previously (Hocking Edwards *et al.* 1995) to allow infusion of either 950 μg/day of LR3IGF-1 or physiological saline into the deep circumflex iliac artery of a discrete skin patch. Animals were maintained, before and after surgery, on lucerne pellets and hay available *ad libitum* at hourly intervals from overhead constant feeders. Feed intake returned to pre-surgery levels within 24h for all animals. During the treatment period the control sheep (infused with physiological saline only) were pair fed to the *ad libitum* intake of the LR3IGF-1 treated sheep. There were six animals in each group.

Blood sampling was achieved through a catheter in the medial saphenous artery using a peristaltic pump at rate of 0.36 g/min, via polyvinylchloride tubing, and collected into polypropylene tubes containing 0.1 mL heparinised (1,000 IU/mL L) saline (Hocking Edwards *et al.* 1995). Insulin and endogenous IGF-1 concentrations in plasma were determined by radioimmunoassay (Kelly *et al.* 1993; Prosser and Davis, 1992) and ceruloplasmin (the major Cu transport protein

found in plasma) by measuring its peroxidase activity (Smith and Wright, 1974). Elemental concentrations (Ca, Cu, Fe, K, Mg, Na, P, Zn) in plasma were determined by simultaneous plasma emission spectroscopy (Lee, 1983). Metabolic measurements in samples from control and LR3IGF-1 treatment animals over the experimental period (pre-infusion, 1, 7, 14, 21 days of infusion) were analysed by analysis of variance using the general linear model procedure for interactions and the repeated measures option (SAS, 1985).

TABLE 1: The effect of the recombinant IGF-1 variant, LR3IGF-1, on some plasma metabolites compared to their pair-fed controls. Overall means (n=6), main effects and first order interactions.

				Effect (P)		
	+LR3IGF-1	Control	LSD	Treatment	Time	Interaction
IGF-1 (ng/mL)	83	79	19	NS	0.002	0.02
Insulin (pg/mL)	400	428	60	NS	0.002	0.05
Copper (µg/mL)	1.6	1.1	0.14	<0.001	<0.001	0.025
Zinc (µg/mL)	0.69	0.83	0.06	<0.001	NS	NS
Iron (µg/mL)	1.4	1.7	0.3	NS	<0.001	NS
Ceruloplasmin (U/L)	48	38	6	0.001	0.002	NS

RESULTS AND DISCUSSION

Feed intake in both groups was approximately 1.2 x maintenance and there was no significant difference in feed intake between control and LR3IGF-1 treated sheep. Total endogenous IGF-1 concentrations in plasma of the control sheep over the trial period were not significantly different over time however there were significant changes ($p<0.05$) in plasma IGF-1 concentrations with time in the LR3IGF-1 sheep (Fig. 1a). After day 7 of the LR3IGF-1 infusion, endogenous IGF-1 had significantly decreased compared to the control sheep, but over the next 2 weeks concentrations increased and by day 21 of treatment, IGF-1 concentrations in plasma were significantly higher ($P<0.05$) than those of the controls. Plasma insulin concentrations showed a similar trend over the 21 day treatment period (Hocking Edwards *et al.* 1995). As with endogenous IGF-1, the overall mean plasma concentrations of insulin were not significantly different between the two treatment groups over the 21 day period. There was however a significant interaction between treatment and time for both plasma IGF-1 and insulin concentrations (Table 1). These linked changes in whole body plasma IGF-1 and insulin in response to the LR3IGF-1 treatment have been ascribed to an acute effect of LR3IGF-1 on insulin, with concomitant changes to endogenous IGF-1, and then these changes in endogenous IGF-1 over-riding those of the LR3IGF-1 treatment in the later periods (Hocking Edwards *et al.* 1995).

Plasma concentrations of the macro-elements (Ca, K, Mg, Na and P) were unchanged over the treatment period and were not significantly different between the groups. However there was a significant treatment effect ($P<0.001$) and treatment x time interaction ($P=0.025$) for the Cu concentration in plasma (Table 1). After 7 days the Cu concentration in plasma of the LR3IGF-1 group increased from 1.06±0.04 to 1.84±0.11 µg/mL compared with 0.94±0.07 and 1.27±0.08 µg/mL for the control group at 0 and 7 days respectively. This difference continued over the remainder of the treatment period (Fig. 1b). Zinc concentrations in plasma significantly ($P<0.001$) decreased in response to LR3IGF-1 treatment (Fig 1c; overall means: 0.69 and 0.83 g/mL for the treatment and control groups respectively). However, unlike the response for Cu, there was no time effect (Table 1) with the difference in plasma Zn concentrations between the LR3IGF-1 treatment group and the control sheep constant over the infusion period. Plasma Fe concentrations declined with time but there was no significant overall treatment effect. Within the treatment group plasma Cu concentration was significantly ($P<0.05$) correlated to endogenous IGF-1 concentration and strongly negatively correlated with the concentration of Fe in plasma. There was no correlation with plasma Zn. Combining both groups plasma Cu and ceruloplasmin peroxidase activity were strongly correlated ($P<0.001$, Fig 2). Not surprisingly the effect of LR3IGF-1 on ceruloplasmin was the same as that for Cu (Table 1) indicating that that the increase in Cu

FIGURE 1: Concentrations (mean ± s.e.) of (a) IGF-1, (b) copper and (c) zinc in plasma of sheep immediately prior to treatment and then at time intervals after the start of infusion of either ● LR3IGF-1 or ○ saline only.

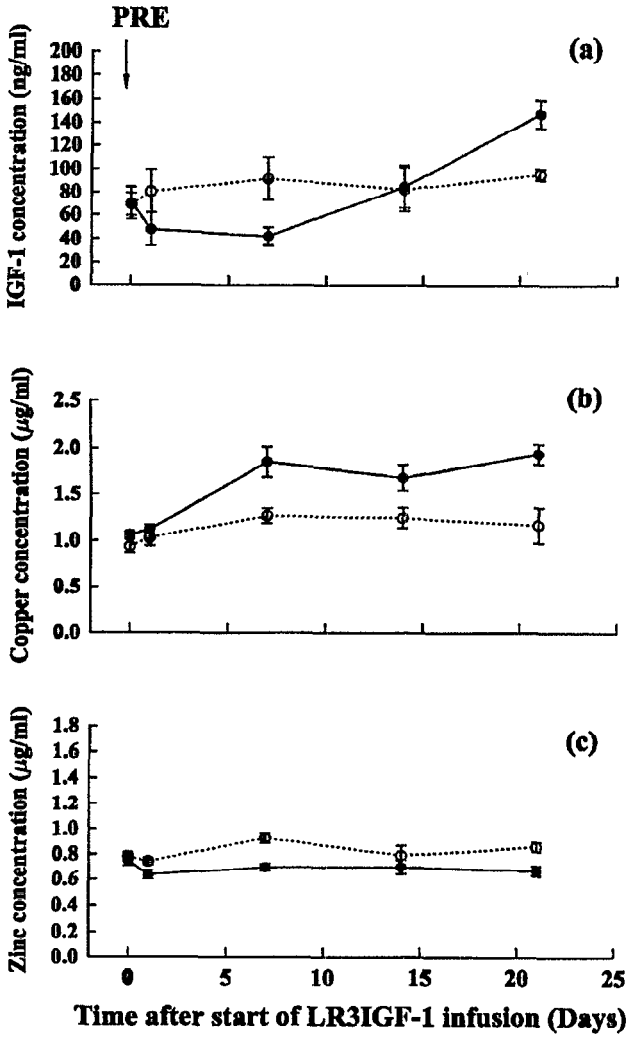
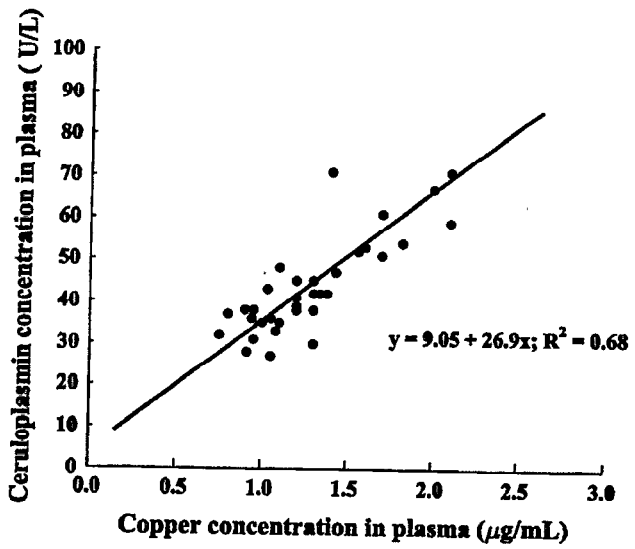


FIGURE 2: Relationship between copper and ceruloplasmin concentrations in plasma of sheep within both LR3IGF-1 and control groups.



concentrations in plasma resulted from a release, presumably from the liver, of increased ceruloplasmin protein. We were unable to analyse liver material which may have indicated remobilisation from the large liver Cu store rather than any change to either the rate of biliary Cu excretion or input from gut absorption. Further work would be required to elucidate the mechanism of the observed response.

The initial responses (over the first 7 days) of the measured plasma metabolites to the LR3IGF-1 infusion are similar to those observed in the diabetic state, in which plasma Zn and insulin are positively correlated, and, in at least one study, associated with an increase in plasma Cu concentration (Schlienger *et al.* 1988). However, after 14 days, endogenous plasma IGF-1 (and insulin) concentrations had increased in the treatment group compared with the pair-fed control sheep (Fig 1a), whereas for the Cu and Zn concentrations in plasma of both treatment and control animals, the relative differences remained similar throughout the treatment period. From these data we are unable to determine whether the effect of continued supplementation with LR3IGF-1 itself on plasma Cu and Zn is a direct and independent effect or a consequence of the resulting changes to endogenous IGF-1 and/or insulin , or to other unmeasured hormonal changes.

CONCLUSION

Clearly chronic endocrine imbalances may result in changes to the tissue distribution of trace elements, such as Cu and Zn, which in turn may contribute to secondary effects, the nature of which could be either beneficial or detrimental. In this study the increase in circulating plasma Cu concentration (concomitant with changes to plasma ceruloplasmin) in response to a chronic infusion of a recombinant IGF-1 analogue was particularly marked (>50 %). Ceruloplasmin is classified as an acute-phase protein (Cousins, 1985) because its synthesis is stimulated by a range of factors including fasting, stress and infection, as well as a range of hormones. However in this study control and treated animals were subjected to identical conditions, therefore differences in ceruloplasmin cannot be attributed to stress conditions. Increased ceruloplasmin-Cu plasma concentrations are of interest because of possible beneficial effects. For example, ceruloplasmin has a possible role as an antioxidant and could be a major scavenger of superoxide radicals in plasma especially when its concentration is elevated (Cousins, 1985). Wiener *et al.* (1985) established a genetic selection in sheep for high and low Cu concentrations in plasma with the improved performance in the high selection line attributed to the elevated ceruloplasmin levels. In another important role ceruloplasmin donates Cu to lysyloxidase, a Cu-enzyme involved in collagen cross-linking, leading to stability and normal bone development. IGF-1 is also implicated in bone proliferation and maturation processes (McGuire *et al.* 1992) and in a recent study in which sheep were given recombinant IGF-1 for 8 weeks significant increases in bone weight per unit length were measured (Cottam *et al.* 1992). Unfortunately in that study Cu plasma concentrations were not determined. However clarifying the mechanisms of these linked effects between Cu and IGF-1, particularly in relation to improved bone development, warrants further research especially under the conditions of marginal supply which characterise Cu nutrition in New Zealand pastoral livestock.

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