

Somatomedin-C and zinc status in rats as affected by Zn, protein and food intake

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1. The objective of the present experiment was to study the level of plasma somatomedin-C (SM-C) and the status of zinc in rats as affected by three levels of Zn given in combinations with two levels of protein.

2. Six groups of rats were fed, for 21 d, on six different diets based on combinations of two levels of dietary protein (low protein, 75 g/kg; high protein, 200 g/kg) and three levels of zinc (low Zn, 0.9 µg/kg; moderate Zn, 55 µg/kg; high Zn, 110 µg/kg). All groups were pair-fed with the group receiving the low-Zn-low-protein diet. An additional group of six rats served as an *ad lib.*-fed control group and was fed on a diet that contained 55 µg Zn/kg and 200 g protein/kg *ad lib.*

3. Body-weight gain and food intake were recorded daily. Rats were killed at the end of the experimental period (21 d). Zn was assayed in plasma, tibia and liver by atomic absorption technique. Plasma SM-C was assayed by radioimmunoassay.

4. In rats given the low-Zn-low-protein diet, the level of plasma SM-C increased in response to the increase in the amount of Zn or Zn and protein in the diet. However, no change was observed when the level of protein alone was increased.

5. Among all groups tested, *ad lib.*-fed rats showed the highest level of plasma SM-C. Thus it may be concluded that a balanced diet combined with adequate food intake is necessary to maintain an optimal level of plasma SM-C.

Somatomedins are peptides of low molecular weight which are regulated by growth hormone (GH; Salmon & Daughaday, 1957), nutrition (Phillips & Young, 1976a; Phillips & Orawski, 1977; Phillips *et al.* 1978; Cossack, 1984) and insulin (Phillips & Orawski, 1977). It has been documented that GH produces growth indirectly through generation of a serum factor(s), somatomedin (SM), which acts directly to produce cartilage proliferation and linear skeletal growth (Daughaday *et al.* 1972). However, low levels of SM with associated growth failure, despite elevation of GH, were noted in Laron-type dwarfism (Laron *et al.* 1966) and in protein-energy malnutrition (Tanner *et al.* 1966; Hintz *et al.* 1978). On the other hand, a normal level of SM associated with a low level of GH was observed in children who were hyperphagic and hyperinsulinaemic after hypothalamic surgery (Costin *et al.* 1976).

The recent establishment of a method(s) for measuring the activity of SM (Van den Brande & Du Caju, 1974; Furlanetto *et al.* 1977) has prompted further investigation of the roles of many factors in growth. However, studies on the effect of some nutrients on the activity of SM in man and experimental animals have not been conclusive. An inverse relation between dietary fat and plasma SM was observed in rats (Reeves *et al.* 1979). Moreover, the level of SM increased with increasing levels of protein and no change was observed in the levels of GH (Reeves *et al.* 1979; Prewitt *et al.* 1982). Most recently, we reported a significant decrease in the level of somatomedin-C (SM-C) in zinc-deficient rats when they were compared with pair-fed, continuously-pair-fed and *ad lib.*-fed rats (Cossack, 1984). In the same study, we observed that plasma SM-C correlated with Zn concentration in the tibia (r 0.79, P < 0.005) and with body-weight gain (r 0.96, P < 0.001). We concluded that growth retardation associated with Zn deficiency could be related to the adverse effect of Zn deficiency on the generation of SM-C, and Zn supplementation reverses this effect.

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Table 1. *Composition of experimental diets** (g/kg diet)

Ingredient	Diet					
	LZLP	MZLP	HZLP	LZHP	MZHP	HZHP
Egg-white solids, spray-dried	93.750	93.750	93.750	225.000	225.000	225.000
Glucose monohydrate (dextrose)	756.246	756.095	756.246	624.996	624.996	624.694
Cellulose powder	30.000	30.000	30.000	30.000	30.000	30.000
Oil, maize	100.000	100.000	100.000	100.000	100.000	100.000
Mineral mixture†	10.000	10.000	10.000	10.000	10.000	10.000
Vitamin mixture‡	10.000	10.000	10.000	10.000	10.000	10.000
Biotin	0.004	0.004	0.004	0.004	0.004	0.004
ZnSO ₄ · 7H ₂ O	0.000	0.151	0.302	0.000	0.151	0.302

* L, low; M, moderate; H, high; P, protein; Z, zinc.

† Supplied (g/kg diet): calcium phosphate, dibasic 19.767, potassium chloride 2.2882, sodium chloride 0.7781, magnesium sulphate 2.4752, MnSO₄ · H₂O 0.1662, FeSO₄ · 7H₂O 0.20, potassium iodate 0.0004, cupric sulphate 0.0151.

‡ Teklad test diets; Teklad, Madison, Wisconsin, supplied (g/kg diet): maize starch 4.66, choline dihydrogen citrate 3.496, ascorbic acid (97.5%) 1.1016, α -tocopherol (500 μ g/g) 0.242, *p*-aminobenzoic acid 0.11, inositol 0.11 and (mg/g diet): biotin 0.441, cyanocobalamin (1 g/l mannitol) 29.74, calcium pantothenate 66.08, pteroylmonoglutamic acid 1.982, menadione 49.56, nicotinic acid 99.12, pyridoxine hydrochloride 22.03, riboflavin 22.03, thiamin hydrochloride 22.03, retinoic acid (500 μ g/g) 39.65, ergocalciferol (500 μ g/g) 4.405.

The increased growth rate in the world population may severely limit the supply of protein. Malnourished populations have often been supplemented with grain sources of protein. It has been shown that poor-quality sources of protein should be given at relatively high levels (compared with high-quality sources) to promote optimal growth (Cossack & Weber, 1983). Moreover, the addition of Zn has been shown to improve the biological value of poor-quality sources of protein (Bolorforooshan & Markakis, 1977). Thus, the objective of the present study was to investigate the changes in growth rate, plasma SM-C and Zn status in rats given two levels of dietary protein in combinations with three levels of Zn.

MATERIALS AND METHODS

Thirty-six white, male, growing rats (35-d-old) of the Holtzman strain (Holtzman Co., Madison, Wisconsin) were given six test diets (Table 1) based on combinations (Table 2) of two levels of protein (low protein (LP), 75 g/kg; high protein (HP), 200 g/kg), and three levels of Zn (low Zn (LZ), 0.9 μ g/kg; medium Zn (MZ), 55 μ g/kg; high Zn (HZ), 110 μ g/kg). The basal diet contained 0.9 μ g Zn/kg by analysis. The animals were housed in individual stainless-steel wire-bottom cages under 12 h light–12 h dark periods in a room at a controlled temperature (22°). Initial body-weights of the rats ranged from 95 to 100 g. The rats were given a standard diet (rat chow, Purina Co., St Louis, MO) for 4 d after which they were randomly allocated to the six experimental diets. The amount of diet given to rats in each group matched the intake of their counterparts of the group given the LZLP diet. An additional group of six rats served as an *ad lib.*-fed control group and was given the MZHP diet *ad lib.* The rats were given free access to distilled–deionized water throughout the experimental period (21 d). Rats were mildly anaesthetized with diethyl ether and blood drawn by heart puncture after which they were killed by decapitation. The blood was collected (using EDTA as anticoagulant) in plastic tubes free of Zn, immediately centrifuged (2000 g) for 10 min and plasma was separated and kept at –60° for SM-C

Table 2. Food intake, body-weight gain, concentration of zinc in tissues and levels of plasma somatomedin-C

(Mean values and standard deviations)

	Dietary protein (g/kg)	Zn content ($\mu\text{g}/\text{kg}$)							
		0.9		55		110		<i>Ad lib.</i> -fed controls	
		LZLP	LZHP	MZLP	MZHP	HZLP	HZHP		
75...	200...	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Food intake (g/rat per d)	75	8.36 ^a	0.8	8.36	—	8.36	—	15.0 ^b	2.6
	200	8.36	—	8.36	—	8.36	—		
Body-wt gain (g/rat per d)	75	0.9 ^a	0.1	1.7 ^b	0.1	1.6 ^b	0.3	5.3 ^d	1.1
	200	1.1 ^a	0.1	2.2 ^c	0.3	2.3 ^c	0.2		
Plasma Zn ($\mu\text{g}/\text{l}$)	75	540 ^a	6.0	1410 ^b	20.0	1470 ^b	12.0	1720 ^c	10.0
	200	540 ^a	10.0	1810 ^c	25.0	1790 ^c	26.0		
Tibia Zn ($\mu\text{g}/\text{g}$ dry wt)	75	116 ^a	8.0	213 ^b	9.0	217 ^b	11.0	273 ^d	12.0
	200	120 ^a	15.0	236 ^c	15.0	250 ^c	19.0		
Liver Zn ($\mu\text{g}/\text{g}$ dry wt)	75	92 ^a	19.0	136 ^b	14.0	136 ^b	16.0	138 ^{bd}	9.0
	200	112 ^c	10.0	150 ^d	15.0	140 ^{bd}	11.0		
Somatomedin-C: units/ml plasma	75	0.90 ^a	0.1	1.24 ^b	0.3	1.22 ^b	0.1	2.3 ^c	0.3
	200	0.89 ^a	0.1	1.37 ^b	0.1	1.20 ^b	0.1		
% of <i>ad-lib.</i> control	75	39.0	—	53.9	—	53.0	—	100	
	200	38.7	—	59.6	—	52.2	—		
% of pair-fed control	75	65.7	—	91.0	—	89.0	—	167.9	
	200	65.0	—	100	—	88.0	—		

^{a-d} Mean values with unlike superscript letters were significantly different; minimum level of significance; $P < 0.05$.

† LZLP group was fed *ad lib.* The intake of the other groups, except the *ad lib.*-fed group, matched the intake of their counterparts of the LZLP group. For details, see p. 164.

determination using a radioimmunoassay method (reagents including SM-C standards and controls were contributed by Nichols Institute, CA, USA, and the basic method is described by Furlanetto *et al.* 1977). A portion of the blood was drawn using Zn-free heparin as recommended for the determination of plasma Zn (Whitehouse *et al.* 1982). Tibia and liver were isolated, cleaned from debris with distilled-deionized water, lyophilized, weighed and digested with nitric acid for the determination of Zn using a flame atomic absorption spectrophotometer (Model 406; Perkin Elmer, Norwalk, Conn.).

Duncan's multiple-range test was employed for statistical analysis (Snedecor & Cochran, 1968).

RESULTS

Table 2 shows body-weight gain, concentrations of Zn in tibia, plasma and liver and the levels of plasma SM-C in rats. Body-weight gains in the groups of rats given Zn-deficient diets (LZLP and LZHP) were significantly ($P < 0.001$) lower when compared with those given high-Zn diets (HZLP and HZHP) regardless of the level of protein in the diet. Increasing the level of protein in the LZLP diet did not result in a significant increase in body-weight (LZLP *v.* LZHP). However, body-weight gain increased significantly ($P < 0.005$) when the level of protein was increased from 75 to 200 g/kg in combination with MZ or HZ (MZLP *v.* MZHP and HZLP *v.* HZHP).

An increase in plasma Zn ($P < 0.005$) concentration was observed in response to the high

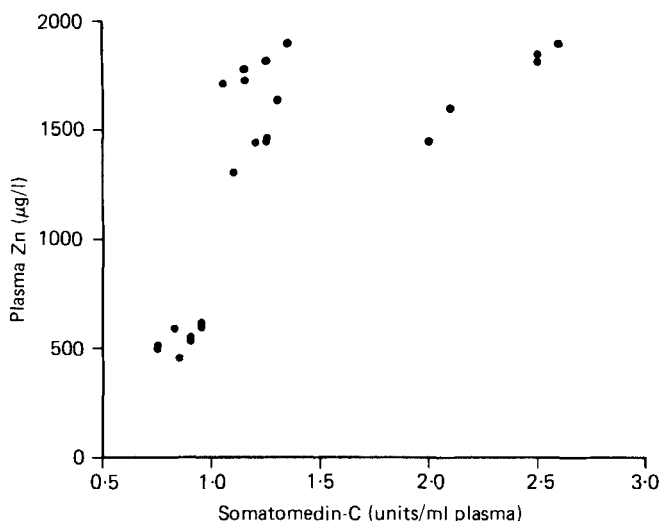


Fig. 1. Relation between the level of plasma somatomedin-C (SM-C) and the concentration of plasma Zn in rats.

level of protein in combination with MZ or HZ. Rats given the MZ diet showed a significant increase ($P < 0.0001$) in the concentration of Zn in plasma regardless of the level of protein. No additional effect was observed when rats were given the HZ diet.

The concentration of Zn in the tibia increased significantly ($P < 0.005$) in response to an increased level of dietary protein in rats given MZLP or HZLP diets (Table 2). The concentration of Zn in the liver increased significantly in response to Zn (LZLP v. MZLP, $P < 0.001$) and also in response to increased level of dietary protein (LZLP v. LZHP, $P < 0.002$). Maximum concentration of liver Zn occurred when both protein and Zn were increased in the diet (MZHP).

Regardless of the level of protein intake, rats given the MZ or HZ diet exhibited significantly higher levels of plasma SM-C ($P < 0.005$) compared with Zn-deficient rats (Table 2). Among pair-fed groups, maximum level of SM-C was observed in the group of rats given 200 g protein/kg diet containing 55 µg Zn/kg (MZHP). In addition, further increase in Zn content to 100 µg/kg (as in HZHP and HZLP groups) did not result in a linear increase in the level of SM-C. Among all groups tested, the *ad lib.*-fed group showed the highest level of SM-C. The relations between the level of plasma SM-C and the concentration of Zn in plasma or tibia are shown in Figs 1 and 2 respectively.

DISCUSSION

The six different diets used in the present study were designed so that the effect of Zn or protein restriction, or both, on the level of plasma SM-C could be tested. This was achieved by comparing plasma SM-C levels in the different experimental groups with those of the pair-fed control (MZHP) and *ad lib.*-fed control groups (Table 2). The difference in the level of SM-C between the *ad lib.*-fed and pair-fed control groups reflected the effect of anorexia, since both groups were given the same diet except that the pair-fed control rats were given a restricted amount of food which matched the intake of their counterparts of the LZLP group. All experimental diets used in the present study were based on the same ingredients, and covered the nutrient requirements of the growing rat (except where Zn deficiency or

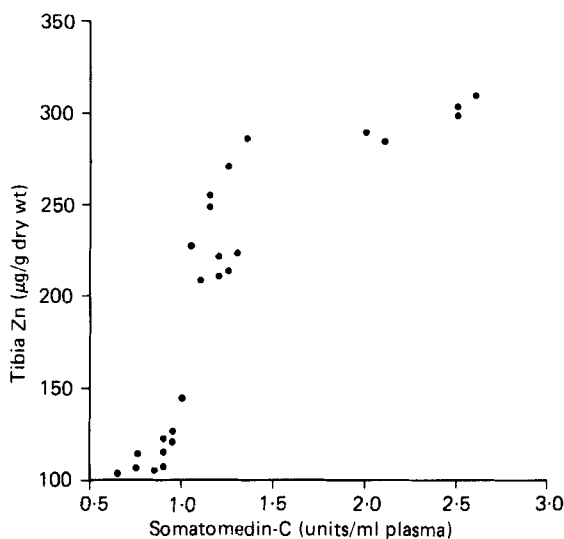


Fig. 2. Relation between the level of plasma somatomedin-C (SM-C) and the concentration of tibia Zn in rats.

protein restriction, or both, were indicated), and have been extensively used in studies involving Zn nutrition and metabolism in rats.

The utilization of Zn and protein are interdependent in animals (Hsue & Anthony, 1975; Magee & Grainger, 1979; Wallwork *et al.* 1983) and man (Sandström & Cederblad, 1980; Cossack & Prasad, 1983). Protein utilization is impaired in Zn-deficient states (Sandstead, 1973; Hsue & Anthony, 1975). On the other hand, the biological availability of Zn is dependent on the source and level of dietary protein (Cossack & Prasad, 1983; Wallwork *et al.* 1983). In the present investigation, body-weight values (Table 2) suggested that in rats given the LZ diet, growth was improved by the addition of Zn regardless of the level of protein in the diet (LZLP *v.* MZLP or HZLP, and LZHP *v.* MZHP or HZHP). Moreover, in rats given the LP diet and adequate Zn (MZLP or HZLP), growth was improved by increasing the level of dietary protein (MZLP *v.* HZHP or HZLP *v.* HZHP). However, increasing the level of protein in the diet did not result in an improvement in the growth of rats given the LZLP diet (LZLP *v.* LZHP). These findings are consistent with the results of Pedersen & Eggum (1983) who gave rats 80, 160 and 240 g casein/kg-based diets with 3, 6, 9 or 50 µg Zn/kg diet.

When rats were given 55 or 110 µg Zn/kg, plasma Zn concentration increased significantly in response to the higher level of protein in the diet. This finding is in contrast to the report of Wallwork *et al.* (1983) who gave rats 60, 110 and 150 g egg-white protein/kg with 6, 12 or 18 µg Zn/kg. This discrepancy may be due to the differences in the levels of Zn and protein being given. Van Campen & House (1974) reported an increased concentration of plasma Zn in rats given 150 g casein/kg in the diet compared with those given 50 g casein/kg; thus, their results are in support of our findings. Concentrations of Zn in the tibia and liver followed a similar trend with regard to the effect of dietary protein or Zn content or both. This effect was more pronounced in the tibia. Increasing the level of Zn or protein, or both, resulted in an increase in the concentration of Zn in the tibia and liver of rats. These findings are in accordance with previous reports (Magee & Grainger, 1979; Pedersen & Eggum, 1983; Wallwork *et al.* 1983).

Dietary composition is one of the most important factors affecting the synthesis of SM in man and experimental animals (Phillips & Young, 1976*a*; Phillips & Orawski, 1977; Prewitt *et al.* 1982; Cossack, 1984) and, thus, influences growth and development. Improvement in growth in response to Zn supplementation has been reported in man (Collip *et al.* 1982; Walraven & Hambidge, 1983; Prasad & Cossack, 1984) and experimental animals (Magee & Grainger, 1979; Wallwork *et al.* 1983). Recently, Cossack (1984) reported that plasma SM-C concentration is dependent on, in addition to other factors, the level of Zn in the diet. Furthermore, plasma SM-C correlated significantly with the Zn status of the rat. In the present study plasma SM-C concentration increased significantly when the Zn-deficient rats were given 55 or 110 μg Zn/kg regardless of the level of dietary protein (Table 2). Increasing the level of dietary protein from 75 to 200 g/kg, in rats given LZ diets, did not result in an increase in the concentration of SM-C in plasma. This finding conflicts with the results of Prewitt *et al.* (1982) who reported a linear increase in plasma SM-C concentration as protein intake increased from 50 to 150 g/kg, and declined when protein increased to 250 and 500 g/kg. This discrepancy may be the result of the difference in the feeding regimen: rats in the present study were pair-fed, while in the experiment of Prewitt *et al.* (1982) rats were fed *ad lib.* Restriction of food intake resulted in a 40% decrease in plasma SM-C concentration in the pair-fed control group (MZHP) as compared with the *ad lib.*-fed control group (Table 2). In this connection, Takano *et al.* (1978) reported a 74% decrease in SM concentration in rats fasted for 24 h compared with controls. Moreover, Phillips & Young (1976*b*), Takano *et al.* (1978), and Yeh & Aloia (1978) postulated that adequate food intake is required for the maintenance of normal levels of SM, and the generation of SM may be modulated by the processing of nutrients, thus providing support for our findings.

The concentration of Zn in the tibia or plasma has been used as an indicator of Zn status in the rat. The present study shows that the level of plasma SM-C was increased in response to the increase in the concentration of Zn in the plasma (Fig. 1) or in the tibia (Fig. 2). However, the relations between the level of plasma SM-C and plasma Zn concentration, and plasma SM-C and the concentration of Zn in the tibia, were not linear.

It may be concluded from the results of the present study that: (1) the level of plasma SM-C was adversely affected due to the restriction of food intake in the rat; (2) increasing the level of protein in the Zn-deficient diet given to growing rats did not result in an increase in the level of SM-C, however, increasing the level of Zn in the low-protein diet resulted in a significant increase in the level of SM-C; (3) a balanced diet combined with adequate food intake is necessary to maintain normal levels of plasma SM-C.

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