Changes in myocardial thyroid hormone metabolism and α-glycerophosphate dehydrogenase activity in rats deficient in iodine and selenium

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Weanling Wistar rats were fed on diets prepared from grain from areas deficient in I and Se where Keshan disease is endemic. Rats were divided into four groups, each of twelve rats, and received a diet supplemented with: I, Se, I+Se or nothing. At 8 weeks after weaning, myocardial α-glycerophosphate dehydrogenase (*EC* 1.1.1.8; α-GPD) activity and indices of Se and thyroid hormone status were determined. The group supplemented with iodine had increased plasma thyroxine levels. There was no difference in plasma triiodothyronine concentration between the groups but triiodothyronine levels in heart were reduced in the Se-supplemented group. Se supplementation increased myocardial glutathione peroxidase activity (*EC* 1.11.1.9) and the type I 5′-deiodinase (*EC* 3.8.1.4) activity in rat liver, but no type I 5′-deiodinase activity was detected in heart. α-GDP activity in heart was increased in the group supplemented with Se, I or both. There was a significant relationship ($P < 0.05$) between myocardial α-GDP activity and plasma thyroxine levels but not between α-GDP and myocardial glutathione peroxidase activity. The results indicate that iodine may be more important than Se in energy metabolism in the myocardium, which may give a new insight for the study of the aetiology of Keshan disease in areas where foodstuffs are deficient in both Se and I.

Selenium: Iodine: Thyroid: Keshan disease

Se deficiency is considered to be one of the main factors predisposing to Keshan disease (Yang *et al.* 1988). This is an endemic cardiomyopathy which occurs in areas where deficiencies of both Se and iodine occur, and is responsive to Se treatment (Ma *et al.* 1993). Plasma thyroxine (T_4) is increased in patients with Keshan disease (Yang *et al.* 1988), but no explanation for this observation was available until the demonstration that type I 5′-deiodinase (*EC* 3.8.1.4 ; IDI) is a Se-containing enzyme (Arthur *et al.* 1990). In rats Se deficiency inhibits conversion of T_4 to triiodothyronine (T_3) in non-thyroid tissues with resultant T_4 accumulation in plasma (Arthur *et al.* 1990).

Rats fed on diets containing grains from Keshan disease areas show reduced activity of α-glycerophosphate dehydrogenase (*EC* 1.1.1.8; α-GPD) in heart. Expression of this enzyme is regulated by T_4 and may be indicative of impaired energy metabolism (Liu *et al.* 1994). The change in α-GPD activity is very similar to that in hypothyroidism caused by I deficiency (Lee & Lardy, 1965). The relative effects of Se and I status on myocardial thyroid hormone metabolism have not been studied. As iodine is essential for thyroid hormone metabolism, we consider that it may play some role in the change of myocardial energy metabolism of patients with Keshan disease. Until now, the aetiological effect of I

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deficiency on Keshan disease has received very little attention. The present study was designed to investigate the effect of I status on the myocardial thyroid hormone and energy metabolism with respect to Se status.

MATERIALS AND METHODS

Animals and diets

Male Wistar weanling rats (from the Laboratory Centre of the Chinese Academy of Medicine), weighing 89 (SD 6) g were assigned to four groups of twelve animals each. The basal diet (Table 1) was based on grains with a low Se and I content obtained from an area of the Sichuan Province in which Keshan disease is endemic. The Se and I contents of the basal diet were 0·006 and 0·06 mg/kg diet respectively (Wang *et al.* 1985; Mahesh *et al.* 1992). Se (0·2 mg Se/kg as Na_2SeO_3) and/or I (0·5 mg I/kg as KIO_3) were added to the basal diet as appropriate to give four diets: (1) control (Se + I +), (2) Se-deficient (Se − I +), (3) I-deficient (SE + I −) and (4) Se- and I-deficient (Se − I −) diet. Each group of rats was given free access to one of the diets for 8 weeks. Distilled water was freely available to all animals. Food intakes were recorded daily and rats were weighed weekly. At the end of the experiment, rats were anaesthetized with diethyl ether, and blood samples were taken from the abdominal aorta. Hearts were removed and washed in ice-cold saline (9 g NaCl/l). Livers were perfused via the portal vein with KCl (150 mM) to remove residual blood. All the tissues removed were frozen immediately in liquid N_2 and then kept at −80° until analysis.

Methods

Thyroid hormones. Plasma T_4 and T_3 concentrations were measured directly by radioimmunoassay (RIA) using RIA kits from North Isotope Co., China. Heart was homogenized and then extracted with methanol–0·02 M-NaCl (400 : 1, v/v) overnight at −4°. After centrifugation at 3000 ***g*** for 30 min, T_3 was assayed in the extracts by RIA using a similar protocol to that used for plasma T_3 (Beckett *et al.* 1992).

Deiodinase activity. Supernatant fractions (0·1 ml) from liver and heart homogenates (approximately 0·1 mg protein after 3000 ***g*** centrifugation for 30 min) were incubated in 0·4 ml 150 mM-potassium phosphate buffer solution (pH 7·5) with 2·5 mM-T_4 in the presence of 4 mM-dithiothreitol at 37° for 2 h. After the addition of 1 ml cold ethanol to

Table 1. *Composition of the basal diet**

Ingredient	g/kg diet
Soyabean	200
Wheat	120
Rice	660
Maize oil	10
I- and Se-free mineral mix†	10
Vitamin A‡	750 μg
Vitamin D‡	6·25 μg

* Soyabean, wheat and rice were obtained from areas where Keshan disease is endemic. No vitamins, apart from those mentioned, were added to the diet.

† Mineral mix contained the following (mg/g mix): $CaCO_3$ 272·8, $K_2HPO_4.3H_2O$ 384·3, $CaHPO_4.2H_2O$ 68·2, $MgSO_4.7H_2O$ 92·8, NaCl 151·9, $FeC_6H_5O_7.5H_2O$ 25, $MnSO_4.4H_2O$ 4·5, $CuSO_4.5H_2O$ 0·27, $ZnCl_2$ 0·23.

‡ From fish-liver oil; values are taken from the supplier's analysis.

stop the reaction, T_3 production was determined in the organic extracts by RIA (Chopra, 1977).

α-Glycerophosphate dehydrogenase activity. Heart was homogenized in ice-cold (0°) 0·25 M-sucrose (1 : 9, w/v). After centrifugation at 1500 *g* for 30 min at 4°, 0·05 ml supernatant fraction was added to 0·6 ml reaction mixture containing 150 mM-potassium phosphate buffer solution (pH 7·6), 15 mM-phosphoglyceric acid and 10 mM-KCN (3 : 2 : 1, by vol.). Deionized water (0·05 ml) was added to the system to a final volume of 0·7 ml. Reaction mixtures were incubated at 38° for 7 min and then 0·3 ml phenazine methosulfate (5 mg/ml) and iodophenyl-nitrophenyl-phenylmonotetrazolium chloride (10 mg/ml) was added to the tube. The change in absorbance at 540 nm was recorded spectrophotometrically (Lee & Lardy, 1965).

Glutathione peroxidase activity. Activity of glutathione peroxidase was measured by an NADPH-coupled method (Wendel, 1981). NADPH, glutathione and glutathione reductase were obtained from Sigma Chemical Co., St Louis, MO, USA.

Selenium content of heart. Weighed portions of heart were digested in 10 ml mixed acid containing 3 ml H_2SO_4, 4 ml $HClO_4$ and 3 ml sodium molybdate solution (30 mmol/l). The content of Se was determined as a fluorimetric complex with 2,3-diaminonaphthalene (Wang *et al.* 1985).

Statistical analysis

ANOVA was used to investigate the effects of Se and I supplementation. Correlation analysis was used to investigate the relationship of α-GPD activity with plasma T_4 and with myocardial glutathione peroxidase activity.

RESULTS

Plasma T_4 concentrations in the two I-supplemented groups were 6–12 times higher than in the two I-deficient groups, while plasma T_3 levels in all groups were unchanged (Table 2). Se supplementation had no effect on plasma T_4 in the I-deficient groups but decreased plasma T_4 levels in the I-supplemented groups. Se supplementation alone reduced myocardial T_3 concentration (Table 2).

Table 2. *Plasma triiodothyronine (T_3) and thyroxine (T_4) levels in rats fed on diets containing grains from areas where Keshan disease is endemic, with (+) or without (−) supplemental selenium and iodine**

(Mean values and standard deviations for twelve rats per group)

	Plasma T_4 (nmol/l)		Plasma T_3 (pmol/l)		Myocardial T_3 (pmol/g heart)	
Group	Mean	SD	Mean	SD	Mean	SD
Se+I+	23·72[b]	4·54	661·9	124·8	3·49[ab]	0·47
Se−I+	59·40[a]	20·90	691·5	122·1	3·69[ab]	0·80
Se+I−	4·37[c]	0·95	752·4	93·9	3·10[b]	0·46
Se−I−	3·91[c]	1·40	606·2	83·4	4·51[a]	1·64

[a,b,c] Mean values within a column not sharing a common superscript letter were significantly different, $P < 0·05$.

* For details of diets and procedures, see Table 1 and p. 672.

Table 3. *Myocardial α-glycerophosphate dehydrogenase (α-GPD) activity, glutathione peroxidase (GPx) activity and selenium content, and hepatic type I 5′-deiodinase (IDI) activity in rats fed on diets containing grains from areas where Keshan disease is endemic, with (+) or without (−) supplemental selenium and iodine**

(Mean values and standard deviations for twelve rats per group)

Group	α-GPD (ΔA/min per g protein)		GPx (U/min per g protein)		Se (μg/g heart)		Hepatic IDI (mmol T_3/min per g protein)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Se+I+	347·3[b]	65·84	211·7[b]	38·06	0·0207[a]	0·0025	2·3[a]	0·67
Se−I+	471·8[a]	101·85	13·2[c]	5·42	0·0028[b]	0·0005	0·5[b]	0·15
Se+I−	190·7[c]	41·02	328·9[a]	72·89	0·0198[a]	0·0017	2·6[a]	0·78
Se−I−	120·8[d]	70·73	9·4[c]	2·58	0·0026[b]	0·0009	0·7[b]	0·47

[a,b,c,d] Mean values within a column with unlike superscript letters were significantly different, $P < 0·05$.

* For details of diets and procedures, see Table 1 and pp. 672–673.

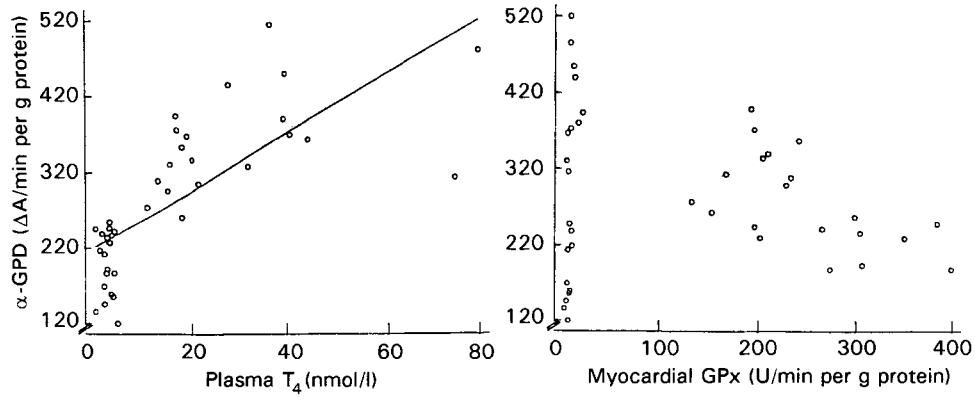

Fig. 1. The relationship between (a) myocardial α-glycerophosphate dehydrogenase (α-GPD) activity and plasma thyroxine (T_4) level and (b) myocardial α-GPD activity and myocardial glutathione peroxidase (GPx) activity. Panel (a): r 0·73989, $P < 0·01$; panel (b): r −0·1163 $P > 0·05$.

Both Se and I supplementation increased myocardial α-GPD activity and the effect was greater in those rats supplemented with I (Table 3). Supplementation with Se but not iodine increased the concentration of Se in heart (about 7-fold) and the activity of myocardial glutathione peroxidase (15–30-fold) and of hepatic IDI (about 4-fold; Table 3), while no IDI activity was measured in rat heart (results not shown).

Multiple correlation analysis showed a strong and significant positive relationship between myocardial α-GPD activity and plasma T_4 concentration, but no significant relationship between activity of α-GPD and that of glutathione peroxidase (Fig. 1).

DISCUSSION

The inhibition of IDI, a Se-containing enzyme, may cause the increase in plasma T_4 level seen in patients with Keshan disease. The relationship between myocardial thyroid hormone metabolism and Se status is still unknown since tissue thyroid hormone levels

differed from those in blood. Many symptoms in patients with Keshan disease, including abnormal energy metabolism, are similar to those of hypothyroidism caused by I deficiency. Furthermore, areas in which Keshan disease is endemic are deficient in both Se and I. We propose, therefore, that I deficiency should be considered in relation to Keshan disease.

In I deficiency the decreased plasma T_4 concentration and comparatively unchanged plasma T_3 level indicate that T_4 was more sensitive to I status. Se deficiency causes a decrease of IDI activity in non-thyroid tissues, and thus inhibits the conversion of T_4 to T_3, leading eventually to T_4 accumulation (Arthur *et al.* 1990). In contrast, we did not observe an increase of plasma T_4 in rats deficient in both Se and I. These results are different from those of Yan *et al.* (1993) but are similar to those of Beckett *et al.* (1993). Since T_4 catabolism is severely decreased in I deficiency, Se deficiency would not produce an increase in T_4 when the production of T_4 is limited by I deficiency. As for plasma T_3, a compensatory mechanism may explain its maintenance during a combined Se and I deficiency when IDI activity is markedly inhibited (Beckett *et al.* 1992).

Only myocardial T_3 was determined since T_3 is the active form of thyroid hormone. Theoretically, myocardial T_3 concentration would be affected by Se status if cardiomyocytes had IDI activity as in liver. Our data showed that there were no significant changes of plasma T_3 level in any group. What was more important was that Se status had no influence on myocardial T_3 concentration in I-sufficient rats. No IDI activity was detected in rat heart of any of the four groups in the present study.

Liu *et al.* (1994) reported decreased myocardial T_3 concentration and inhibition of IDI activity in rats fed with grain from an area where Keshan disease is endemic. However, many other reports indicate that IDI appears in rat liver, kidney and muscle in addition to the thyroid gland (Arthur *et al.* 1990). Until now, there has been no direct evidence for the existence of deiodinases in rat heart. If that is so, myocardial T_3 would be related to plasma T_3. Since it has been well established that plasma T_3 level remains stable during Se deficiency, Se deficiency should not produce any significant change in myocardial T_3. Our present data support this speculation. Recently, however, mRNA for type II iodothyronine deiodinase has been found in human heart (Croteau *et al.* 1995). This may explain why Se and I deficiencies are related to Keshan disease.

α-GPD is a crucial enzyme for energy metabolism, and its gene expression is modified by thyroid hormone (Lee & Lardy, 1965). Myocardial enzyme metabolism in patients with Keshan disease is low, a characteristic phenomenon very similar to hypothyroidism (Yang *et al.* 1988). In the present study, low α-GPD activity was observed in the two I-deficient groups, which coincided with the decrease in plasma T_4. Liu *et al.* (1994) observed a decrease in α-GPD correlated with a decrease in myocardial T_3, and ascribed it to Se deficiency. A strong correlation was found between α-GPD activity and plasma T_4 level, an index for I status, and no significant relationship with glutathione peroxidase activity, an index for Se status (Fig. 1). Since no myocardial IDI activity was detected and a comparatively unchanged plasma T_3 concentration was found, we infer that the abnormal energy metabolism in heart of rats fed with grain from areas where Keshan disease is endemic may be due more to deficiency of I rather than that of Se. In fact, iodine could influence protein catabolism in some tissues (Wang *et al.* 1991). I supplementation has been shown to enhance the current activity of K ion channels in cardiomyocytes deficient in Se and I (Bao *et al.* 1997). Iodine may have its own biological effect besides being a factor for synthesis of thyroid hormone (Verma *et al.* 1990).

Malic enzyme (*EC* 1.1.1.40) activity has been shown to be modified by thyroid hormone in a similar way to that of α-GPD in Se-deficient rat liver (Vadhanavikit &

Ganther, 1994). These authors ascribed the increased activity of malic enzyme (or NADPH-generating enzyme) in Se deficiency to the increased demand for NADPH for glutathione metabolism. The lack of relationship between α-GPD and glutathione peroxidase activity in our present study does not provide direct support for this theory. From our present study, it can be concluded that iodine has more influence on myocardial energy metabolism that does Se, and it is proposed that iodine deficiency plays an important role in the development of Keshan disease.

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