

## Effect of dietary proteins on the plasma immunoreactive insulin-like growth factor-1/somatomedin C concentration in the rat

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Plasma immunoreactive insulin-like growth factor-1/somatomedin C (IR-IGF-1) was determined in rats fed for 1 week on a protein-free diet, or diets containing gluten, gluten supplemented with lysine and threonine, maize-gluten meal (with arginine), maize-gluten meal (with arginine) supplemented with tryptophan and lysine, or casein. IR-IGF-1 concentration was higher in the arterial plasma of rats fed on a diet containing casein at 120 g/kg diet (4-8 U/ml) than in rats fed on a protein-free or a low-casein (50 g/kg diet) diet (1.5-2 U/ml). The plasma of rats fed on gluten or maize-gluten meal as the protein source showed intermediate values. However, giving a diet containing an amino acid mixture as recommended by the National Research Council (1978) but deprived of lysine or tryptophan did not affect significantly the plasma IR-IGF-1 concentration. Total IGF-1 concentration (which was measured immunologically after extraction of the plasma with acid-ethanol) was also lower in the rats fed on the protein-free diet than in those fed on the casein (120 g/kg diet) diet. The ratio IR-IGF-1:total IGF-1 was higher in the rats fed on the casein diet (120 g casein/kg diet) than in those fed on the protein-free diet. The results suggest an important influence of IR-IGF-1 or IR-IGF-1:total IGF-1 ratio on protein anabolism and nutrition. IR-IGF-1 and total IGF-1 were found in the fractions of molecular weights 40 kDa and 150 kDa after gel filtration of rat plasma. A larger amount of IGF-1 was recovered in the fraction of 150 kDa in the rats fed on the casein diet. <sup>125</sup>I-IGF-1 added to the plasma of rats fed on the protein-free diet was found mainly in the fraction of 40 kDa after gel-filtration. On the other hand, <sup>125</sup>I-IGF-1 added to the plasma of rats fed on the gluten or casein diets was mainly recovered in the free IGF-1 fraction. The results suggest that IGF-binding protein(s) of molecular weight about 40 kDa was not saturated with IGF-1 in the rats fed on the protein-free diet. The results indicate the important role of IGF-1 and its binding proteins in the regulation of protein metabolism in rats.

### Insulin-like growth factor-1: Protein synthesis: Rat

It is widely accepted that the amino acid balance of dietary protein (nutritional quality of protein) affects greatly the assimilation of dietary proteins into body proteins (Allison, 1964). If a dietary protein is deficient in certain essential amino acids, growth of animals is retarded and there is marked wastage of dietary nitrogen. This implies that the nutritional quality of protein may affect the plasma concentrations of hormones that enhance the assimilation of dietary proteins into body proteins.

Two hormones are known to play important roles in protein anabolism. One is insulin and the other is growth hormone (GH) (Munro, 1964; Waterlow *et al.* 1978).

Insulin has been shown to enhance assimilation of dietary proteins into body proteins by stimulating amino acid transport into muscle and enhancing muscle protein synthesis (Manchester, 1970*a*; Waterlow *et al.* 1978). The important role of insulin in protein

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metabolism is also deduced from the observation that N assimilation is impaired in diabetic animals (Waterlow *et al.* 1978). Plasma concentrations of insulin are also known to be affected by malnutrition (Lunn *et al.* 1973; Anthony & Faloona, 1974; Smith *et al.* 1975; Kabadi *et al.* 1976; Edozien *et al.* 1978; Dollet *et al.* 1985).

GH also facilitates protein anabolism (Manchester, 1970*b*; Waterlow *et al.* 1978). In hypophysectomized rats, growth is retarded concomitantly with decreased plasma concentration of GH (Waterlow *et al.* 1978). Recently, part of the action of this hormone, particularly the activity in promoting tissue protein synthesis, has been postulated to be mediated by insulin-like growth factor-1/somatomedin C (IGF-1) (Daughaday, 1983), a hormone that is mainly synthesized and secreted by the liver under the regulation of GH (Binoux *et al.* 1982; Schwander *et al.* 1983). Plasma concentrations of IGF-1 are regulated by both GH and the nutritional status of animals. Good nutrition raises the plasma concentration of this hormone and poor- or undernutrition reduces the concentration (Reeves *et al.* 1979; Clemmons *et al.* 1981, 1985; Prewitt *et al.* 1982; Maes *et al.* 1984). In undernourished children raised plasma GH concentration is sometimes observed, probably because of the impaired feedback regulation of the secretion of GH or growth hormone releasing factor by the reduced plasma IGF-1 concentration (Yamashita & Melmed, 1986; Reichlin, 1988).

IGF-1 circulates in plasma in association with binding proteins that affect the measurement of IGF-1 by radioimmunoassay. Although the values determined in unextracted plasma are only approximately 25–30% of the actual content (Bala *et al.* 1983), they correlate well with the biological activity of IGF-1 (Chatelain *et al.* 1983). Furthermore, the saturation level of binding proteins also seems to be affected by GH (White *et al.* 1981; Cohen & Blethen, 1983).

Although many nutritional factors have been shown to regulate the plasma concentration of IGF-1, no detailed studies have been performed on the effect of quantity and quality of dietary proteins on the plasma concentration of IGF-1.

In this context, we have attempted to elucidate whether the plasma concentration of IGF-1 and the condition of association of this hormone with binding proteins in plasma are related to the quantity and nutritional quality of dietary proteins.

## MATERIALS AND METHODS

### *Animals*

Male Wistar rats were used throughout the experiments and five rats were used for each treatment. Initial body-weights and changes in body-weight during the experimental periods were recorded. The rats were purchased from Shizuoka Agricultural Cooperative for Laboratory Animals (Hamamatsu, Japan) and kept in wire-bottomed cages in a room maintained at 22° with a 12 h light (06.00–18.00 hours)–12 h dark (18.00–06.00 hours) cycle. Water and conventional laboratory pellets (Oriental Yeast Co., Tokyo, Type MF) were available *ad lib.* until the rats received the experimental diets. The experimental diets were given from 10.00 to 18.00 hours for 1 week. After changing to this feeding schedule, rats lost body-weight during the first 2 or 3 d because of the restriction in feeding time. They then gained weight if they received adequate amounts of good quality protein. Rats ate about half their daily feed within 1 h after feeding. On day 8, when blood samples were taken, the rats were fed at 10.00 hours and blood samples were taken at 11.30 hours, except during time-course experiments when the effect of time-period after feeding was investigated. When changes in the immunoreactive IGF-1 (IR-IGF-1) concentrations after feeding were studied, rats were fed from 10.00 to 12.00 hours and blood samples were obtained at 10.00, 11.00, 12.00, 14.00 and 18.00 hours. The blood obtained at 10.00 hours

(i.e. just before feeding the rats) was referred to as the zero-time sample. This meal-feeding schedule was planned in order to exclude the possibility of an increase or decrease in IR-IGF-1 concentration after food ingestion. However, time-course experiments indicated that the plasma IR-IGF-1 concentration did not change significantly after a meal (S. Takahashi, T. Noguchi and H. Naito, unpublished results). Thus, we have assumed that the meal-feeding schedule is not necessary for investigation of the effect of dietary treatments on plasma IR-IGF-1 concentration.

#### *Diets*

The composition of the diets employed in the present investigations are shown in Table 1; they were: protein-free (PF); gluten (G); gluten+lysine and threonine (GLT); maize-gluten meal+arginine (CGMA); maize-gluten meal+arginine, lysine and tryptophan (CGMALT); and casein+methionine at 50+0.8 (5C), 120+2.0 (C), 140+2.3 (14C) and 170+2.8 (17C) g/kg. Besides the feeds shown in Table 1, diets containing amino acid mixtures as the only source of nitrogen were also prepared. In this case, essential amino acids were added to the diet according to the requirement level recommended by the National Research Council (1978). Glutamic acid was used to adjust the total amino acid level in the diet to 120 g/kg diet. The mixture of amino acids thus prepared was referred to as the complete amino acid mixture. The lysine- and tryptophan-deficient diets were prepared by replacing lysine or tryptophan in the complete amino acid mixture by glutamic acid, and for lysine- or tryptophan-excess diets lysine or tryptophan was added at double the recommended amounts at the expense of glutamic acid.

#### *Blood sampling*

Rats were anaesthetized by injection of 50 mg pentobarbital/kg body-weight, and arterial blood was collected into a plastic tube by severing the carotid artery. Blood coagulation was prevented with Na<sub>2</sub>EDTA as indicated for the assay of IGF-1 (see p. 524). Blood samples were quickly chilled on ice and centrifuged at 3000 g for 10 min. The plasma was kept at -20° until required for analysis.

#### *Determination of IR-IGF-1 before and after acid extraction*

An IGF-1 assay kit (Nichols Institute Diagnostics, California; the anti-IGF-1 is polyclonal) was purchased from Japan Radioisotope Association. Plasma samples were diluted to an appropriate volume with buffer and the immunoreactive hormone concentration was determined. A calibration curve was obtained for every assay. Rat plasma diluted at several stages showed curves typical of the radioimmunoassay system. It has not been confirmed that the radioimmunoassay kit for human IGF-1 is also applicable to rat IGF-1. However, the amino acid sequence of rat IGF-1 differs from human IGF-1 in only three amino acid residues (Shimatsu & Rotwein, 1987; H. Kato, T. Noguchi and H. Naito, unpublished results), thus the assay kit was tentatively assumed to be equally reactive with rat or human IGF-1. The results were expressed as units of IGF-1 equivalent to human IGF-1.

As mentioned previously, most of the IGF-1 in plasma is bound to binding proteins and only a part of it is immunoreactive in native plasma. However, extraction of plasma with acid-ethanol isolates almost all the bound IGF-1 from the binding proteins and IGF-1 becomes freely immunoreactive after extraction. In the present experiments, the method of Baxter *et al.* (1983) and Daughaday *et al.* (1980) was employed to convert the bound IGF-1 into freely immunoreactive IGF-1, as follows. Plasma samples (10  $\mu$ l) were mixed with 40  $\mu$ l 2 M-hydrochloric acid-ethanol mixture (1:7, v/v) and mixed well. The mixture was kept for 30 min at room temperature and centrifuged at 2000 g for 30 min at 4°. For radioimmunoassay, the supernatant fraction (10  $\mu$ l) was mixed with 1.4 ml phosphate

Table 1. *The composition of the experimental diets (g/kg)*

Diets ... Ingredients*	Protein- free (PF)	Gluten (G)	Gluten + lysine + threonine (GLT)	Maize- gluten meal + arginine (CGMA)	Maize- gluten meal + arginine + lysine + tryptophan (CGMALT)	Casein + methionine			
						5C	C	14C	17C
$\beta$ -Maize starch	850	720	719	643	643	799	728	708	677
Gluten	—	120	120	—	—	—	—	—	—
Maize-gluten meal (crude protein (nitrogen $\times$ 6.25) 600 g/kg)	—	—	—	200	200	—	—	—	—
Casein	—	—	—	—	—	50	120	140	170
L-Glutamic acid	—	10	—	4.5	1.9	—	—	—	—
L-Lysine monohydrochloride	—	—	9	—	2.0	—	—	—	—
L-Threonine	—	—	2	—	—	—	—	—	—
L-Arginine	—	—	—	2.75	2.75	—	—	—	—
L-Tryptophan	—	—	—	—	0.6	—	—	—	—
L-Methionine	—	—	—	—	—	0.8	2.0	2.3	2.8

\* In addition to these components, all diets contained (g/kg): 50 soya-bean oil, 50 cellulose, 40 mineral mixture and 10 vitamin mixture.

The mineral and vitamin mixtures (obtained from Oriental Yeast Co., Tokyo) were prepared according to Rogers & Harper (1965).

buffer containing 300 mg Tris base/l (this mixture could be stored at 4° for at least 1 week). The supernatant fraction was used for radioimmunoassay. The supernatant fraction (100  $\mu$ l) contained 1  $\mu$ l sevenfold diluted plasma. The amount of IGF-1 determined without acid-ethanol extraction will be referred to as IR-IGF-1 and that determined after acid-ethanol extraction of plasma as total IGF-1. The method for determination of total IGF-1 after acid-ethanol extraction was proved to be valid for human and rat plasma (Daughaday *et al.* 1980, 1982). Many investigators have employed the latter method. However, Mesiano *et al.* (1988) showed that the acid-ethanol extraction method is not appropriate in the case of ovine plasma. Mesiano *et al.* (1988) employed a monoclonal antibody for the determination of IGF-1. In our case, an assay kit with a polyclonal antibody was used, and although the acid-ethanol treatment method was compared with the acid-gel-filtration method of Mesiano *et al.* (1988), variable results among assays were not obtained for several samples of rat plasma (far less variation as compared with the variation among the animals). This good reproducibility may be due to the species specificity of the binding proteins or to the difference in the properties of the monoclonal and polyclonal antibodies. Thus, the 'total IGF-1' in the present paper should be considered to be 'immunologically determined IGF-1 after acid-ethanol extraction.'

#### *Sephacryl S-200 gel filtration of protein-bound IGF-1 in plasma*

The method proposed by Cohen & Blethen (1983) was used. In brief, a plastic column (15  $\times$  840 mm) was packed with Sephacryl S-200 gel (Pharmacia), which was previously equilibrated at 4° with 0.1 M-Tris hydrochloride containing 5 mM-EDTA (pH 7.4). Plasma samples (500  $\mu$ l) were diluted with the Tris hydrochloride buffer to 2 ml and applied to the column. The column was eluted with the same buffer at a flow rate of 0.15 ml/min. Fractions (1.4 ml) were collected and used for the determination of IR-IGF-1. The IR-IGF-1 in each fraction was also determined before and after extraction with acid-ethanol. This

Table 2. Immunoreactive insulin-like growth factor-1 (IR-IGF-1) concentration before and after acid-ethanol extraction (total IGF-1) in arterial plasma of rats fed on a protein-free diet (PF), or diets containing 120 g gluten/kg (G), gluten supplemented with lysine and threonine (GLT), maize-gluten meal with arginine (CGMA), maize-gluten meal with arginine, supplemented further with lysine and tryptophan (CGMALT), or casein (C)

(Values are means with their standard errors for five rats per group)

Diet	Final body wt* (g)		IR-IGF-1 (U/ml) (before acid-ethanol extraction (A))		Total IGF-1 (U/ml) (after acid-ethanol extraction (B))		A/B	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
PF	123.0 <sup>a</sup>	1.2	1.61 <sup>a</sup>	0.31	17.4 <sup>a</sup>	1.4	0.09 <sup>a</sup>	0.01
G	133.6 <sup>b</sup>	5.0	3.76 <sup>abc</sup>	0.61	23.1 <sup>ab</sup>	1.8	0.16 <sup>abc</sup>	0.02
GLT	144.4 <sup>c</sup>	3.4	4.53 <sup>bc</sup>	0.56	19.3 <sup>a</sup>	2.5	0.24 <sup>c</sup>	0.03
CGMA	135.8 <sup>b</sup>	1.7	3.40 <sup>ab</sup>	0.63	23.8 <sup>ab</sup>	3.5	0.14 <sup>ab</sup>	0.01
CGMALT	137.8 <sup>b</sup>	2.5	5.83 <sup>cd</sup>	1.15	25.8 <sup>ab</sup>	2.6	0.23 <sup>bc</sup>	0.05
C	153.0 <sup>d</sup>	1.9	7.37 <sup>d</sup>	0.98	30.7 <sup>b</sup>	5.9	0.24 <sup>c</sup>	0.04

<sup>a, b, c, d</sup> Values in columns with unlike superscript letters were significantly different ( $P < 0.05$  or less) by Duncan's (1955) multiple range test.

\* The initial body-weight of the rats was 136.9 (SE 1.3) g.

gel filtration was performed on the plasma of each rat. Molecular markers were blue dextran (Pharmacia),  $\beta$ -amylase (EC 3.2.2.1; 200 kDa), bovine serum albumin (66 kDa), ribonuclease A (EC 3.1.4.22; 13.7 kDa) and aprotinin (6.5 kDa).

#### *In vitro binding of <sup>125</sup>I-IGF-1 to plasma proteins*

Plasma samples (100  $\mu$ l) obtained from rats fed on diets C or PF were mixed with 3  $\mu$ l <sup>125</sup>I-IGF-1 (300 000 counts/min, tracer amount of IGF-1, dissolved in 0.14 M-NaCl-0.01 M-phosphate buffer containing bovine serum albumin at 10 g/l, pH 7.5) and diluted to 2 ml with 0.05 M-Tris hydrochloride (pH 7.4) containing bovine serum albumin (10 g/l). This mixture was kept at 4° for 16 h and fractionated by Sephacryl S-200 column chromatography as described above. IGF-1 was labelled with <sup>125</sup>I according to Greenwood *et al.* (1963), employing chrolamin-T. The total radioactivities in the four fractions were calculated by dividing the chromatogram (see Fig. 2, p. 530) and the nutritional significance of the association of radioactive IGF-1 with plasma proteins was analysed. This procedure was applied to the plasma of each rat.

#### *Determination of radioactivity*

Radioactivity of <sup>125</sup>I-IGF-1 was determined with a  $\gamma$ -counter (Aloka Auto Well Gamma System ARC-500).

#### *Statistical analyses*

The results were analysed statistically according to Duncan's (1955) multiple range test.

### RESULTS

Table 2 shows IR-IGF-1 and total IGF-1 concentrations in the plasma of rats fed on diets PF, G, GLT, CGMA, CGMALT and C. The plasma IR-IGF-1 and total IGF-1 concentrations were highest in the rats fed on diet C and lowest in those fed on diet PF.

Table 3. Immunoreactive insulin-like growth factor-1 (IR-IGF-1) concentration in arterial plasma of rats fed on amino acid diets containing an essential amino acid mixture simulated to the requirement reported by the National Research Council (NRC) (1978), or amino acid mixtures deficient in lysine (NRC-Lys), deficient in tryptophan (NRC-Trp), or amino acid mixtures supplemented with twice the NRC recommended amount of lysine (NRC+Lys) or tryptophan (NRC+Trp). The rats were fed on these diets for 1 week

(Values are means with their standard errors for five rats per group)

Diet	Final body-wt* (g)		IR-IGF-1† (U/ml)	
	Mean	SE	Mean	SE
NRC	187.6 <sup>b</sup>	4.6	4.21	0.71
NRC-Lys	156.2 <sup>a</sup>	1.6	3.75	0.70
NRC-Trp	150.2 <sup>a</sup>	2.3	2.92	0.25
NRC+Lys	195.8 <sup>b</sup>	7.7	4.14	1.06
NRC+Trp	193.4 <sup>b</sup>	5.8	4.37	0.54

<sup>a, b</sup> Values in columns with unlike superscript letters were significantly different ( $P < 0.05$  or less) by Duncan's (1955) multiple range test.

\* The initial body-weight of the rats was 169.0 (SE 3.9) g.

† There were no statistical differences ( $P > 0.05$ ) in IR-IGF-1 among the dietary groups.

Supplementation of diet CGMA with tryptophan and lysine (diet CGMALT) increased plasma IR-IGF-1 concentration. However, addition of lysine and threonine to diet G (diet GLT) did not raise the IR-IGF-1 concentration significantly. The ratio IR-IGF-1:total IGF-1 was significantly higher in diet C-fed rats than in those fed on diets PF or CGMA. The physiological significance of these results will be discussed later.

Table 3 shows the IR-IGF-1 concentration in rats fed on the diets containing the NRC complete, lysine- or tryptophan-deficient, and lysine- or tryptophan-excess amino acid mixtures. Omission of lysine or tryptophan from the complete mixture did not affect the IR-IGF-1 concentration significantly. Furthermore, supplementation with excess amounts of either tryptophan or lysine did not bring about any effects on the IR-IGF-1 concentrations. Therefore, the difference in IR-IGF-1 among the dietary protein groups (Table 2) is presumably due not to a single limiting-amino acid deficiency but to the total amino acid balance of dietary proteins.

The plasma IR-IGF-1 concentration did not change significantly after a meal of either diet G or diet GLT (values not shown). This means that the previously mentioned findings were not the effect of taking a meal but an effect of the diet itself.

Table 4 shows the effect of dietary casein level on plasma IR-IGF-1 concentrations. The plasma concentration of IR-IGF-1 was significantly higher in the rats fed on adequate amounts of casein than in those fed on deficient amounts of casein (compared with the recommended level of dietary protein, National Research Council, 1978).

Typical gel filtration patterns of the plasma samples of the rats fed on diet PF, G and C are shown in Fig. 1. IR-IGF-1 was recovered in the fractions with apparent molecular weights of approximately 150 and 40 kDa. These results show that almost equal amounts of rat IGF-1 are found in the 150 and 40 kDa fractions. Fig. 1 also shows the total activity of IR-IGF-1 in the fractions of Sephacryl S-200 gel chromatography. The total activity of the fractions of both 150 and 40 kDa was larger than that of IR-IGF-1, showing that only a part of IGF-1 in these fractions is immunoreactive as described previously. The IR-IGF-1 and total IGF-1 activities in the plasma of three groups of rats were estimated by dividing the fractions as shown in Fig. 1 and summing the activities in the fractions. As shown in

Table 4. *The effect of dietary casein on arterial immunoreactive insulin-like growth factor-1 (IR-IGF-1) concentrations in rats fed on graded levels of casein in the diet. The rats were fed on these diets for 1 week.*

(Values are means with their standard errors for five rats per group)

Dietary casein level (g/kg diet)	Final body-wt* (g)		IR-IGF-1 (U/ml)	
	Mean	SE	Mean	SE
0	122.5 <sup>a</sup>	1.5	1.86 <sup>a</sup>	0.19
50	126.6 <sup>a</sup>	2.8	1.60 <sup>a</sup>	0.12
120	137.6 <sup>b</sup>	4.4	3.88 <sup>b</sup>	0.40
140	140.4 <sup>b</sup>	3.2	4.74 <sup>b</sup>	0.54
170	138.4 <sup>b</sup>	2.5	4.08 <sup>b</sup>	0.86

<sup>a, b</sup> Values in columns with unlike superscript letters were significantly different ( $P < 0.05$  or less) by Duncan's (1955) multiple range test.

\* The initial body-weight of the rats was 139.3 (SE 1.7) g.

Table 5, IR-IGF-1 in the 150 kDa fraction divided by the total IGF-1 (the activity of IGF-1 after the treatment of the plasma with acid-ethanol) correlated well with the growth of the rats. Therefore, the activity of IR-IGF-1 bound to the binding protein of 150 kDa is probably significant in the assimilation of dietary proteins into body proteins.

There were at least four fractions which bound radio-iodinated IGF-1 (Fig. 2). The approximate molecular weights of the fractions were: 150, 65, 40 and 15 kDa. The radioactivity in the fraction of 65 kDa was probably that bound to serum albumin in the original plasma, and that bound to bovine serum albumin added before gel filtration and added to the gel filtration buffer. Serum albumin presumably binds <sup>125</sup>I-IGF-1 non-specifically because this protein was present abundantly in the original plasma and in the gel filtration samples. When <sup>125</sup>I-IGF-1 was mixed with bovine serum albumin and applied to the column, only peaks corresponding to 65 (peak B) and 15 kDa (peak D) were observed (results not shown). This suggests that <sup>125</sup>I-IGF-1 bound to serum albumin is eluted as peak B and free <sup>125</sup>I-IGF-1 as peak D. A protein of 40 kDa in the plasma of the PF diet-fed rats bound relatively large amounts of <sup>125</sup>I-IGF-1 in vitro (Fig. 2(c)). In contrast, a large portion of the radio-iodinated IGF-1 was found as a free form (peak D) when <sup>125</sup>I-IGF-1 was incubated with the plasma obtained from C-fed rats (Fig. 2(a)). These conclusions were supported statistically by the analyses of the sum of the radioactivity in the fractions shown in Fig. 2 (Table 6). The physiological significance of these results will be discussed.

#### DISCUSSION

Insulin has been assumed to be a most important hormone in protein anabolism (Manchester, 1970a; Waterlow *et al.* 1978). The plasma concentration of insulin has been reported to decrease during malnutrition (Lunn *et al.* 1973; Anthony & Faloon, 1974; Smith *et al.* 1975; Kabadi *et al.* 1976; Edozien *et al.* 1978; Dollet *et al.* 1985). The present investigations showed that IGF-1 is also important in protein metabolism. Our results confirmed in rats the previous reports on IGF-1 which showed a close relationship between the plasma IGF-1 concentration (or IGF concentration assayed biologically) and N balance of animals (Reeves *et al.* 1979; Prewitt *et al.* 1982; Maes *et al.* 1984; Yahya *et al.* 1986) and humans (Clemmons *et al.* 1981, 1985).

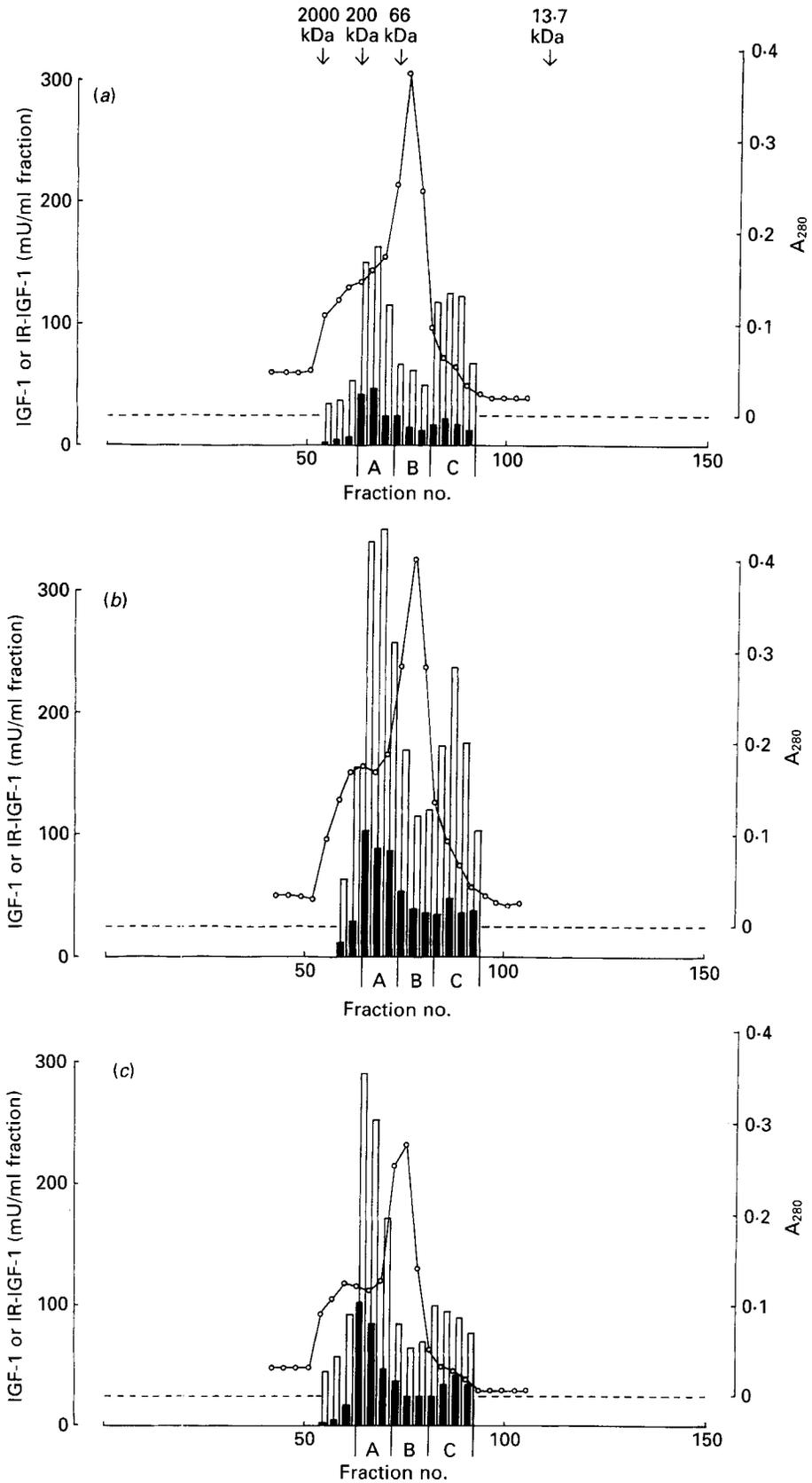


Fig. 1. For legend see opposite.

Table 5. Summary of the data on immunoreactive insulin-like growth factor-1 (IR-IGF-1) in the fractions of plasma of rats fed on a protein-free diet, or diets containing casein or gluten

(The IR-IGF-1 in the fractions of plasma after gel filtration was divided by the sum of IGF-1 (IR-IGF-1 determined after extraction of plasma with acid-ethanol) of each fraction and expressed taking the total IGF-1 as 100. The results shown in Fig. 1 were used for this table. Values are means with their standard errors for five rats per group)

Dietary group	IR-IGF-1 in each fraction/total IGF-1									
	Final body-wt* (g)		Fraction A		Fraction B		Fraction C		Sum of IR-IGF-1 (fractions A + B + C)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Protein-free	123.5 <sup>a</sup>	1.2	10.7 <sup>a</sup>	0.9	5.8	0.9	11.5 <sup>ab</sup>	3.7	28.0 <sup>ab</sup>	4.8
Gluten	138.3 <sup>b</sup>	2.3	12.7 <sup>a</sup>	0.4	4.0	0.8	8.6 <sup>a</sup>	1.3	25.3 <sup>a</sup>	1.7
Casein	154.8 <sup>c</sup>	3.1	20.3 <sup>b</sup>	1.5	6.9	1.5	16.0 <sup>b</sup>	2.6	43.2 <sup>b</sup>	4.1

<sup>a, b</sup> Values in columns with unlike superscript letters were significantly different ( $P < 0.01$  or  $0.05$  in the case of fraction C) by Duncan's (1955) multiple range test. The difference in fraction B was not statistically significant ( $P < 0.05$ ).

\* The initial body-weight of the rats was 140.7 (SE 0.7) g.

The structure and function of IGF-1 are similar to those of insulin and proinsulin (Honegger & Blundell, 1983; Froesch & Zapf, 1985). IGF-1 enhances glucose (Berhanu & Olefsky, 1981; Zapf *et al.* 1981; Beguinot *et al.* 1985) and amino acid (Kaplowitz *et al.* 1984) transport into cells and, like insulin, induces cell division (Bockus *et al.* 1983). However, this hormone is secreted primarily by the liver (Binoux *et al.* 1982; Schwander *et al.* 1983) and is relatively stable in plasma, as compared with insulin (Kaufmann *et al.* 1978). Although insulin circulates as the free form in plasma, IGF-1 is bound to plasma proteins known as IGF binding proteins (Kaufmann *et al.* 1978; for review, see Ooi & Herington, 1988; Zapf & Froesch, 1988).

The present results show that the plasma concentration of IR-IGF-1 was higher in diet C-fed rats (4–8 U/ml) than in those fed on diet PF or on a low-protein diet (1.5–2 U/ml). Giving poor-quality protein sources caused a decrease in IR-IGF-1. However, the effect of the nutritional quality of dietary proteins was not as marked as that of protein deficiency. This was also confirmed by an experiment employing an amino acid mixture. Giving a lysine-deficient or tryptophan-deficient diet did not bring about a significant decrease in plasma IR-IGF-1 after 1 week.

As described previously, IGF-1 is found in plasma mostly combined with binding proteins. A portion of the total IGF-1 in plasma is known to be immunoreactive. However, if IGF-1 is extracted from plasma with a mixture of acid and ethanol, almost all the IGF-1 in plasma becomes fully immunoreactive IGF-1. The results obtained by determining total IGF-1 showed that the ratio IR-IGF-1 : total IGF-1 was significantly higher in diet C-fed rats than in those fed on diet PF. The value for the ratio for plasma obtained from diet G-fed rats was close to that of diet PF-fed rats. We assume at present that the ratio is physiologically important for the following reasons.

At least five molecular species of binding protein have been reported in human plasma (Hardouin *et al.* 1987). The molecular weights of these binding proteins vary from 24 to 42

Fig. 1. Typical Sephacryl S-200 gel filtration profiles of plasma from rats fed on (a) protein-free, (b) gluten and (c) casein diets. (□), Total insulin-like growth factor-1 (IGF-1) (see p. 528); (■), immunoreactive insulin-like growth factor-1 (IR-IGF-1). (○—○) Absorption at 280 nm ( $A_{280}$ ). ↓, The positions of elution of the molecular markers, with molecular weights shown.

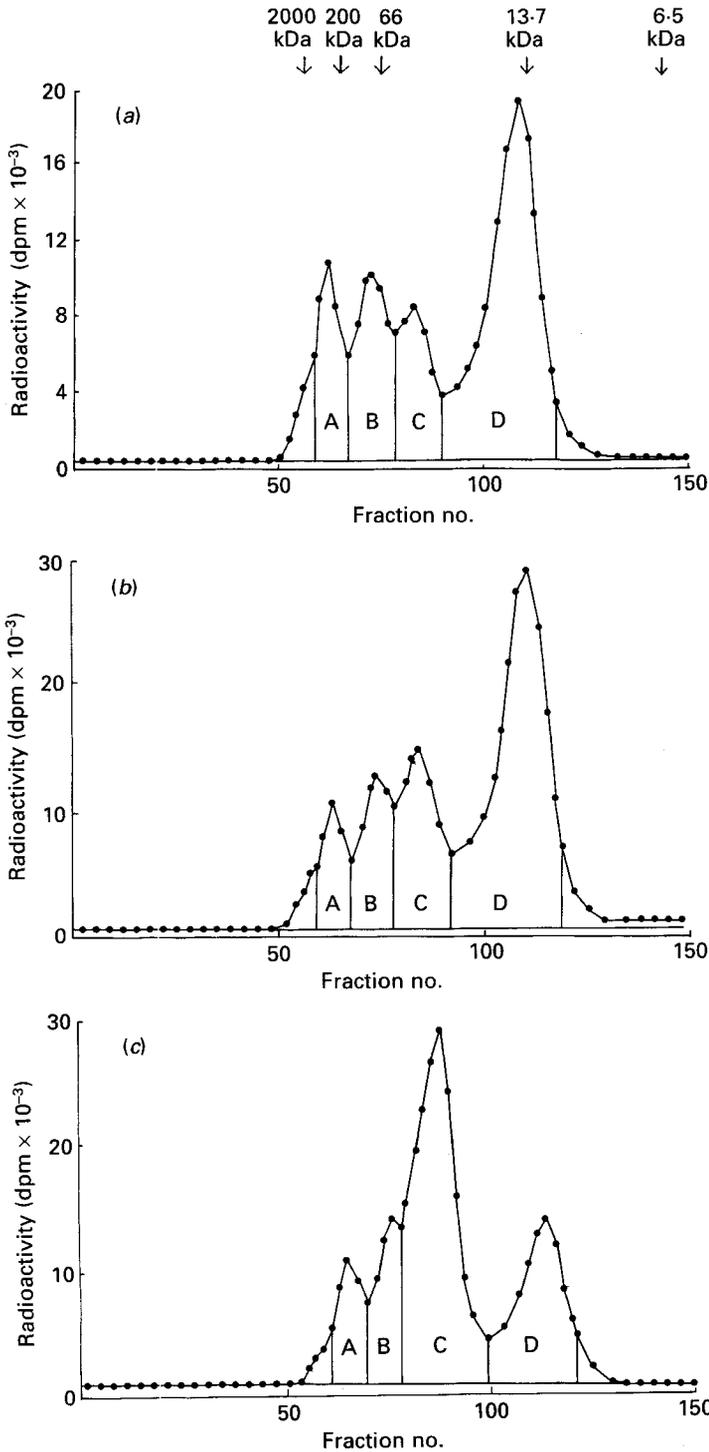


Fig. 2. Typical gel filtration profiles of plasma mixed with  $^{125}\text{I}$ -insulin-like growth factor-1 ( $^{125}\text{I}$ -IGF-1). The plasma was obtained from rats fed on (a) casein, (b) gluten or (c) protein-free diets. After mixing with  $^{125}\text{I}$ -IGF-1, the plasma was chromatographed on Sephacryl S-200 and the radioactivity (disintegrations/min (dpm)  $\times 10^{-3}$ ) in each fraction was determined. The chromatogram was divided into four fractions as shown in the figure and the radioactivity in each area was calculated by summing the radioactivity of each fraction.  $\downarrow$  The elution positions of the molecular markers, with molecular weights shown.

Table 6. Summary of the data on radioactive insulin-like growth factor-1 (IGF-1) (<sup>125</sup>I-IGF-1) bound to the IGF binding proteins in the plasma of rats fed on a protein-free diet or diets containing casein or gluten.

(The results shown in Fig. 2 were used for this table. The total radioactivity found in fractions A, C and D was taken to be 100 and the distribution of the radioactivity in each fraction was calculated. The radioactivity in fraction B was not included because this radioactivity was possibly bound to plasma albumin. Values are means with their standard errors for five rats per group)

Dietary group	Final body-wt* (g)		Distribution of <sup>125</sup> I-IGF-1 added to plasma						Total radioactivity (disintegrations/min × 10 <sup>-3</sup> )	
	Mean	SE	Fraction A		Fraction C		Fraction D		Mean	SE
Protein-free	123.5 <sup>a</sup>	1.2	9.8	1.2	54.4 <sup>a</sup>	2.2	35.8 <sup>a</sup>	3.4	417	101
Gluten	138.3 <sup>b</sup>	2.3	10.8	1.1	31.1 <sup>b</sup>	4.8	58.1 <sup>b</sup>	5.3	586	44
Casein	154.8 <sup>c</sup>	3.1	14.1	1.9	13.6 <sup>c</sup>	3.0	72.3 <sup>c</sup>	4.7	400	36

<sup>a, b, c</sup> Values in columns with unlike superscript letters were significantly different ( $P < 0.01$  or  $0.05$  in the case between casein and gluten in fraction D) by Duncan's (1955) multiple range test. The differences in fraction A and total radioactivity were not statistically significant ( $P < 0.05$ ).

\* The initial body-weight of the rats was 140.7 (SE 0.7) g.

kDa. However, when rat plasma is subjected to gel-filtration, two peaks, one approximately 150 kDa and the other about 40 kDa, are usually found (Fig. 1). The peak of 40 kDa may not be composed of a single binding protein-IGF-1 complex but a mixture of a number of binding proteins with IGF-1. Furthermore, the peak of 150 kDa is suggested to be composed of IGF-1, one or more binding proteins (of molecular weight 53 kDa if the molecular species of rat binding protein are similar to those of man, Baxter (1988)), and some other components, for example the acid-labile subunit reported by Baxter (1988). These findings indicate that IGF-1 may form various kinds of complexes with binding proteins and other factors. The observation that dietary factors affect the IR-IGF-1:total IGF-1 ratio may mean that IGF-1 makes different kinds of complexes with binding proteins according to different nutritional conditions. For example, if plasma IGF-1 is mainly bound to one kind of binding protein under good nutritional conditions, and the complex is more immunoreactive than the other binding protein-IGF-1-other component complexes, the plasma of rats fed on adequate amounts of good-quality protein will show a high IR-IGF-1:total IGF-1 ratio. The results shown in Fig. 1 and Table 5 favour this hypothesis. Another possibility is that some plasma factors inhibit the reaction of binding protein-IGF-1 complex(s) with the antibody of IGF-1. If the factors are produced in larger amounts under poor compared with good nutritional conditions, the IR-IGF-1:total IGF-1 ratio will be higher under good nutritional conditions. Of the many working hypotheses these are the only two possibilities which explain the high IR-IGF-1:total IGF-1 ratio in the plasma of rats fed on adequate amounts of casein.

<sup>125</sup>I-IGF-1, when it is injected intravenously, has been shown to be bound first to a protein of 40 kDa, then transferred to that of 150 kDa, and finally degraded (Kaufmann *et al.* 1977; they used the old term non-suppressible insulin-like activity, NSILA-S). However, when <sup>125</sup>I-IGF-1 is added to the plasma its main portion is bound to the protein of 40 kDa. The latter observation was confirmed in the present experiments. Furthermore, it was elucidated that a large portion of added <sup>125</sup>I-IGF-1 remained unbound in the plasma of well-fed rats. This form of IGF-1 was not found in circulating plasma of rats fed on either diet C or diet PF. As described previously, there are at least two possible explanations to these results. The first one is that IGF-1 binding protein of 40 kDa

increases in malnourished animals and decreases in well-fed animals. Therefore, a relatively large amount of IGF-1 is bound to the 40 kDa binding protein in malnourished animals. The second possibility is that plasma binding-proteins of rats fed on well-balanced diets are mostly saturated by endogenous IGF-1. In our experiments, plasma IGF-1 binding-protein of 40 kDa of rats fed on diet PF was not saturated by endogenous IGF-1 and had room to bind the added  $^{125}\text{I}$ -IGF-1. The combination of these two hypotheses should also be considered. The present observations that the rats fed on diet C contained larger amounts of IR-IGF-1 or total IGF-1 in the 40 kDa fraction (Fig. 1 and Table 5) favours the second hypothesis.

The relatively low radioactivity in the binding protein of 150 kDa (Fig. 2(a, c)) may be due to the saturation of this protein by IGF-1 or to the inability of transfer of IGF-1 from a protein of 40 kDa to that of 150 kDa. Some papers have shown changes in plasma concentrations of IGF-1 binding proteins from humans (Baxter & Martin, 1986; Baxter & Cowell, 1987; Hardouin *et al.* 1987; for review, see Ooi & Herington, 1988). Careful determination of the amount of the binding proteins and the rate of saturation of the binding proteins will elucidate the physiological meaning of the present observations.

The present observations suggest an important role for IGF-1 in protein anabolism and nutrition, as pointed out by Millward (1985). Further studies will provide more details of the association of IGF-1 with different kinds of binding protein under various nutritional conditions.

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