Correlation between the urinary excretion of acid-soluble peptides, fractional synthesis rate of whole body proteins, and plasma immunoreactive insulin-like growth factor-1/somatomedin C concentration in the rat

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The relations between the urinary excretion of acid-soluble peptide (ASP)-form amino acids, the rate of whole body protein synthesis and plasma immunoreactive insulin-like growth factor-1/somatomedin C concentration were investigated in rats. The urinary ASP-form leucine plus valine excretion correlated well with the rate of whole body protein synthesis and with the plasma immunoreactive insulin-like growth factor-1 concentration. The results provide further evidence for the hypothesis that urinary excretion of ASP is an excellent index of the status of protein metabolism in animals.

Insulin-like growth factor-1: Protein synthesis: Acid-soluble peptides: Rat

Previously we reported that the urinary excretion of acid-soluble peptides (ASP) is potentially an excellent index of whole body protein degradation (Noguchi *et al.* 1982, 1988). Based on observations of the relationship between urinary excretion of ASP and total nitrogen under various nutritional conditions, we proposed a hypothesis for the assessment of protein metabolism. The hypothesis contained two assumptions: the first was that urinary excretion of ASP is proportional to the rate of whole body protein degradation, the second was that the ratio of urinary N:ASP is linearly correlated with the efficiency of dietary N utilization at the recommended requirement level of dietary protein. (i.e. If the dietary protein level exceeded the recommended level, the ratio increased according to the increase in dietary protein content, even in the rats fed on the casein diets.)

It has been shown by several authors that the fractional rate of tissue (for example, muscle) or whole body protein synthesis (or fractional rate of tissue growth or protein gain) is positively correlated with the fractional rate of their protein degradation (i.e. tissues which are actively synthesizing their proteins also degrade their proteins actively) if animals are not suffering from special degenerative diseases or are not given drugs such as glucocorticoid or thyroid hormones (Millward *et al.* 1975, 1976; Millward & Waterlow, 1978; Nishizawa *et al.* 1978; Reeds *et al.* 1980; Bates & Millward, 1981; MacDonald & Swick, 1981; Goldspink & Kelly, 1984). For example, this relationship is impaired in muscle during starvation, in experimentally induced diabetic animals, or after glucocorticoid administration. Under those conditions, the fractional rate of protein synthesis decreased or was unchanged and that of protein degradation increased (Millward *et al.*

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1976; Millward & Waterlow, 1978; Nishizawa *et al.* 1978; Conde, 1979; MacDonald & Swick, 1981; Odedra & Millward, 1982; Odedra *et al.* 1983).

If our previous hypothesis is valid, urinary excretion of ASP (which is supposed to reflect the rate of whole body protein degradation) and the rate of whole body protein synthesis should be correlated in normal animals. The first purpose of the present investigations was to examine this assumption.

Recently, the important role of insulin-like growth factor-1/somatomedin C (IGF-1) in protein anabolism has been demonstrated by many authors (Reeves *et al.* 1979; Clemmons *et al.* 1981, 1985; Prewitt *et al.* 1982; Maes *et al.* 1984; Yahya *et al.* 1986). This hormone is known to be produced primarily in the liver (Binoux *et al.* 1982; Schwander *et al.* 1983) in response to pituitary growth hormone and the nutritional status of men or animals (Daughaday, 1983; Yamashita & Melmed, 1986; Reichlin, 1988). The concentration of plasma IGF-1 is high under good nutrition and low in starvation or protein deprivation. Based on these findings, we suggest that urinary excretion of ASP may be correlated with the plasma concentration of IGF-1 under various nutritional conditions, particularly as the rate of whole body protein synthesis is correlated with that of whole body protein degradation, as described previously. The second purpose of the present investigations was to elucidate the relationship between the urinary excretion of ASP and plasma immunoreactive IGF-1 concentration.

MATERIALS AND METHODS

Animals and diets

Male Wistar rats (body-weight 90 g) were fed on a casein diet for 4 d, at which time they were divided into three groups of ten rats each. The first group received the same casein diet, the second group a gluten diet and the third group a protein-free diet. After 5 d (2 d for the casein group), urine samples from half the rats of each dietary group were collected for 24 h. After the collection period (i.e. on the 6th day for the gluten and protein-free groups and the 3rd day for the casein group), these rats were injected with [U-^{14}C]tyrosine (118 μCi/kg body-weight) and L-alanyl-L-tyrosine (1·7 mmol/kg body-weight) intraperitoneally. Arterial blood was obtained on the same day from the remaining half of each group by incision of the carotid artery under light diethyl ether anaesthesia. Blood clotting was prevented with EDTA as recommended for the immunoreactive IGF-1 assay (Nichols Institute Diagnostics assay kit, see p. 517).

The casein diet contained the following ingredients (g/kg): casein 120, L-methionine 2, mineral mixture 40, vitamin mixture 10, soya-bean oil 50, cellulose powder 50, β-maize starch 728. The gluten diet was prepared by replacing casein with wheat gluten and L-methionine by β-maize starch. The protein-free diet was prepared by replacing casein and L-methionine by β-maize starch. The vitamin and mineral mixtures, which were formulated according to Rogers & Harper (1965), were obtained from Oriental Yeast Co. (Tokyo). The diets and water were given *ad lib.*

The body-weight (g) of the rats was initially 90 (SE 1·1), and after 4 d was 120 (SE 1·7). The body-weights (g) of the three groups of rats at the time of [^{14}C]tyrosine injection and blood sampling were 125 (SE 1·7), 123 (SE 2·4) and 109 (SE 0·8) for the casein, gluten and protein-free groups respectively.

Determination of the rate of whole body protein synthesis

The method of Garlick & Marshall (1972) and Garlick *et al.* (1980), partially modified by Funabiki *et al.* (1986), was employed for the estimation of the rate of whole body protein synthesis. In brief, 20 min after injection of [^{14}C]tyrosine (118 μCi L-[U-^{14}C]tyrosine with

1·7 mmol unlabelled L-alanyl-L-tyrosine/kg body-weight), the rats were killed by exanguination under light diethyl ether anaesthesia. The whole body was frozen in liquid N_2. The frozen body was stored at −80° until required for analysis. The whole body was ground using a meat chopper and free tyrosine was obtained by extracting three times with trichloroacetic acid (50 g/l; TCA), using 1 ml TCA/g tissue for each extraction. The residue from extraction was retained for the determination of radioactivity in the protein. The protein fraction was hydrolysed with 6 M-hydrochloric acid at 105° for 24 h in a sealed and evacuated tube. After hydrolysis, HCl was removed by evaporation using a rotary evaporator, and the resulting amino acid mixture was used to obtain tyrosine. Tyrosine was isolated from the hydrolysate and the TCA extract as described by Funabiki *et al.* (1986). Radioactivity in tyrosine was determined as described previously (Takahashi *et al.* 1985). The amount of tyrosine was determined fluorometrically (Waalkes & Udenfriend, 1957) after converting tyrosine into tyramine using tyrosine decarboxylase (*EC* 4.1.1.25).

Determination of immunoreactive IGF-1

Plasma immunoreactive IGF-1 was determined using an assay kit (Nichols Institute Diagnostics, California); the assay kit was prepared for human IGF-1 and values were expressed as the unit equivalent to human IGF-1. However, there is no evidence on which to base the assumption that cross-reactions of rat IGF-1 and human IGF-1 with the anti-human IGF-1 antibody are equivalent. However, only three amino acid residues in rat IGF-1 are different from human IGF-1 (Shimatsu & Rotwein, 1987; H. Kato, A. Okoshi, Y. Miura and T. Noguchi, unpublished results), thus the previously stated assumption may be correct.

Determination of urinary ASP

Urinary excretion of ASP was measured as described previously (Noguchi *et al.* 1988).

Statistical analyses

The relationship between the urinary excretion of ASP-form leucine plus valine and the rate of whole body protein synthesis was analysed by applying linear regression. The differences among the means were analysed by Tukey's Q test (Snedecor & Cochran, 1967).

RESULTS

Fig. 1 shows the relationship between the urinary ASP-form leucine plus valine excretion and the rate of whole body protein synthesis, indicating a linear correlation.

Table 1 shows values for plasma immunoreactive IGF-1 concentration, urinary excretion of ASP-form leucine plus valine and the rate of whole body protein synthesis in rats given casein, gluten or protein-free diets. The results show that all values were highest in casein-fed rats and lowest in rats fed on the protein-free diet. The values for gluten-fed rats were found to be between those for rats fed on the casein and protein-free diets. However, the differences in the rate of whole body protein synthesis between the gluten-fed rats and those fed on the protein-free diet or on the casein diet were not statistically significant.

DISCUSSION

The present investigations clearly showed that the rate of urinary excretion of ASP is an excellent index of protein metabolism of rats. A correlation was established with whole body protein synthesis (or whole body protein degradation if our assumption is the case, see p. 515) and plasma immunoreactive IGF-1 concentration. However, we suggest that the correlation between rate of urinary excretion of ASP and fractional synthesis rate of whole

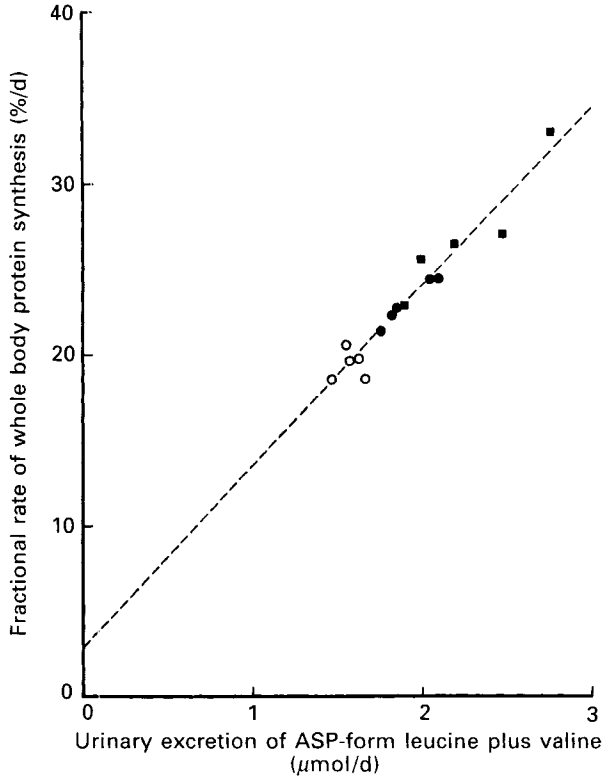

Fig. 1. The relationship between the urinary excretion of acid-soluble peptide (ASP)-form leucine plus valine and the rate of whole body protein synthesis for groups of rats fed on a casein (■), gluten (●) or protein-free diet (○) (for details of the diets, see p. 516). Each point represents an observation for one rat. The values were fitted to a linear regression (----), $Y = 10{\cdot}7X + 2{\cdot}6$, where Y is the fractional rate of whole body protein synthesis (%/d) and X is the urinary excretion of ASP-form leucine plus valine (μmol/d). Standard deviation of the regression coefficient was 1·03 and standard deviation from the regression was 1·33. The regression was highly significant ($P < 0{\cdot}01$). For details of procedures, see pp. 515–516.

Table 1. *Urinary excretion of acid-soluble peptide (ASP)-form leucine plus valine, the rate of whole-body protein synthesis, and plasma immunoreactive insulin-like growth factor-1 (IR-IGF-1) concentration in rats fed on a casein, gluten or protein-free diet*

	Urinary excretion of ASP-form leucine plus valine (μmol/d)		Fractional rate of whole body protein synthesis (%/d)		Plasma IR-IGF-1 (units/ml)	
Diet	Mean	SE	Mean	SE	Mean	SE
Casein	2·2[a]	0·2	26·8[a]	2·5	6·1[a]	0·1
Gluten	1·9[b]	0·1	22·8[ab]	0·7	3·7[b]	0·6
Protein-free	1·6[c]	0·0	19·7[b]	0·4	1·5[c]	0·2

[a, b, c] Values within columns with unlike superscript letters were significantly different ($P < 0{\cdot}05$ or less) by Tukey's Q test (Snedecor & Cochran, 1967).

body protein will be obtained only when whole body protein turnover is normal. Under normal conditions, the fractional synthesis rate of whole body proteins generally shows a positive correlation with the fractional degradation rate of whole body proteins, as described previously. As discussed previously (see p. 515, and Nam *et al.* 1990), this correlation will not be obtained when animals are suffering from specific degenerative diseases, are starved, or are given drugs such as glucocorticoids or thyroid hormones. Under these conditions, excretion of ASP will be enhanced, or even if it is not enhanced, the synthesis of whole body proteins will be severely impaired. We have shown that abnormal excretion of urinary ASP is found under these conditions (Nam *et al.* 1990). In streptozotocin-induced diabetes, urinary excretion of ASP is greatly increased, with a concomitant increase in urinary N:ASP (Nam *et al.* 1990). However, when dexamethasone was administered to adrenalectomized rats, urinary ASP excretion greatly increased, and urinary N:ASP did not change significantly (Nam *et al.* 1990). In the former case, the correlation between the rate of whole body protein synthesis and degradation may be impaired and in the latter case it may be unchanged.

The correlation between ASP excretion and plasma immunoreactive IGF-1 concentrations also favours our assumption that ASP is an excellent index of protein metabolism in rats. The concentration of IGF-1 reflects the status of N assimilation under various nutritional or physiological conditions (Reeves *et al.* 1979; Clemmons *et al.* 1981, 1985; Prewitt *et al.* 1982; Maes *et al.* 1984; Yahya *et al.* 1986). The present results suggest intimate physiological relationships among IGF-1 concentration, whole body protein synthesis (or possibly degradation) and ASP excretion. In other words, the present investigations clearly showed the validity of our hypothesis that urinary ASP is an excellent index of protein metabolism in rats and probably in humans. During normal growth, urinary ASP excretion will reflect IGF-1 concentration, and in turn the growth rate of the animals. Therefore, by determining urinary ASP, the rate of synthesis (or degradation, if our assumption is true, see p. 515) of whole body proteins may be predicted. However, an abnormal increase in ASP could suggest the presence of a degenerative disease. Furthermore, an abnormally high N:ASP could suggest impaired utilization of dietary N or re-utilization of endogenous N. This index could be more sensitive than the rate of urinary ASP excretion per unit body-weight or per unit metabolic body size.

As discussed previously (Noguchi *et al.* 1988), there are many individuals whose status of protein metabolism (the whole body protein turnover rate or the efficiency of utilization of dietary proteins) should be assessed. The present results provide further physiological evidence to support our previous proposal for the assessment of protein metabolic status from the urinary excretion of ASP.

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