Quantity as Well as Quality of Dietary Protein Affects Serine Dehydratase
Gene Expression in Rat Liver

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Summary Weanling rats were fed respective diets diverse in protein source and content
for a full week, and hepatic serine dehydratase (SDH) was examined for its gene expression
and activity induction attendant on high protein intake. The protein sources used were
three kinds of milk casein, codfish meat, and wheat gluten. The body weight gain (% aug-
mentation/wk) increased with increasing protein intake and reached a plateau in both milk
casein- and codfish meat-fed rats by protein intake above 2.5 g/100 g BW/d; however, the
body weight gain continued to increase albeit at a slower rate in wheat gluten-fed rats. Quite
similar tendencies were also seen in nitrogen balance. The ascent of SDH activity induction
and its causal gene expression were characterized as codfish meat>milk casein>>wheat
gluten in order of response to protein intake near or more than 4 g/100 g BW/d. The differ-
ence in SDH gene expression among these dietary proteins was substantiated by a confirma-
tion experiment in which six rats of each group were fed 25% or 50% protein diets under
the same conditions as above. Hence, the quantity as well as quality of dietary protein
turned out to have an influence on SDH gene expression.

Key Words serine dehydratase, nitrogen balance, protein-nutritive value, gene expres-
sion, activity induction

It is a well-known fact that the activity induction of
serine dehydratase (SDH; EC 4.2.1.13) in rat liver is
brought about by consecutive ingestion of dietary pro-
tein over the nutritional requirement (1–5). In the case
of excessive protein intake, surplus amino acids are
mostly catabolized in the liver and amino- or amide-ni-
trogen is excreted as urea into the urine (6), whereupon
a functionally hypertrophied liver is occasionally at-
tended with cell proliferation or a rise in the activity
of metabolic enzymes (1, 2, 5). Among them is SDH capa-
cable of converting L-serine to pyruvic acid for gluco-
neogenesis. It is, however, impossible for L-serine, or a com-
bination of L-threonine and glycine otherwise, to pre-
dominate in the amino acids arising from protein diges-
tion. Meanwhile, there is abundant [not a little—this
means “much”] information on the regulation of SDH
induction by glucagon, glucocorticoid, and insulin
(7–10). This inference has been derived from hormonal
situations under high-protein (i.e., low-carbohydrate
feeding), fasting, or diabetic conditions, but is merely
substantiated by experiments with immature rats given
relevant hormones in enormous doses (8, 9). As for the
dietary adaptation of SDH, its causal high protein in-
take provides a physiologically interesting feature in
some respects. A number of experiments have been car-
rried out in this connection thus far (1–5, 7–9), but it is
difficult to make a comparison among past data be-
cause of the diversity in growing stage, feeding period,
eating antecedents, and/or criteria for measured val-
ues.

At the outset of up-to-date reexaminations into the
mechanism of SDH induction, the present paper deals
with the activity induction and gene expression of SDH
in weanling rats fed a variety of diets different in protein
source and content over a period of 1 wk.

MATERIALS AND METHODS

Chemicals. Chemicals of analytical grade were pur-
chased from commercial sources and used without fur-
ther purification.

Animals and diets. Seventy-four weanling male rats
of the Sprague-Dawley strain were obtained from Nihon
S.L.C. (Hamamatsu, Japan) and housed in hanging
stainless wire cages in an air-conditioned facility with a
half-day light/dark cycle (fluorescent lighting between
8:00 and 20:00). After a few days’ acclimatization, the
weanling rats were divided into three groups differenti-
ated by the feeding of dietary protein: groups of three
(milk casein or wheat gluten group) or four (codfish
meat group) were allowed free access to the respective
diets (difference in protein content shown in Table 1).
Table 1. Composition of the experimental diets (%).

|                  | Milk casein<sup>1</sup> (n=18) | Codfish meat<sup>1</sup> (n=32) | Wheat gluten<sup>1</sup> (n=24) |
|------------------|-------------------------------|-------------------------------|---------------------------------|
| Protein<sup>2</sup> | [5.10, 15, 20, 25, 50]         | [3.6, 9, 12, 15, 20, 25, 50]    | [5.10, 15, 20, 25, 30, 40, 50]   |
| α-Cornstarch<sup>2</sup> | [79, 74, 69, 64, 59, 34]       | [81, 78, 75, 72, 69, 64, 59, 34]| [79, 74, 69, 64, 59, 54, 44, 34] |
| Soybean oil<sup>3</sup> | 5                             | 5                             | 5                               |
| Mineral premix<sup>4</sup> | 5                             | 5                             | 5                               |
| Vitamin premix<sup>5</sup> | 1                             | 1                             | 1                               |
| Cellulose powder<sup>5</sup> | 5                             | 5                             | 5                               |

<sup>1</sup> Bovine milk casein and wheat gluten were purchased from Oriental Yeast Co. (Tokyo, Japan) and Nacalai Tesque, Inc. (Kyoto, Japan), respectively. Codfish meat was a gift of Nihon Suisan Kaisha, Ltd. (Tokyo, Japan). Protein contents ranged from 3 or 5% to 50%, among which the differences were corrected by the addition of α-cornstarch.

<sup>2</sup> Purchased from Nippon Starch Chemical Co., Ltd., Osaka, Japan.

<sup>3</sup> A gift of Fuji Oil Co., Osaka, Japan.

<sup>4</sup> AIN-93G likeness, products of Oriental Yeast Co.

<sup>5</sup> Purchased from Nacalai Tesque, Inc.

The feeding period was a full week. Food intake and body weight were measured on alternative days. On the last day, two days’ feces and urine accumulated to that time were collected to assess nitrogen balance. The rats were then sacrificed and their livers excised subjected to deep-freezing. In a confirmation experiment, a total of 36 weanling rats were subdivided into six groups (n=6), which were individually fed either a 25% or 50% protein diet different in protein source, and after one-week’s time the livers were taken out for characterization of the activity induction and gene expression of SDH.

This experimental design was approved by the Animal Experiment Committee of Kyoto Prefectural University, and the rats were handled according to the Guidelines for Care and Use of Laboratory Animals.

Nitrogen balance and protein intake. Nitrogen contents in diets, feces and urine were determined according to the semi-micro Kjeldahl method, as usual. Apparent nitrogen balance was for convenience obtained on the basis of these data by subtracting excretal nitrogen from dietary nitrogen intake. When necessary, dietary nitrogen intake was changed into protein intake using a conversion factor of 6.38 for milk casein, 6.25 for codfish meat, and 5.83 for wheat gluten.

Activity measurement of SDH. The activity of SDH was measured as previously described (8). In brief, the reaction was initiated by adding 0.1 mL of clarified liver homogenate to 0.5 mL of 400 mM L-serine and 0.5 mM pyridoxal phosphate in pH 8 phosphate buffer, allowed to stand at 37°C for 10 min, and terminated with 1.5 mL of 0.012% dinitrophenylhydrazine in 2N HCl followed by colorimetric measurement at 510 nm with 4 mL of 2N NaOH. A unit of the activity corresponds to the amount of SDH capable of catalyzing pyruvate formation at 1 nmol/min under the above reaction conditions. Protein determination conformed to the Lowry-Folin procedure.

Amplification of SDH mRNA (RT-PCR). Frozen liver was crushed to pieces together with a guanidinium-phenol-chloroform method (11), from which total RNA was quickly extracted without any considerable impairment. A fixed quantity of total RNA (1 μg) was converted to cDNA with the aid of reverse transcriptase (RT) in 19 μL of RT buffer containing a mixture of dATP, dCTP, dGTP, and dTTP (1 mM), 0.5 units of ribonuclease inhibitor and 100 pmol of random primer via incubation at 32°C for 10 min, 42°C for 20 min, 99°C for 5 min, and 4°C for cooling in a prescribed manner. Next, its quarter was mixed with 20 μL of polymerase chain reaction (PCR) buffer containing 1.25 units of Taq polymerase and 0.1 mM paired primers for SDH such as 5'-CTCCTGGAAGAGCTGAAGG-3' (sense) and 5'-CCACCAAGATCTTCTCATCG-3' (antisense) followed by amplification for SDH mRNA under conditions of 25–30 cycles at 94°C for 30 s, 55°C for 2 s, and 68°C for 25 s. Parallel to this reaction, a housekeeping gene, β-actin mRNA, was also amplified for the normalization of signals using 5'-CTACATGACTGCTGGTGG-3' (sense) and 5'-TACACAGTCTACAGTCG-3' (antisense) as paired primers. The resulting PCR products were electrophoresed in 1.2% agarose gel together with molecular weight markers for reference in the usual way, each band being visualized under UV irradiation after staining with ethidium bromide. The relevant bands were quantitated by image analysis with “Scanalytics” software (CPST, Billerica, MA, USA).

Statistical analysis. Values were obtained as the means±SD for six rats in the confirmation experiment, which were analyzed by Tukey’s test following ANOVA. Calculations were carried out using JMP4.0.51 for Macintosh (SAS Institute Inc., Cary, NC, USA). The differences among groups were considered significant at p<0.05.

RESULTS

Figure 1 illustrates a relationship between protein intake (g/100 g BW/d) and body weight gain (% augmentation/wk) with regard to dietary proteins such as milk casein, codfish meat, and wheat gluten. The increments nearly reached a plateau for protein intake at a little more than 2 g/100 g BW/d in both the milk casein- and codfish meat-fed groups. Such was not the case with the wheat gluten group, which still showed an increase

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Fig. 1. Growth of weaning rats fed respective diets different in protein source and content over a period of 1 wk. Body weight gain was plotted against its corresponding protein intake. Protein intake was determined based on the Kjeldahl nitrogen content of the diets and the amount of consumption. Each circle represents the value measured for each rat.

Fig. 2. Relation of nitrogen balance to protein intake. Feeding conditions were the same as in Fig. 1. Nitrogen balance was estimated from the difference between dietary nitrogen and excretal nitrogen. Each circle represents the value measured for each rat.

Fig. 3. Activity induction of hepatic SDH in response to protein intake. The same rat livers as in Figs. 1 and 2 were used for activity measurement of SDH. Each circle represents the value measured for each rat.

The changes in nitrogen balance (mg/100 g BW/d) present upon protein intake as to the respective protein sources are likewise depicted in Fig. 2. The slopes of linear plots for milk casein and codfish meat resembled each other to a great degree, while the angle for that of wheat gluten was only half that of the former two. These situations were faithfully reflected in the growth curves.

Figure 3 makes a comparison among the three groups given milk casein, codfish meat, and wheat gluten as protein sources, respectively, with regard to the SDH activity levels of their relevant individuals. As for the SDH activity, its induction in the casein-fed group became conspicuous by protein intake at more than 4 g/100 g BW/d corresponding to the 50% casein diet. Neither the 25% nor 50% casein diet had a protein intake range from 3 to 4 g/100 g BW/d. Alternatively, protein intake in the fish meat-fed group increased at 2.5 g/100 g BW/d, and reached a level twice as high as on the account of its lysine-deficient quality.

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the casein-fed group, more than 4g/100g BW/d. On the contrary, the SDH activity level in gluten-fed rats was less than that in casein- or fish meat-fed rats even in the case of high protein intake, being only half or one-third of the latter in association with the poor nutritive value of gluten. On looking at amplified SDH mRNA (Fig. 4), the variations of activity and mRNA levels were exactly alike in shape to one degree or another among dietary groups. Interestingly, both casein and fish meat, but not gluten, brought about a steep rise in SDH mRNA as the result of protein over-intake beyond requirements for growth.

In order to substantiate the above findings, a confirmation experiment was carried out where a total of 36 weanling rats were separated into six groups, each fed a different protein source and protein content selected from fragmentary information in the preceding experiment. Table 2 shows the results measured concerning body weight gain, food intake, nitrogen balance and tissue weight to check the degree of growth for each group. There were no significant differences in food intake and tissue weight among these six groups, while the gluten-fed group was inferior to the other dietary groups in terms of body weight gain and nitrogen balance, but vice versa in urinary nitrogen excretion (e.g., 296±31 mg/100 g BW/d in 25% wheat gluten group against 155±18 and 146±21 mg/100 g BW/d in 25% milk casein and codfish meat groups, respectively). The results of SDH activity and mRNA measurements are represented with rod-like graphs in Fig. 5A statistically significant distinction was observed for SDH activity and its gene expression between both groups given 25% and 50% protein-based diets irrespective of the varied protein sources. It thus turned out that codfish meat would function most effectively in SDH induction, followed by milk casein, and wheat gluten is somewhat inferior to them in this respect, although all differences among the dietary groups at the same protein level were not significant.

**DISCUSSION**

In so far as immature rats are concerned, a significant rise in SDH induction was ascertained by 1-wk feeding of diets with a 50% protein content. Its responsiveness was not only dependent on high protein intake but also on age-related decrease in protein requirement to a considerable extent (data not shown). In other words, immature rats require abundant protein intake for their growth, but mature rats, being fully grown, do not require so much protein with the exception of metabolic compensation. In this study, we examined the response of SDH induction to high protein intake in wean-
Fig. 5. Difference in activity induction and gene expression of hepatic SDH among dietary groups. Weanling rats were fed 25% or 50% protein-based diets diverse in protein source, and a week later, were examined for SDH activity induction (A) and gene expression (B). Values are the means±SD for six rats; those not sharing a common superscript letter are significantly different at p<0.05.

A couple of simple explanations may be presently offered for the reason why high protein intake elevates SDH gene expression linked with activity induction: one is that surplus amino acids over the protein requirement need to undergo metabolism as soon as possible, and the other is that endocrine hormones themselves or thereby mediated active factors take part in the gene amplification of metabolic enzymes. Among previous views to meet the former was a "substrate induction" theory by Nakagawa et al. (12), who had observed the activity induction of SDH in rats given a semi-purified diet containing only L-serine besides indispensable amino acids as nitrogen sources. Recently, however, it is doubtful that the individual amino acids serve as inductive signals for their relevant metabolic enzymes in higher animals.

Redundantly, the serine content of gluten (48.6 mg/g protein) is slightly lower than that of casein (53.4 mg/g protein), but higher than that of codfish meat (42.0 mg/g protein). Nevertheless, codfish meat and casein were superior to gluten in SDH induction. The pro-
tein-nutritive value of gluten, in which l-lysine serves as the first limiting amino acid, is no more than one-third of the requirement for growing rats under moderate conditions. Accordingly, the growth curve was no match for that of the casein- or codfish meat-fed groups. Plant protein is generally said to be inferior in digestibility to animal protein, but there was virtually no appreciable difference between casein and wheat gluten in vivo digestion (13). It is noteworthy in this connection that a number of digestive products from gluten were metabolized and excreted into the urine, daily amounting to twice the urinary nitrogen of the casein- or codfish meat-fed groups.

The second limiting amino acid in gluten is l-threonine, which was at a protein level of 27.8 mg/g, relative to the amino acid content in casein and codfish meat (43-44 mg/g protein). Threonine concentration in blood circulating the body is kept within limits because of its importance as a neurotransmitter in the brain (14). For this reason, threonine may possibly behave like a signal for protein over-intake. Actually, the continuance of high protein intake seems to cause a decrease, rather than increase, in the blood threonine concentration by virtue of enhanced SDH (1, 2, 4, 15). SDH acts directly on threonine to form α-ketobutyric acid (16), so it is called T-SDH at times. More decisive evidence against the possibility is the observation that much supplemental threonine can temporarily elevate its postprandial concentration in the blood, but never contribute to the active induction of T-SDH (14).

An unusual elevation of T-SDH activity is also brought about by fasting or diabetic situations (9) besides superfluous protein intake. In extreme cases, excess protein can be replaced by low carbohydrates. Accordingly, a virtual insulin shortage is characteristic of hormone secretion in common with these situations. Recently, it has become apparent that insulin depresses transcription via its linkage with the cis-element (e.g., IRS, in the 5'-upstream region of phosphoenolpyruvate carboxykinase (PEPCK) or insulin-like growth factor 1 binding protein (IGFBP-1) gene) (17-19). Similarly, T-SDH is restrained in terms of its gene expression under insulinotropic conditions. Nevertheless, IRS has not been identified to date (20). Such a hypoinsulinemia may safely be referred to as relative hyperglucagonemia, if any, in the opposite sense. The conception of high protein intake raising the ratio of glucagon to insulin has been generally accepted without exact verification, but there is no information on glucagon recognition at a specific site in the 5'-upstream region of T-SDH gene. In relation to transcriptional regulation, much attention is brought back to the occurrence of deoxyribonuclease-hypersensitive sites within the 6 kb upstream region of SDH gene due to high protein intake found by Ogawa et al. (3). This fact dimly hints at the possibility that signals at transcriptional stages are triggered by the very super-nutrition of combined amino acids rather than limited amino acids arising from high protein intake. The response to protein of poor quality may be accounted for by reference to the example of insulin-like growth factor 1 (IGF-1) gene expression ranked low in gluten- or zein-fed rats as compared to casein-fed rats (21, 22).

Even so, in what manner super-nutrition gives rise to the gene expression and activity induction of T-SDH remains to be further investigated in many aspects. Elucidation of the mechanism would lead to a significant gain in information regarding signal transduction by nutrients.

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