Difference of Plasma Amino Acids Following Casein or Soy Protein Intake: Significance for Differences of Serum Lipid Concentrations

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Summary There were significant differences of postprandial plasma concentrations for 8 amino acids (Cys, Val, Met, Leu, Tyr, Lys, Trp, and Arg) depending on whether pigs consumed a meal containing casein or isolated soy protein. The postprandial plasma amino acid pattern conformed with the amino acid composition of the dietary protein (except for Ser). The data, however, do not allow to conclude unambiguously, whether specific amino acids are responsible for the difference of serum cholesterol following casein or soy protein intake. Significant differences between casein- and soy-fed rats were observed regarding total and free plasma thyroxine and triiodothyronine concentrations. This observation can explain the accompanying different serum cholesterol concentrations. The different thyroid hormone concentrations were not paralleled by differences in TSH levels suggesting that dietary proteins affect thyroid function at the thyroid gland.

Key Words casein, soy protein isolate, serum cholesterol, thyroid hormones, TSH, amino acids

Introduction Numerous laboratories have observed that animal or vegetable protein cause different serum cholesterol concentrations if fed to rabbits or rats (1). The finding surprises the biochemist because no obvious links between amino acid and cholesterol metabolism are known which may explain this response of lipid metabolism to the exchange of dietary protein.

Interestingly, this response is characterized by a marked species specificity. Rodents respond, whereas pigs(2) and primates(3) do not. It is particularly important to recognize that normal humans do not have different serum lipids independent of whether animal or vegetable protein is consumed, as documented by the carefully controlled studies of van Raaij et al.(4, 5).

Moreover, female rats respond more than male animals(6) and young, growing animals are more prone to show protein-induced differences of serum lipids than adult animals(7). Also other dietary components as fiber and the kind of carbohydrate (dimeric vs. polymeric(8)) interfere with this peculiar metabolic response.

These observations have led in the past several investigators to propose biochemical mechanisms of how dietary proteins may affect serum lipids. The mechanisms proposed so far may be divided in two major categories (Fig. 1): either gastro-intestinal or metabolic. Gastro-intestinal mechanisms affect the entero-hepatic circulation of either cholesterol or bile acids and cause thereby protein-dependent differences of steroids lost via fecal excretion. Metabolic mechanisms involve the protein-induced differences of plasma amino acid or hormone concentrations, which may then modulate tissue cholesterol turnover. It is the purpose of this study to explore whether differences of plasma amino acids following consumption of casein or soy protein may cause different serum cholesterol concentrations.

Methods Pig experiments Six adult female Göttingen miniature pigs, aged about 1 year and weighing 34–35 kg, were used. The pigs were fitted with a permanent silicon catheter in the v. jugularis externa 2 weeks before the experiment. They were
Fig. 1. Categories of physiological functions affected by dietary casein or soy protein isolate.

individually housed in a room maintained at 20±2°C and 60–70% humidity.

Diet Water was provided ad libitum and food was given in 2 equal amounts at 600 and 1600h. The pigs were given 390–410g dry matter per day of a semipurified diet to maintain constant body weight, i.e. daily energy intake = \(0.44 \text{ MJ metabolizable energy} \times (\text{kg body weight})^{-0.75}\). The diet (Table 1) imitated the gross composition of a western style diet for humans, providing 18, 52, and 30% of energy from protein, carbohydrate and fat, respectively. According to the chemical analysis of the diet crude protein content \((N \times 6.25)\) in dry matter was 20.0 wt% (casein) and 20.1 wt% (soy protein isolate). Crystalline cholesterol (1%, wt/wt) was added to the diet in order to create a metabolic state where casein causes higher serum cholesterol levels than soy protein (9) which is not the case on a low cholesterol diet (10).

Analytical procedures Plasma samples were obtained by centrifugation at 3600 × g for 15 min. Plasma amino acids were measured by ion exchange chromatography using an amino acid analyzer (LC 5001, Biotronik, Munchen, West Germany) after deproteinization with sulfosalicylic acid, following the method of Spackman et al. (11). Plasma cholesterol was determined enzymatically according to Roschlau et al. (12).

Rat experiments Animals, weighing 82 g were fed a semisynthetic diet for 17 days ad libitum. The diet contained 10 wt% protein, 5 wt% soy oil and 74.8 wt% sucrose. At the end of this period blood samples were obtained by exsanguination. Plasma hormone concentrations were analyzed by radio-immunoassay. Free thyroid hormones were determined with a low-affinity antibody method (Mallinckrodt Diagnostika, Dietzenbach, FRG, SPAC ET). The rationale for this method is discussed extensively in Ref. (13). We compared the results of this method with equilibrium dialysis. A satisfying agreement of both methods shows that free thyroid hormone concentrations have been determined accurately (14).

Plasma TSH following casein or soy protein In a separate nutrition experiment plasma TSH concentrations were studied in pigs.

For this purpose 2 groups of 10 growing male
Göttingen miniature pigs (weight 10.4-15.9 kg) were kept for 5 weeks on a semisynthetic diet based either on casein- or soy-protein isolate. The diet contained 14 energy % protein, 32 energy % fat (P/S ratio 0.43), 54 energy % carbohydrate (maize starch/sucrose = 55/45) and 0.06 wt% cholesterol. On the morning of day 1, 15, and 35 of the experiment plasma samples were obtained after an overnight fast by venipuncture (v. jugularis) and analyzed for TSH levels as follows: Since a commercial assay for porcine TSH was not available serum porcine TSH was measured with an assay developed in our laboratory (p-TSH-RIA). This RIA was established first by radiolabelling p-TSH with I-125 using a modified chloramine-T-method. Labeling efficacy and immunologic potency of p-TSH were proven by binding tests and competition with so-called cold antigen against a specific anti-p-TSH antibody. In general, all labeling procedures yielded a binding of p-TSH to the antibody of 32±2% without presence of cold antigen. Secondly, the final dilution of the first antibody was evaluated to be 1:50,000. Third, a second antibody against anti-rabbit IgG (for the first antibody rabbits were immunized) was added after an overnight incubation.

The standard preparations (known amounts of p-TSH) were established in a TSH-free human serum (= assay matrix). The lower detection limit amounted to 0.3 μU/ml. p-TSH, iodination grade, from porcine pituitary, lyophilized, essentially salt-free (M.W. 32,000) was obtained from UCB-Bio-products S.A., B-1420 Braine-l’Alleud/Belgium.

Results and Discussion

Plasma amino acids There are several nutritional studies reporting on the effect of single amino acids on plasma lipids (15). Table 2 gives a survey on studies related to this problem. Kritchovsky suggested that the lysine/arginine ratio, which is low in plant (soy protein 0.88) and high in most animal proteins (casein 2.04), determines the protein effect on serum cholesterol and the degree of atherosclerosis (16, 17). His view was supported by Sugano et al. (18) who also found a positive correlation between the lysine/arginine ratio and serum cholesterol in rats. Though an addition of lysine to soy protein increased the serum cholesterol level and degree of atheromata, the addition of arginine to casein gave equivocal results (16).

When other investigators supplemented soy protein with lysine or casein with arginine, respectively, this gave no conclusive effects (19-24). Numerous studies have been performed which show that also other amino acids beside lysine or arginine play a role in determining serum cholesterol levels (Table 2). Particular attention was paid to the effect of methionine, which is lower in soy protein than in casein. In many cases a serum cholesterol lowering effect of methionine supplements was observed (for a recent review see Ref. (1)) though, when added in relatively large amounts to a soy protein diet, it elevated serum cholesterol levels (25-27).

For the concept that differences of amino acid composition of dietary proteins are responsible for differences of serum lipids it is of interest whether plasma amino acids show protein-dependent differences. For this purpose we have studied the circadian changes of plasma amino acids following the consumption of casein or soy protein in a balanced diet. Adult Göttingen miniature pigs were fed an euenergetic semisynthetic diet for 7 weeks. Postprandial plasma amino acids measured after the morning meal of the last day are shown in Table 3. It may be recognized that there were protein dependent significant differences of cystine-cys-

| Amino acid | Rise | Decrease | No change |
|------------|------|----------|-----------|
| Met        | 10   | 10       | 5         |
| Arg        | 2    | 3        | 3         |
| Tyr        | 1    | 1        | 1         |
| Val        |      |          | 2         |
| Leu        | 1    | 2        |           |
| Ala        |      | 1        | 1         |
| Asp-X      |      |          | 1         |
| Gly        |      | 5        | 2         |
| Lys        | 3    | 3        | 5         |
| Cys        | 2    | 9        |           |
| Trp        | 2    | 1        | 1         |
| Ser        |      |          | 1         |
| Ileu       |      | 1        | 1         |
| Phe        |      |          | 1         |
| Glu-X      |      | 3        | 1         |
| His        | 1    |          |           |
| Thr        | 1    | 2        |           |
| Taurine    |      | 3        |           |
Table 3. Plasma amino acid concentrations of minipigs consuming a diet containing either casein or soy protein isolate.

|               | Casein | Soy  | (μmol/liter) |
|---------------|--------|------|-------------|
|               | X³     | SEM  | X³          | SEM    |
| Methionine    | 86.44  | 8.64 | 44.01       | 5.56   |
| Arginine      | 177.81 | 26.90| 251.33      | 33.88  |
| Tyrosine      | 238.56 | 15.55| 184.68      | 23.03  |
| Valine        | 731.19 | 57.60| 526.85      | 66.08  |
| Tryptophan    | 69.10  | 7.90 | 75.40       | 5.50   |
| Leucine       | 468.56 | 28.67| 364.26      | 34.76  |
| Lysine        | 409.38 | 62.50| 309.16      | 32.92  |
| Cystine-Cysteine| 55.19 | 2.56 | 67.73       | 5.16   |

¹ Values are means of all concentrations observed at 60 min and 120 min postprandially. All values showed protein-dependent differences at either °p<0.01 or °p<0.05; n=6. t-test, paired.

Fig. 2. Comparison of ratios of single amino acids in the dietary proteins with the corresponding ratios of postprandial plasma levels. For example, methionine content of soy isolate is 0.4 of that of casein. Left column shows ratio in the diet: soy isolate versus casein. Right column shows ratio in the plasma: after soy isolate feeding versus casein feeding. The ratio was averaged from the early (0–3 h) postprandial amino acid concentrations expressed in μmol/liter.

Met, Arg, Tyr, Val, Leu, Ala, Asx, Gly, Lys, Cys, Trp, Ser, Ile, Phe, His, Thr

* Mean of 12 analyses in the first 3 hours postprandially
proline not analysed in plasma

Moreover, nearly all these differences were concordant with the amino acid composition of the proteins consumed (Fig. 2). The only exception was serine. The surprisingly low plasma concentrations of serine following casein may be due to a low degradation and absorption of phosphorylated peptide sequences (28) which are rich in this amino acid (29).

It becomes obvious also from Fig. 2 that all amino acids (except serine) responded qualitatively as predicted from the amino acid composition of the protein consumed. However, this is not always the case in quantitative terms, as demonstrated by arginine, cysteine-cystine, alanine and glycine. Therefore it is not possible to predict venous amino acid concentrations from the amino acid composition of the protein consumed. In summary, the data presented herein do not allow to conclude unambiguously whether specific amino acids and which amino acids are responsible for the serum
cholesterol modulating effect. Neither the considerable number of amino acids being different as reported herein nor the multitude of contradictory reports by others on single amino acids affecting serum cholesterol (Table 2) favour the hypothesis that one or several amino acid(s) cause protein-induced hypercholesterolemia.

Endocrine response to different dietary proteins

Clinical observations have since long shown that several endocrine disorders are accompanied by abnormal serum lipid concentrations. This knowledge is listed in Table 4. This has led several laboratories to investigate the endocrine response to dietary casein and soy protein. We have observed that dietary soy protein induced significantly higher plasma total thyroxine concentrations if compared to casein (30). We wondered whether free thyroid hormone levels paralleled total thyroxine levels and therefore the following experiment with rats was performed.

In this experiment casein-fed rats had the same food consumption as soy-fed animals and the protein efficiency ratios conformed with earlier reports. The corrected protein efficiency ratio (PER) values amounted to 2.5 and 1.60 for casein and soy protein isolate, respectively. Significant differences between casein- and soy-fed animals were observed regarding total and free thyroxine and triiodothyronine concentrations (Fig. 3). It remains to be established whether these differences can be observed in man consuming either casein or vegetable protein in a tightly controlled nutritional experiment.

Table 4. Hormonal effects on serum cholesterol levels.

| Hormone          | Effect     | Reference               |
|------------------|------------|-------------------------|
| Insulin          | LDL decrease | Lopes-Virella et al., 1981 |
| HDL increase     |            | Sadur and Eckel, 1983   |
| Decrease         |            | Bennion et al., 1977    |
| No effect        |            | Briones et al., 1984    |
| Glucagon         | Decrease   | Aubry et al., 1974      |
| Growth hormone   | Decrease   | Friedmann et al., 1970  |
| Cortisol         | Increase   | Troxler et al., 1977    |
| Thyroid hormones | Decrease   | Agdeppa et al., 1979    |
|                  |            | Hansson et al., 1983    |

Mechanisms for different thyroid hormone levels

Higher plasma triiodothyronine and thyroxine concentrations may be either due to the stimulatory action of the pituitary gland via higher TSH levels (secondary hyperthyroidism) or to an effect exerted on the thyroid gland directly which would be
accompanied by low TSH levels (primary hyperthyroidism). The former of these two possible mechanisms seemed particularly attractive because it is known that brain serotonin concentrations can be influenced by plasma amino acids (31) and that serotonin affects TRH release (32).

Such a mechanism might have been operative because those amino acids affecting brainstem serotonin showed protein-dependent changes indeed in our experiments. Figure 4 demonstrates that the ratio of plasma amino acids affecting brain serotonin (31) was indeed different between casein and soy protein consuming animals. We have measured therefore plasma TSH levels in casein and soy-fed minipigs in order to distinguish between the 2 above mechanisms.

Table 5 shows the porcine plasma TSH concentrations at the beginning and during the experiment. There was no statistically significant difference following 2 or 5 weeks independent of whether casein or soy protein was consumed (Table 5). From this finding we conclude that the higher thyroxine and triiodothyronine levels following soy protein intake are not due to a stimulatory action of the pituitary. The dietary proteins exert very probably their differential effect at the level of the thyroid gland. It remains to be established by future research how this is mediated biochemically.

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Table 5. TSH plasma concentrations following casein or soy isolate intake.

| Diet         | Weeks of intake |
|--------------|-----------------|
|              | 0               | 2               | 5               |
| Casein       | 1.45 ± 0.33     | 1.85 ± 0.27     | 1.59 ± 0.27     |
| Soy isolate  | 1.35 ± 0.29     | 1.17 ± 0.30     | 1.30 ± 0.20     |
| p*           | 0.47            | 0.056           | 0.22            |

Plasma TSH (µU/ml)

The values were averaged and SEM was calculated after transformation to logarithmic values.

* Rank-test, unpaired.

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