Activation of skeletal muscle protein breakdown following consumption of soyabean protein in pigs

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Diets with protein of inferior quality may increase protein breakdown in skeletal muscle but the experimental results are inconsistent. To elucidate the relationship, pigs were fed isoenergetic and isonitrogenous diets based on soyabean-protein isolate or casein for 15 weeks, with four to six animals per group. A higher plasma level of urea (2.5-fold the casein group value, \( P < 0.01 \)), higher urinary N excretion (2.1-fold the casein group value, \( P < 0.01 \)), a postabsorptive rise in the plasma levels of urea, 3-methylhistidine and isoleucine in soyabean protein-fed pigs suggested recruitment of circulatory amino acids by protein breakdown in peripheral tissues. Significant differences between dietary groups were detected in lysosomal and ATP-dependent proteolytic activities in the semimembranosus muscle of food-deprived pigs. A higher concentration of cathepsin B protein was found, corresponding to a rise in the cathepsin B activity, in response to dietary soyabean protein. Muscle ATP-stimulated proteolytical activity was 1.6-fold the casein group value \( P < 0.03 \). A transient rise in the level of cortisol (2.9-times the casein group value, \( P < 0.02 \)) occurred in the postprandial phase only in the soyabean group. These data suggest that the inferior quality of dietary soyabean protein induces hormonally-mediated upregulation of muscle protein breakdown for recruitment of circulatory amino acids in a postabsorptive state.

Amino acid limitation: Protein degradation: Energy retention

An improvement of protein quality by supplementation with essential amino acids increased both protein synthesis and degradation in swine (Salter et al. 1990; Kerr & Easter, 1995). These reports contrast with studies that did not find significant changes in protein turnover by amino acid supplementation in pigs (Fuller et al. 1987) and rats (Garlick & Grant, 1988). The mechanism underlying the action of dietary proteins with inferior quality is incompletely understood, so that the biological background of the differing results remains to be clarified. Hormones such as insulin and glucocorticoids have been reported to be involved in the response to changes in the amino acid supply (Seve & Ponter, 1997). Recent studies have suggested that liver and muscle protein synthesis and degradation (Fereday et al. 1998; Patti et al. 1998) are the targets of the hormonally induced regulation to match changes in diet.

The conflicting results mentioned earlier may arise from different methods of measuring protein synthesis and degradation (Grizard et al. 1995; Smith et al. 1998). However, interactions among hormonal and dietary effects can also provide ambiguous results, since insulin-induced inhibition of proteolysis seems to be sensitive to amino acid supply (Fereday et al. 1998). Thus, the aim of the present study was a direct analysis of some proteolytic activities in skeletal muscle in relation to plasma levels of amino acids in response to diets based on soyabean protein or casein.

Materials and methods

Materials

Nutrients were purchased from Deutsches Milchkontor GmbH Hamburg, Interfood, Bad Homburg, and Schleicher and Schüll, Dassel, Germany. Catheters were from Cook, Mönchengladbach, Germany. Rabbit antisera to cathepsin B and cystatin C and cathepsin B standard were from Calbiochem-Novabiochem GmbH, Bad Soden, Germany; proteinase inhibitors from Alexis, Grünberg, Boehringer-Mannheim, and Serva, Heidelberg, Germany; the calpain substrate, tert-butoxycarbonyl-L-leucyl-L-methionine-7-amino-4-chloromethylcoumarin; the cathepsin substrates,
by heat treatment under mild alkaline conditions. The diet (with casein or soyabean-protein isolate as the only independent variable) provided 2.5-times the maintenance requirement of metabolizable energy, i.e. 1850 kJ ME/kg body weight$^{0.62}$ per d, corresponding to approximately 110 g DM/d and kg body weight$^{0.62}$. The casein diet was supplemented with 11.5 g methionine, 5.8 g threonine and 4.6 g tryptophan/kg dietary protein.

**Energy and nitrogen balance**

The energy balance was measured by indirect calorimetry based on the C and N balances. Measurements were performed in four open-circuit respiration chambers using four successive 24 h periods with daily collection of urine and faeces separately, stored at 0–3°C until analyses. The concentration of O$_2$, CO$_2$ and CH$_4$, the temperature, relative humidity and the rate of air flow were recorded on-line every 10 min. Standing and lying periods were monitored by a video camera. N and DM in feed, faeces and urine were determined by the Kjeldahl method and by oven-drying at 101°C for 48 h respectively. The energy contents of feed, freeze-dried faeces and urine were analysed by an adiabatic bomb calorimeter (C 400; Janke & Kunkel GmbH, Staufen, Germany) followed by gravimetric determination of CO$_2$ for calculation of the C content in feed and faeces as described by Hoffmann et al. (1993).

**Analyses**

Food amino acids were measured by automatic ion-exchange chromatography (Biochrom 20; Pharmacia-Biotech Europe GmbH, Freiburg, Germany) following acid hydrolysis (6 M-HCl, 134°C, 2 h) in an autoclave, cysteine and methionine after oxidation with peroxyformic acid, tryptophan after alkaline hydrolysis with NaOH. Acid-soluble amino acids in plasma or muscle were determined by ion-exchange chromatography after precipitation of protein (sulfosalicylic acid, 0.25 M final concentration, 20 min on ice) and centrifugation (4000 g, 10 min, 4°C) using plasma or muscle extracts. The latter were prepared by homogenization (Ultra Turrax; Janke & Kunkel GmbH) of minced muscle (maximal speed, repeated homogenization for 2 min with 30 s intervals) on ice in five volumes 5 mM-PBS (pH 7.0)/g tissue and subsequent centrifugation (4000 g, 10 min, 4°C). Glycogen was measured in the homogenate (omitting centrifugation) by α-amylase-catalysed release of glucose, detecting glucose before and after digestion. Standard methods were also used for the analysis of glucose (Sigma Diagnostics, catalogue no. 315), of aqueous-soluble protein (Bradford, 1976), of albumin (Sigma Diagnostics, catalogue no. 631) and of urea-N (Sigma Diagnostics, catalogue no. 535). Commercially available enzyme immunoassays were used for assaying cortisol (catalogue no. MDKC01, Milenia, Bad Nauheim, Germany), thyroxine and triiodothyronine (catalogue no. 3894 and 1615, DRG Diagnostica, Bad Nauheim, Germany) and insulin (no. 43914809, Wako Pure Chemical Industries, Bad Nauheim, Germany).
Western blot analysis

Muscle was homogenized as described earlier using a buffer consisting of: 20 mM-N-(2-hydroxyethyl) piperazine-N′-(2-ethanesulfonic acid) (Hepes) pH 7.3, 10 mM-NaCl, 10 mM-KCl, 2 mM-MgSO₄, 0.5 mM-EDTA, 1 mM-1,4-dithiothreitol, 100 ml glycerol/1. After centrifugation (110 000 g, 30 min, 4°C) the protein concentration of the supernatant was determined and the supernatant fraction was stored (–80°C). After thawing, the supernatant fraction was mixed with non-reducing electrophoresis loading buffer (1:2, v/v), incubated for 2 min at 90°C, and electrophoresis and blotting was performed as described by Löhke et al. (1993, 1998) using 40 μg protein per lane. An enhanced chemiluminescence kit (Amersham, Braunschweig, Germany) and a chemiluminescence analyser (Fluor-S Multi Manager; BioRad, Hamburg, Germany) were used to visualize and to quantify the protein bands.

Proteolytic activities

Cathepsin B activity in muscle fibres was detected by flow cytometry (EPICS Elite, Coulter, Krefeld, Germany) as described by Löhke et al. (1995). This technique enables a single cell fluorescence analysis of fibres separate from other cells arising from digestion of muscle tissue by collagenase (Löhke et al. 1995, 2000). The cathepsin B activity was analysed in 20 mM-potassium phosphate (pH 6.5) to inhibit the activity as described by Towatari et al. (1998) as expected. However, the postprandial differences were 20 μM-CA-074) (Towatari et al. 2000). The cathepsin B activity was measured in initial rate conditions, incubating the cells for 20 min at 37°C according to a preliminary experiment, indicating linearity in the fluorescence increase during the first 20 min of an incubation period using the substrate in approximately saturated concentration (30 μM). The specificity of the test was examined using the cell-permeable cathepsin B inhibitor N-(1-L,3-trans-propylcarbamoyloxirane-2-carbonyl)-L-isoleucyl-L-proline (CA-074) (Towatari et al. 2000), dissolved in dimethylsulfoxide. The final concentrations were 20 μM CA-074 (a maximal-effective dose) and 1.4 mM-dimethylsulfoxide. Cathepsin H activity was assayed with 7-amino-4-chloromethylcoumarin-arginine in PBS, pH 7.0, using N-((L-3-trans-ethoxy carbonyloxirane-2-carbonyl)-l-leucyl-3-methylbutylamidine (E-64d) (100 μM) to inhibit the activity as described by Towatari et al. (1991). Calpain activities were determined with tert-butylxycarbonyl-leucine-methionine-7-amino-4-chloromethylcoumarin as a cell-permeable fluorogenic substrate following a method described by Rossier et al. (1993). ATP-dependent proteolysis was measured in the cytosolic fraction of the muscle using azocasein as substrate according to a slightly modified method (Glickman et al. 1998). The homogenization buffer consisted of: 20 mM-

N-(2-hydroxyethyl) piperazine-N′-(2-ethanesulfonic acid) (pH 7.8), 20 mM-NaCl, 10 mM-KCl, 2 mM-MgSO₄, 0.5 mM-EDTA, 1 mM-1,4-dithiothreitol, 100 ml glycerol/1. After centrifugation (110 000 g, 30 min, 4°C), the protein concentration of the supernatant was determined with the Bradford reagent. The supernatant fraction was mixed (1:2, v/v) with azocasein (10 mg/ml) dissolved in homogenization buffer, then 0.01 volumes 5 mM-potassium phosphate (pH 6.5) or ATP (50, 100, 200 mM) dissolved in this buffer were added. After an incubation for 60 min at 37°C, the reaction was stopped on ice. Bovine serum albumin (5 mg/ml final concentration) and TCA (0.37 M final concentration) were added. After 15 min on ice, the precipitate was spun off (4000 g, 10 min, 4°C), and the staining of the supernatant fraction was read at 405 nm. Controls (the same procedure as described earlier but with buffer instead of homogenate) were subtracted. Specificity of the ubiquitin-linked proteolytical pathway (Dick et al. 1996) was examined by clasto-lactacystin β-lactone dissolved in 1,4-dithiothreitol with final concentrations of 2 μM-clasto-lactacystin β-lactone and 2 mM-1,4-dithiothreitol.

Statistical analysis

A computer-aided Jandel scientific statistical package (version 1.02 1994, Erkrath, Germany) was used. Variances were calculated by ANOVA (animals within diets) and orthogonal contrasts were used for the test of differences between dietary groups in serial data (time-dependent levels of amino acids). Differences between dietary groups were evaluated to be statistically significant by t test or as indicated by the tests for normality and equal variances. Paired t test was used to compare temporal differences within dietary groups.

Results

Nitrogen and energy balance

The soyabean-protein diet significantly reduced the N deposition (0.75-fold the casein group value) and increased the urinary N excretion (P = 0.01) 2.1-fold the casein group value (Table 1). The energy balance data in Table 2 show that only the protein-energy retention markedly responded to the soyabean-protein diet with a 1.5-fold decrease (P = 0.01 v. the casein value). The striking increase in urinary N excretion was associated with a strong elevation of the plasma urea level (Fig. 1) in similar magnitude (2.5-fold the casein group value, P = 0.001). The postabsorptive urea level increased (paired t test, P = 0.02) only in the soyabean group (Fig. 1). These data indicate a increase in net overall rate of protein breakdown and amino acid oxidation respectively (Grofte et al. 1998; Young et al. 2000).

Temporal course of circulatory amino acid levels

A dietary deficit in essential amino acids (Fig. 2(c)) caused a postprandial imbalance in circulatory amino acid levels (Fig. 2(a)) as expected. However, the postprandial differences
in amino acids markedly deficient in soyabean protein not only disappeared postprandially but differences occurred in the circulatory concentration of other amino acids (Fig. 2(b)). Details of this postabsorptive response are illustrated in Fig. 3 for lysine and threonine, moderately deficient in soyabean-protein isolate (Fig. 2(c)), and isoleucine, which is supplied in similar concentrations by soyabean protein- and casein-based diets (Fig. 2(c)).

The compensatory postabsorptive response (Fig. 3) can be the consequence of reduced absorption through, or increased release of amino acids from, tissues into the circulation with the prerequisite of an increase in proteolytic activities. The latter hypothesis was examined because of the increase in net overall protein breakdown using soyabean-protein diet-induced rise in plasma urea level and in N excretion as indicators (Table 1 and Fig. 1).

### Acid-soluble muscle amino acids

Skeletal muscle can recruit amino acids released by protein breakdown (Cannon et al. 1991; Mackenzie et al. 1993). In addition, amino acids by themselves affect proteolytic activities (Mortimore & Pösö, 1987). Hence, we looked in food-deprived pigs for the concentration of acid-soluble amino acids in the semimembranosus muscle representative for porcine hindleg muscles (Fig. 4). In contrast to other amino acids, the concentrations of lysine, threonine, and phenylalanine were found to be significantly lower (Fig. 4(a)) in soyabean protein-fed pigs (P ≤ 0.03 v. the casein group), raising the possibility that the muscle contributes to maintenance of the circulatory postabsorptive level of these amino acids. The muscle of soyabean protein-fed pigs contained histidine and its derivative, 3-methylhistidine, in higher concentration (Fig. 4(b)) than the muscle of the casein group (P = 0.04 and P = 0.01 respectively), suggesting myofibrillar protein breakdown contributed to the dietary response.

### Proteolytic activities in the semimembranosus muscle

All three major proteolytic activities in the muscle, the lysosomal, Ca$^{2+}$- and ATP-dependent pathways, can degrade myofibrillar proteins as reported for calpains (Mortimore & Pösö, 1987), lysosomal cathepsin B (Hirao et al. 1984), and proteasomes (Attaix et al. 1998). ATP-dependent proteolysis of azocasein differed between the dietary groups (Fig. 5). In the presence of 2 mM-ATP, the rate of substrate hydrolysis was 1.6-fold higher in the muscle from the soyabean-protein group than from the casein group (P = 0.02). The activity was significantly inhibited by elastolactacycin β-lactone, a selective inhibitor of proteasome activities (Fenteany et al. 1995; Dick et al. 1996). In contrast to Ca$^{2+}$-dependent protolysis (data not reported), significant differences were also detected between the dietary groups in the activity of lysosomal cathepsins B and H (Fig. 6). The cell permeable inhibitor of cathepsin B (N-(L-3-trans-propylcarbamoyloxirane-2-carbonyl)-L-isoleucyl-L-proline (CA-074)) (Towatari et al. 1991), and of cathepsin H (N-(L-3-ethoxycarbonylloxirane-2-carbonyl)-L-leucyl-3-methylbutylamida (E64d)) used in a dose of ≥100 μM (Towatari et al. 1991), significantly reduced the hydrolysis of the substrates for these cathepsins (Fig. 6), indicating that these enzymes contributed to a major portion of substrate hydrolysis.

### Table 1. Nitrogen balance in pigs fed soyabean protein- or casein-based diets* (Mean values and standard deviations)

<table>
<thead>
<tr>
<th>Protein</th>
<th>Weight gain (g/d)</th>
<th>N intake (g N/d)</th>
<th>Urine (g N/d)</th>
<th>Faeces (g N/d)</th>
<th>N deposition (g N/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean SD</td>
<td>Mean SD</td>
<td>Mean SD</td>
<td>Mean SD</td>
<td>Mean SD</td>
</tr>
<tr>
<td>Casein</td>
<td>6 358 ± 33</td>
<td>14.2 ± 0.1</td>
<td>3.3 ± 0.4</td>
<td>6.9 ± 0.6</td>
<td>0.01 ± 0.08</td>
</tr>
<tr>
<td>Soyabean</td>
<td>4† 337 ± 17</td>
<td>15.4 ± 0.3</td>
<td>6.0 ± 0.7</td>
<td>2.2 ± 0.2</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td>P value‡</td>
<td>0.06</td>
<td>0.07</td>
<td>0.01</td>
<td>0.08</td>
<td>0.01</td>
</tr>
</tbody>
</table>

* For details of diets and procedures, see p. 448.
† Two pigs were omitted due to health problems.
‡ F test, casein- v. soyabean protein-based diet.

### Table 2. Energy balance in pigs fed soyabean protein- or casein-based diets* (Mean values and standard deviations)

<table>
<thead>
<tr>
<th>Protein</th>
<th>Protein intake (g/kg BW$^{0.62}$ per d)</th>
<th>ER (kJ/kg BW$^{0.62}$ per d)</th>
<th>Protein ER (kJ/kg BW$^{0.62}$ per d)</th>
<th>Fat ER (kJ/kg BW$^{0.62}$ per d)</th>
<th>Heat (kJ/kg BW$^{0.62}$ per d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean SD</td>
<td>Mean SD</td>
<td>Mean SD</td>
<td>Mean SD</td>
<td>Mean SD</td>
</tr>
<tr>
<td>Casein</td>
<td>6 8.0 ± 0.3</td>
<td>742 ± 53</td>
<td>119 ± 2</td>
<td>623 ± 52</td>
<td>1074 ± 57</td>
</tr>
<tr>
<td>Soyabean</td>
<td>4† 8.4 ± 0.1</td>
<td>720 ± 55</td>
<td>82 ± 7</td>
<td>638 ± 48</td>
<td>1108 ± 33</td>
</tr>
<tr>
<td>P value‡</td>
<td>0.07</td>
<td>0.11</td>
<td>0.01</td>
<td>0.16</td>
<td>0.09</td>
</tr>
</tbody>
</table>

ER, energy retention; BW, body weight.
* For details of diets and procedures, see p. 448.
† Two pigs were omitted due to health problems.
‡ F test, casein- v. soyabean protein-based diet.
Expression of cathepsin B and cystatin C proteins

Immunoblot data demonstrating marked differences in cathepsin B forms between the dietary groups are shown in Fig. 7. The cathepsin B protein with relative molecular mass $\geq 30,000$ (Fig. 7, line I) corresponds to the enzyme precursor, with relative molecular mass 27,000–30,000 to active dimers (Fig. 7, line II), and with relative molecular mass 22,000–25,000 (Fig. 7, line III) to one of the subunits of the enzyme (Baricos et al. 1988). In contrast, differences among the dietary groups in cystatin C, one of the major natural inhibitors of cathepsins (Barrett et al. 1986; Leonardi et al. 1996), were not found (Fig. 8).

Response of cortisol levels to the diets

Lysosomal cathepsins have been reported to be positively regulated by glucocorticoids (Hong & Forsberg, 1995; Inubishi et al. 1996). Hence, we looked for dietary effects on cortisol concentrations. A transiently prolonged postprandial rise in the cortisol level was evident in pigs fed the soyabean-protein diet (Fig. 9). The cortisol concentration following the soyabean-protein meal was 2.9-times the value of the casein group ($P = 0.01$) at this time.

Discussion

The results demonstrate that dietary soyabean protein induced an increase in several muscle proteolytic activities.

Fig. 1. Plasma urea nitrogen in response to soyabean protein- (B) or casein- (A) based diets. Values are means for six pigs per group with standard deviations shown by vertical bars. For details of diets and procedures, see p. 448. a,b Mean values with unlike superscript letters were significantly different ($0-1$ h v. $20-23$ h within the soyabean-fed group, $P = 0.02$; soyabean-fed group v. casein-fed group, $P \leq 0.001$ (paired t test).

![Urea-N graph](https://www.cambridge.org/core/graphics/54.70.40.11, on 07 Apr 2019 at 10:39:15, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms)
and, as a corollary, a rise in muscle 3-methylhistidine. They also show an elevation of plasma urea and urinary N excretion and an altered pattern of diurnal plasma amino acids in response to the soyabean-protein diet. The postprandial differences between soyabean protein- and casein-fed pigs in the plasma concentrations of essential amino acids disappeared and the isoleucine level increased in the postabsorptive period. These results are indicative of a decrease in the utilization and/or of recruiting the levels of these amino acids by protein degradation. There are two ultimate sources of essential amino acids for cells, the external milieu and endogenous protein degradation. In contrast to leucine, valine, and most other essential amino acids, isoleucine is not markedly deficient in soyabean protein. The liver is not the major site for catabolism of branched-chain amino acids, whereas the skeletal muscle oxidizes significant amounts (Schneible et al. 1981). Thus, under isoenergetic and isonitrogenous conditions as well as lower N deposition but sufficient dietary supply of isoleucine, the postabsorptive rise in circulatory isoleucine seems to reflect excessive isoleucine arising from endogenous protein breakdown in protein-storing tissues, such as skin, gut and skeletal muscle. These tissues recruit, at least in part, their protein stores postprandially, as indicated
by the postprandial course of the isoleucine level, which was similar to the other essential amino acids. The muscle was found to be one of the responsive tissues. A coordinate activation of the ATP-dependent and the lysosomal proteolysis was observed. The latter was indicated by an increase in the expression of cathepsin B and in the activities of cathepsins B and H. The soyabean-protein diet also induced myofibrillar protein degradation using muscle 3-methylhistidine level as an indicator. However, 3-methylhistidine is readily converted into dipeptides in porcine tissues (Harris & Milne, 1987). Therefore, the difference may also arise from different concentrations of constituents of these dipeptides such as β-alanine. Results from previous studies suggest that myofibrillar proteins require an initial attack by Ca²⁺-dependent proteases in the cytosol in order to be degraded within autophagic vacuoles (Mortimore & Pösö, 1987). More recently, proteasome-mediated proteolysis has also been shown to be responsible for myofibrillar protein breakdown (Mansoor et al. 1996). Differences in the rate of cleaving a substrate by calpain action were not detected between the dietary groups. Thus, involvement of this pathway in the proteolytic response to the soyabean-protein-isolate diet remains to be clarified. In contrast, ATP-dependent and lysosomal proteolytic activities were found to be elevated by the soyabean diet. The data are consistent with results indicating stimulated protein turnover in the muscle of pigs following soyabean-protein infusion in comparison with a casein infusion (Deutz et al. 1998). In turn, these results contrast somewhat with the concept that skeletal muscle protein synthesis is depressed while initially skeletal muscle protein degradation rate remains unchanged, thereby resulting in a net catabolic state when feeding a poor-quality-protein diet (Fuller et al. 1987; Garlick et al. 1980; Garlick & Grant, 1988).

**Fig. 5.** ATP-dependent proteolytic activity in *semimembranosus* muscles of pigs fed soyabean protein-(●, ○) or casein-(■, □) based diets. Azocasein was used as substrate for ATP-responsive protein-degrading activity in muscle cytosolic fraction, inhibitable by 2 μM clastolactacycin β-lactone (○, □), an inhibitor of proteasome activity. Acid-soluble stain (azogroups-containing digest) after incubation of azocasein with muscle cytosol was expressed as stain/mg cytosolic protein. Absorption. Values are means with standard deviation shown by vertical bars. For details of diets, see p. 448. Mean values were significantly different (t test): soyabean-fed (n 4) v. casein-fed (n 6), ††P = 0.04; †††P = 0.03; ††††P = 0.02.

**Fig. 6.** Activity of (a), cathepsin B and (b), cathepsin H in muscle cells of pigs fed soyabean protein-(●, ○) casein-(■, □) based diets. Hydrolysis of fluorogenic substrates in concentrations indicated on the abscissa were measured in myofibres by flow cytometry. Fluorescence was converted into concentration by calibration with fluorescent standard leads. AMC-Arg, 7-amino-4-methylcoumarin, benzoxylcarbonyl-L-arginyl-L-arginine amide; CAMC-Arg, 7 amino-4-chloromethylcoumarin, arginine. Values are means with standard deviations shown by vertical bars. For details of diets, see p. 448. Mean values were significantly different for all reactions without inhibitors: soyabean-fed v. casein-fed, P<0.05.)
Soyabean-derived products may contain antinutritional factors, some of which are thought to suppress carcinogenesis (Hawrylewicz et al. 1995; Kennedy, 1995). The soyabean protein used in the diet was commercially isolated by heat treatment under mild alkaline conditions, destroying heat-labile factors, including trypsin inhibitor activity and lectins (Liener, 1994, 1995). Protease inhibitors exert their antinutritional effect by causing pancreatic hypertrophy and hyperplasia while lectins act on intestinal mucosa, inhibiting growth by interfering with the absorption of nutrients. In contrast, heated soyabean flour did not induce a pancreatic response (Nakai et al. 1992). Impaired absorption of nutrients is expected to induce differences in balance data among the dietary groups. Neither N intake, retention of energy and fat, nor heat production, a measure of metabolic rate, nor faecal N excretion differed between the groups. Hence, interference with absorption of nutrients is unlikely to cause the lower N deposition and the changes in plasma amino acids and urea as a consequence of feeding dietary soyabean protein.

Deficient essential amino acids, especially methionine, have been reported to act as antinutritional factors and methionine supplementation reversed some adverse effects of soyabean protein on metabolism (Moundras et al. 1995). Moreover, starvation or malnutrition, including reduced amino acid supply, can affect food intake, amino acid metabolism, and expression of glucocorticoid-responsive proteolytic enzymes by altering the function of the hypothalamic–pituitary–adrenal axis (Suemaru et al. 1986; Chua et al. 1991; Woodward et al. 1991). Meal intake is a strong stimulus for this axis, inducing glucocorticoid secretion (Hansen et al. 1997). Therefore, the altered cortisol response demonstrated here seems to be the consequence of hypothalamic sensitization due to transient and, in the case of methionine, permanent amino acid deficit. Apart from the effects of glucocorticoids, a decrease in amino acid concentration by itself is known to induce coordinate activation of proteolytic activities (Mortimore & Pösö, 1987; Belkhou et al. 1994; Fereday et al. 1998). Some amino acids can participate in the regulation of gene expression by interacting with amino acid response elements in promoters of responsive genes. Pleiotropic effects arise from a change in the expression of transcription factors, which regulate the expression of a cascade of genes. Amino acid limitation has been reported to affect the expression of a transcription factor, which interacts with C/enhancer binding protein, i.e. transcription factors of the group of CCAAT/enhancer binding proteins (Bruhat et al. 1997; Jousse et al. 1999). One of these C/enhancer binding protein, C/enhancer binding protein-α, plays a central role in the maintenance of energy homeostasis by regulating the expression of genes encoding leptin (He et al. 1995) and transporters of insulin-responsive glucose uptake (Kaestner et al. 1990). Glucose transport is energized by pathways also used in amino acid transport mediated by system A of the skeletal muscle (Bonadonna et al. 1993; Tsakiridis et al. 1995; Hajduch et al. 1997). System A transports both glucogenic and some essential amino acids, including methionine, which is deficient in soyabean protein. Thus, amino acid limitation predicts C/enhancer binding protein-α-regulated response which affects cellular transport processes and, as a corollary, the absorption of amino acids from circulatory pool. Moreover, genes encoding some lysosomal enzymes,
including cathepsins L and H, contain both C/enhancer binding protein and cyclic nucleotide response elements (Ishidoh et al. 1989). Therefore, these genes are targets of signals such as catecholamines and some proteohormones, whose effects are mediated by membrane receptors capable of signal transduction by altering intracellular levels of cyclic nucleotides (cAMP, cGMP). Glucocorticoids can increase the density and the activity of cAMP-producing receptors (Reynisdottir et al. 1993). Thus, the rise in muscle cathepsin H activity could be a consequence of the markedly elevated cortisol level in concert with changes in cyclic nucleotide-mediated signal transduction following a soyabean-protein diet.

Apart from Ca\(^{2+}\)-dependent and lysosomal proteolysis, the ATP-dependent proteasome proteolytic pathway is thought to be of major importance in the breakdown of skeletal muscle proteins (Attaix et al. 1998). Glucocorticoids have been reported either to exert no effects (Hong & Forsberg, 1995) or to activate proteasome-mediated proteolysis (Mansoor et al. 1996), while cytokines have been consistently reported to induce lysosomal (Cannon et al. 1991; Hall-Angeras et al. 1991; Hong & Forsberg, 1995), and proteasome-mediated protein breakdown in skeletal muscles (Attaix et al. 1998). Hyperinsulinaemia and hyperaminoacidaemia reduce the expression of ubiquitin mRNA in skeletal muscle (Larbaud et al. 1996). However, ubiquitination and proteasomal degradation are not obligatorily coupled. Some enzymes, and the model substrate casein, are degraded by proteasomes in ubiquitination-independent fashion (Driscoll & Goldberg, 1990; Murakami et al. 1992). Therefore, we used an assay that measured ATP-stimulated hydrolysis of azocasein, a reaction independent of ubiquitination, in the absence and presence of clastolactacystin β-lactone, a compound known to selectively inhibit proteasome-mediated proteolysis (Fenteany et al. 1995; Dick et al. 1996). Despite a low cleavage rate, significant differences were detected between soyabean and casein groups, suggesting the proteasome pathway as a target of regulatory responses to amino acid limitation in soyabean-protein diet.

Collectively, the results indicate that dietary soyabean protein can induce an amino acid imbalance. Deficient amino acids are known to trigger regulatory responses, including an activation of muscle protein degradation, which could contribute to a recruitment of circulatory amino acids. Glucocorticoids are probably involved in the proteolytic stimulation. These responses are ultimately driven by amino acid limitation in soyabean-protein diet. Glucocorticoids are probably involved in the proteolytic stimulation. These responses are ultimately driven by amino acid limitation in soyabean-protein diet. Glucocorticoids are probably involved in the proteolytic stimulation. These responses are ultimately driven by amino acid limitation in soyabean-protein diet. Glucocorticoids are probably involved in the proteolytic stimulation. These responses are ultimately driven by amino acid limitation in soyabean-protein diet.

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