Tissue- and substrate-dependent responses of oxidative phosphorylation to dietary protein level in chicks

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The ADP:O values in both cardiac and hepatic mitochondria have significantly decreased with an increase in protein level after 7, 14 and 21 d of feeding (Toyomizu *et al.* 1992). The present studies were undertaken to clarify tissue-specific effects of dietary protein levels on oxidative phosphorylation in the liver, kidney, skeletal muscles and small intestine and to characterize oxidative metabolism with diverse substrates in the liver. Chicks were fed on semi-purified diets of different protein levels (7, 25, 43 and 61% of metabolizable energy content) for 21 d. The responses of protein levels to oxidative phosphorylation showed tissue-dependency; although liver mitochondria of chickens fed on higher-protein diets exhibited reduced ADP:O values and state 3, neither changes in ADP:O value nor state 3 and state 4 rates were observed in the isolated mitochondria from kidney and skeletal muscles. Small intestinal mucosal mitochondria from chickens fed on a high (61%)-protein-energy diet showed significantly reduced ADP:O value and respiratory control ratio when compared with medium-protein-energy diets (25 and 43%). In liver mitochondria showing the most sensitive dependency to the levels of dietary protein, the ADP:O value decreased with increasing protein levels when pyruvate + malate- or glutamate-requiring complexes I, III and IV of the electron transport chain were used as substrates, but it did not change when succinate-requiring complexes II, III and IV or ascorbate + tetramethyl-*p*-phenylenediamine requiring complex IV was used. These results imply that impaired oxidative phosphorylation capacities with increasing dietary protein levels may be associated with functional damage to the respiratory chain for electron flow from NAD-linked substrates to the ubiquinone pool.

Dietary protein: Oxidative phosphorylation: Tissue specificity: Chicken

We have previously shown that the level of dietary protein is an important determinant of oxidative phosphorylation with pyruvate + malate as substrates in rat heart mitochondria (Toyomizu & Clandinin, 1993). When the dietary protein and fat levels were altered, feeding 70% protein-energy diets reduced the ADP:O value compared with the 30% protein-energy level, but no difference was observed between low-fat and high-fat groups. Further, the impairment of oxidative phosphorylation in rats fed on a high-protein diet was supported by our finding with liver and heart mitochondria in chickens (Toyomizu *et al.* 1992). A parallel correlation between ADP:O values for liver mitochondria and body fat was also observed in the chickens fed on diets with different levels of protein, implying that the reduction in oxidative phosphorylation may partly contribute to the decrease in body fat in chickens.

However, compensatory mechanisms, including increased oxidative phosphorylation in the other tissues, could be proposed for the higher-protein-fed groups, considering that

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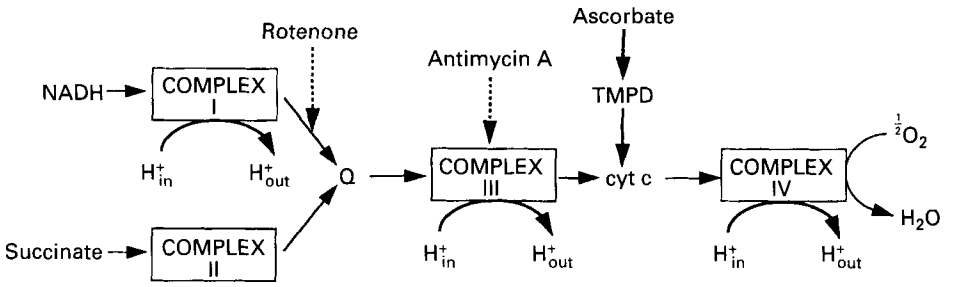

Fig. 1. The electron transport chain. Q, ubiquinone; cyt c, cytochrome c. The electron transport complexes (located in the inner mitochondrial membrane) are complex I (NADH:ubiquinone oxidoreductase; *EC* 1.6.5.3), complex II (succinate: ubiquinone oxidoreductase; *EC* 1.3.99.1), complex III (ubiquinol:ferricytochrome-c oxidoreductase; *EC* 1.10.2.2), and complex IV (ferrocytochrome-c:oxygen oxidoreductase; *EC* 1.9.3.1–2). ⟶, The electron flow through the electron transport chain; ⋯▶, specific inhibitors for each complex (rotenone for complex I and antimycin A for complex III). Different substrates were used to study the characteristics of O_2 consumption: pyruvate, malate, and L-glutamate, which generate intramitochondrial NADH; succinate, which provides electrons and the ubiquinone level; and ascorbate + tetramethyl-*p*-phenylenediamine (TMPD), which reduces the respiratory chain at the cyt c level.

various ages, dietary conditions, etc. may have variable effects on different tissues. In particular, a form of tissue-specific protein metabolism has been reported. It has been emphasized that there are no significant differences in fractional protein synthesis in rat liver between weaning and senility, while the rates progressively decline in the kidney, intestine and whole body throughout life (Goldspink & Kelly, 1984; Goldspink *et al.* 1984). Reeds (1989) pointed out the significance of tissue-dependency of protein turnover in as much as changes in whole-body protein synthesis when feeding a dietary supplement of carbohydrate were not necessarily reflected in those in protein synthesis of the hindquarter tissue. In terms of mitochondrial oxidative activity it has already been observed that the liver does not respond as well as the heart or muscle to different fat composition in diet (Houtsmuller *et al.* 1970; Christophersen & Bremer, 1972) and to synthetic glucocorticoid (Martens *et al.* 1991). In rats the thermogenic responses with uncoupling to cold adaptation and hyperphagia induced by feeding a balanced, palatable cafeteria diet (diet-induced thermogenesis) were shown specifically in brown adipose tissue (Rothwell *et al.* 1983; Rothwell & Stock, 1987; Trayhurn *et al.* 1987) but not other tissues, namely liver and muscle. On the other hand, in birds an effector of diet-induced thermogenesis analogous to the brown adipose tissue of some mammals has yet to be identified (Johnston, 1971). In this way there was no direct evidence for the existence of thermogenic responses with uncoupling effects of oxidative phosphorylation except in liver and heart in the chicken.

Other compensatory changes may occur in the other site-entrance for substrates except pyruvate + malate: if one site is somewhat less active the other entry points would be used to a greater extent. Recent studies showed that oxidation of the NAD-linked substrate was less sensitive to glucocorticoid than that of the FAD-linked one (Martens *et al.* 1991), and that the inhibition of the uncoupled state by arachidonic acid was more marked in NAD-linked than in FAD-linked respiration (Takeuchi *et al.* 1991).

The present study was conducted, therefore, to clarify tissue-specific effects of dietary protein levels on the oxidative phosphorylation in the liver, kidney, skeletal muscles and small intestine. Further study was carried out to elucidate the utilization of various substrates requiring complexes I, III and IV, complexes II, III and IV and complex IV in the respiratory chain (Fig. 1) in liver mitochondria of chickens fed on diets with different protein levels.

Table 1. *Composition of experimental diets* (g/kg)*

Protein levels as metabolizable energy (% total energy)...	7	25	43	61
Isolated soya-bean protein†	47·12	201·23	354·62	507·33
Soya-bean meal†	23·42	100·02	176·26	252·16
L-methionine†	0·86	3·67	6·48	9·27
L-lysine monohydrochloride†	0·37	1·59	2·80	4·01
Soya-bean oil	46·41	46·74	47·06	47·40
Yellow maize‡	160·33	115·22	70·35	25·69
α-Maize starch‡	240·49	172·84	105·52	38·53
Glucose‡	400·82	288·06	175·87	64·21
Cellulose	16·95	13·79	10·53	7·29
Calcium phosphate, dibasic	27·89	25·20	22·53	19·87
Calcium carbonate	13·11	12·59	12·06	11·55
Potassium chloride	8·61	5·76	2·92	0·00
Sodium chloride	3·88	3·60	3·32	3·05
Trace mineral mixture§	5·40	5·39	5·37	5·36
Vitamin mixture§	4·32	4·31	4·30	4·29
Total	1000·00	1000·00	1000·00	1000·00
Metabolizable energy (MJ/kg), calculated	14·01	13·97	13·93	13·90

* All the diets contained the same amount of cellulose, fat, minerals, and vitamins per unit metabolizable energy.

† Protein consisted of an isolated soya-bean protein–soya-bean meal–L-methionine–L-lysine monohydrochloride (657:326:12:5, by wt) mixture.

‡ Carbohydrate consisted of yellow maize–α-maize starch–glucose (2:3:5, by wt) mixture.

§ See Akiba & Matsumoto (1978).

MATERIALS AND METHODS

Animals and diets

Male chicks (Arbor Acres or Cobb) were obtained from a commercial hatchery (Ishida Poultry and Egg's Co. Ltd, Nagaoka 940, Japan) at 1 d of age. They were housed in electrically-heated batteries and provided with water and a commercial starter diet *ad lib.* for the first 13 d. The chicks were randomly divided into four groups. They were housed individually in wire cages under controlled light (14 h light and 10 h dark) and temperature ($25 \pm 2°$). In each of several series of experiments, four experimental diets providing protein at 7, 25 (control diet), 43, and 61% of total energy (PME) were formulated on a metabolizable energy (ME) basis by substituting the carbohydrate (CME) at a constant fat level (FME; Table 1). The carbohydrate source was a yellow maize–α-maize starch–glucose (2:3:5, by wt) mixture. The fat source was soya-bean oil. The protein source was an isolated soya-bean protein–soya-bean meal–L-methionine–L-lysine monohydrochloride (657:326:12:5, by wt) mixture. All the diets contained the same amount of cellulose, fat, minerals and vitamins on a per MJ metabolizable energy basis. Four or five chicks from each group at 21 d of the feeding regimen were killed by cervical dislocation.

Isolation of mitochondria

Liver and kidney mitochondria were prepared according to the methods of Hoppel *et al.* (1979). Briefly, the liver and kidney were rinsed, blotted, weighed, minced, and washed with cold MSM buffer containing 220 mM-mannitol, 70 mM-sucrose, 5 mM-3-(N-mor-

pholino)propanesulphonic acid (Mops), pH 7.4. A suspension (100 g/l) of the minced liver in cold MSM buffer containing 2 mM-EDTA was homogenized in a Potter–Elvehjem homogenizer with a loose-fitting pestle. Nuclei and cell debris were removed by centrifugation at 400 ***g*** for 10 min, and mitochondria were isolated by centrifugation of the resulting supernatant fraction at 7000 ***g*** for 10 min. The resulting mitochondrial pellet was washed twice with MSM buffer and finally the pellet was suspended with MSM buffer.

Skeletal muscle mitochondria were isolated from *pectoralis profundus* or *biceps femoris* by the procedure of Lee *et al.* (1979). The minced tissue was suspended in the Chappell–Perry medium (Chappell & Perry, 1954) containing 100 mM-KCl, 50 mM-Tris hydrochloride, 1 mM-ATP, 5 mM-$MgCl_2$, 1 mM-EDTA, pH 7·5. This suspension was treated with Nagarse, and homogenized with an Ultra-Turrax (Janke & Kunkel GmbH, Germany). The homogenate was centrifuged at 600 ***g*** for 10 min, and the supernatant fraction was centrifuged at 14000 ***g*** for 10 min. The pellet was suspended in a medium containing 100 mM-KCl, 50 mM-Tris hydrochloride, 0·2 mM-ATP, 1 mM-$MgCl_2$, 0·2 mM-EDTA, 10 g albumin/l, pH 7·5. The suspension was centrifuged at 7000 ***g*** for 10 min, then the resulting pellet was resuspended in the modified Chappell-Perry medium described previously but without albumin. This was centrifuged at 3500 ***g*** for 10 min, and finally the pellet was resuspended with 0·25 M-sucrose.

Small intestinal mucosal mitochondria were prepared by the method of Lawrence & Davies (1986). The mucosal scrapings from the intestine was stirred with the DEAE-cellulose suspension. This suspension consisted of 10 g DEAE cellulose in isolation medium A containing 70 mM-sucrose, 220 mM-mannitol, 2 mM-N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid (Hepes), 0·5 mM-ethylene glycol bis(β-aminoethyl-ether)-N,N,N′,N′-tetraacetic acid (EGTA), 0·1 mM-phenylmethylsulphonyl fluoride (PMSF), 3·7 g albumin/l, pH 7·4. The mucosal suspension was homogenized using a Potter–Elvehjem homogenizer. This homogenate was centrifuged at 750 ***g*** for 10 min. The resulting supernatant fraction was recentrifuged at 10000 ***g*** for 7 min. The crude mitochondrial pellet was suspended in isolation medium A. This suspension was centrifuged at 14000 ***g*** for 7 min. The resulting pellet was resuspended in isolation medium B containing 70 mM-sucrose, 220 mM-mannitol, 2 mM-Hepes, 12 g albumin/l (pH 7·4). The suspension was centrifuged at 14000 ***g*** for 7 min and finally the pellet was suspended with isolation medium B.

All procedures were performed at 0–4°.

Measurement of oxygen consumption rate

O_2 consumption was measured polarographically with a Clark electrode no. 5331 (Yellow Springs Instrument Co. Inc., Ohio, USA) by using a YSI model 5300 O_2 monitor linked to a recorder (U-228; Nippon Denshi Kagaku Co. Ltd, Kyoto, Japan). The incubation medium for liver or kidney mitochondria containing 80 mM-KCl, 50 mM-Mops, 5 mM-KH_2PO_4, 1 mM-EGTA, 1 g albumin/l, pH 7·0 (Hoppel *et al.* 1979). The medium for skeletal muscle mitochondria consisted of 150 mM-sucrose, 25 mM-Tris hydrochloride, and 10 mM-KH_2PO_4 pH 7·5 (Lee *et al.* 1979). The medium for small intestinal mucosal mitochondria contained 70 mM-sucrose, 220 mM-mannitol, 2 mM-Hepes, 0·75 mM-EDTA, 0·50 mM-EGTA, 2·5 mM-$MgCl_2$, 2·5 mM-KH_2PO_4, 1·3 g albumin/l, pH 7·4 (Lawrence & Davies, 1986).

Substrate concentrations were 10 mM-pyruvate + 2·5 mM-malate, 10 mM-L-glutamate, 10 mM-succinate, or 0·5 mM-tetramethyl-*p*-phenylenediamine (TMPD) + 5·0 mM-ascorbate; other additions were 10 mM-malonate, 3·75 μM-rotenone or 0·4 μM-antimycin A. The system was equilibrated with mitochondria at 37°; then the rate of O_2 consumption was determined. The state 3 respiratory rate was initiated by 220 nmol ADP, the state 4 respiratory rate after exhaustion of ADP, respiratory control ratios and ADP:O values

were determined on third and subsequent cycles as described by Chance & Williams (1956) and Chappell (1964). The exact concentration of added ADP was determined spectrophotometrically (Jaworek *et al.* 1974). All determinations were made without undue lapse of time after isolation of mitochondria. The solubility of O_2 at 37° was assumed to be 0·39 μg atoms O_2/ml (Clandinin, 1978). To avoid bias we measured O_2 consumption of each mitochondrial preparation from the four dietary groups according to a systematically randomized order that was different on each day. Oxidation rate was expressed in ng O_2/mg mitochondrial protein per min. Protein was measured by a colorimetric method (Lowry *et al.* 1951), except for small intestinal mucosal protein determination which was carried out by the Bio-Rad dye-binding procedure (Bio-Rad GmbH, Munich, Germany).

Statistical procedure

With one-way analysis the effect of treatment on mitochondrial functions was examined to separate the effect of dietary protein levels. The significance level for individual group comparisons was $P < 0.05$ using Duncan's least significant difference multiple-range test (SAS Institute Inc., 1985).

RESULTS

Body-weight gain and food consumption

Representative body weights and feed consumption of chickens are shown in Fig. 2. The body-weight gain increased to a maximum level with 43% protein-energy diet and decreased thereafter. No significant differences in body-weight gain was observed in chicks fed on 43% protein-energy diet when compared with 25% protein energy diet. Similar results were obtained with all the experiments. Food intake expressed as metabolizable energy for chicks fed on a 7% protein-energy diet especially was lower than that for chicks fed on 43% protein-energy diet when compared with 25% protein-energy diet. Similar their food intake to satisfy their energy requirements (Hill & Dansky, 1954; Powell *et al.* 1972). Here, the chicks fed on a 7% protein-energy diet could not adjust their intake to compensate for differences in dietary content. It is quite possible that body size might not become large enough so that stomach distension would be a limiting factor in food intake (Forbes, 1986).

Oxidative phosphorylation in the liver, kidney, skeletal muscles, and small intestinal mucosa

Table 2 shows the effects of dietary protein level on oxidative phosphorylation in the liver, kidney, skeletal muscles, *pectoralis profundus* and *biceps femoris*, and small intestinal mucosa. The rates of O_2 uptake and ADP:O values observed were similar in magnitude to those previously reported for rat liver, kidney, skeletal muscles, and small intestinal mucosa (Lee *et al.* 1979; Lawrence & Davies, 1986; Toyomizu *et al.* 1992). Although the rates of state 3 and ATP synthesized in liver with pyruvate+malate plus malonate significantly decreased with increasing protein levels in diet, changes were not observed in the kidney, skeletal muscles, and small intestinal mucosa. The rate of state 4 oxidation was not affected by dietary protein in the liver, kidney or skeletal muscles, but it was affected in intestinal mucosa, where the rate for chickens fed on a 61% protein-energy diet significantly increased compared with groups fed on the lower-protein diets. Respiratory control ratios in all tissues were significantly unchanged by dietary protein level except when glutamate+malate were used as substrates in small intestinal mucosa. Determination of ADP:O values in mitochondria also indicate differences in the response to protein level among tissues; that is, no changes in ADP:O values were observed in the isolated mitochondria from the kidney or skeletal muscles, whereas liver mitochondria exhibited a

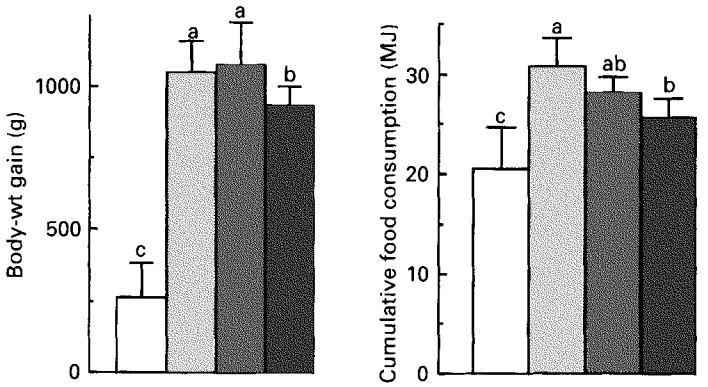

Fig. 2. Effect of dietary protein level on body-weight gain and cumulative food consumption over 21 d of feeding period: □, 7%; ▨, 25%; ▩, 43%; ■, 61% total energy of diet provided as protein. Differences in means were tested by Duncan's multiple comparison test. Values are means and standard deviations represented by vertical bars. Means with different superscript letters were significantly different ($P < 0.05$).

reduced ADP:O value with increasing protein level, and intestinal mucosal mitochondria of chickens fed on a 61% protein-energy diet showed a significantly reduced ADP:O value compared with 25 and 43% protein-energy diets.

Oxidative phosphorylation with diverse substrate

As illustrated in Fig. 1, the electron transport chain transports electrons from NADH or FADH to O_2 and produces a transmembranous proton gradient used for the generation of ATP (Hatefi, 1985). Determination of oxidative phosphorylation was investigated in intact liver mitochondria isolated from chickens fed on diets of different protein levels for 21 d (Table 3). There were no differences in state 3 and state 4 oxidation rates, in the amount of ATP synthesized, nor in the respiratory control index for pyruvate + malate, glutamate, succinate, and ascorbate + TMPD as substrates among groups fed at various dietary protein levels. However, ADP:O values were significantly reduced with increasing dietary protein level for pyruvate + malate and glutamate as substrates requiring complexes I, III and IV of the electron transport chain, but they showed no changes for either succinate-requiring complexes II, III and IV or for ascorbate + TMPD-requiring complex IV. Similar results for ADP:O values for pyruvate + malate or succinate as substrates were obtained with liver mitochondria prepared using an isolation medium differing from the medium used in the present experiment from animals under the same condition of diets and environment (values not shown). The reduction in ADP:O values for a group fed on a 61% protein-energy diet was approximately 20% for pyruvate + malate and 10% for glutamate when compared with a group fed on a 7% protein-energy diet.

DISCUSSION

We have already shown that the ADP:O values in both cardiac and hepatic mitochondria are significantly decreased with increasing protein levels after 7, 14, and 21 d of feeding (Toyomizu *et al.* 1992). However, it can not be seen whether changes of this magnitude in both hepatic and cardiac mitochondrial oxidative phosphorylation activity would be sufficient enough to reconcile decreases in both weight of and percentage of carcass fat with increasing dietary protein level. In fact, Webster (1981) pointed out the significance of analysis on an organ-by-organ basis of differences in heat production between nutritional

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Table 2. *Oxidative activity of isolated liver, skeletal muscle and small intestinal mucosa mitochondria from chicks fed on diets containing different levels of dietary protein for 21 d**

(Mean values for four to five birds)

Source of mitochondria	Substrate†	Dietary PME (%)	State 3 (ng atom/min per mg protein)	State 4 (ng atom/min per mg protein)	Respiratory control ratio	ADP:O	ATP synthesized (nmol/min per mg protein)
Liver	10 mM-pyruvate + 2·5 mM-malate	7	142[a]	35	3·95	2·74[a]	371[a]
		25	124[ab]	27	4·69	2·43[b]	289[b]
		43	123[ab]	34	3·28	2·34[b]	269[b]
		61	108[b]	30	3·42	2·29[b]	234[b]
		SEM (16 df)	10	3	0·46	0·07	21
Kidney	10 mM-pyruvate + 2·5 mM-malate	7	148	39	3·60	2·69	377
		25	146	39	4·11	2·89	397
		43	153	36	3·92	2·66	375
		61	135	37	3·62	2·78	362
		SEM (12 df)	12	3	0·37	0·11	25
Skeletal muscle (*pectoralis profundus*)	10 mM-pyruvate + 2·5 mM-malate	7	348	79	4·67	2·88	1007
		25	321	67	4·88	3·09	968
		43	302	59	5·18	2·79	841
		61	292	64	4·80	2·78	818
		SEM (12 df)	28	7	0·49	0·17	80
Skeletal muscle (*biceps femoris*)	10 mM-pyruvate + 2·5 mM-malate	7	427	103	4·17	2·75	1174
		25	465	120	3·84	2·72	1251
		43	405	80	5·28	2·82	1153
		61	391	113	4·56	2·71	1032
		SEM (12 df)	94	28	0·54	0·08	237
Small intestinal mucosa	10 mM-pyruvate + 2·5 mM-malate	7	860	316[b]	2·71	2·17	1820
		25	1100	374[b]	2·97	2·42	2601
		43	1091	389[b]	2·94	2·35	2429
		61	1211	554[a]	2·15	2·01	2397
		SEM (12 df)	183	53	0·33	0·17	384
	10 mM-glutamate + 2·5 mM-malate	7	1178	383[b]	2·96[ab]	2·19[ab]	2540
		25	1197	387[b]	3·19[a]	2·36[a]	2743
		43	1154	360[b]	3·36[a]	2·39[a]	2556
		61	1575	616[a]	2·46[b]	1·82[b]	2797
		SEM (12 df)	251	55	0·42	0·38	502

[ab] Means with different superscript letters were significantly different ($P < 0·05$).
PME, metabolizable energy supplied as protein, expressed as a percentage of total energy.
* For details of dietary treatments, see Table 1 and p. 461.
† All substrates require complexes I, III and IV of the electron transport chain.
§ Respiratory control ratio was calculated as the ratio of state 3: state 4 oxidation rates.

Table 3. *Oxidative activity with diverse substrates of isolated liver from chicks fed on diets containing different levels of dietary protein for 21 d experimental periods**

(Mean values for four to five birds)

Electron transport complexes required		Dietary PME (%)	State 3 (ng atom/min per mg protein)	State 4 (ng atom/min per mg protein)	Respiratory control ratio†	ADP:O	ATP synthesized (nmol/min per mg protein)
Complexes I, III, IV:	10 mM-pyruvate + 2·5 mM-malate + 10 mM-malonate	7	178	47	3·57	2·46[a]	424
		25	153	42	3·58	2·20[ab]	331
		43	189	44	4·00	2·16[b]	394
		61	233	48	4·50	2·00[b]	434
		SEM (15 df)	19	4	0·30	0·10	47
	10 mM-L-glutamate	7	79	20	4·21	2·83[a]	218
		25	92	16	5·18	2·84[a]	250
		43	99	17	5·73	2·82[a]	242
		61	95	17	5·20	2·58[b]	242
		SEM (15 df)	7	2	0·53	0·08	18
Complexes II, III, IV	10 mM-succinate + 3·75 mM-rotenone	7	263	51	4·85	2·05	511
		25	231	59	3·62	1·85	430
		43	242	66	3·69	1·70	414
		61	277	61	4·28	1·82	505
		SEM (16 df)	41	7	0·36	0·13	88
Complex IV	5 mM-ascorbate + 0·5 mM-TMPD + 0·4 μM-antimaycin A	7	629	476	1·33	1·19	740
		25	815	599	1·37	1·04	840
		43	924	711	1·32	0·99	896
		61	635	440	1·45	1·23	762
		SEM (16 df)	130	103	0·05	0·07	128

[ab] Means with different superscript letters were significantly different ($P < 0·05$).

PME, metabolizable energy supplied as protein, expressed as a percentage of total energy.

* For details of dietary treatments, see Table 1 and p. 461.

† Respiratory control ratio was calculated as state 3:state 4 oxidation rates.

conditions. Differences in response of thermogenesis to low-protein diets have been found between brown adipose tissue and liver (Rothwell *et al.* 1983). We conducted the present study, therefore, to determine whether dietary protein levels affect mitochondrial oxidative phosphorylation in the kidney, skeletal muscles and small intestinal mucosa as well as in the liver.

Mitochondria isolated from the small intestine, skeletal muscle and kidney exhibited, respectively, 8·9, 2·6, and 1·2 times higher O_2 uptake in state 3 oxidation than those from the liver. In the present study the effects of dietary protein on oxidative phosphorylation were shown to be tissue-specific, with differences between liver, kidney, skeletal muscle and small intestinal mucosa. Consistent with our previous studies (Toyomizu *et al.* 1992), in liver mitochondria ADP:O value and ATP synthesis significantly decreased with increasing dietary protein level. A similar reduction in ADP:O was observed with mitochondria isolated from small intestinal mucosa in chicks fed on a high-protein diet. It is conceivable that reduced oxidative phosphorylation in livers and small intestines in chickens fed on a high-protein diet might be caused by metabolites from dietary protein such as amino acids and their derivatives. This is partly supported by findings with chickens fed *ad lib.* by Tinker *et al.* (1986), who reported that the liver removed a number of amino acids mainly supplied by the diet and the major metabolite fluxes were across the liver. Therefore, oxidative phosphorylation capacity in the kidney or skeletal muscle would be less affected by the protein level in the diet than that of the liver and intestinal mucosa. Alternatively, the responsiveness of oxidative phosphorylation in different tissues to a certain chemical compound, which is generated as a result of eating a high-protein diet, could vary. In support of this hypothesis, the effect of hormones on mitochondrial energy metabolism was shown to vary with different organs (Martens *et al.* 1991).

On the other hand, feeding a low-protein diet also reduced ADP:O value in the mucosal mitochondria (Table 2). It has been reported that fat malabsorption is often present in protein–energy malnutrition (Holemans & Lambrechts, 1955; Gomez *et al.* 1956). Truswell (1975) pointed out that the most important reason for fat malabsorption was mucosal atrophy. This lower oxidative phosphorylation capacity by treatments with low-protein diets might induce such a mucosal atrophy.

The present study also established that changes in oxidative phosphorylation capacity relating to dietary protein level were dependent on a variety of mitochondrial substrates. As illustrated in Fig. 1, the electron transport chain is located in the inner mitochondrial membrane. The step-by-step transfer of electrons from NADH or FADH to O_2 produces a transmembranous proton gradient used for the generation of ATP (Hatefi, 1985). The decreased ADP:O values with increasing protein level were observed in liver mitochondria isolated from chicks when malate + pyruvate plus malonate were used as the NAD-linked substrates. In the case of another NAD-linked substrate, glutamate, ADP:O values were also similarly reduced with increasing dietary protein. On the other hand, in the case of an alternative site of entry for substrates, such as succinate plus rotenone or ascorbate + TMPD plus antimycin A, the oxidative phosphorylation capacities were not affected by dietary protein level (Table 3). These results suggested that impaired oxidative phosphorylation with NAD-linked substrates in chicks fed on high-protein diets may be induced by a defect in the process of the electron flow from NAD-linked substrates and NADH dehydrogenase (*EC* 1.6.5.3) to the ubiquinone pool, and complex I, given that malate + pyruvate or glutamate require complexes I, II and IV of the electron transport chain but that succinate requires complexes II, III and IV, and ascorbate + TMPD require complex IV.

The inner membranes of intact mitochondria are normally impermeable to NADH, which is oxidized on the matrix face of the membrane by a FMN-containing component, NADH dehydrogenase (Nicholls, 1981). In the present experiment exogenous NADH,

regardless of dietary treatment, did not enhance basal mitochondrial respiration in the liver (result not shown), indicating that the decreased ADP:O value with increasing dietary protein level might not be caused by the structural damage to the inner membrane.

In conclusion, the response of mitochondrial energy metabolism to dietary protein level is tissue-specific, with the difference being between the sensitive tissues (liver, small intestinal mucosa) and the insensitive tissues (kidney, skeletal muscle). In addition, it is possible that the decreased oxidative phosphorylation capacities with increasing dietary protein level may be associated with functional damage of the respiratory chain for only the electron flow from NAD-linked substrates and NADH dehydrogenase to the ubiquinone pool.

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REFERENCES

Akiba, Y. & Matsumoto, T. (1978). Effects of force-feeding and dietary cellulose on liver lipid accumulation and lipid composition of liver and plasma in growing chicks. *Journal of Nutrition* **108**, 739–748.

Chance, B. & Williams, G. R. (1956). The respiratory chain and oxidative phosphorylation. *Advances in Enzymology* **17**, 65–134.

Chappell, J. B. (1964). The oxidation of citrate, isocitrate and *cis*-aconitate by isolated mitochondria. *Biochemical Journal* **90**, 225–237.

Chappell, J. B. & Perry, S. V. (1954). Biochemical and osmotic properties of skeletal muscle mitochondria. *Nature* **173**, 1094–1095.

Christophersen, B. O. & Bremer, J. (1972). Erucic acid – an inhibitor of fatty acid oxidation in the heart. *Biochimica et Biophysica Acta* **280**, 506–514.

Clandinin, M. T. (1978). The role of dietary long chain fatty acids in mitochondrial structure and function. Effects on rat cardiac mitochondrial respiration. *Journal of Nutrition* **108**, 273–281.

Forbes, J. M. (1986). *The Voluntary Food Intake of Farm Animals*, pp. 86–113. London: Butterworth & Co. Ltd.

Goldspink, D. F. & Kelly, F. J. (1984). Protein turnover and growth in the whole body, liver and kidney of the rat from the foetus to senility. *Biochemical Journal* **217**, 507–516.

Goldspink, D. F., Lewis, S. E. M. & Kelly, F. J. (1984). Protein synthesis during the developmental growth of the small and large intestine of the rat. *Biochemical Journal* **217**, 527–534.

Gomez, F., Ramos-Galvan, R., Cravioto, J., Frenk, S., Vazquez Santaella, J. & de la Pena, C. (1956). Fat absorption in chronic severe malnutrition in children. *Lancet* **ii**, 121–122.

Hatefi, Y. (1985). The mitochondrial electron transport and oxidative phosphorylation system. *Annual Review of Biochemistry* **54**, 1015–1069.

Holemans, K. & Lambrechts, A. (1955). Nitrogen metabolism and fat absorption in malnutrition and in kwashiorkor. *Journal of Nutrition* **56**, 477–494.

Hill, F. W. & Dansky, L. M. (1954). Studies of the energy requirements of chickens. 1. The effect of dietary energy level on growth and feed consumption. *Poultry Science* **33**, 112–119.

Hoppel, C., DiMarco, J. P. & Tandler, B. (1979). Riboflavin and rat hepatic cell structure and function: mitochondrial oxidative metabolism in deficiency states. *Journal of Biological Chemistry* **254**, 4164–4170.

Houtsmuller, U. M. T., Struijk, C. B. & Van der Beek, A. (1970). Decrease in rate of ATP synthesis of isolated rat heart mitochondria induced by dietary erucic acid. *Biochimica et Biophysica Acta* **218**, 564–566.

Jaworek, D., Gruber, W. & Bergmeyer, H. U. (1974). Adenosine-5′-diphosphate and adenosine-5′-monophosphate. In *Methods of Enzymatic Analysis*, pp. 2127–2131. [H. U. Bergmeyer, editor]. New York: Academic Press.

Johnston, D. W. (1971). The absence of brown adipose tissue in birds. *Comparative Biochemistry and Physiology* **40A**, 1107–1108.

Lawrence, C. B. & Davies, N. T. (1986). A novel, simple and rapid method for the isolation of mitochondria which exhibit respiratory control, from rat small intestinal mucosa. *Biochimica et Biophysica Acta* **848**, 35–40.

Lee, C. P., Martens, M. E., Jankulovska, L. & Neymark, M. A. (1979). Defective oxidative metabolism of myodystrophic skeletal muscle mitochondria. *Muscle and Nerve* **2**, 340–348.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry* **193**, 265–275.

Martens, M. E., Peterson, P. L. & Lee, C. P. (1991). In vitro effects of glucocorticoid on mitochondrial energy metabolism. *Biochimica et Biophysica Acta* **1058**, 152–160.

Nicholls, D. G. (1982). *Bioenergetics, An Introduction to the Chemiosmotic Theory*, pp. 99–132. London: Academic Press.

Powell, T. S., Douglas, C. R., Stonerock, R. H. & Harms, R. H. (1972). Feed intake of hens fed various levels of energy from feed and/or sucrose-water. *Poultry Science* **51**, 1851.
Reeds, P. J. (1989). Regulation of protein turnover. In *Animal Growth Regulation*, pp. 183–189 [D. R. Campion, G J. Hausman and R. J. Martin, editors]. New York and London: Plenum Press.
Rothwell, N. J. & Stock, M. J. (1987). Effect of environmental temperature on energy balance and thermogenesis in rats fed normal or low protein diets. *Journal of Nutrition* **117**, 833–837.
Rothwell, N. J., Stock, M. J. & Tyzbir, R. S. (1983). Mechanisms of thermogenesis induced by low protein diets. *Metabolism* **32**, 257–261.
SAS Institute Inc. (1985). *SAS User's Guide*: *Statistics*, version 5, pp. 433–506. Cary, NC: SAS Institute Inc.
Takeuchi, Y., Morii, H., Tamura, M., Hayaishi, O. & Watanabe, Y. (1991). A possible mechanism of mitochondrial dysfunction during cerebral ischemia: inhibition of mitochondrial respiration activity by arachidonic acid. *Archives of Biochemistry and Biophysics* **289**, 33–38.
Tinker, D. A., Brosnan, J. T. & Herzberg, G. R. (1986). Interorgan metabolism of amino acids, glucose, lactate, glycerol and uric acid in the domestic fowl (*Gallus domesticus*). *Biochemical Journal* **240**, 829–836.
Toyomizu, M. & Clandinin, M. T. (1993). Effects of dietary protein and fat level on oxidative phosphorylation in rat heart mitochondria. *British Journal of Nutrition* **69**, 97–102.
Toyomizu, M., Kirihara, D., Tanaka, M., Hayashi, K. & Tomita, Y. (1992). Dietary protein level alters oxidative phosphorylation in heart and liver mitochondria of chicks. *British Journal of Nutrition* **68**, 89–99.
Trayhurn, P., Ashwell, M., Jennings, G., Richard, D. & Stirling, D. M. (1987). Effect of warm or cold exposure on GDP binding and uncoupling protein in rat brown fat. *American Journal of Physiology* **252**, E237–E243.
Truswell, A. S. (1975). Carbohydrate and lipid metabolism in protein-calorie malnutrition. In *Protein-Calorie Malnutrition*, pp. 119–141 [R. E. Olson, editor]. New York: Academic Press.
Webster, A. J. F. (1981). The energetic efficiency of metabolism. *Proceedings of Nutritional Society* **40**, 121–128.

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