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

BY MASAHIRO TANAKA, TERU ISHIBASHI, KATSUYUKI OKAMOTO AND MASAAKI TOYOMIZU*†

Animal Nutrition, Department of Agriculture and Graduate School of Science and Technology, Niigata University, 2-8050 Ikarashi, Niigata, 950-21, Japan

(Received 7 July 1992 – Accepted 9 October 1992)

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 higherprotein 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-proteinenergy 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-pphenylenediamine 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

† For reprints. Present address: Animal Nutrition, Faculty of Agriculture, Tohoku University, 1-1, Tsutsumidori-Amamiyamachi, Sendai, 981 Japan.



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). \rightarrow , The electron flow through the electron transport chain; \rightarrow , specific inhibitors for each complex (rotenone for complex I and antimycin A for complex III). Different substrates were used to study the characteristics of O₂ consumption: pyruvate, malate, and L-glutamate, which generate intramitochondrial NADH; succinate, which provides electrons and the ubiquinone level; and ascrobate + 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.

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	

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

* 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.

 \ddagger 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 $(25 \pm 2^{\circ})$. 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-

461

M. TANAKA AND OTHERS

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-MgCl₂, 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-MgCl₂, 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 DEAEcellulose 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'-2ethanesulphonic 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-KH₂PO₄, 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-KH₂PO₄ 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-MgCl₂, 2·5 mm-KH₂PO₄, 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, $3.75 \,\mu$ M-rotenone or $0.4 \,\mu$ M-antimycin A. The system was equilibrated with mitochondria at 37°; then the rate of O₂ 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

463

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 μ g 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: \Box , 7%; \boxtimes , 25%; \boxtimes , 43%; \blacksquare , 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

https://doi.org/10.1079/BJN19930140 Published online by Cambridge University Press

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*

	birds)
•	five
	t0
\$	four
	for
	values
~	(Mean

Liver $10 \text{ mW-pyrtwate} + 25 \text{ mW-mdate}$ $7 = 142^{\text{m}} = 35 = 3-95 = 2.4^{\text{m}} = 337^{\text{m}} = 337 = 339 = 2.9^{\text{m}} = 239^{\text{m}} = 239^{m$	Liver $[1, 0]$ $[0]$ mw-pyruvate $+25$ mw-malate 7 $[1, 2^{24}]$ $[2, 27]$ $[2, 3$	Liver 10 mM-pyruvate + 25 mM-malate 7 142 ⁴ 35 395 274 ⁴ 35 274 ⁴ 35 274 ⁴ 35 274 ⁴ 32 24 ³⁰ 31 23 ⁴⁰ 30 342 239 ⁴ 324 23 ⁴¹ 328 234 ⁴¹ 328 344 328 344 328 344 328 344 328 344 328 344 328 344 328 344 328 344 328 344 328 344 328 348 348 348 348 348 348 348 348 348 34	Source of mitochondria	Substrate†	Dietary PME (%)	State 3 (ng atom/min	State 4 per mg protein)	Respiratory control ratio	ADP:0	AIF synthesized (nmol/min per mg protein)
Kidney [10] 23^{40} 21^{40} 27^{40} 24^{40} 29	25 124° 27 4.60 2.4° 2.8° Kidney 10 mw-pyruvate + 25 mw-malate 7 100° 3 9.45 2.4° 2.8° 2.3°	25 124^{th} 27 460 243^{th} Kidney 10 323 234^{th} 27 460 243^{th} Skidney 10 323 344 323 234^{th} 293 243^{th} Skidney 10 10^{th} 393 346 299 346 299 Skelatal muscle 10 1123^{th} 316 392 234^{th} 292 Skelatal muscle 10 1123^{th} 317^{th} 392^{th} 299^{th} 279^{th} <	Liver	10 mm-pyruvate + 2·5 mm-malate	7	142 ^a	35	3.95	2·74ª	371 ^a
time 123 th 24 235 234 234 235 23	Kidney I 123 ¹⁰ 34 3.28 2.34° 2.3	43 123 ^{mb} 34 3.28 2.34 ^{mb} Kidney 100 ^{mb} 3 3 3.42 2.29 ^{mb} Kidney 100 ^{mb} 3 3 3.42 2.29 ^{mb} 3 Skeletal muscle 10 108 ^{mb} 3 3.42 2.29 ^{mb} 3 Skeletal muscle 10 113 3 3.42 2.94 ^{mb} 2.66 Skeletal muscle 10 113 3 3.7 3.66 2.69 <i>Qectoralis profundus</i> 10 113 3 3.7 3.66 2.78 Skeletal muscle 10 113 3.8 3.7 3.67 2.78 <i>Osceps femoris</i> 61 12 3.321 67 4.88 2.79 <i>Osceps femoris</i> 10 mw-pyruvate + 25 mw-malate 7 4.27 1013 2.78 <i>Osceps femoris</i> 10 mw-pyruvate + 25 mw-malate 7 4.27 10.3 2.71 2.71 <i>Osceps femoris</i> 10 <td< td=""><td></td><td>5 4</td><td>25</td><td>124^{ab}</td><td>27</td><td>4.69</td><td>$2.43^{\rm b}$</td><td>289^h</td></td<>		5 4	25	124^{ab}	27	4.69	$2.43^{\rm b}$	289 ^h
61 108 ⁺ 30 342 2.29° 234° 23° Kidney 10 mw-pyruvate + 25 mw-malate 7 146 39 344 239 375 Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 146 39 346 077 21 Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 348 79 346 79 347 365 375 375 Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 348 79 447 288 1007 Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 348 79 447 273 818 Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 427 103 818 3007 Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 447 278 818 3007 Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 447 209 548 277 1174 Skeletal muscle 10 mw-pyruv	Kidney 61 108 ⁺ 30 342 2.29 ⁺ 234 ⁺ Kidney 10 39 446 077 21 25 135 37 360 265 375 5 146 39 441 299 377 5 135 37 362 266 375 61 135 321 67 487 303 365 5 321 67 348 309 968 307 301 325 5 321 67 348 309 968 309 968 61 233 321 67 488 309 968 317 biceps femoris 10 mu-pyruvate + 25 mw-malate 7 445 120 341 273 1174 biceps femoris 10 331 316 273 323 345 273 1123 biceps femoris 10 346 273	Kidney [6] [08" 30 342 229" 239" Kidney 10 mw-pyruvate + 25 mw-malate 7 146 39 340 266 Zs 146 135 37 366 266 266 Gi 135 37 36 342 289 266 Vectoralis profundus) 10 mw-pyruvate + 25 mw-malate 7 338 37 365 342 289 Vectoralis profundus) 10 mw-pyruvate + 25 mw-malate 7 338 37 367 348 279 Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 332 37 367 348 279 Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 332 32 367 348 279 Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 348 273 041 Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 348 271 217 217 Skeletal muscle 10 mw-pyruvate +			43	123^{ab}	34	3.28	2.34^{b}	269^{b}
Kidney 10 3 0.46 007 21 Kidney 10 148 39 266 377 Keleal muscle 13 153 37 362 375 Skeleal muscle 10 123 362 375 362 375 Skeleal muscle 10 123 348 79 467 288 302 Skeleal muscle 10 12 348 79 467 288 302 Skeleal muscle 10 12 348 79 467 279 818 Skeleal muscle 10 12 348 79 467 279 818 Skeleal muscle 10 127 312 61 223 61 323 302 518 279 818 302 Skeleal muscle 10 127 212 212 212 2123 2123 2123	Kidney 10 3 0.46 007 21 Kidney 10 mw-pyruvate + 25 mw-malate 7 148 39 340 266 377 Skeletal muscle 10 113 37 362 278 367 375 Skeletal muscle 10 113 37 362 278 367 375 375 Skeletal muscle 10 12 33 37 362 375 365 375 Skeletal muscle 10 12 34 79 447 288 100 Skeletal muscle 10 28 302 59 518 303 548 279 841 Skeletal muscle 10 28 302 59 518 300 568 317 Skeletal muscle 10 32 341 277 288 300 518 303 548 273 1174 Skeletal muscle 10 346 271	Kidney 10 3 0.46 007 Kidney 10 3 0.46 007 269 43 133 36 392 266 266 43 133 36 392 266 266 61 135 37 362 278 266 <i>Vectordis profundus</i> 10 13 37 362 279 261 25 321 61 12 348 79 467 288 101 Skeletal muscle 10 12 348 79 467 288 279 266 <i>Vectordis profundus</i> 61 292 64 488 279 271 117 Skeletal muscle 10 23 302 59 518 279 271 271 <i>biceps femoris</i> 61 232 64 488 273 117 275 112 272 112 272 272 112			61	$108^{\rm h}$	30	3.42	2.29 ^b	234 ^b
Kidney $10 \text{ mk-pyruvate} + 25 \text{ mk-malate}$ 714839360260377 25 113 35 36 397 397 397 397 25 61 135 36 392 266 372 61 135 37 362 278 362 61 135 37 362 278 302 56 7 321 67 488 309 966 377 322 67 488 309 967 967 488 302 968 10 12 232 64 480 278 $86M$ (12 df) 28 7 249 917 88 10 10 28 7 247 103 $86M$ (12 df) 28 103 384 277 2117 10 10 391 113 366 277 2117 10 10 391 113 316^9 271 217 1032 10 10 391 113 316^9 271 227 1174 10 10 384 271 237 242 1032 10 10 386 271 237 237 237 10 10 380 271 217 227 221 10 10 380 271 217 227 221 10 10 380 274 236 221 <td>Kidney10mA-pyruvate $+25$ mM-malate714839360269377251463636363736373637256113536373637365812135363736373658131353637963799611233637011257732167483009689612926448027881861292644802788186129264480273814(biceps femoris)9451203842711179945120384271117805810028120384271117994512038427121711799451203842712171251910139428271217125191094382324623724291094382324923724291094282712172372429109438237242201237910943823724220123</td> <td>Kidney 10 mk-pyruvate + 25 mk-malate 7 148 39 360 269 25 135 37 362 278 911 289 911 289 911 289 911 289 911 289 911 289 911 289 911 289 911 289 911 289 911 289 911 289 911 289 911 289 911 279 011 289 279 011 289 279 011 289 279 011 286 279 011 273 012 011 273 011 273 011 273 011 273 011 273 011 273 011 273 011 273 011 273 011 273 011 274 273 101 274 273 101 274 273 111 10 274 271 211 211 274 271 211<</td> <td></td> <td></td> <td>sem (16 df)</td> <td>10</td> <td>3</td> <td>0-46</td> <td>0.07</td> <td>21</td>	Kidney10mA-pyruvate $+25$ mM-malate714839360269377251463636363736373637256113536373637365812135363736373658131353637963799611233637011257732167483009689612926448027881861292644802788186129264480273814(biceps femoris)9451203842711179945120384271117805810028120384271117994512038427121711799451203842712171251910139428271217125191094382324623724291094382324923724291094282712172372429109438237242201237910943823724220123	Kidney 10 mk-pyruvate + 25 mk-malate 7 148 39 360 269 25 135 37 362 278 911 289 911 289 911 289 911 289 911 289 911 289 911 289 911 289 911 289 911 289 911 289 911 289 911 289 911 289 911 279 011 289 279 011 289 279 011 289 279 011 286 279 011 273 012 011 273 011 273 011 273 011 273 011 273 011 273 011 273 011 273 011 273 011 273 011 274 273 101 274 273 101 274 273 111 10 274 271 211 211 274 271 211<			sem (16 df)	10	3	0-46	0.07	21
	$ \begin{array}{c cccc} & & & & & & & & & & & & & & & & & $	25 146 39 411 289 61 153 37 362 278 61 135 37 362 278 <i>pectoralis profinatus</i> 10 mw-pyruvate + 25 mw-malate 7 348 79 467 288 <i>pectoralis profinatus</i> 10 mw-pyruvate + 25 mw-malate 7 321 67 488 309 5 55 302 59 518 279 011 Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 427 103 272 103 <i>biceps femoris</i> 10 mw-pyruvate + 25 mw-malate 7 425 103 2417 273 <i>biceps femoris</i> 10 mw-pyruvate + 25 mw-malate 7 425 103 2417 272 <i>biceps femoris</i> 10 mw-pyruvate + 25 mw-malate 7 425 103 271 217 mucosa 10 mw-pyruvate + 25 mw-malate 7 405 103 246 271 217 mucosa 10 mw-pyruvate + 25 mw-malate 7 405 103 246 271 217 217 <td>Kidney</td> <td>10 mm-pyruvate + 2·5 mm-malate</td> <td><u> </u></td> <td>148</td> <td>39</td> <td>3-60</td> <td>2.69</td> <td>377</td>	Kidney	10 mm-pyruvate + 2·5 mm-malate	<u> </u>	148	39	3-60	2.69	377
	43 by the second (1 d) 13 (1	43 153 36 392 266 61 135 37 362 278 Skeletal muscle 10 mM-pyruvate + 25 mM-malate 7 348 79 467 281 (pectoralis profundus) 12 348 79 467 288 1 Skeletal muscle 10 mM-pyruvate + 25 mM-malate 25 321 67 488 309 Skeletal muscle 10 mM-pyruvate + 25 mM-malate 7 342 70 949 017 bicops femoris) 10 mM-pyruvate + 25 mM-malate 7 427 103 344 279 bicops femoris) 10 mM-pyruvate + 25 mM-malate 7 465 120 344 277 11 Small intestinal 10 mM-pyruvate + 25 mM-malate 7 465 271 275 11 mucosa 10 mM-pyruvate + 25 mM-malate 7 465 271 275 275 for one 316 316 271 275 275 271 271	'n		25	146	39	4·11	2-89	397
	61 multiple 13 multiple 37 multiple 362 multiple 37 multiple 362 multiple 37 multiple 362 multiple 37 multiple 362 multiple 37 multiple 362 multiple 37 multiple 37 multiple 362 multiple 37 multiple 37 multiple 362 multiple 37 multiple <	61 135 37 362 278 Skeletal muscle 10 mM-pyruvate + 25 mM-malate 7 348 79 4467 288 (pectoralis profundus) 25 321 67 488 309 279 Skeletal muscle 10 mM-pyruvate + 25 mM-malate 7 322 59 518 279 Skeletal muscle 10 mM-pyruvate + 25 mM-malate 7 427 103 417 275 Skeletal muscle 10 mM-pyruvate + 25 mM-malate 7 427 103 4117 275 biceps femoris) 10 mM-pyruvate + 25 mM-malate 7 425 103 411 271 11 Small intestinal 10 mM-pyruvate + 25 mM-malate 7 405 297 271 217 Mucosa 10 mM-glutamate + 25 mM-malate 7 405 272 11 stand 11 mestinal 10 mM-glutamate + 25 mM-malate 7 1103 244 217 217 217 217 217 217 217 217<			43	153	36	3.92	2.66	375
Skeletal muscle (pectoralis profundus)10 mM-pyruvate + 25 mM-malate71230.370.1125 25 321 67 487 288 1007 43 300 59 518 2.79 841 61 222 64 4.80 2.78 818 61 292 64 4.80 2.78 818 58 17 292 64 4.80 2.78 818 61 292 64 4.80 2.78 818 61 292 64 4.80 2.78 818 61 292 64 4.80 2.78 818 $8m$ (12 df) 28 17 427 103 441 272 $(biceps femoris)$ 43 10 28 47 103 441 272 $(biceps femoris)$ 10 94 286 221 292 249 $8mall intestinal10109423729211748mall intestinal10942372342212218mall intestinal10942372342212378mall intestinal101139412722212218mall intestinal109412382242212218mall intestinal10860316^{0}2242212248mall intestinal10$	Skeletal muscle 10 mk-pyruvate + 25 mk-malate 7 348 7 677 288 101 25 (pectoralis projondus) (0 mk-pyruvate + 25 mk-malate 7 348 7 64 480 279 841 Skeletal muscle 10 mk-pyruvate + 25 mk-malate 7 348 7 649 278 279 841 Skeletal muscle 10 mk-pyruvate + 25 mk-malate 7 427 103 2417 278 279 841 277 2114 Skeletal muscle 10 mk-pyruvate + 25 mk-malate 7 427 103 247 277 273 1174 818 Skeletal muscle 10 mk-pyruvate + 25 mk-malate 7 425 103 247 277 273 1174 Small intestinal 10 mk-pyruvate + 25 mk-malate 7 427 103 247 273 271 1023 Small intestinal 10 mk-pyruvate + 25 mk-malate 7 425 271 2	Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 348 79 646 288 10 mw-pyruvate + 25 mw-malate 7 348 79 467 288 10 mw-pyruvate + 25 mw-malate 7 348 79 467 288 309 Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 348 79 467 288 309 518 279 61 278 309 278 309 071 278 309 071 309 071 273 101 273 101 273 101 273 101 273 101 273 101 273 101 275 11 275 11 275 11 275 11 275 11 275 11 275 12 12 12 12 12 12 12 12 271 275 11 275 12 12 12 12 12 12 12 12 12 12 12 12<			61	135	37	3-62	2.78	362
Skeletal muscle10 mk-pyruvate + 25 mk-malate7348794672881007(pectoralis projendas)01292644883099683216129264488309968581029264480277881858102926448027788185810292644802778818581029264480277881858102911134417277311745861391113445277311516139111344527110325861316*27120122375810316*2712173103261316*27121731032591113389*297237592574211389*27121737551190374*297237751197389*2712173751197389*2712173751197389*2712173751197389*2712173751197389*2712173751197389*2712173751197389*273219*751197389*274219*751197 <td>Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 348 79 467 288 1007 $pectoralis projundus)$ 61 292 64 488 309 98 $pectoralis projundus)$ 61 292 64 488 309 98 Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 427 103 411 275 1174 Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 445 120 384 275 1174 Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 445 120 344 272 1174 Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 465 120 344 272 1124 Small intestinal 10 mw-pyruvate + 25 mw-malate 7 86 316'' 271 123 Small intestinal 10 mw-pyruvate + 25 mw-malate 7 860 316'' 271 237 Small intestinal 10 mw-pyruvate + 25 mw-malate 7 860 316''<!--</td--><td>Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 348 79 467 288 1 (pectoralis profundus) (0 mw-pyruvate + 25 mw-malate 7 321 67 488 340 (pectoralis profundus) 25 321 67 488 340 Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 427 103 417 275 Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 427 103 417 275 1 Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 425 103 445 272 1 1 Small intestinal 10 mw-pyruvate + 25 mw-malate 7 465 120 538 273 1 1 Mucosa 316 113 316 271 1 1 271 217 1 272 1 2 mucosa 10 316 23 316 274 203 201 2 2 2 2 <td< td=""><td></td><td></td><td>sem (12 df)</td><td>12</td><td>ę</td><td>0.37</td><td>0-11</td><td>25</td></td<></td></td>	Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 348 79 467 288 1007 $pectoralis projundus)$ 61 292 64 488 309 98 $pectoralis projundus)$ 61 292 64 488 309 98 Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 427 103 411 275 1174 Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 445 120 384 275 1174 Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 445 120 344 272 1174 Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 465 120 344 272 1124 Small intestinal 10 mw-pyruvate + 25 mw-malate 7 86 316'' 271 123 Small intestinal 10 mw-pyruvate + 25 mw-malate 7 860 316'' 271 237 Small intestinal 10 mw-pyruvate + 25 mw-malate 7 860 316'' </td <td>Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 348 79 467 288 1 (pectoralis profundus) (0 mw-pyruvate + 25 mw-malate 7 321 67 488 340 (pectoralis profundus) 25 321 67 488 340 Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 427 103 417 275 Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 427 103 417 275 1 Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 425 103 445 272 1 1 Small intestinal 10 mw-pyruvate + 25 mw-malate 7 465 120 538 273 1 1 Mucosa 316 113 316 271 1 1 271 217 1 272 1 2 mucosa 10 316 23 316 274 203 201 2 2 2 2 <td< td=""><td></td><td></td><td>sem (12 df)</td><td>12</td><td>ę</td><td>0.37</td><td>0-11</td><td>25</td></td<></td>	Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 348 79 467 288 1 (pectoralis profundus) (0 mw-pyruvate + 25 mw-malate 7 321 67 488 340 (pectoralis profundus) 25 321 67 488 340 Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 427 103 417 275 Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 427 103 417 275 1 Skeletal muscle 10 mw-pyruvate + 25 mw-malate 7 425 103 445 272 1 1 Small intestinal 10 mw-pyruvate + 25 mw-malate 7 465 120 538 273 1 1 Mucosa 316 113 316 271 1 1 271 217 1 272 1 2 mucosa 10 316 23 316 274 203 201 2 2 2 2 <td< td=""><td></td><td></td><td>sem (12 df)</td><td>12</td><td>ę</td><td>0.37</td><td>0-11</td><td>25</td></td<>			sem (12 df)	12	ę	0.37	0-11	25
	$(pectoralis profundus)$ 25 321 67 488 300 968 (12 dr) 302 392 64 488 309 941 Skeletal muscle $10 \text{ mM-pyruvate} + 25 \text{ mM-malate}$ 7 427 103 417 275 1174 Skeletal muscle $10 \text{ mM-pyruvate} + 25 \text{ mM-malate}$ 7 465 120 384 277 1174 Skeletal muscle $10 \text{ mM-pyruvate} + 25 \text{ mM-malate}$ 7 465 120 384 277 1174 Small intestinal $10 \text{ mM-pyruvate} + 25 \text{ mM-malate}$ 7 860 316° 271 1032 Small intestinal $10 \text{ mM-pyruvate} + 25 \text{ mM-malate}$ 7 113 456 271 1032 Small intestinal $10 \text{ mM-pyruvate} + 25 \text{ mM-malate}$ 7 113 456 271 1032 Small intestinal $10 \text{ mM-pyruvate} + 25 \text{ mM-malate}$ 7 113 389° 271 210° 272 210° 239° 201° 201° 201°	(pectoralis profundus) 25 321 67 488 309 61 292 64 480 278 309 58 59 518 279 61 278 309 58 61 292 64 480 278 309 58 53 302 59 518 279 017 7 049 017 275 11 275 11 275 11 275 11 275 11 275 11 275 11 275 120 384 272 11 275 11 275 11 275 11 275 11 275 11 275 11 275 271 271 271 271 271 271 271 271 271 271 271 271 271 272 11 275 11 275 297 274 208 274 208 274 208 274 208 276 201 236 236 236 236 236	Skeletal muscle	10 mm-pyruvate + 2·5 mm-malate	2	348	62	4.67	2.88	1007
4330259518279841612926448027881888729264480278818Skeletal muscle10 mM-pyruvate + 25 mM-malate74271034172751174(biceps femoris)254051203842721251 43 3011134172751174 61 3911134452721251 61 3911134452711032 880 5281033442721032 880 1103911134562711032 880 3162712712171820 880 3162712712171820 880 3162712712171820 880 3162712712171820 880 1240138274272242 880 1241183532942362743 880 12411835362798274236 1001 3873387219627432362743 10 117838723627432362743 10 11783872362798254023982540 10 1178387236279827432362743 10 1178387336923627	43 302 59 518 279 841 61 292 64 480 2778 818 Skeletal muscle 10 mm-pyruvate + 25 mm-malate 7 427 103 417 275 1174 (biceps femoris) 25 465 120 384 272 1251 3mall intestinal 10 mm-pyruvate + 25 mm-malate 7 405 80 578 282 1173 Small intestinal 10 mm-pyruvate + 25 mm-malate 7 405 80 578 237 1133 Small intestinal 10 mm-pyruvate + 25 mm-malate 7 1100 316° 271 1133 Incosa 25 1100 316° 271 217 1133 Incosa 26 1100 316° 274 2001 237 Incosa 316° 274 237 237 237 237 Incosa 1100 316° 274 237 237 237 Incosa 1110 386° 274 236° 2743 236° 27	43 302 59 5-18 279 61 292 64 480 278 58 10 28 10 417 275 1 (biceps femoris) 28 103 417 275 1 (biceps femoris) 29 545 103 417 275 1 (biceps femoris) 29 578 28 271 1 1 272 1 1 (biceps femoris) 391 113 456 271 1 1 272 1 1 28 273 1 1 273 271 1 1 1 1 386 273 384 275 1 272 271 1 272 271 271 271 271 271 271 271 273 271 271 273 274 275 271 217 274 272 271 271 271 271 271 271 271 271 271 271 271 271 271 271 <t< td=""><td>(pectoralis profundus)</td><td>5</td><td>25</td><td>321</td><td>67</td><td>4·88</td><td>3.09</td><td>968</td></t<>	(pectoralis profundus)	5	25	321	67	4·88	3.09	968
	6129264480278818Skeletal muscle10 mw-pyruvate + 2.5 mw-malate74271034172751174(biceps femoris)254651203.842.72115381(biceps femoris)43405805.282.821174(biceps femoris)3911134.552.711032Small intestinal10 mw-pyruvate + 2.5 mw-malate7860316°2.711032Small intestinal10 mw-pyruvate + 2.5 mw-malate7860316°2.712.972.97Nucosa10 mw-pyruvate + 2.5 mw-malate71100374°2.972.972.972.42Incosa10 mw-glutamate + 2.5 mw-malate71178389°2.942.972.422.60110 mw-glutamate + 2.5 mw-malate71178383°2.942.732.942.972.942.972.942.942.9510 mw-glutamate + 2.5 mw-malate71178383°2.942.752.942.972.942.972.942.972.942.9510 mw-glutamate + 2.5 mw-malate711783.83°2.942.972.942.972.942.972.942.972.942.972.942.972.942.972.942.972.942.952.942.952.942.972.942.972.942.972.942.972.942.972.942.	61 292 64 480 278 1 Skeletal muscle 10 mm-pyruvate + 2:5 mm-malate 7 0.49 0.17 275 1 (biceps femoris) 25 465 103 4.17 2.75 1 25 465 103 4.17 2.75 1 26 405 80 5.28 2.82 2 2 61 391 1113 4.56 2.71 1 2 7 391 1113 4.56 2.71 1 1 860 316 316 2.31 2.94 2.35 2 <td< td=""><td>5 4</td><td></td><td>43</td><td>302</td><td>59</td><td>5.18</td><td>2:79</td><td>841</td></td<>	5 4		43	302	59	5.18	2:79	841
	Skeletal muscle10mw-pyruvate + 2.5 mw-malate70.490.1780 25 465 103 4.17 2.75 1174 105 103 405 80 528 1174 105 113 405 80 528 1174 105 113 405 80 528 1174 105 113 405 80 528 1174 105 113 405 80 528 1133 10 113 456 271 1032 100 316^{4} 271 208 237 1100 374^{4} 297 271 1032 1100 374^{4} 271 237 2429 1100 374^{4} 271 237 2429 1100 374^{4} 273 297 2429 1100 374^{4} 273 291 2397 10 mucosa 1211 554^{4} 271 232 10 mucosa 1211 554^{4} 271 232 10 mucosa 255 1190 234^{4} 235 240 10 mucosa 1178 383^{2} 297 271 2397 10 mucosa 1178 383^{2} 296^{4} 271 239^{4} 10 mucosa 1211 1178 387^{4} 276^{4} 299^{4} 10 mucosa 1197 387^{4} 276^{4} 299^{4} 274^{4} 10 mucosa 117	Skeletal muscle 10 mw-pyruvate + 2.5 mw-malate 7 0.49 0.17 25 465 103 4.17 2.75 1 61 391 113 4.56 2.71 1 13 391 113 4.56 2.71 1 10 10 mw-pyruvate + 2.5 mw-malate 7 860 316^{0} 2.71 2.72 1 10 mw-pyruvate + 2.5 mw-malate 7 860 316^{0} 2.71 2.17 2.17 10 mw-pyruvate + 2.5 mw-malate 7 860 316^{0} 2.71 2.17 2.17 10 mw-glutamate + 2.5 mw-malate 7 1100 374^{0} 2.71 2.17 2.11			61	292	64	4.80	2.78	818
Skeletal muscle10 mm-pyruvate + 2.5 mm-malate74271034.172.751174(biceps femoris)254651203.842.721251 43 405805.282.821153 61 3911134.562.711032sem (12 df)94280.540.08237Small intestinal10 mm-pyruvate + 2.5 mm-malate71100374°2.712.17sem (12 df)94280.540.082.37sem (12 df)1100374°2.712.171820mucosa251100374°2.972.422601mucosa1001389°2.942.352.942.397sem (12 df)183389°2.942.352.9173.8410 mucosa1211554°2.152.172.171820nucosa1211554°2.152.19°2.3972.39710 mucosa1211554°2.16°2.19°2.19°2.546117389°2.153.19°2.19°2.94°2.19°2.94°10 mucosa1178387°3.19°2.19°2.94°2.94°2.94°117387°3.19°2.16°2.17°2.94°2.94°2.94°117387°3.19°2.19°2.19°2.94°2.94°2.94°1181575616°2.46°1.82°2.39°2.94°	Skeletal muscle10 mM-pyruvate + 25 mM-malate74271034.17 2.75 1174(biceps femoris)254651203.84 2.72 1251(biceps femoris)61391113 4.56 2.711032553842.721153 2.52 251103261391113 4.56 2.711032 2.37 51100316°2.712.17180mucosa251100374°2.972.37611211554*2.172.35243611211554*2.172.392.367100m-glutamate + 2.5 mM-malate71178383°2.942.3671010374°2.942.35243243101191554*2.172.35243611211554*2.19°2.36*2.4310 mM-glutamate + 2.5 mM-malate71178387°2.99*2.36*611575610*2.46*1.82°2.74371197387°2.99*2.39°2.566611575610*2.96*2.19*2.39*71197387°2.99*2.99*2.743811543.00°2.7450.17387°2.99*71197387°2.7450.173.9*2.743811972.95*0.172.9*	Skeletal muscle 10 mM-pyruvate + 25 mM-malate 7 427 103 4.17 2.75 1 (biceps femoris) 10 mM-pyruvate + 25 mM-malate 25 465 120 3.84 2.72 1 (biceps femoris) 391 1113 4.56 2.71 1 Small intestinal 10 mM-pyruvate + 2.5 mM-malate 7 860 316 ^b 2.71 2.71 1 Small intestinal 10 mM-pyruvate + 2.5 mM-malate 7 860 316 ^b 2.71 2.71 1 mucosa 374 ^b 2.97 3.89 ^b 2.97 2.97 2.42 2.01 mucosa 38 ^b 1091 389 ^b 2.94 2.35 2.01 2.35 2.97 2.97 2.97 2.97 2.97 2.94 2.96 2.96 ^m 2.19 ^m 2.96 ^m 2.19 ^m 2.36 ^m			sem (12 df)	28	7	0.49	0-17	80
	(biceps femoris) 25 465 120 384 2.72 1251 61 391 113 94 28 2.71 1032 81 391 113 4.56 2.71 1032 82 384 2.71 1032 82 10 349 2.71 1032 83 10 316 2.71 217 120 84 25 1100 374° 2.71 120 94 1211 564 2.77 120 384 95 1091 389° 2.97 2.42 2601 961 1211 554* 2.17 1297 240 910 389° 2.94 2.35 249 297 910 178 383° 2.96* 274 297 910 183 53 2.96* 2.74 297 910 177 383° 2.96* 2.74 2.97 910 177 387 2.96* 2.74 2.97	(biceps femoris) 25 465 120 3.84 2.72 1 61 391 113 4.56 2.71 1 80 5.28 2.82 2.82 2.82 2.82 81 10 mucosa 391 113 4.56 2.71 1 81 11 94 28 0.54 0.08 0.08 0.08 0.08 0.08 2.91 1 1 82 10 94 2.8 0.53 0.08 0.54 0.08 0.08 0.08 0.08 0.08 0.08 0.08 0.08 0.08 0.08 0.08 0.08 0.08 0.08 0.01 0.08 0.01 0.08 0.01 0.03 0.01 0.03 0.01 0.03 0.01 0.03 0.01 0.03 0.01 0.03 0.01 0.03 0.01 0.01 0.03 0.01 0.03 0.01 0.03 0.01 0.03 0.01 0.03 0.01 0.03 0.01 0.03 0.01 0.03 0.03 0.01	Skeletal muscle	10 mm-pyruvate + 2·5 mm-malate	L .	427	103	4.17	2.75	1174
43405805-282.821153613911134-562.711032SEM (12 df)94280.540.08237Small intestinal10 mw-pyruvate + 2.5 mw-malate7860316°2.711032251100374°2.972.422601612111554°2.171820sem (12 df)183339°2.942.3392.152.16334°2.942.39710 mw-glutamate + 2.5 mw-malate71178383°2.16°2.19°2.540611211554°2.19°2.36°2.71338410 mw-glutamate + 2.5 mw-malate71178387°2.19°2.540551197387°3.19°2.19°2.56°611575616°2.46°1.82°2743550.420.36°2.36°2.7132.97°550.420.387°2.36°2.79°2.79°550.420.387°2.39°2.79°2.79°550.420.387°0.387°2.748°1.78°550.420.387°0.420.38°279°550.420.387°0.38°2.748°1.79°550.420.387°0.38°0.38°2.743°550.420.387°0.420.38°279°550.420.38°0.420.38°2797 <t< td=""><td>43405805-282.821153613911134-562.7110328mall intestinal10 mM-pyruvate + 2.5 mM-malate7860316°2.711032221100314°2.772.171820911138.60316°2.772.601921091389°2.942.352601931091389°2.942.352429941211554°2.192.3972397951107383°2.966°2.19°°2.743961178383°2.966°2.19°°2.743973.1973.19°2.36°2.743951197387°3.96°2.19°°2.743961157616°2.46°1.82°2.743973.66°3.74°3.66°2.36°2.743981157616°2.46°1.82°2.743981555611575616°2.46°1.82°982.052.560.38°2.36°2.743982.55611575616°2.46°1.82°982.662.512.550.330.420.38°2.74398157555616°2.36°2.7432.74398157555616°2.36°2.7432.74398157555616°2.7450.38°2.743</td></t<> <td>43 405 80 5-28 2-82 61 391 113 4-56 2-71 1 8mall intestinal 10 mM-pyruvate + 2:5 mM-malate 7 860 316^b 2-71 2-17 1 mucosa 25 1100 374^b 2-71 2-17 2-17 1 mucosa 25 1100 374^b 2-97 2-71 2-17 2-17 2 mucosa 25 1100 374^b 2-97 2-72 2 2 10 mucosa 25 1100 374^b 2-97 2-17 2 2 11 12 1211 554^a 2-15 2-15 2 <t< td=""><td>(biceps femoris)</td><td></td><td>25</td><td>465</td><td>120</td><td>3-84</td><td>2·72</td><td>1251</td></t<></td>	43405805-282.821153613911134-562.7110328mall intestinal10 mM-pyruvate + 2.5 mM-malate7860316°2.711032221100314°2.772.171820911138.60316°2.772.601921091389°2.942.352601931091389°2.942.352429941211554°2.192.3972397951107383°2.966°2.19°°2.743961178383°2.966°2.19°°2.743973.1973.19°2.36°2.743951197387°3.96°2.19°°2.743961157616°2.46°1.82°2.743973.66°3.74°3.66°2.36°2.743981157616°2.46°1.82°2.743981555611575616°2.46°1.82°982.052.560.38°2.36°2.743982.55611575616°2.46°1.82°982.662.512.550.330.420.38°2.74398157555616°2.36°2.7432.74398157555616°2.36°2.7432.74398157555616°2.7450.38°2.743	43 405 80 5-28 2-82 61 391 113 4-56 2-71 1 8mall intestinal 10 mM-pyruvate + 2:5 mM-malate 7 860 316 ^b 2-71 2-17 1 mucosa 25 1100 374 ^b 2-71 2-17 2-17 1 mucosa 25 1100 374 ^b 2-97 2-71 2-17 2-17 2 mucosa 25 1100 374 ^b 2-97 2-72 2 2 10 mucosa 25 1100 374 ^b 2-97 2-17 2 2 11 12 1211 554 ^a 2-15 2-15 2 <t< td=""><td>(biceps femoris)</td><td></td><td>25</td><td>465</td><td>120</td><td>3-84</td><td>2·72</td><td>1251</td></t<>	(biceps femoris)		25	465	120	3-84	2·72	1251
	61391113 4.56 2.71 1032Small intestinal10 mw-pyruvate + 2.5 mw-malate7860 316^{b} 2.71 1032 25 1100 374^{b} 2.97 2.17 1820 25 1100 374^{b} 2.97 2.42 2601 261 1211 554^{a} 2.15 2429 2397 860 316^{b} 2.16 2.42 2601 2742 860 316^{b} 2.94 2.35 2429 61 1211 554^{a} 2.15 201 2397 $86M$ 1201 88 1191 839^{b} 2.94 2.35 $10 mw-glutamate + 2.5 mw-malate71178383^{b}2.96^{ab}2.19^{ab}2540251197387^{b}3.19^{a}2.36^{a}2.99^{a}27438m12 df1575616^{a}2.96^{ab}2.99^{a}27438m12 df387^{b}3.90^{b}2.36^{a}27438m12 df376^{b}2.99^{a}27438m12 df3.76^{a}2.99^{a}27438m12 df3.6^{b}2.96^{a}2.99^{a}27438m12 df3.76^{b}2.99^{a}2.99^{a}27438m12 df2.16^{a}2.96^{a}2.99^{a}279^{a}8m2.10^{a}2.10^{a}2.91^{a}2.9^{a}$	61 391 113 4:56 2:71 1 Small intestinal 10 mM-pyruvate + 2:5 mM-malate 7 860 316 ^b 2:71 2:17 1 mucosa 25 1100 374 ^b 2:97 2:42 2 mucosa 25 1100 374 ^b 2:97 2:42 2 61 1211 554 ^a 2:94 2:35 2 2 860 1211 554 ^a 2:15 2 2 2 10 mM-glutamate + 2:5 mM-malate 7 1178 387 ^b 2:94 2:35 2 2 10 mM-glutamate + 2:5 mM-malate 7 1178 387 ^b 2:96 ^{ab} 2:19 ^{ab} 2 1178 387 ^b 3:67 ^b 3:76 ^{ab} 2:36 ^{ab} 2:96 ^{ab} 2:94 ^{bb} 2:96 ^{ab} 2:94 ^{bb} <			43	405	80	5.28	2.82	1153
	Small intestinal10 mM-pyruvate + 2.5 mM-malate794280.540.08237 25 1100316 ^b 2.712.171820mucosa251100374 ^b 2.972.422601 43 1091389 ^b 2.942.352429 61 1211534 ^a 2.152.01384 $8m$ (12 df)183534 ^a 2.152.01384 $8m$ (12 df)183534 ^a 2.152.01237 $8m$ (12 df)183534 ^a 2.19 ^{ab} 2.99 ^{ab} 2.94 ^{ab} 2.743 10 mM-glutamate + 2·5 mM-malate71178387 ^b 3.19 ^{ab} 2.99 ^{ab} 2.94 ^{ab} 2.743 61 1157616 ^a 3.19 ^{ab} 2.96 ^{ab} 2.19 ^{ab} 2.7432743 $8m$ (12 df)1575616 ^a 2.94 ^{bb} 2.99 ^{ab} 2.99 ^{ab} 2.743 $8m$ (12 df)2.51550.420.38 ^{ab} 2.99 ^{ab} 2.745 $8m$ (12 df)2.51550.420.38 ^{ab} 2.99 ^{ab} 2.79 ^{ab} 2.79 ^{ab} $8m$ Means with different superscript letters were significantly different ($P < 0.05$).0.420.38 ^{ab} 2.743	Small intestinal 10 mM-pyruvate + 25 mM-malate 7 860 316 ^b 2.71 2.17 1 mucosa 25 1100 374 ^b 2.97 2.42 2 mucosa 25 1100 374 ^b 2.97 2.42 2 mucosa 26 1100 374 ^b 2.97 2.42 2 nucosa 236 ^b 1011 554 ^a 2.94 2.35 2 2 10 mM-glutamate + 2.5 mM-malate 7 1178 383 ^b 2.96^{ab} 2.19^{ab} 2.96^{ab} 2.19^{ab} 2.96^{ab}			61	391	113	4.56	2-71	1032
Small intestinal10 mm-pyruvate + 25 mm-malate7860 316^{b} 2.71 2.17 1820mucosa 25 1100 374^{b} 2.97 2.42 2601 mucosa 43 1091 389^{b} 2.94 2.35 2429 61 1211 554^{a} 2.94 2.397 2397 $8EM$ (12 df) 183 53 0.17 384 10 mm-glutamate + 2.5 mm-malate7 1178 383^{b} 2.96^{b} 2.19^{b} 2540 43 1157 360^{b} 3.36^{a} 2.96^{b} 2.99^{a} 2743 55 1197 387^{b} 3.19^{a} 2.36^{a} 2743 7 1178 387^{b} 3.19^{a} 2.36^{a} 2743 61 1575 616^{a} 2.46^{b} 1.82^{b} 2773 $8EM$ (12 df) 251 556 0.42 0.38 279^{a} $8EM$ (12 df) 251 55 0.42 0.38 279^{a}	Small intestinal10 mm-pyruvate + 2.5 mm-malate7860316°2.712.171820mucosa251100374°2.972.422601mucosa431001389°2.972.422601611211554°2.942.3524295611211554°2.942.332397611211554°2.942.3524295545742.17183530.1738450.mm-glutamate + 2.5 mm-malate71178387°2.96°*2.19°2540611575616°3.74°2.46°1.82°274355611575616°3.36°2.97°274350.420.383.60°3.36°2.97°27435m1575616°2.46°1.82°277327435m1575616°2.36°2.96°2.97°27735m0.420.380.420.38°50727655mxmmake2.5560.420.38°50727655mxmmake2.5560.420.38°270727655mxmmake2.46°1.82°277327655mxmmakexmmake2.46°1.82°27735mxmmakexmmakexmmakexmmake2.46°1.82°5mxmmakexmmakexmmakexmmakexmmake2.96° <t< td=""><td>Small intestinal 10 mw-pyruvate + 25 mw-malate 7 860 316^b 271 217 1 mucosa 25 1100 374^b 297 242 2 mucosa 43 1091 389^b 294 235 2 61 1211 554^a 215 201 2 2 10 mw-glutamate + 2.5 mw-malate 7 1178 383^b 2.96^{ab} 2.19^{ab} 2 25 1197 387^b 3.19^{ab} 2.36^{ab} 2.19^{ab} 2 61 1575 616^{ab} 3.60^b 3.36^{ab} 2.39^{ab} 2.39^{ab} 2 8ab Maane with different enveccrient letters were significant (V AffPrent (P > 005) 0.42 0.38^{ab} 2.39^{ab} 2</td><td></td><td></td><td>sem (12 df)</td><td>94</td><td>28</td><td>0-54</td><td>0.08</td><td>237</td></t<>	Small intestinal 10 mw-pyruvate + 25 mw-malate 7 860 316 ^b 271 217 1 mucosa 25 1100 374 ^b 297 242 2 mucosa 43 1091 389 ^b 294 235 2 61 1211 554 ^a 215 201 2 2 10 mw-glutamate + 2.5 mw-malate 7 1178 383 ^b 2.96 ^{ab} 2.19 ^{ab} 2 25 1197 387 ^b 3.19 ^{ab} 2.36 ^{ab} 2.19 ^{ab} 2 61 1575 616 ^{ab} 3.60 ^b 3.36 ^{ab} 2.39 ^{ab} 2.39 ^{ab} 2 8ab Maane with different enveccrient letters were significant (V AffPrent (P > 005) 0.42 0.38 ^{ab} 2.39 ^{ab} 2			sem (12 df)	94	28	0-54	0.08	237
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	mucosa 25 1100 374^{b} 2.97 2.42 2601 61 1211 554^{a} 2.97 2.42 2601 53 1091 389^{b} 2.94 2.35 2429 61 1211 554^{a} 2.15 2.01 2397 10 mw-glutamate + 2.5 mw-malate 7 1178 333^{b} 2.96^{ab} 2.19^{ab} 2.540 43 1197 387^{b} 3.19^{a} 2.36^{a} 2743 61 1575 616^{a} 2.46^{b} 1.82^{b} 2743 3.6^{a} 2.39^{a} 2743 5.0 0.42 0.38 502	mucosa 25 1100 374 ^b 297 242 2 61 1211 584 ^a 294 235 2 61 1211 584 ^a 2.15 201 2 861 12 df) 183 53 0.33 0.17 2 97 2.15 2.16 2.94 2.35 2 98 1211 554 ^a 2.15 201 2 98 383 ^b 2.96 ^{ab} 2.19 ^{ab} 2 10 1178 383 ^b 2.96 ^{ab} 2.19 ^{ab} 25 1197 387 ^b 3.19 ^a 2.36 ^{ab} 2.9 ^{ab} 61 1575 616 ^{ab} 3.46 ^{bb} 1.82 ^{bb} 2.39 ^{ab} 8 ^{ab} Maane with different enveccrint latters wave significantly different (P < 0.05)	Small intestinal	10 mm-pyruvate + 2·5 mm-malate	7	860	316^{b}	2-71	2.17	1820
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	43 1091 389 ^b 294 2.35 2429 61 1211 554 ^a 2.15 2.01 2397 8EM (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 ^{ab} 2.36 ^{ab} 2743 43 1154 360 ^b 3.19 ^{ab} 2.36 ^{ab} 2743 61 1575 616 ^{ab} 2.39 ^{ab} 2556 2743 8.m (12 df) 2.51 55 0.42 0.38 ^b 2.36 ^{ab} 2797 8.b<	43 1091 389 ^b 2.94 2.35 2 61 1211 554 ^a 2.15 201 2 85M 1211 554 ^a 2.15 201 2 85M 1211 554 ^a 2.15 201 2 85M 1211 554 ^a 2.15 201 2 10 <mw-glutamate+2.5<mw-malate< td=""> 7 1178 387^b 3.19^a 2.96^{ab} 2.19^{ab} 2 25 1197 387^b 3.19^a 2.36^{ab} 2.36^{ab} 2.36^{ab} 2.36^{ab} 2 61 1575 616^{ab} 2.46^{bb} 1.82^{bb} 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 3 2 6 2 2 2 2 2 2 2 2 2 2 3 2 3 2 3 2 3 3 2 3 2 3 2 3 3 3 3 3<</mw-glutamate+2.5<mw-malate<>	mucosa		25	1100	374^{b}	2.97	2.42	2601
	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	61 1211 554 ^a 2-15 2-01 2 8EM (12 df) 183 53 0-33 0-17 1178 383 ^b 2-96 ^{ab} 2-19 ^{ab} 2 25 1197 387 ^b 3-19 ^a 2-36 ^a 2 43 1154 360 ^b 3-19 ^a 22 ³ 6 ^a 2 61 1575 616 ^a 2-46 ^b 1:82 ^b 2 8EM (12 df) 251 55 0-42 0-38			43	1001	389 ^b	2.94	2-35	2429
	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$			61	1211	554 ^a	2.15	2.01	2397
10 mM-glutamate +2.5 mM-malate 7 1178 38.3 ^b 2.96 ^{ab} 2.19 ^{ab} 2540 25 1197 38.7 ^b 3.19 ^a 2.36 ^a 2743 43 1154 360 ^b 3.36 ^a 2.36 ^a 2743 61 1575 616 ^a 2.46 ^b 1.82 ^b 2797 8m (12 df) 251 55 0.42 0.38 502	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	10 mm-glutamate + 2·5 mm-malate 7 1178 383 ^b 2·96 ^{ab} 2·19 ^{ab} 2 25 1197 387 ^b 3·19 ^a 2·36 ^{ab} 2·36 ^{ab} 2 43 1154 360 ^b 3·36 ^{ab} 2·36 ^{ab} 2 2 61 1575 616 ^{abb} 2·36 ^{abb} 2·39 ^{abb} 2 ^{abb} Maone with different currescript latters wave significantly different (P > 0.05) 0·42 0·38			SEM (12 df)	183	53	0-33	0.17	384
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 sew (12 df) 251 55 0·42 0·38 502	²⁵ 1197 387^{b} $3\cdot 19^{a}$ $2\cdot 36^{a}$ 2743 43 1154 360^{b} $3\cdot 36^{a}$ $2\cdot 39^{a}$ 2743 61 1575 616^{a} $2\cdot 46^{b}$ $1\cdot 82^{b}$ 2797 ³⁵ Means with different superscript letters were significantly different ($P < 0.05$).	25 1197 387 ^b 3·19 ^a 2·36 ^a 2 43 1154 360 ^b 3·36 ^a 2·39 ^a 2 61 1575 616 ^a 2·46 ^b 1·82 ^b 2 8EM (12 df) 2.51 5.5 0·42 0·38 ^{ab} Maone with different enters were significantly different (P > 0.05)	·	10 mm-glutamate + 2.5 mm-malate	2	1178	383 ^b	2.96^{ab}	2.19^{ab}	2540
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	43 1154 360^{b} $3:36^{a}$ $2:39^{a}$ 2556 61 1575 616^{a} 2.46^{b} $1:82^{b}$ 2797 8EM (12 df) 251 55 0.42 0.38 502 who means with different superscript letters were significantly different ($P < 0.05$).	43 1154 360 ^b 3:36 ^a 2:39 ^a 2 61 1575 616 ^a 2:46 ^b 1:82 ^b 2 82M (12 df) 251 55 0:42 0:38)	25	1197	387^{b}	3.19ª	2.36^{a}	2743
61 1575 616 ^a 2.46 ^b 1.82 ^b 2797 SEM (12 df) 251 55 0.42 0.38 502	61 1575 616^{a} 2.46^{b} 1.82^{b} 2797 SEM (12 df) 251 55 0.42 0.38 502 * ^b Means with different superscript letters were significantly different ($P < 0.05$).	61 1575 616 ^a 2.46 ^b 1.82 ^b 2 SEM (12 df) 251 55 0.42 0.42 0.38 ^{ab} Maone with different envecednt letters were significantly different (P > 0.05)			43	1154	360^{b}	3.36^{a}	2.39^{a}	2556
SEM (12 df) 251 55 0-42 0-38 502	$s_{\rm EM} (12 \text{ df}) \qquad 251 \qquad 55 \qquad 0.42 \qquad 0.38 \qquad 502$	SEM (12 df) 251 55 0:42 0:38 ^{ab} Maone with different envecement letters were significantly different (P > 0.05)			61	1575	616^{a}	2.46^{b}	1.82^{b}	2797
	^{ab} Means with different superscript letters were significantly different ($P < 0.05$).	^{ab} Maone with different curvescript latters were significantly different $(P > 0.05)$			seм (12 df)	251	55	0-42	0-38	502
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 completes 1. III and 1V of the electron transnort chain					-			

DIETARY PROTEIN AND OXIDATIVE PHOSPHORYLATION

able 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*
Tał	

466

(Mean values for four to five birds)

Electron transport complexes required		Dietary PME (%)	State 3 (ng atom/min pe	State 4 r mg protein)	Respiratory control ratio†	ADP:O	synthesized (nmol/min p mg protein
Complexes I, III, IV:	10 mm-pyruvate + 2.5 mm-malate	7	178	47	3-57	2-46 ^a	424
	+ 10 mM-malonate	25	153	42	3.58	2.20^{ab}	331
		43	189	44	4-00	2.16^{b}	394
		61	233	48	4.50	$2.00^{\rm b}$	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	66	17	5.73	2.82ª	242
		61	95	17	5.20	2.58^{b}	242
		SEM (15 df)	7	7	0-53	0-08	18
Complexes II, III, IV	10 mm-succinate +	7	263	51	4.85	2-05	511
	3.75 mm-rotenone	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 +	2	629	476	1-33	1·19	740
4	0-4 µm-antimaycin A	25	815	599	1.37	1-04	840
	•	43	924	711	1.32	66-0	896
		61	635	440	1-45	1-23	762
		SEM (16 df)	130	103	0.05	0-07	128

M. TANAKA AND OTHERS

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,

M. TANAKA AND OTHERS

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.

Financial support was provided by a grant-in-aid (no. 02856072) for Scientific Research from the Ministry of Education, Science and Culture, Japan.

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* **40**A, 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.