Effects of dietary protein and fat level on oxidative phosphorylation in rat heart mitochondria

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The effect of dietary protein and fat levels on cardiac mitochondrial oxidative phosphorylation was assessed polarographically. Weanling rats were fed on semi-purified diets containing different protein levels (10, 30, 50 and 70%) on a gross energy basis (PGE) for 9, 23 and 58 d. Cardiac mitochondria isolated from rats fed on a 70% PGE diet for 23 d exhibited significantly reduced ADP:oxygen (ADP:O) values compared with mitochondria from rats fed on a low-protein diet. Feeding low-protein diets for 58 d increased the ADP:O value. When the dietary fat level was altered to provide (% PGE:% fat-energy): 30:14, 30:30, 70:14, 70:30, feeding 70% PGE diets reduced the ADP:O value compared with the 30% PGE level, but no difference was observed between low-fat and high-fat groups. These results indicate that the impaired ADP:O value for rats fed on very-high-protein diets was not due to the dietary fat level but that the level of dietary protein is an important determinant of oxidative phosphorylation in rat heart mitochondria.

Protein intake: Fat intake: Oxidative phosphorylation: Rat

Treatment of morbid obesity often involves the use of high-protein, very-low-energy diets (Schemmel *et al.* 1983). Some biological responses to high-protein diets have been investigated. When animals were fed on a high-protein diet, fasting blood glucose and plasma insulin concentrations were significantly elevated in rats (Usami *et al.* 1982) and the absolute rate of whole-body protein synthesis decreased in chicks (Kita *et al.* 1989). Stereological analysis of ultrathin sections illustrated that the high-protein diet induced a significant increment in the density and size of hepatocyte mitochondria (Zaragoza *et al.* 1987). However, little is known regarding the effect of high-protein diets on bioenergetic function or mitochondrial function. In the present study two experiments were undertaken: the first experiment involved clarifying the effect of dietary protein level on oxidative phosphorylation of cardiac mitochondria, while the second study was designed to determine whether or not the impaired oxidative phosphorylation of rats fed on high-protein diets would be improved by addition of dietary fat.

MATERIALS AND METHODS

Animals and diets. Male Sprague–Dawley Weanling rats obtained from the University of Alberta at 3 weeks of age (52 (SE 3) g) were housed individually under a controlled 12 h light–dark cycle at 21 ± 1°. After a 6 d adaptation period, animals were fed on a semi-

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

	Expt 1				Expt 2			
	10% PGE	30% PGE	50% PGE	70% PGE	MPLF	MPHF	HPLF	HPHF
Casein	68·5	220·3	395·9	601·4	220·3	247·6	601·4	690·3
Fat*	53·4	57·3	61·8	67·0	57·3	138·0	67·0	164·9
Dextrose	518·4	409·7	284·0	37·0	409·7	328·8	136·9	0·0
Maize starch	259·2	204·8	142·0	68·5	204·8	164·4	68·5	0·0
Cellulose	37·3	40·0	43·1	46·8	40·0	45·0	46·8	53·7
Vitamin Mix†	8·4	9·0	9·7	10·5	9·0	10·1	10·5	12·1
Mineral Mix‡	45·4	48·7	52·5	57·0	48·7	54·8	57·0	65·4
Choline	2·3	2·5	2·7	2·9	2·5	2·8	2·9	3·3
Inositol	5·3	5·6	6·1	6·6	5·6	6·3	6·7	7·6
L-methionine	1·9	2·0	2·2	2·4	2·0	2·3	2·4	2·7
On gross energy basis:								
% PGE	10·0	30·0	50·0	70·0	30·0	30·0	70·0	70·0
% FGE	14·0	14·0	14·0	14·0	14·0	30·0	14·0	30·0
% CGE	76·0	56·0	36·0	16·0	56·0	40·0	16·0	0·0

% PGE, % FGE and % CGE, percentage gross energy from dietary protein, fat and carbohydrate respectively; MPLF, medium-protein low-fat diet; MPHF; medium-protein high-fat diet; HPLF, high-protein low-fat diet; HPHF, high-protein high-fat diet.

* Fat consisted of a 80:13:7 (by wt) mixture of beef tallow, safflower oil and linseed oil.

† Robblee & Clandinin (1984).

‡ The mixture (Robblee & Clandinin, 1984) was modified to add 6·33 g K_2HPO_4, 52 mg $MnSO_4.H_2O$ to 100 g mixture.

purified experimental diet (Table 1) and water *ad lib*. Body weight was recorded weekly and food intake was recorded every 2 or 3 d. Gross energy provided by carbohydrate, fat and protein was determined by bomb calorimetry to be 14·7, 39·5 and 22·0 kJ/g respectively. All diets contained the same amounts of cellulose, minerals and vitamins per 100 kJ energy-yielding nutrients. In Expt 1 four experimental diets of 10, 30 (standard diet), 50 and 70% protein-energy (PGE) level were formulated on a gross energy (GE) basis by substituting the carbohydrate level (CGE) at a constant fat (FGE) level (Table 1). In Expt 2 four experimental diets providing medium-protein–low-fat (MPLF), medium-protein–high-fat (MPHF), high-protein–low-fat (HPLF), and high-protein–high-fat (HPHF) were formulated on a GE basis by substituting protein and fat for carbohydrate (Table 1).

Measurement of mitochondrial respiration. Four rats from each group were killed by cervical dislocation at 9, 23 and 58 d of feeding in Expt 1. Twelve rats from each group were killed at 23 d of feeding in Expt 2. Hearts were immediately excised for mitochondrial isolation (Clandinin, 1978). The purity and stability of these mitochondrial preparations have been characterized (Clandinin, 1978). Oxidation rates of isolated mitochondria were measured polarographically at 37° using an O_2 monitor. Rates of utilization, expressed in ng O_2/mg mitochondrial protein per min were measured with 10 mM-pyruvate, 2 mM-malate and 10 mM-malonate as substrates for oxidative phosphorylation in the reaction mixture described previously by Clandinin (1978). The ADP concentration was determined spectrophotometrically (Jaworek *et al.* 1974). Respiratory rates, respiratory control indices and ADP:O ratios were calculated according to Chance & Williams (1956). Protein was measured by a calorimetric method (Lowry *et al.* 1951).

Statistical procedures. The effect of treatment on mitochondrial functions was first examined by two-way analysis of variance to separate the effect of dietary protein level from the effect due to the duration of feeding in Expt 1. The effect of dietary protein and

fat levels was similarly examined in Expt 2 with a statistical package SAS (Statistical Analysis System Institute, 1992). Significance level for individual group comparisons was $P < 0{\cdot}05$ using Duncan's least significant difference multiple-range test (Statistical Analysis System Institute, 1992).

RESULTS AND DISCUSSION

Body-weight gain for rats fed on 10 and 70% PGE diets for 23 and 58 d was lower than that for rats fed on a 30% PGE diet (Expt 1, Table 1). Rats fed on high-protein diets grew at a slower rate than rats fed on medium-protein diets, particularly for animals fed on high-fat diets (Expt 2). No significant effect of dietary protein on energy consumption was observed, but rats fed on the medium-protein high-fat diet consumed more than animals fed on the medium-protein low-fat diet. These findings support those of Expt 1 and earlier observations with mice fed on diets of different protein levels (Toyomizu *et al.* 1988) and fat levels (Toyomizu *et al.* 1991).

The rate of O_2 uptake and ADP:O ratio observed (Table 2) were similar to those previously reported (Clandinin, 1978, 1979). Determination of mitochondrial respiration did not indicate a difference in state 3 or state 4 O_2 uptake rate and ATP synthesized between either rats fed on diets of different protein levels for 9, 23 or 58 d, or between rats fed on diets of different fat levels. These results indicate that the oxidative rate is not affected by dietary protein and fat levels.

The ADP:O ratio was not significantly different for mitochondria isolated from rats for all treatments after 9 d of feeding (Fig. 1(A)). The reduction in ADP:O ratio after feeding diets for 23 d was significant for animals fed on a 70% PGE diet compared with groups fed on the lower-protein diets (10, 30 or 50% PGE). Mitochondria isolated from rats fed on a 10% PGE diet for 58 d exhibited an increased ADP:O ratio compared with rats fed on the higher-protein diets.

Most investigations of the proton stoichiometries of oxidative phosphorylation have ignored dietary effects. However, the effect of dietary composition on bioenergetic function is important since uncoupling of mitochondrial oxidative phosphorylation progressively decreases metabolic energy available from protein and fat relative to that available from glucose (Livesey, 1984). Little study has been done on the effects of excess dietary protein on mitochondrial function, although responses to a protein-deficient diet (Williams, 1971) and an essential fatty acid-deficient diet (Rafael *et al.* 1984) were examined. The present results show that the level of dietary protein is an important determinant of oxidative phosphorylation in rat heart mitochondria.

Dietary fat level as well as fat sources also affect bioenergetic function in mitochondria. Rats fed on a diet containing very-long-chain fatty acids show a decline in the oxidation of substrates at coupling sites I and II as well as a decrease in ATP synthesis in heart mitochondria associated with changes in composition of mitochondrial structure (Clandinin, 1978). Dietary fat level and the polyunsaturated:saturated fatty acid ratio alter ATPase (*EC* 3.6.1.34) activity in cardiac mitochondria and change mitochondrial structure (Robblee & Clandinin, 1984). Therefore, we investigated whether the reduced oxidative phosphorylation in mitochondria of rats fed on a high-protein diet is altered by increasing dietary fat content.

Mitochondria from rats fed on high-protein diets for 23 d showed significantly reduced ADP:O ratios when compared with medium-protein diets, but the impaired ADP:O ratio of rats fed on the high-protein diet was not improved by addition of dietary fat (Fig. 1(B)). To the authors' knowledge, virtually no information is available to confirm a direct effect of the amount of carbohydrate on mitochondrial functions. Earlier studies demonstrated that although fatty acid compositional differences were observed in hepatic mitochondria

Table 2. *Expts 1 and 2. Cumulative feed consumption, body-weight gain and oxidative activity of isolated cardiac mitochondria, with pyruvate and malate as substrate, from rats fed on diets of different levels of dietary protein and fat*†

Diet	Duration of feeding (d)	Feed consumption (MJ)	Body-wt gain (g)	Oxygen uptake rates (ng atom/min per mg protein)		ATP synthesized (nmol/min per mg protein)	Respiratory control index‖
				State 3	State 4		
Expt 1‡¶							
% PGE							
10	9	2·2	40·7[b]	585	151	1400	3·92
30	9	2·4	66·9[a]	658	186	1590	3·64
50	9	2·21	62·6[a]	593	164	1500	3·66
70	9	2·18	59·7[a]	586	171	1380	3·52
% PGE							
10	23	6·48	109[b]	551	138	1350	3·99
30	23	6·93	166[a]	527	126	1280	4·20
50	23	6·51	159[a]	541	146	1310	3·74
70	23	6·67	137[ab]	521	139	1190	3·78
% PGE							
10	58	18·86	284[b]	546	144	1410	3·82
30	58	20·72	359[a]	593	165	1440	3·61
50	58	19·43	321[ab]	585	152	1430	3·88
70	58	20·38	327[ab]	664	168	1510	3·95
Pooled SE				39·4	12·8	103	0·227
Statistical significance of:							
Diet		—	—	NS	NS	NS	NS
Days		—	—	*	**	*	NS
Diet × days		—	—	NS	NS	NS	NS
Expt 2§¶							
MPLF	23	7·20[b]	164[a]	462	147	1110	3·16
MPHF	23	7·63[a]	170[a]	480	150	1160	3·21
HPLF	23	7·40[ab]	159[ab]	463	159	1030	2·93
HPHF	23	7·42[ab]	149[b]	475	151	1060	3·15
Pooled SE		0·129	3·96	25·8	8·18	60·4	0·132
Statistical significance of:							
PGE		NS	***	NS	NS	NS	NS
FGE		*	NS	NS	NS	NS	NS
PGE × FGE		*	**	NS	NS	NS	NS

% PGE and % FGE, percentage gross energy from dietary protein and fat; MPLF, medium-protein low-fat diet; MPHF, medium-protein high-fat diet; HPLF, high-protein low-fat diet; HPHF, high-protein high-fat diet; NS, not significant.

[ab] Differences between individual means were analysed by Duncan's multiple-range test. Mean values with unlike superscript letters were significantly different ($P < 0·05$):

* $P < 0·10$, ** $P < 0·05$, *** $P < 0·01$.

† For details of diets, see Table 1.

‡ Mean values for four rats.

§ Mean values for twelve rats for growth data, and for six samples of two pooled hearts for O_2 consumption data.

‖ Calculated as state 3:state 4 oxidation rates.

¶ Expt 1 df 36 for state 3 and state 4 O_2 uptake rates, for ATP synthesized and for respiratory control index. Expt 2 df 44 for feed consumption and body-weight gain, and 20 for state 3 and 4 O_2 uptake rates, for ATP synthesis and for respiratory control index.

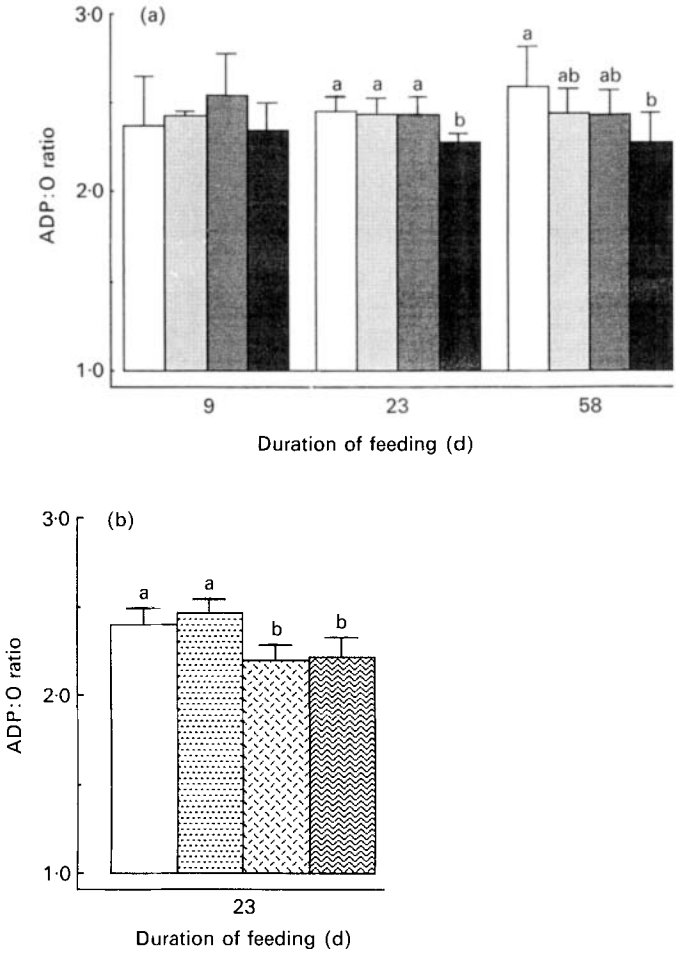

Fig. 1. Expts 1 and 2. Responses of ADP:oxygen (ADP:O) ratio to (a) dietary protein level (% PGE; percentage gross energy from protein) at different feeding periods (Expt 1), and (b) dietary fat level (medium protein (MP), high protein (HP), high fat (HF), low fat (LF); Expt 2) in rat heart mitochondria. Expt 1: (□), 10% PGE; (▨), 30% PGE; (■), 50% PGE; (■), 70% PGE. Expt 2: (□), MPLF; (▨), MPHF; (▨), HPLF; (▨), HPHF. Values are means for four rats (Expt 1) and for six samples of two pooled hearts (Expt 2) in each group with their standard errors represented by vertical bars. Differences in means were tested by Duncan's multiple-range test. Means with the same superscript letter for the same duration of feeding were not significantly different ($P < 0.05$). Pooled SE were 0·079 (df 36) and 0·037 (df 20) for Expts 1 and 2 respectively. For details of diets, see Table 1.

of rats fed on a 650 g sucrose/kg or a 650 g maize starch/kg diet, these differences had little effect on the membrane-associated, succinate-supported respiration in liver (Wander & Berdanier, 1985). The present study showed no change in ADP:O ratio when dietary fat was substituted for carbohydrate. These results imply that carbohydrate sources or levels do not affect mitochondrial respiration. Therefore, it may be concluded that change in ADP:O ratio in the present study was mainly due to changes in dietary protein rather than fat or carbohydrate level. However, the mechanism involved in this effect of protein remains to be clarified.

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