DIETARY-INDUCED MODIFICATION OF CALCIUM TRANSPORT IN MITOCHONDRIA ISOLATED FROM FLIGHT-MUSCLE OF DEVELOPING SHEEP BLOWFLY *LUCILIA CUPRINA*

Fyfe L. Bygrave and Robyn L. Smith

Department of Biochemistry, Faculty of Science,
Australian National University, Canberra, A.C.T. 2600, Australia

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SUMMARY: *The activity of mitochondrial calcium transport in flight-muscle of the sheep blowfly Lucilia cuprina is shown to depend on the nature of the diet upon which the adult fly is maintained. The finding raises the possibility that calcium transport in mitochondria from other tissues and species also might be influenced by the dietary intake.*

Numerous biochemical and physiological events in a range of cell species and tissues are influenced by calcium (Ca) ions and it has been advocated for some time that these events often may be controlled in part by the intracellular concentration of ionized Ca. Much evidence supports the view that the Ca transport system located in the mitochondrial inner membrane plays a major role in controlling cell Ca (reviewed in refs. 1-3). Particularly compelling evidence stems from observations that mitochondrial Ca transport changes markedly during development in insect flight-muscle and mammalian tissue(4,5), undergoes transient changes after hormonal perturbation of rat liver *in vivo*(6) and may have undergone permanent changes in certain tumour lines(7,8).

In extending this line of research, we considered the possibility that mitochondrial Ca transport activity might be influenced also by the dietary intake. The developing insect system described previously(4) appeared to have particular attributes to enable these ideas to be tested. We report here

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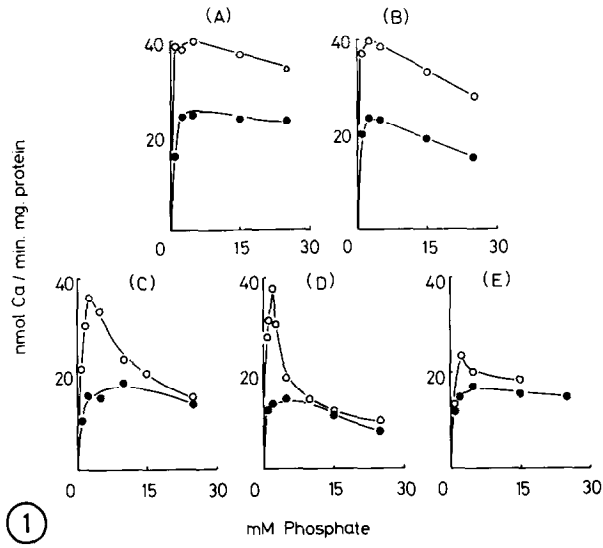
results of experiments in which Ca transport was compared in flight-muscle mitochondria isolated from two groups of adult flies. One group was maintained on sugar alone (Diet A) and the other on a diet supplemented with protein (Diet B). We show that Ca transport in flight-muscle mitochondria from flies maintained on Diet A is significantly greater than that in flies maintained on Diet B.

EXPERIMENTAL: *Lucilia* larvae and pupae, kindly provided by the Division of Entomology, C.S.I.R.O., Canberra, were reared and maintained as described previously(4). Upon emergence, adult flies were placed in muslin-covered glass jars and allowed to feed on Diets A or B. Diet A consisted of sugar cubes (a product of C.S.R.). Diet B was "Farex" baby food, (a product of Glaxo Australia Ltd.) with the following composition : carbohydrates, 75.2%; protein, 13.8%; fat, 3%; minerals, 4.0% and moisture, 4%. Water was provided in liberal quantities with each diet. Flies were maintained at 28°C throughout all stages of the life-cycle. Mitochondria were isolated from the flight-muscle of approximately 80 flies as previously described(4) at the developmental stages indicated in legends to figures. Ca transport was measured using the Ruthenium Red-quench technique(9). Details are described in the legends to figures.

RESULTS AND DISCUSSION: Inorganic phosphate (phosphate) induces marked concentration-dependent effects on Ca transport in *Lucilia* mitochondria(10). Data in Fig. 1 show the influence of phosphate concentration on Ca transport by *Lucilia* flight-muscle mitochondria isolated from adults that had been maintained for different periods on Diets A and B. A large difference in Ca transport activity between the two groups is seen as early as 6 hours after emergence (Fig. 1a). A similar pattern of response



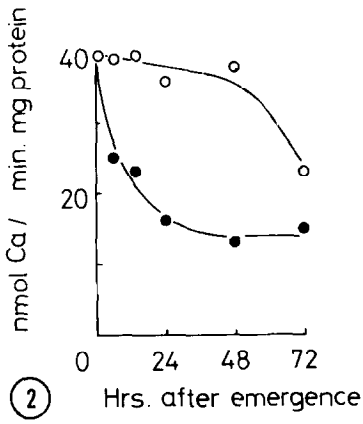


Fig.1. Ca transport measured as a function of phosphate concentration in flight-muscle mitochondria of Lucilia maintained on different diets. Measurement of Ca transport was made in reaction media containing in a final vol. of 1.0ml: 100mM KCl, 25mM α-glycerol phosphate and varying concentrations of inorganic phosphate as indicated. The final pH and temp. were 7.4 and 25°C, respectively. Mitochondria (1 mg. protein) obtained from flight-muscle of flies maintained on diet A (○) or diet B (●) for the periods indicated, were added and after a 1 min. pre-incubation, 50μM Ca^{2+} (containing approx. 0.3μCi $^{45}Ca^{2+}$) was added to initiate the reaction. At 30 sec. intervals, 100μl of reaction medium was transferred to Eppendorf tubes containing 0.5mM ethanedioxybis-(ethylamine) tetraacetic acid (EGTA) plus 5μM Ruthenium Red. The tubes were centrifuged and portions of the supernatant counted for radioactivity (see ref. 9). The ages of the flies from which mitochondria were isolated were (a) 6 hr; (b) 15 hr.; (c) 24 hr.; (d) 48 hr. and (e) 72 hr.

Fig.2. Changes during development of mitochondrial Ca transport measured in the presence of 2.5mM phosphate. Data are derived from Fig. 1 and show Ca transport activity with 2.5mM phosphate at the different developmental stages in mitochondria from flies maintained on diet A (○) or B (●).

of Ca transport to phosphate concentration is obtained but Ca transport activity is depressed about 50% in those mitochondria isolated from flies maintained on Diet B. At 15 hours after emergence the response pattern is also similar (Fig. 1b) but

high phosphate concentrations begin to inhibit Ca transport as previously reported(10). The marked inhibition of mitochondrial Ca transport by phosphate concentrations greater than 2.5mM is apparent by 24 hours in flies maintained on Diet A (Fig. 1c); Ca transport activity is depressed at all concentrations of phosphate in mitochondria of flies maintained on Diet. B. This feature is accentuated by 48 hours (Fig. 1d) and it is not until 72 hours after emergence that Ca transport at 2.5mM phosphate by mitochondria of flies maintained on Diet A, begins to decline (Fig. 1c).

Data in Fig. 2 derived from those in Fig. 1 show how Ca transport, measured in the presence of 2.5mM phosphate, changes during development in flies maintained on the two diets. Those maintained on Diet B clearly exhibit reduced transport activity early in development while those maintained on Diet A retain maximal activity for at least 48 hours.

Other experiments, data of which are not presented, indicated that the changes to Ca transport occurred in flies of both sexes. Also the respiratory activities in the presence or absence of ADP were similar in both types of mitochondria. Thus the differences seen appear not to reflect differences in the energy transducing systems responsible for driving Ca transport and occur independently of the sex of the fly.

The above findings are significant in that they provide clear evidence which shows, we believe for the first time, that the dietary regimen can influence mitochondrial Ca transport activity. While the data do not permit conclusions to be drawn about the molecular details of the dietary-induced changes, we feel that further exploitation of this system will provide such information. Since mitochondrial Ca transport

in insect flight-muscle and mammalian tissues share many properties(1,4) we consider these findings may have quite general relevance.

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REFERENCES

1. Bygrave, F.L., Current Topics in Bioenergetics, 6, 259-318 (1977).
2. Baker, P.F., Soc. Exp. Biol. Symp., 30, 67-88 (1976).
3. Carafoli, E. and Crompton, M., Soc. Exp. Biol. Symp., 30, 89-115 (1976).
4. Bygrave, F.L., Daday, A.A. and Doy, F.A., Biochem. J., 146, 601-608 (1975).
5. Bygrave, F.L. and Ash, G.R., FEBS Letters, 80, 271-274 (1977).
6. Dorman, D.M., Barritt, G.J. and Bygrave, F.L., Biochem. J. 150, 389-395 (1975).
7. Thorne, R.F.W. and Bygrave, F.L., Biochem. J., 144, 551-558 (1974).
8. Bygrave, F.L., in Control Mechanisms in Cancer (W.E. Criss, T. Ono and J.R. Sabine, eds.) pp. 411-423, Raven Press, New York (1976).
9. Reed, K.C. and Bygrave, F.L., Biochem. J. 140, 143-155 (1974).
10. Smith, R.L. and Bygrave, F.L., Biochem. J. in press.