A SELECTIVE DECREASE IN MITOCHONDRIAL GLYCEROL PHOSPHATE ACYLTRANSFERASE ACTIVITY IN LIVERS FROM STREPTOZOTOCIN-DIABETIC RATS

Edna J. BATES and David SAGGERSON

Department of Biochemistry, University College London, Gower Street, London WC1E 6BT, England

Received 30 September 1977

1. Introduction

The glycerol phosphate pathway of fatty acid esterification is one of the biosynthetic routes to glycerolipids. Glycerol phosphate acyltransferase (GPAT), is a possible site of control of this process, being the first committed enzyme. Little is known about regulation of glycerolipid synthesis although recent studies in adipose tissue indicate the possibility of acute hormonal regulation of GPAT activity [1,2]. In order to establish a basis for studies of short-term insulin action on hepatic GPAT in the intact animal the effect of mild diabetes on the mitochondrial and microsomal enzymes has been investigated. Effects of insulin on GPAT in the perfused rat liver are presented [3]. A statistically inconclusive study of the effect of starvation on hepatic GPAT has been made [4], but there do not appear to be any previous reports of the effect of diabetes. The present results show an interesting divergence in the responses of the mitochondrial and microsomal activities.

2. Materials and methods

Male Sprague Dawley rats (120–130 g) were subcutaneously injected with streptozotocin (100 mg/kg) dissolved in 50 mM citrate, 0.15 M NaCl buffer, pH 4.0. Every 24 h for six days each animal received a subcutaneous injection of 1.6 IU protamine zinc insulin. Insulin was then withdrawn and animals showing a strongly positive 'Clinistix' test for urine glucose were taken to be diabetic and killed on the fourth day thereafter. Control animals received only a single injection of citrate–NaCl buffer at 120–130 g body wt. Growth curves of diabetic rats during insulin maintenance were comparable with controls. All animals had constant access to food and water.

Rats were killed by a blow on the head and blood collected from the mouth into a heparinised syringe. Analysis of glucose [5] and non-esterified fatty acids [6] were performed on samples of plasma. The entire liver was homogenised in 80 ml ice-cold 0.25 M sucrose, 10 mM Tris–chloride, 1 mM EDTA, pH 7.4 and centrifuged at 620 × *g* for 10 min. The supernatant was then centrifuged at 7250 × *g* for 10 min and the resulting mitochondrial pellet washed twice with 30 ml homogenising buffer by resuspension and centrifugation at 9200 × *g* for 10 min. The washed mitochondria were then resuspended in 0.25 M sucrose, 10 mM Tris–chloride, 1 mM EDTA dithiothreitol, pH 7.4, at 5–6 mg protein/ml. Microsomes were obtained by centrifuging the 7250 × *g* postmitochondrial supernatant at 105 000 × *g* 1 h and the pellet resuspended in 0.25 M sucrose, 10 mM Tris–chloride, 1 mM EDTA, 1 mM dithiothreitol, pH 7.4, at 3–5 mg protein/ml.

Glutamate dehydrogenase (EC 1.4.1.2) was assayed at 25°C by the method [7] after treatment of samples with 0.1% (v/v) Triton X-100. NADP-cytochrome *c* reductase (EC 1.6.2.4) was assayed at 25°C by the method [8]. Glucose 6-phosphatase (EC 3.1.3.9) was assayed at 37°C in final vol. 3.9 ml containing 90 mM sodium acetate buffer, pH 6.3, 5.1 mM glucose 6-phosphate and the phosphate released measured by the method [9]. These enzyme activities were all measured in whole homogenates, mitochondria and microsomes.

Acyl CoA, L-glycerol 3-phosphate-*o*-acyltransferase (EC 2.3.1.15) was assayed at 30°C in final vol. 1.0 ml

containing 120 mM KCl, 50 mM Tris–chloride buffer, pH 7.4, 0.6 μCi L-[U-^{14}C]glycerol 3-phosphate, 65 μM palmitoyl CoA, 0.7 mM dithiothreitol and the indicated L-glycerol 3-phosphate concentration. In addition, 1.75 mg or 6 mg of fatty acid-poor albumin were added to microsomal and mitochondrial assays, respectively. The reaction was initiated with 0.1 ml microsomes or mitochondria and performed for 3 min during which incorporation of carbon-14 was linear with time. The reaction was terminated with 2 ml water-saturated butanol and radioactive incorporation into butanol-soluble products determined as described [10]. One unit of enzyme activity represents the conversion of 1 μmol substrate to products/min at the specified temperature.

Protein in whole homogenates, mitochondria or microsomes was determined by a biuret method [11] modified by the addition of 1% (w/v) deoxycholate. DNA in fresh homogenates was measured by the method [12].

Statistical analysis of data was performed using the Student's t test modified by Bessel's correction factor for small samples

3. Results and discussion

Table 1 shows general characteristics of the diabetic animals and their controls. In addition, details of the three marker enzymes used as reference activities are tabulated. Plasma from diabetic animals had considerably elevated glucose levels but no significant increases in non-esterified fatty acid suggesting that the rats were unlikely to be ketotic.

Recent studies in adipose tissue [13] have suggested that long chain fatty acyl CoA synthetase may be regulated by insulin. Therefore it was considered inadvisable to employ a fatty acyl CoA generating system dependent on endogenous fatty acyl CoA synthetase for the assay of GPAT. Accordingly palmitoyl CoA was used as the acyl substrate. A saturated rather than unsaturated acyl entity was chosen to

Table 1
General characteristics of control and diabetic animals

	Control (5 animals)	Diabetic (7 animals)	P
Body weight (g)	194 ± 4	156 ± 3	<0.001
Liver weight (g)	7.9 ± 0.3	6.2 ± 0.2	<0.001
Total liver DNA (mg)	32.6 ± 1.3	34.4 ± 1.3	n.s.
Total liver protein (g)	1.60 ± 0.05	1.56 ± 0.03	n.s.
Plasma glucose (mg/100 ml)	113.2 ± 8.8	369.2 ± 24.2	<0.001
Plasma non-esterified fatty acid (mM)	0.70 ± 0.11	0.88 ± 0.08	n.s.
Glutamate dehydrogenase activity:			
units/liver	1509 ± 78	1571 ± 76	n.s.
units/mg DNA	46.3 ± 2.0	46.1 ± 1.6	n.s.
units/mg mitochondrial protein	2.55 ± 0.10	2.68 ± 0.15	n.s.
NADP cytochrome *c* reductase activity:			
units/liver	75.9 ± 11.2	77.3 ± 3.3	n.s.
units/mg DNA	2.31 ± 0.28	2.28 ± 0.13	n.s.
units/mg microsomal protein	0.13 ± 0.01	0.19 ± 0.01	<0.01
Glucose 6-phosphatase activity:			
units/liver	87.9 ± 4.4	195.2 ± 14.2	<0.001
units/mg DNA	2.71 ± 0.17	5.71 ± 0.33	<0.001
units/mg microsomal protein	0.15 ± 0.02	0.53 ± 0.03	<0.001

All values are expressed as means ± SEM

n.s. indicates $P > 0.05$

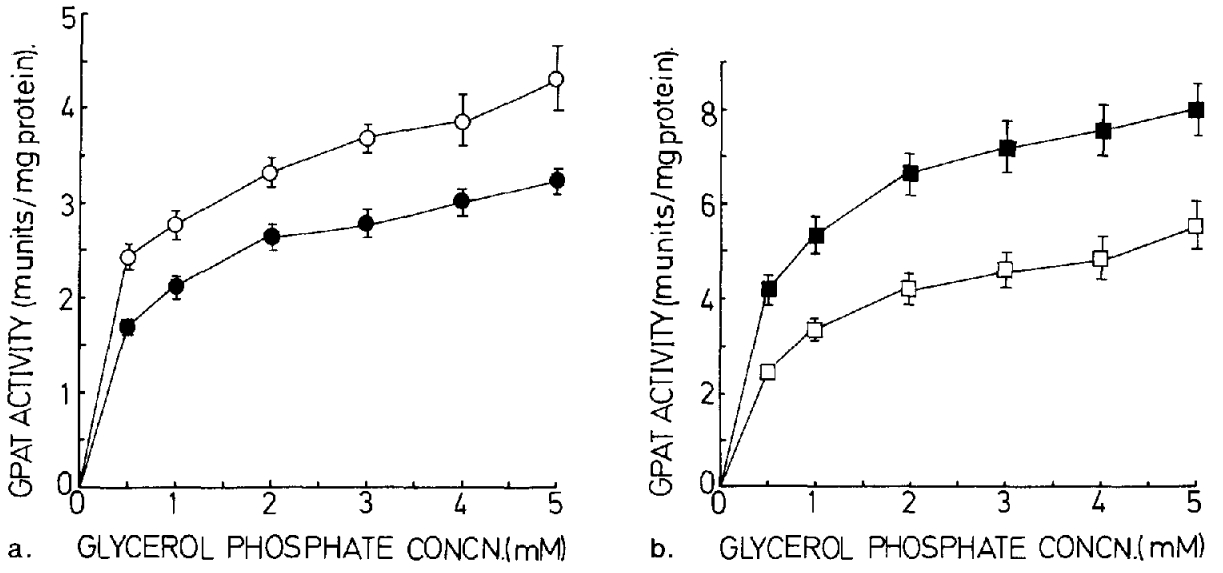

Fig.1. Specific activities of mitochondrial and microsomal GPAT/mg protein. (1a.) Mitochondrial activities: (○) control; (●) diabetic. (1b.) Microsomal activities: (□) control; (■) diabetic.

ensure high acylation rates in both mitochondria and microsomes [14,15]. To obtain optimum assay conditions for GPAT it was necessary to use different albumin concentrations in mitochondria and microsomes.

Fig.1a shows that mitochondrial GPAT specific activity expressed per mg protein was decreased in diabetic animals. This decrease was significant at all glycerol phosphate concentrations ($P < 0.001, 0.01, 0.02, 0.01, 0.05, 0.02$ for 0.5, 1, 2, 3, 4 and 5 mM glycerol phosphate respectively) and varied between 20 and 29%, the mean being 24%. However, microsomal GPAT specific activity per mg protein was increased by 44 – 73% in diabetic animals ($P < 0.01$ in all cases except $P < 0.02$ at 5 mM glycerol phosphate) the mean increase being 58% (fig.1b). Since total liver protein was not changed in diabetic rats (table 1) we presume that this could reflect a selective decrease in microsomal fraction protein.

From the whole homogenate contents of marker enzymes and DNA, together with the GPAT and marker enzyme content of subcellular fractions, mitochondrial and microsomal GPAT activity per total mg liver DNA can also be calculated. This treatment corrects for incomplete recoveries of mitochondria and microsomes and we consider it more reliable than expression of activity per protein. Fig.2 shows that mitochondrial GPAT activity also decreased per mg

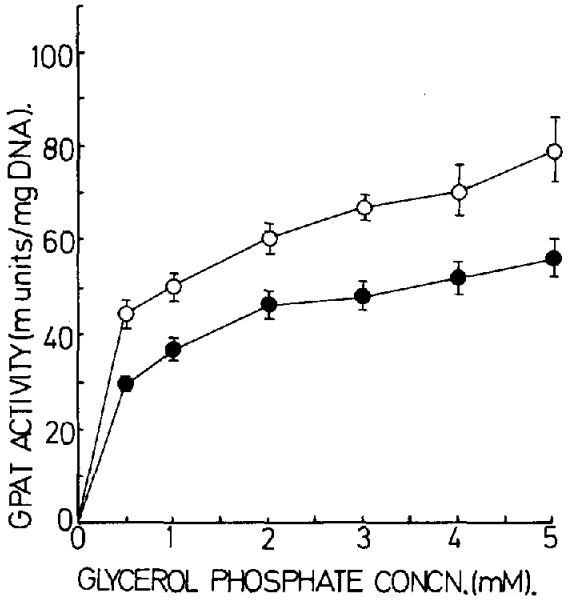

Fig.2. Activity of mitochondrial GPAT expressed/mg total liver DNA. Mitochondrial activities were calculated using glutamate dehydrogenase as marker: (○) control; (●) diabetic.

DNA in diabetics ($P < 0.01, 0.01, 0.05, 0.01, 0.05, 0.05$ for 0.5, 1, 2, 3, 4 and 5 mM glycerol phosphate respectively). This decrease varied between 23 and 33%, the mean being 28%. Since total liver DNA and glutamate dehydrogenase were unchanged in diabetes (table 1), a real decrease in the total mitochondrial GPAT activity per liver had occurred. On the other hand microsomal GPAT activity per mg DNA was essentially unchanged by diabetes. Using NADP-cytochrome *c* reductase as marker a small but non-significant increase in GPAT activity was apparent in diabetic livers, whereas with glucose 6 phosphatase as marker diabetes apparently caused a small but non-significant decrease in microsomal GPAT.

Contamination of mitochondrial GPAT with an invariant microsomal GPAT activity could lead to some underestimation of the percentage decrease in the mitochondrial activity due to diabetes. This is in fact small for two reasons. Firstly, the percentage of glucose 6 phosphatase in the mitochondrial fraction was only 10.5 ± 1.6% and 6.8 ± 0.5% in controls and diabetics respectively. Corresponding contamination of microsomes by glutamate dehydrogenase was 20.9 ± 2.0% and 14.6 ± 2.8% in controls and diabetic livers. Secondly, cross contaminating GPAT activities are measured under quite suboptimal conditions due to the use of different albumin concentrations in the mitochondrial and microsomal assays.

These results suggest a selective response of the mitochondrial GPAT to mild diabetes. It is noteworthy that the very different responses seen with thiol reagents [3,15,16] could suggest that the mitochondrial and microsomal GPAT activities reside in different enzymes. Whether the decrease in mitochondrial GPAT reflects an acute regulatory response or some gross change in amount of enzyme protein is unclear at present. The effect of insulin on GPAT activity in perfused rat liver seen in the accompanying paper [3] however suggests that acute regulation by insulin may occur. A mitochondrial GPAT activity varying in response to insulin is obviously of relevance to interrelated control of fatty acid esterification and oxidation.

Acknowledgements

We are grateful to the Medical Research Council for a studentship to E. J. B. and a project to D.S. Also we thank Miss C. Carpenter for technical assistance.

References

[1] Sooranna, S. R. and Saggerson, E. D. (1976) FEBS Lett. 64, 36–39.
[2] Sooranna, S. R. and Saggerson, E. D. (1976) FEBS Lett. 69, 144–148.
[3] Bates, E. J., Topping, D. L., Sooranna, S. R., Saggerson, D. and Mayes, P. A. (1977) FEBS Lett. 84, 225–228.
[4] Van Tol, A. (1974) Biochim. Biophys. Acta 357, 14–23.
[5] Slein, M. W. (1965) in: Methods in Enzymatic Analysis (Bergmeyer, H. U. ed) Academic Press, pp. 117–123, New York, London.
[6] Itaya, K. and Ui, M. (1965) J. Lipid Res. 6, 16–20.
[7] Martin, B. R. and Denton, R. M. (1970) Biochem. J. 117, 861–877.
[8] Phillips, A. H. and Langdon, R. G. (1962) J. Biol. Chem. 237, 2652–2660.
[9] Lowry, O. H. and Lopez, J. A. (1946) J. Biol. Chem. 162, 421–428.
[10] Daae, L. N. W. and Bremer, J. (1970) Biochim. Biophys. Acta 210, 92–104.
[11] Gornall, A. G., Bardawill, C. S. and David, M. M. (1949) J. Biol. Chem. 177, 751–766.
[12] Burton, K. (1956) Biochem. J. 62, 315–323.
[13] Jason, C. J., Polokoff, M. A. and Bell, R. M. (1976) J. Biol. Chem. 251, 1488–1492.
[14] Daae, L. N. W. (1972) Biochim. Biophys. Acta 270, 23–31.
[15] Haldar, D. and Pullman, M. E. (1975) Fed. Proc. Fed. Am. Soc. Exp. Biol. 34, 632.
[16] Monroy, G., Rola, F. H. and Pullman, M. E. (1972) J. Biol. Chem. 247, 6884–6894.