THE MODIFYING EFFECT OF MANGANESE ON THE ENZYMIC PROFILES AND PATHWAYS OF CARBOHYDRATE METABOLISM IN RAT LIVER AND ADIPOSE TISSUE DURING DEVELOPMENT

Najma Zaheer Baquer, John S. Hothersall, A.L.Greenbaum and Patricia McLean.

Courtauld Institute of Biochemistry,
The Middlesex Hospital Medical School,
London, W1P 5PR,
and
Department of Biochemistry,
University College London,
London, WC1E 6BT,
Great Britain.

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SUMMARY : The effect of Mn^{2+} on the pattern of emergence of enzymes in rat liver and adipose tissue was studied in weaned rats given a milk diet (high fat) or sucrose-casein diet (high carbohydrate) for three weeks. Addition of Mn^{2+} to the high fat diet was associated with induction of key glycolytic, lipogenic and pentose pathway enzymes in both liver and adipose tissue; parallel increases were found in the incorporation of [1-^{14}C] glucose into lipid and CO_2. Mn^{2+} induced a change in the profile of enzyme activity similar in pattern to that found in rats given a high sucrose diet or that produced by insulin treatment. Mn^{2+} appears partially to overcome the regulatory feed-back mechanisms of the high fat diet and to provide a signal for the coordinated increase of glucose catabolic and lipogenic processes.

A critical developmental stage, involving considerable changes in hepatic enzyme profile, occurs at the time of weaning in the switchover from a diet rich in fat (milk) to one relatively high in carbohydrate. Certain enzymes associated with the regulation of carbohydrate metabolism and lipogenesis are considerably increased in rat liver at this period of time [see reviews 1,2]; these include glucokinase, glucose 6-phosphate dehydrogenase, pyruvate kinase, malic enzyme and ATP-citrate lyase [1,3-5]. The interplay of both dietary and hormonal factors in enzyme emergence has been emphasized by a number of authors [2,6,7].

The present study shows that manganese ions can have a powerful influence on the dietary regulation of the pattern of emergence of enzymes in the period following weaning and that

this metal ion can largely override the feedback control imposed by a relatively high fat diet and that this signal produces a series of changes closely similar to those found after administration of a high carbohydrate diet or of insulin.

METHODS

Animals : Young male rats of the albino strain, taken at 21 days after birth, were divided into four groups and given one of the following diets each of which were similar in their protein and vitamin contents, iron and copper supplementation, but differed in the proportion of fat and carbohydrate and in their Mn^{2+} content. The vitamin and mineral supplementation/100g. dry diet was : Vit.A, 4 µg; Vit.D_2, 300 units; Vit.E, 10 mg; thiamine hydrochloride, 100 µg; riboflavin, 400 µg; pyridoxine, 100 µg; choline chloride, 30 mg; pantothenic acid, 6 mg; $CuSO_4$, 1 mg; $FeSO_4$, 20 mg; NaCl, 1 g.

Fat diet 24% fat, 39% carbohydrate, 37% casein.

Fat diet + Mn^{2+} As above + 3 mg $MnSO_4$/100 g. diet.

Carbohydrate diet 58% sucrose, 5% fat (corn oil), 37% casein.

Carbohydrate diet + Mn^{2+} As above + 3 mg. $MnSO_4$/100g.diet.

The rats were maintained on this diet for 3 weeks and all groups were limited to the amount of food consumed by the fat diet group. Growth curves were approximately parallel for these groups.

Preparation of homogenates : The medium contained 0.25M sucrose, 20mM triethanolamine buffer at pH 7.4 and 0.1 mM dithiothreitol. Liver homogenates were prepared using 4 volumes medium : 1 part tissue with a Potter-Elvehjem homogeniser. Adipose tissue homogenates were prepared in the same medium with the addition of 5 mg. fatty acid-free albumin/ml, added to protect phosphofructokinase, and fatty acid synthetase from inactivation [8]. 'Soluble fraction', refers to high speed supernatant fractions prepared by centrifugation at 105,000g for 45 min. Fractions were dialysed for 1 hr against the same medium as that used for extraction except for the omission of the albumin.

RESULTS

The effect of Mn^{2+} in reversing the well-known suppression of

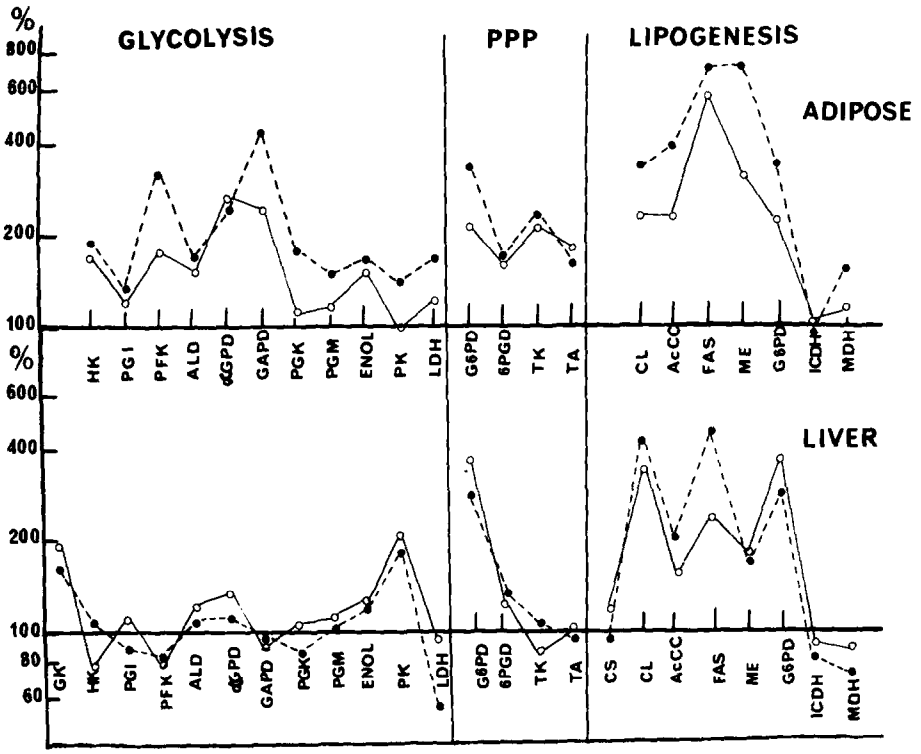

FIG. 1. The effect of Mn^{2+} supplementation and of substitution of high carbohydrate for fat on enzyme profiles of the livers and adipose tissue from rats fed a high fat (milk) diet.

The values for enzyme activities of the rats fed the high fat (milk) diet are taken as 100% and the values from the animals on modified diets expressed as a percentage of these. Liver values are expressed as activities/g. tissue and adipose tissue as activities/pair epididymal fat pads.

○——○ Fat diet + Mn^{2+} v Fat diet.

●- - -● Carbohydrate diet v Fat diet.

ABBREVIATIONS : GK, glucokinase; HK, hexokinase; PGI, phosphoglucoseisomerase; ALD, aldolase; αGPD, α-glycerophosphate dehydrogenase; GAPD, glyceraldehyde 3-phosphate dehydrogenase; PGK, phosphoglycerate kinase; PGM, phosphoglyceromutase; ENOL, enolase; PK, pyruvate kinase; LDH, lactate dehydrogenase; G6PDH, glucose 6-phosphate dehydrogenase; 6PGDH, 6-phosphogluconate dehydrogenase; TK, transketolase; TA, transaldolase; CS, citrate synthase; CL, citrate lyase; AcCC, acetyl CoA carboxylase; FAS, fatty acid synthetase; ME, malic enzyme; G6PDH, glucose 6-phosphate dehydrogenase; ICDH, isocitrate dehydrogenase, NADP-linked; MDH, malate dehydrogenase.

the lipogenic enzymes, and their satellite systems, by high fat diets is shown in Fig. 1, where it may also be seen that the pattern of the enzyme change produced by this metal ion is remarkably similar to that obtained when animals are transferred from a fat diet to one relatively high in carbohydrate. The similarity applies to both liver and adipose tissue except for a few enzymes (e.g. α-glycerophosphate dehydrogenase and malic

Table 1. Relative effects of manganese and high carbohydrate diet on the flux of glucose through alternative pathways of metabolism in liver and adipose tissue.

	Fat diet	Fat diet + Mn^{2+}	Carbohydrate diet
	ADIPOSE TISSUE (μg atoms glucose carbon/2 fat pads/hr)		
$^{14}CO_2$ formation			
[1-^{14}C]glucose	0.752±0.075	1.30 ±0.210*	1.38 ±0.18**
[6-^{14}C]glucose	0.262±0.035	0.227±0.025	0.284±0.062
^{14}C-Lipid formation			
[1-^{14}C]glucose	0.551±0.056	0.783±0.099*	1.24 ±0.21*
[6-^{14}C]glucose	0.715±0.070	1.112±0.152*	2.81 ±0.42**
	LIVER (μg atoms glucose carbon/g/hr)		
$^{14}CO_2$ formation			
[1-^{14}C]glucose	1.76 ±0.08	2.55 ±0.19*	4.63 ±0.46**
[6-^{14}C]glucose	1.35 ±0.11	1.90 ±0.10*	2.47 ±0.25*
^{14}C-Lipid formation			
[1-^{14}C]glucose	0.19 ±0.02	0.28 ±0.03	1.19 ±0.14**
[6-^{14}C]glucose	0.31 ±0.03	0.54 ±0.03*	1.86 ±0.29**

200 mg liver slices or 100 mg adipose tissue were incubated in 4.5 ml Krebs-Ringer bicarbonate medium containing 20mM glucose and 0.5μC ^{14}C glucose for 1 hr. Values given are the mean ± SEM, the asterisks denote : *, $P < 0.05$; **, $P < 0.01$.

Table 2. Effect of manganese and insulin *in vitro* on the utilization of [1-^{14}C] glucose by adipose tissue.

	Conversion of [1-^{14}C] glucose (μg atoms glucose carbon/g/hr)	
	$^{14}CO_2$	^{14}C-Lipid
Glucose	5.05 ± 0.41	4.70 ± 0.47
Glucose + insulin	24.1 ± 1.29	23.6 ± 3.22
Glucose + Mn^{++}	10.3 ± 1.2	9.46 ± 1.1
Glucose + insulin + Mn^{++}	37.6 ± 3.73	32.1 ± 3.05

The flasks contained 200 mg adipose tissue in 4.5 ml Krebs-Ringer bicarbonate medium containing 20 mM glucose, 0.5 μC [1-^{14}C] glucose with addition of 1 unit of insulin and/or $MnSO_4$, 1mM final concentration. Gas phase O_2/CO_2; 95/5; time of incubation 1 hr. Adipose tissue from 6 week old albino rats fed the stock cube diet (MRC86) were used; each value is the mean ± SEM of 6 experiments.

enzyme) which have a special role to play in adipose tissue [9]. The stimulating effect of Mn^{2+} on lipogenesis and glucose oxidation is also shown by the incorporation of specifically labelled glucose into $^{14}CO_2$ and ^{14}C-lipid (Table 1), although in these experiments the effect of Mn^{2+} is not so marked as that found in the rats fed a carbohydrate (sucrose) diet. Both treatments produced a coordinated increase of enzymes functionally related to lipogenesis, viz. glucokinase, pyruvate kinase, ATP-citrate lyase, acetyl CoA carboxylase, fatty acid synthetase and malic enzyme. Addition of Mn^{2+} to the carbohydrate diet caused no further increment in either enzyme activities or isotope incorporations above that found with sucrose alone.

Table 2 shows the effects on glucose oxidation and incorporation into lipids of incubating isolated epididymal fat pads in the presence of either Mn^{2+}, insulin or Mn^{2+} + insulin. It is apparent from the data presented in this Table that both agents cause an increased oxidation of the carbon-1 of glucose and an increase of its incorporation into lipid and that the effects of these two agents are additive.

DISCUSSION

Continuation of a high fat diet for three weeks after the end of weaning prolongs the period of suppression of the enzymes related to lipogenesis both in the liver and adipose tissue, while transfer to a diet in which the fat content has been drastically reduced and the carbohydrate component increased causes the expected [2,10] emergence of the lipogenic enzymes. The point of major interest emerging from Fig. 1 and Table 1 is that the addition of Mn^{2+} can evoke a response in the group given a fat diet similar in profile, and almost equal in amplitude, to that elicited in the group given a sucrose diet Thus, this metal ion would appear to override the regulatory mechanisms normally operating to control the process of lipogenesis in a situation where plentiful lipid is available.

The profile of change in the pair-fed, Mn^{2+} - treated animals is so closely similar to that produced by sucrose (Fig. 1), by insulin treatment or by the administration of a high carbohydrate diet after starvation [10-14] that the possibility that Mn^{2+} acts via an increase in the secretion, release or binding of insulin must be considered. Some support for the view that the response to the presence of Mn^{2+} may be insulin-mediated may be derived from the observation that the addition of Mn^{2+} to the diet of the fat-fed rats is followed by a significant increase in the activity of glucokinase, an enzyme which, it is generally held, is induced by glucose and insulin [15, 16].

The changes observed would also be consistent with an effect of Mn^{2+} in lowering the tissue cAMP since this nucleotide has been reported to suppress the formation of a number of enzymes which are shown here to be raised in the presence of Mn^{2+} [17,18]. Mn^{2+} has been reported to be an activator of both adenyl cyclase and phosphodiesterase [see 19]. However, *in vitro* activation of opposing reactions gives no guideline to the expected change in an *in vivo* situation. Preliminary observations [unpublished, M.Sapag-Hagar], suggest that the steady state level of cAMP in the liver after three weeks on the diet does not change with Mn^{2+} addition to the diet. This does not preclude earlier, transient changes.

A third possibility is that the presence of Mn^{2+} augments the activity of Mn^{2+} - dependent enzymes by increasing their stability. However, the change in enzyme profile is not limited

to enzymes with a specific requirement for Mn^{2+}, such as acetyl CoA carboxylase and malic enzyme, nor are all Mn^{2+} - requiring enzymes elevated by Mn^{2+} - treatment (e.g. NADP-linked isocitrate dehydrogenase). It is, perhaps noteworthy that many of the liver enzymes which have been markedly increased in the present experiments have a requirement for, or are activated by, metal ions, e.g. Mg^{2+}, and, for many of these, Mn^{2+} can replace Mg^{2+} as the activating ion (glucose 6-phosphate and 6-phosphogluconate dehydrogenases [20], ATP-citrate lyase [21], hepatic pyruvate kinase, phosphofructokinase and hexokinase [22]). Glucokinase was exceptional in not being activated by Mn^{2+} [22].

Attention has already been drawn to the dichotomy between the *in vitro* effects of Mg^{2+} and Mn^{2+}, where these two metals may substitute as activators for an extensive series of enzymes, and those occurring *in vivo* where the mammalian organism differentiates sharply between Mg^{2+} and Mn^{2+} [23].

In vitro studies reveal that Mn^{2+} and insulin have additive effects on the oxidation of glucose and on lipid synthesis, even in the presence of saturating levels of insulin (200 mU/ml) and with 20 mM glucose (Table 2), and it would thus appear that the initial activation by these two agents is independent. An independent action of Mn^{2+} and insulin on adenyl cyclase or on cAMP-phosphodiesterase [19] would be consistent with these data as would an action in increasing the rate of entry of glucose into adipocytes. The possible role of Mn^{2+} in increasing the conversion of GSH to GSSG may also be of importance [24] as this conversion would serve the dual function of releasing glucose 6-phosphate dehydrogenase from an NADPH inhibition [24] and of stimulating the pentose phosphate pathway by provision of $NADP^+$, a combined "push-pull" mechanism.

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REFERENCES

1. Walker, D.G. In 'The Biochemistry of Development' (Ed. P.F Benson) p.77 (Spastics International Medical Publications, London, 1971).
2. Greengard, O., Essays in Biochemistry, 7, 159 (1971).

3. Vernon, R.G. and Walker, D G. Biochem.J., 106, 321 (1968).
4. Ballard, F J. and Hanson, R.W. Biochem.J., 102, 952 (1967).
5. Middleton, M.C. and Walker, D.G. Biochem.J , 127, 721 (1972).
6. Vernon, R.G and Walker, D G. Biochem.J., 106, 331 (1968).
7. Zaragoza-Hermans, N.M. European J Biochem., 38, 170 (1973).
8. Baquer, N.Z. and McLean, P. Biochem.Biophys.Res.Commun. 48, 729 (1972).
9. McLean, P., Greenbaum, A.L. and Gumaa, K.A. European J. Biochem., 20, 277 (1972).
10. Gibson, D M., Lyons, R.T., Scott, D F. and Muto, Y. Advances in Enzyme Reg. 10, 187 (1972).
11. Young, J.W., Shrago, E. and Lardy, H.A. Biochemistry 3, 1687 (1964).
12. Weber, G., Singhal, R.L., Stamm, N.B., Lea, M.A and Fisher, E.A. Advances in Enzyme Reg. 4, 59 (1966).
13. McLean, P., Brown, J. and Greenbaum, A.L. In 'Carbohydrate Metabolism and its Disorders' (Ed. F.Dickens, P.J Randle, and W.J.Whelan) Vol. 1, p.397 (Academic Press, London and New York, 1968).
14. Lowenstein, J.M. Biochem.Soc.Symposium, 27 (Ed. T.W. Goodwin) p.61 (Academic Press, London and New York, 1968).
15. Salas, M., Vinuela, E. and Sols, A. J.biol.Chem. 238, 3535 (1963).
16. Niemeyer, H., Perez, N. and Codoceo, R. J.biol.Chem. 242, 860 (1967).
17. Rudack, D., Davie, B. and Holten, D. J.biol.Chem. 246, 7823 (1971).
18. Ureta, T., Radojkovic, J. and Niemeyer, H. J.biol.Chem. 245, 4819 (1970).
19. Robinson, G.A., Butcher, R.W and Sutherland, E.W. In 'Cyclic AMP', p.72 (Academic Press, New York, 1971).
20. Glock, G.E. and McLean, P. Biochem.J. 55, 400 (1953).
21. Srere, P.A. J.biol.Chem., 236, 50, (1961).
22. Wimshurst, J.M. and Manchester, K.L. FEBS Letters 27, 321 (1972).
23. Cotzias, G.C. Fed.Proc., 20, 98 (1961).
24. Eggleston, K.V. and Krebs, H.A. Biochem.J. 138, 425 (1974).