Fructose 2,6-bisphosphate in livers of genetically obese rats

Louis HUE

Hormone and Metabolic Research Unit, University of Louvain and International Institute of Cellular and Molecular Pathology, Avenue Hippocrate 75, B-1200 Brussels, Belgium

and Gérard van de WERVE and Bernard JEANRENAUD

Laboratoires de Recherches Métaboliques, Avenue de la Roseraie 64, CH-1211 Geneva 4, Switzerland

(Received 17 June 1983/Accepted 8 August 1983)

Livers of genetically obese (*fa/fa*) rats, starved for 24 h, contained more fructose 2,6-bisphosphate, glucose 6-phosphate, fructose 6-phosphate and glycogen, and more pyruvate kinase and phosphofructokinase 2 activities, than livers of control lean rats. These changes may be explained in terms of cyclic AMP concentration, which was decreased in livers of obese starved rats.

In previous studies (Hue & van de Werve, 1982; Claus & Pilkis, 1982) it has been shown that the amount of fructose 2,6-bisphosphate in livers of genetically obese mice was greater than in livers of control lean mice. This was particularly evident when the animals were starved; under these conditions, fructose 2,6-bisphosphate content was 6–7-fold higher in livers of obese mice than in livers of lean controls (Hue & van de Werve, 1982). Since fructose 2,6-bisphosphate stimulates phosphofructokinase (Van Schaftingen *et al.*, 1981a), this enhanced [fructose 2,6-bisphosphate] may contribute to keep glycolysis active in the livers of these animals and thus provide lipogenesis with an increased supply of pyruvate.

A similar situation may exist in livers of genetically obese rats of the Zucker strain (*fa/fa*). By contrast with lean controls, isolated hepatocytes from these rats are known to glycolyse and to synthesize fatty acids even after a period of starvation (McCune *et al.*, 1981). The metabolic profile is therefore similar to that in livers of genetically obese mice, and it was our purpose to study the mechanisms that control fructose 2,6-bisphosphate accumulation in the livers of these rats. These mechanisms are the concentration of fructose 6-phosphate, the substrate of phosphofructokinase 2, and cyclic AMP, which controls the state of activity of phosphofructokinase 2 and fructose 2,6-bisphosphatase via the cyclic AMP-dependent protein kinase (for reviews, see Hers & Van Schaftingen, 1982; van de Werve & Jeanrenaud, 1982; Hers & Hue, 1983).

Materials and methods

Animals

Genetically obese (*fa/fa*) male rats and their lean littermate controls (FA/?) were purchased from the Centre de Sélection et d'Élevage d'Animaux de Laboratoire (CNRS, Orléans, France). The animals were 11 weeks old and were fed *ad libitum* with standard laboratory chow or starved overnight before the experiment; four groups of animals were studied, each of four rats. They were maintained in constant-temperature (23°C) animal quarters with a fixed (12 h) light cycle.

The rats were anaesthetized by an intraperitoneal injection of sodium pentobarbital (60 mg/kg); after 15 min the abdomen was opened, and another 15 min later the liver was freeze-clamped between aluminium blocks precooled in liquid N₂ (Wollenberger *et al.*, 1960). Liver samples were kept frozen (–80°C) until further processing. Blood samples (1 ml) were collected from the vena cava.

Measurement of metabolites and enzyme activities

Concentrations of glucose (Huggett & Nixon, 1957), lactate (Hohorst, 1963b), glucose 6-phosphate and fructose 6-phosphate (Hohorst, 1963a), fructose 2,6-bisphosphate (Van Schaftingen *et al.*, 1982), glycogen (Hue *et al.*, 1975), plasma insulin (Herbert *et al.*, 1965) and, in purified extracts (van de Werve *et al.*, 1974), cyclic AMP (Gilman, 1970) were measured as indicated.

Pyruvate kinase activity was measured as described previously (Feliu *et al.*, 1977) at low

Table 1. Concentrations of glycogen, glucose 6-phosphate (Glc-6-P), fructose 6-phosphate (Fru-6-P), fructose 2,6-bisphosphate (Fru-2,6-P₂) and cyclic AMP in livers and concentrations of lactate in blood of normal and obese (*fa/fa*) rats

Values shown are means $\pm$ s.e.m. for four animals in each group. The difference between the corresponding control and obese rats is indicated by * ($P < 0.05$), ** ($P < 0.0125$) and *** ($P < 0.0025$).

Animals	Concn. in liver					Blood lactate (mM)
	Glycogen (mg/g)	Glc-6-P (nmol/g)	Fru-6-P (nmol/g)	Fru-2,6-P ₂ (nmol/g)	Cyclic AMP (nmol/g)	
Control fed	56 $\pm$ 2	82 $\pm$ 13	34 $\pm$ 5	4.6 $\pm$ 0.4	0.78 $\pm$ 0.08	1.83 $\pm$ 0.09
Control starved	1.9 $\pm$ 0.4	26 $\pm$ 5	12 $\pm$ 4	0.7 $\pm$ 0.3	1.03 $\pm$ 0.08	1.29 $\pm$ 0.09
Obese fed	58 $\pm$ 4	134 $\pm$ 17*	46 $\pm$ 9	7.1 $\pm$ 0.9*	0.87 $\pm$ 0.04	2.46 $\pm$ 0.17**
Obese starved	16 $\pm$ 3***	75 $\pm$ 14**	34 $\pm$ 7*	4.4 $\pm$ 1.2**	0.66 $\pm$ 0.09**	1.88 $\pm$ 0.08***

(0.3 mM) and saturating (5 mM) concentrations of phosphoenolpyruvate, with irrelevant modifications. The ratio of activity of pyruvate kinase at low to that at high concentration of substrate gives an indication of the proportion of the enzyme in the active form (Feliu *et al.*, 1976; Claus *et al.*, 1979).

To measure phosphofructokinase 2 activity, liver samples were homogenized at 0°C in 4 vol. of 0.1 M-KF/5 mM-potassium phosphate/50 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid], pH 7.1. After centrifugation for 15 min in an Eppendorf Microfuge, the activity of phosphofructokinase 2 in the supernatant was assayed for 30 min at 30°C in the presence of 0.5 mM-fructose 6-phosphate, 1.5 mM-glucose 6-phosphate, 5 mM-MgATP, 5 mM-potassium phosphate, 50 mM-KCl, 60 mM-KF and 50 mM-Hepes, pH 7.1. The reaction was stopped by the addition of 1 vol. of 0.1 M-NaOH and the mixture was heated for 10 min at 80°C. Fructose 2,6-bisphosphate was measured as described by Van Schaftingen *et al.* (1982).

Results

Despite the high insulinaemia found in 11-week-old obese (*fa/fa*) rats (10.3 $\pm$ 2.4 ng/ml, versus 1.0 $\pm$ 0.3 ng/ml in controls), the blood sugar of the obese rats (8.1 $\pm$ 0.7 mM) was more elevated than that of the controls (5.8 $\pm$ 0.4 mM), indicating a state of insulin resistance.

We have measured [fructose 2,6-bisphosphate] in livers of rats which were either fed or starved for 24 h. To document changes in glycolytic rate, we have also measured the concentration of plasma lactate, hepatic glycogen and hexose 6-phosphates as well as the activities of pyruvate kinase and phosphofructokinase 2. The results are shown in Tables 1 and 2. When livers of fed rats were compared, parameters such as glycogen, cyclic AMP and fructose 6-phosphate were the same, whereas glucose 6-phosphate, fructose 2,6-bisphosphate and plasma lactate as well as pyruvate kinase

Table 2. Activity of pyruvate kinase and phosphofructokinase 2 in livers of normal and obese (*fa/fa*) rats

Values shown are means $\pm$ s.e.m. for four animals in each group. Pyruvate kinase activity was measured at 0.3 mM- ($v_{0.3}$) and 5 mM-phosphoenolpyruvate (V_5) and phosphofructokinase 2 at 0.5 mM-fructose 6-phosphate as explained in the Materials and methods section. The difference between the corresponding control and obese rats is indicated by * ($P < 0.05$) and ** ($P < 0.0125$).

Animals	Pyruvate kinase activity (μ mol/min per g of liver)			Phosphofructokinase (nmol/min per g of liver)
	$v_{0.3}$	V_5	$100 \times \frac{v_{0.3}}{V_5}$	
Control fed	13 $\pm$ 1	41 $\pm$ 2	31.0 $\pm$ 0.7	2.8 $\pm$ 0.6
Control starved	9 $\pm$ 2	28 $\pm$ 4	30.6 $\pm$ 1.1	0.5 $\pm$ 0.2
Obese fed	18 $\pm$ 2*	60 $\pm$ 7*	30.5 $\pm$ 0.6	4.7 $\pm$ 0.6*
Obese starved	21 $\pm$ 4*	54 $\pm$ 7**	38.8 $\pm$ 2.6*	2.3 $\pm$ 0.6*

and phosphofructokinase 2 activities were increased in the obese group. When livers from starved obese rats were compared with livers of lean controls, the differences became more marked: it appeared that in livers of obese rats (i) the concentration of hexose 6-phosphates, fructose 2,6-bisphosphate, glycogen and blood lactate was several times greater, whereas the concentration of cyclic AMP was less than 70% of that in controls, and (ii) the activity of pyruvate kinase measured at both low and saturating concentrations of substrate was higher than in the controls, and the ratio of these two activities indicated that more enzyme was in the active form; finally, the activity of phosphofructokinase 2 was 4 times that in lean controls.

Discussion

The relatively large concentrations of liver glycogen and hexose 6-phosphates and the high activity of

pyruvate kinase and phosphofructokinase 2 found in starved obese rats suggest that glycolysis is active. This confirms a previous observation by McCune *et al.* (1981). The high plasma [lactate] found may also result from an increased hepatic glycolysis and impaired gluconeogenesis as well as from an increased extrahepatic production. Thus the metabolic state of livers from starved obese rats is similar to that of normal fed rats and does not support the existence of 'insulin resistance'. In contrast, glucose production measured in intact fed obese rats by the euglycaemic-clamp technique was resistant to insulin (Terretaz & Jeanrenaud, 1983).

Our data also indicate that, as in livers of genetically obese mice (Hue & van de Werve, 1983; Claus & Pilkis, 1982), the factor responsible for this active glycolysis is probably fructose 2,6-bisphosphate. In hepatocytes incubated under aerobic conditions, the rate of lactate production is indeed well correlated with [fructose 2,6-bisphosphate] (Hue, 1982).

What are the mechanisms responsible for the maintenance of high [fructose 2,6-bisphosphate] in livers of starved obese rats? A high steady state of [hexose 6-phosphate] and, as a consequence, of [fructose 2,6-bisphosphate] might result from the hyperglycaemia present in the starved obese rats, but is not the only explanation, since high [fructose 2,6-bisphosphate] was also found in livers of starved obese mice, which had the same blood glucose as lean controls (Hue & van de Werve, 1982). The low [cyclic AMP] that is observed in livers of starved obese rats, and which is in agreement with a previous observation (Lavine *et al.*, 1975), offers an explanation for the metabolic state of these livers. It may explain the maintenance of elevated [glycogen], which would keep [hexose 6-phosphate] relatively high and thus favour the synthesis of fructose 2,6-bisphosphate (Hue *et al.*, 1981). It could also cause an increase in the activity of phosphofructokinase 2, as this enzyme is inactivated by cyclic AMP-dependent protein kinase (Van Schaftingen *et al.*, 1981b; El-Maghrabi *et al.*, 1982; Furuya *et al.*, 1982). The observed enhanced activity may result not only from a low [cyclic AMP] but also from an increase in total amount of the enzyme in the liver, or from both. Our data do not allow us to discriminate between the two possibilities. On the other hand, since cyclic AMP-dependent protein kinase can inactivate pyruvate kinase (Engström, 1978), a low [cyclic AMP] may also explain that the proportion of pyruvate kinase in the active form was higher in livers of starved obese rats than in controls. In addition, the total content of pyruvate kinase was increased, in agreement with previous observations (Willms *et al.*, 1970; Spydevold *et al.*, 1978). In contrast, it was decreased during starvation in lean controls, as previously reported (Krebs & Eggleston,

1965). It should be noted that, in livers of starved control rats, the proportion of pyruvate kinase in the active form is not significantly decreased despite a high [cyclic AMP]. We have no explanation for this finding, which is in contradiction with several other reports (Feliu *et al.*, 1977; van Berkel *et al.*, 1977).

In conclusion, the low [cyclic AMP] in livers of starved obese rats orientates the metabolism towards an inhibition of glycogenolysis and gluconeogenesis, and a stimulation of glycolysis and lipogenesis. The high [fructose 2,6-bisphosphate] observed in livers of fed obese rats cannot be explained in terms of cyclic AMP; it may result from a long-term adaptation, such as an increase in the content of phosphofructokinase 2.

L. H. is Maître de Recherches du Fonds National de la Recherche Scientifique. We thank Mrs. Y. Mazzola for having typed this manuscript. This work was supported by grants 3.951.080 and 3.851.0.83 of the Swiss National Science Foundation, Berne, Switzerland, and by a grant-in-aid from Nestlé S.A., Vevey, Switzerland.

References

- Claus, T. H. & Pilkis, S. J. (1982) *Biochem. Biophys. Res. Commun.* **109**, 664–668
- Claus, T. H., El-Maghrabi, M. R. & Pilkis, S. J. (1979) *J. Biol. Chem.* **254**, 7855–7862
- El-Maghrabi, M. R., Claus, T. H., Pilkis, J. & Pilkis, S. J. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 315–319
- Engström, L. (1978) *Curr. Top. Cell. Regul.* **13**, 29–51
- Feliu, J. E., Hue, L. & Hers, H. G. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2762–2766
- Feliu, J. E., Hue, L. & Hers, H. G. (1977) *Eur. J. Biochem.* **81**, 609–617
- Furuya, E., Yokoyama, M. & Uyeda, K. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 325–329
- Gilman, A. G. (1970) *Proc. Natl. Acad. Sci. U.S.A.* **67**, 305–312
- Herbert, V., Lau, K. S., Gottlieb, C. W. & Bleicher, S. J. (1965) *J. Clin. Endocrinol. Metab.* **25**, 1375–1384
- Hers, H. G. & Hue, L. (1983) *Annu. Rev. Biochem.* **52**, 617–653
- Hers, H. G. & Van Schaftingen, E. (1982) *Biochem. J.* **206**, 1–12
- Hohorst, H. J. (1963a) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 134–138, Academic Press, New York and London
- Hohorst, H. J. (1963b) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 266–270, Academic Press, New York and London
- Hue, L. (1982) *Biochem. J.* **206**, 359–365
- Hue, L. & van de Werve, G. (1982) *FEBS Lett.* **145**, 263–266
- Hue, L., Bontemps, F. & Hers, H. G. (1975) *Biochem. J.* **152**, 105–114
- Hue, L., Blackmore, P. F. & Extton, J. H. (1981) *J. Biol. Chem.* **256**, 8900–8903
- Huggett, A. St. G. & Nixon, D. A. (1957) *Biochem. J.* **66**, 12P

- Krebs, H. A. & Eggleston, L. V. (1965) *Biochem. J.* **94**, 3c–4c
- Lavine, R. L., Voyles, N., Perrino, P. V. & Recant, L. (1975) *Endocrinology* **97**, 615–620
- McCune, S. A., Durant, P. J., Jenkins, P. A. & Harris, R. A. (1981) *Metab. Clin. Exp.* **30**, 1170–1179
- Spydevold, S. O., Greenbaum, A. L., Baquer, N. & McLean, P. (1978) *Eur. J. Biochem.* **89**, 329–339
- Terrettaz, J. & Jeanrenaud, B. (1983) *Endocrinology* **112**, 1346–1351
- van Berkel, T. J. C., Kruijt, J. K. & Koster, J. F. (1977) *Eur. J. Biochem.* **81**, 423–432
- van de Werve, G. & Jeanrenaud, B. (1982) in *Liver in Metabolic Diseases* (Bianchi, L., Gerok, W., Landmann, L., Sickinger, K. & Stalder, G. A., eds.), MTP Press, Lancaster, in the press
- van de Werve, G., Van den Berghe, G. & Hers, H. G. (1974) *Eur. J. Biochem.* **41**, 97–102
- Van Schaftingen, E., Jett, M. F., Hue, L. & Hers, H. G. (1981a) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 3483–3486
- Van Schaftingen, E., Davis, D. R. & Hers, H. G. (1981b) *Biochem. Biophys. Res. Commun.* **103**, 362–368
- Van Schaftingen, E., Lederer, B., Bartons, R. & Hers, H. G. (1982) *Eur. J. Biochem.* **129**, 191–195
- Willms, B., Ben-Ami, P. & Söling, H. D. (1970) *Horm. Metab. Res.* **2**, 135–141
- Wollenberger, A., Ristau, O. & Schoffa, G. (1960) *Pflugers Arch. Gesamte Physiol. Menschen Tiere* **270**, 399–412