Increased concentration of fructose 2,6-bisphosphate in livers of genetically obese mice

Louis Hue and Gérald van de Werve*

Laboratoire de Chimie Physiologique, Université de Louvain and *International Institute of Cellular and Molecular Pathology, UCL 75 39, avenue Hippocrate 75, B-1200 Brussels, Belgium*

Received 1 July 1982

Fructose 2,6-bisphosphate *Glycolysis* *Lipogenesis* *Obesity* *Phosphofructokinase* *(C57BL/65-ob/ob mice)*

1. INTRODUCTION

Enhanced lipogenesis has been observed in livers of genetically obese mice [1,2]. This might be one of the mechanisms responsible for the increased fat deposition which occurs in these animals. Liver glycolysis provides C3 units for the synthesis of lipids and thus is an important component of the control of lipogenesis. A defective allosteric regulation of phosphofructokinase has been observed in livers of genetically obese mice [3]. The defect, consisting of a decreased sensitivity towards ATP inhibition, might be explained by the presence of an excessive amount of fructose 2,6-bisphosphate in livers of these animals. Indeed, fructose 2,6-bisphosphate, the newly discovered stimulator of PFK [4,5], is capable of relieving inhibition by ATP [6,7]. It is formed in livers exposed to glucose and is degraded after the administration of glucagon or upon starvation [4,5]. An elevated fructose 2,6-bisphosphate content in livers of obese mice may explain an enhanced glycolysis and consequently may offer a partial explanation for the increased lipogenesis observed in the livers of these animals.

This work was undertaken to verify this hypothesis by measuring the content of fructose 2,6-bisphosphate in livers of obese mice.

* Present address: Laboratoire de Recherches Métaboliques, Université de Genève, Faculté de Médecine, 64 avenue de la Roseraie, 1205, Geneva, Switzerland

2. MATERIALS AND METHODS

Male obese (C57BL/6J-ob/ob) mice and their control lean littermates (obtained from the Jackson Laboratories, Bar Harbor, Maine, USA) were fed ad libitum, or starved, for 15 h overnight before the experiment. Mice which were 25 weeks old were chosen since it is known that their glycemia is not different from the value in control lean mice [8]. After 15 min of anesthesia (pentobarbital, 0.1 mg/g body weight intraperitoneally) the abdomen was opened, blood samples were taken and the livers were quickly removed and frozen [9]. Liver samples were stored at $-20°C$ until further processed.

Livers were perfused at 37°C without recirculation with a Krebs–Ringer bicarbonate medium containing 10 mM glucose and 2.5% albumin. The medium was in equilibrium with O_2/CO_2 (19:1). After 30 min of perfusion, the livers were quick-frozen and further processed as indicated below.

2.1. *Measurement of phosphofructokinase and fructose 2,6-bisphosphate*

Phosphofructokinase activity was measured in extracts of livers as described previously [4]. The assay was performed in the presence of 0.1 mM AMP, 1.5 mM Mg-ATP, 1 mM NH_4Cl, 5 mM $MgCl_2$, 5 mM P_i, 100 mM KCl, 50 mM HEPES at pH 7.1 and various concentrations of fructose 6-phosphate. Fructose 2,6-bisphosphate was measured in heat-treated extracts by its ability to stimulate phosphofructokinase; details of the method

Published by Elsevier Biomedical Press
00145793/82/0000–0000/$2.75

are given elsewhere [4,10]. An aliquot of each liver sample was also acid-treated to destroy fructose 2,6-bisphosphate (pH 2, 20 min at 20°C). After neutralization, aliquots were tested to determine their ability to stimulate phosphofructokinase. This value, which represents stimulation by compounds other than fructose 2,6-bisphosphate, was subtracted from that obtained before the acid treatment. The difference represents the stimulation due to fructose 2,6-bisphosphate. To express the values obtained in nmol of fructose 2,6-bisphosphate/g of tissue, the increase in phosphofructokinase activity measured upon addition of heat-treated liver extracts, was compared with that obtained with pure fructose 2,6-bisphosphate (prepared as in [11] by Dr E. Van Schaftingen).

2.2. *Other methods*

Blood samples were deproteinized with equimolar amounts of $ZnSO_4$ and $Ba(OH)_2$ [12] and glucose was measured in the supernatant [13]. For the determination of glycogen, frozen livers were treated for 15 min at 100°C in the presence of 1 M KOH. After neutralization with 1.5 N acetic acid, glycogen was enzymically hydrolyzed into glucose [14] which was then measured [13].

3. RESULTS

The effect of substrate concentration on the activity of phosphofructokinase present in crude liver extracts was measured. Results (fig.1) indicate that, in liver extracts of fed animals, phosphofructokinase from obese mice displayed a slightly greater affinity for fructose 6-phosphate than did the enzyme from livers of lean controls. The difference

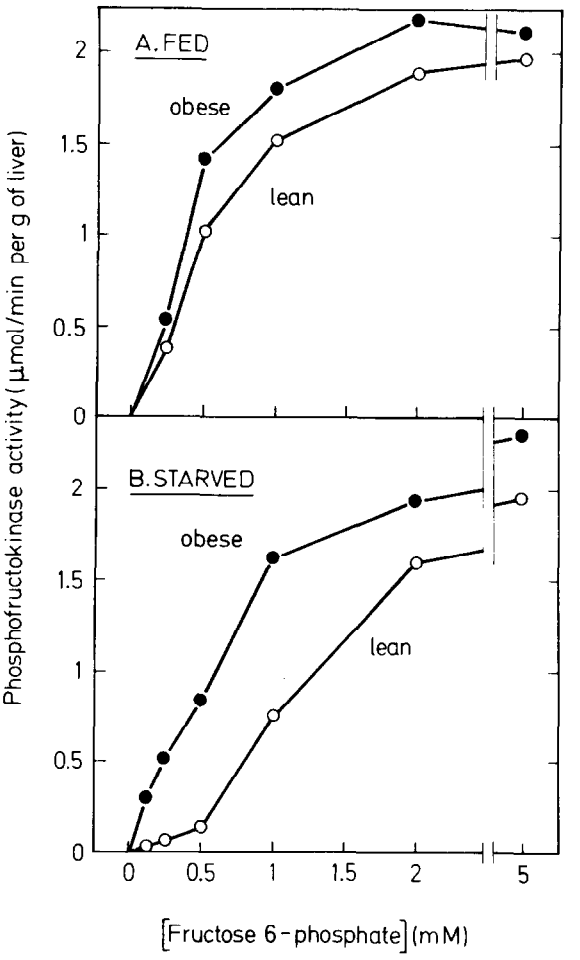

Fig.1. Influence of fructose 6-phosphate concentration on the phosphofructokinase activity present in liver extracts of lean and obese mice. The activity was measured as indicated in Materials and Methods.

Table 1

Glycaemia, glycogen and fructose 2,6-bisphosphate content of livers of lean and obese mice

	Glucose (mM)	Glycogen (mg/g liver)	Fructose 2,6-bisphosphate (nmol/g of liver)
Fed lean	8.1±0.4	47 ±3	4.8±0.8
Fed obese	8.5±1.1	42 ±0.4	9.2±1.1
Starved lean	5.9±0.5	5.2±0.9	0.7±0.1
Starved obese	6.5±0.6	24 ±2	4.6±0.3

Values are means ± SEM for 3 animals

was much larger when the saturation curve of phosphofructokinase from starved obese mice was compared with that of starved lean mice. Since these experiments were performed in crude extracts of livers, the difference observed might be explained by the presence of different concentrations of fructose 2,6-bisphosphate in extracts of livers from obese and lean animals. The liver content of fructose 2,6-bisphosphate was thus measured in livers of fed and starved obese and lean mice. Glycaemia and liver glycogen content were also measured. Data are presented in table 1. There was no difference in glycaemia between control and obese mice. The glycogen content was the same in livers of fed obese and lean mice but, in livers of starved obese mice, it was 5-fold higher than in lean controls and represented about 50% of the value found in fed animals. The concentration of fructose 2,6-bisphosphate was almost twice as large in livers of fed obese mice as in livers of fed controls; in livers of starved obese mice the content was more than 6-fold higher than in starved lean controls.

Since these values were obtained from intact anaesthetized animals, it was of interest to see whether the differences between obese and lean mice persisted in isolated liver preparations and was due solely to hepatic factors rather than extrahepatic influences such as the presence of hormones. Fructose 2,6-bisphosphate was therefore measured in livers of starved animals which were perfused for 30 min in the presence of 10 mM glucose. The results (table 2) demonstrated that the difference persisted after perfusion of the liver, i.e., in the absence of hormones and that conditions required for maintaining this difference were present in the isolated liver.

Table 2

Fructose 2,6-bisphosphate content of livers of lean and obese starved mice after 30 min of perfusion with 10 mM glucose

	Fructose 2,6-bisphosphate (nmol/g of liver)
Starved lean	0.8 ± 0.1
Starved obese	2.7 ± 0.6

Values are means ± SEM for 3 preparations

In livers of younger (12 week-old) fed mice, the difference in fructose 2,6-bisphosphate content between control and obese mice was less important than with 25 week-old mice, the value for the obese animals being only 1.4-fold larger than that of lean controls (results not shown).

4. DISCUSSION

Results reported here demonstrate the presence of fructose 2,6-bisphosphate in livers of mice. The concentrations measured are in the same order of magnitude as those previously reported for rat liver in vivo and for isolated rat hepatocytes [15–17]. In addition our results clearly show that livers of obese mice contain more fructose 2,6-bisphosphate than their control littermates. The most striking difference was observed in livers of starved animals. In livers and hepatocytes from starved rats, the content of fructose 2,6-bisphosphate is several fold lower than in livers of fed animals [4,15]. This difference was also observed here in livers of lean mice. However, livers of starved obese mice still contained concentrations of fructose 2,6-bisphosphate which were at least 6-fold higher than those of lean mice. This difference in fructose 2,6-bisphosphate content might explain the difference in the saturation curve of phosphofructokinase for fructose 6-phosphate (fig.1). Since fructose 2,6-bisphosphate is a potent positive effector of phosphofructokinase which relieves inhibition by ATP, the difference in fructose 2,6-bisphosphate content observed may also explain the decreased sensitivity towards ATP inhibition which has been previously reported [3]. In a strain of genetically diabetic mice an opposite situation has been observed, namely an increased sensitivity of liver phosphofructokinase towards ATP inhibition [18]. It is not known whether the livers of these diabetic mice contain a decreased concentration of fructose 2,6-bisphosphate as is the case for alloxan diabetic rats [19,20].

The presence of relatively increased concentrations of fructose 2,6-bisphosphate in livers of starved obese mice could contribute to keep an active glycolysis in the livers of these animals. Therefore the increased concentration of fructose 2,6-bisphosphate might also contribute to the increased lipogenesis by providing an increased supply of lactate and pyruvate. This would indeed

give an explanation for the increased hepatic lipogenesis observed in obese mice during starvation [1].

What are the mechanisms involved in the maintenance of an elevated fructose 2,6-bisphosphate in livers of starved obese mice? Glucose and hormones such as glucagon are known to modulate fructose 2,6-bisphosphate content [4,5,17]. Since there was no difference in glycaemia between lean and obese animals, this factor is probably not responsible for the difference. On the other hand, the immediate importance of hormonal factors can be ruled out since the difference persisted in perfused livers, i.e., in the absence of hormones. The presence of glycogen and of an active glycogenolysis is another factor which may lead to an accumulation of fructose 2,6-bisphosphate by providing fructose 6-phosphate for the synthesis of fructose 2,6-bisphosphate [15]. One may therefore speculate that, because of the higher concentration of glycogen in livers of starved obese mice, the concentration of hexose 6-phosphates is kept at a higher steady state level and so may cause the accumulation of fructose 2,6-bisphosphate. Such a difference, although small, has already been observed [21]. The mechanisms involved in the maintenance of a high glycogen content in livers of obese mice during starvation are however unclear. A decrease in the cyclic AMP content of livers of starved obese mice [22] might be of relevance.

ACKNOWLEDGEMENTS

L. Hue is Maître de Recherches of the Fonds National de la Recherche Scientifique. This work was supported by the Belgian Fonds de la Recherche Scientifique Médicale and the U.S. Public Health Service (grant AM 9235).

REFERENCES

[1] Hems, D.A., Rath, E.A. and Verrinder, T.R. (1975) Biochem. J. 150, 167–173.
[2] Jeanrenaud, B., Freychet, P., Assimacopoulos-Jeannet, F., Le Marchand, Y. and Karakash, C. (1977) Biochem. Soc. Trans. 5, 890–894.
[3] Katyare, S.S. and Howland, J.L. (1974) FEBS Lett. 43, 17–19.
[4] Van Schaftingen, E., Hue, L. and Hers, H.G. (1980) Biochem. J. 192, 887–895.
[5] Van Schaftingen, E., Hue, L. and Hers, H.G. (1980) Biochem. J. 192, 897–901.
[6] Van Schaftingen, E., Jett, M.F., Hue, L. and Hers, H.G. (1981) Proc. Natl. Acad. Sci. USA 78, 3483–3486.
[7] Uyeda, K., Furuya, E. and Luby, L.J. (1981) J. Biol. Chem. 256, 8394–8399.
[8] Garthwaite, T.L., Martinson, D.R., Tseng, L.F., Hagen, T.C. and Menahan, L.A. (1980) Endocrinology 107, 671–676.
[9] Wollenberger, A., Ristau, O. and Schoffa, G. (1960) Pflügers Arch. Gesamte Physiol. Menschen Tiere 270, 399–412.
[10] Hue, L., Van Schaftingen, E. and Blackmore, P.F. (1981) Biochem. J. 194, 1023–1026.
[11] Van Schaftingen, E. and Hers, H.G. (1981) Eur. J. Biochem. 117, 319–323.
[12] Weichselbaum, T.E. and Somogyi, M. (1941) J. Biol. Chem. 140, 5–20.
[13] Huggett, A.S.G. and Nixon, D.A. (1957) Biochem. J. 66, 12 P.
[14] Hue, L., Bontemps, F. and Hers, H.G. (1975) Biochem. J. 152, 105–114.
[15] Hue, L., Blackmore, P.F. and Exton, J.H. (1981) J. Biol. Chem. 256, 8900–8903.
[16] Hue, L., Blackmore, P.F., Shikama, H., Robinson-Steiner, A. and Exton, J.H. (1982) J. Biol. Chem. 257, 4308–4313.
[17] Hers, H.G. and Van Schaftingen, E. (1982) Biochem. J. 206, 1–12.
[18] Hosey, N.M., Chatterjee, T., Cohen, A.J., Stein, A.L., Kemp, R.G. and Marcus, F. (1980) Proc. Natl. Acad. Sci. USA 77, 2497–2499.
[19] Neely, P., El-Maghrabi, M.R., Pilkis, S.J. and Claus, T.H. (1981) Diabetes 30, 1062–1064.
[20] Kuwajima, M. and Uyeda, K. (1982) Biochem. Biophys. Res. Commun. 104, 84–88.
[21] Elliott, J., Dade, E., Salmon, D.M.W. and Hems, D.A. (1974) Biochim. Biophys. Acta 343, 307–323.
[22] Lavine, R.L., Voyles, N., Perrino, P.V. and Recant, L. (1975) Endocrinology 97, 615–620.