Vol. 140, No. 1, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 15, 1986 Pages 419-426

GLYCOLYSIS AND LIPID SYNTHESIS IN BROWN ADIPOSE TISSUE DURING AGEING IN THE RAT

Morag L.F. Simpson*, Gregory J. Cooney+,ξ Ian D. Caterson+ and Eric A. Newsholme*

+Department of Endocrinology, Royal Prince Alfred Hospital, Missenden Road, Camperdown, N.S.W. 2050, Australia and *Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX132U, United Kingdom

Received July 30, 1986

The maximal activity of the enzymes hexokinase and 6-phosphofructokinase were measured in interscapular brown adipose tissue during ageing in the rat. The activity of these glycolytic enzymes increased markedly during the suckling-weaning transition (20-22 days of age), but from 30 to 100 days of age the activities per gram wet weight and per mg protein decreased. The rate of lipogenesis in brown adipose tissue was also increased over the suckling-weaning transition and the contribution of glucose to this fatty acid synthesis was greater in weaned animals especially under the influence of insulin.

The importance of brown adipose tissue (BAT) as a major site of non-shivering thermogenesis in mammals is now well established (1). The synthesis of triglyceride from glucose is also quantitatively important in BAT (2-4). The role of lipogenesis in BAT is probably to maintain a store of triglyceride for thermogenesis as the rate of lipogenesis is increased during cold acclimation (3). That glucose is the major precursor for BAT lipogenesis during cold-acclimation is suggested from the finding that the activities of the key glycolytic enzymes hexokinase, 6-phosphofructokinase and

ξAddress for correspondence.

0006-291X/86 $1.50

pyruvate dehydrogenase are markedly increased during this condition (5,6).

It has been reported that in suckling rats and mice the rate of lipogenesis in BAT is low (7,8). To examine the possible importance of glycolysis in BAT at this time of development the maximal activities of hexokinase and 6-phosphofructo-kinase have been measured during the suckling period, the suckling weaning-transition and during ageing to 100 days. To determine whether changes in the maximum activity of key glycolytic enzymes can indicate changes in the rate of lipogenesis from glucose in BAT the incorporation of ^{3}H from $^{3}H_2O$ and ^{14}C from [U-^{14}C] glucose into the fatty acid moiety of triglyceride was studied in suckling and weaned rats. The effect of insulin administration on incorporation of [U-^{14}C] glucose and $^{3}H_2O$ into fatty acid in BAT was also studied.

METHODS

The animals used in this study were obtained from the animals house of the Department of Biochemistry, University of Oxford, U.K., and the Bosch animal house, University of Sydney, N.S.W., Australia. They were housed at 21°C and weaned at 20 days of age. Weaned rats were supplied ad libitum with water and laboratory chow (Rat and Mouse Kubes, Allied Feeds, Rhodes, N.S.W., Australia; or rat and mouse No.1 beta diet, B.P. Nutrition Ltd., Wilham, Sussex, U.K.).

Chemicals and enzymes were obtained from Boehringer Ltd. (Lewes, Sussex, U.K.) and radiochemicals were from Amersham International, U.K. Insulin (Actrapid) was obtained from Novo, Copenhagen, Denmark.

At the required age rats were killed by cervical dislocation, the interscapular BAT removed and homogenised in a medium containing 60mM Tris, 1mM EDTA and 5mM MgCl at pH 8.2. Blood was collected from the chest cavity for determination of specific radioactivity of $^{3}H_2O$ in plasma. Hexokinase was assayed radiochemically (9) and 6-phosphofructokinase spectrophotometrically (10).

For the measurement of lipogenesis and ^{14}C incorporation into fatty acids in BAT of suckling rats (16 days of age and 30g body weight), the animals were removed from the mother 3 hours before the start of the experiment; for weaned rats (30 days of age and 60g body weight) food was removed 3 hours before the commencement of the experiment which was always between 11 a.m. and 12 noon. $^{3}H_2O$ (50 mCi/kg) and [U-^{14}C] D glucose (100 mCi/kg) were injected i.v. through

the tail vein. When the effect of insulin (1.0 U/kg) was investigated it was injected i.v. simultaneously with the radioactive compounds. The volume of injection did not exceed 0.2 ml.

Animals were sacrificed 1 hour after injection and tissue lipid extracted and saponified as previously described (11). The rates of fatty acid synthesis are expressed as ug atoms ^{3}H incorporated into tissue fatty acid/hr/g fresh weight. The incorporation of ^{14}C from [U-^{14}C] glucose in fatty acid is expressed as a percentage of the total ^{14}C injected per gram fresh weight of BAT. Protein was measured using a Biorad reagent with gamma globulin as the standard.

RESULTS

The increase in weight of the interscapular BAT with age correlates well with increased body weight both during the suckling-weaning transition and during normal growth to 60 days of age after which there is a decrease in the proportion of BAT to body weight (fig 1). In contrast the activity of hexokinase increased dramatically during the suckling-weaning period (Fig. 2a) as did the activity of 6-phosphofructokinase (Fig. 2b). The changes in activity were not due to changes in the triglyceride content of BAT because the same increases were observed if the results are expressed as activity per mg protein (Fig. 3). There was

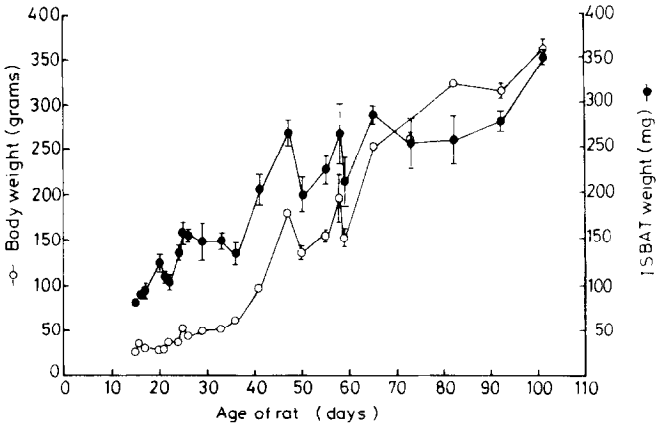

FIGURE 1

Increase in body weight and interscapular brown adipose tissue weight of the rat with age. Each point represents the mean ± S.E.M. for 4-5 separate animals ○ body weight (g) ● interscapular BAT weight (mg).

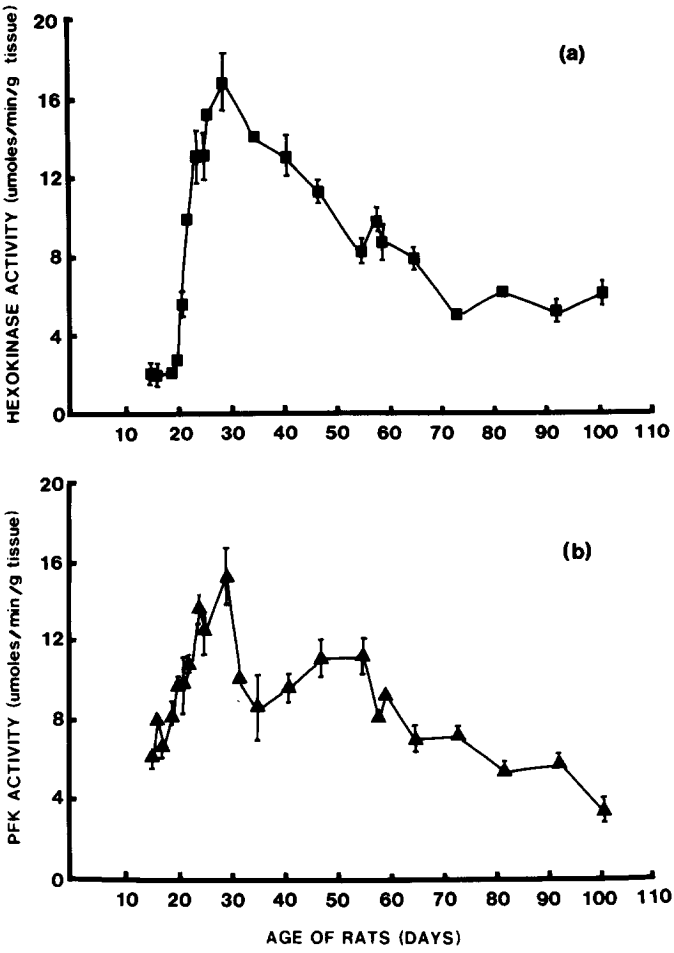

FIGURE 2

Changes in the activity per gram fresh weight of hexokinase and 6-phosphofructokinase in interscapular BAT of the rat with age.

Assays were carried out as described in the Methods section. Each point represents the mean ± S.E.M. for 4-5 separate animals. ■ hexokinase; ▲ 6-phosphofructokinase.

also a gradual decrease in the specific activity of both enzymes after 30 days of age.

The rate of synthesis of fatty acids was higher in BAT of weaned rats compared to suckling rats (Table 1), and the effect of insulin on lipogenesis in suckling and weaned rats was different. In suckling rats insulin stimulated

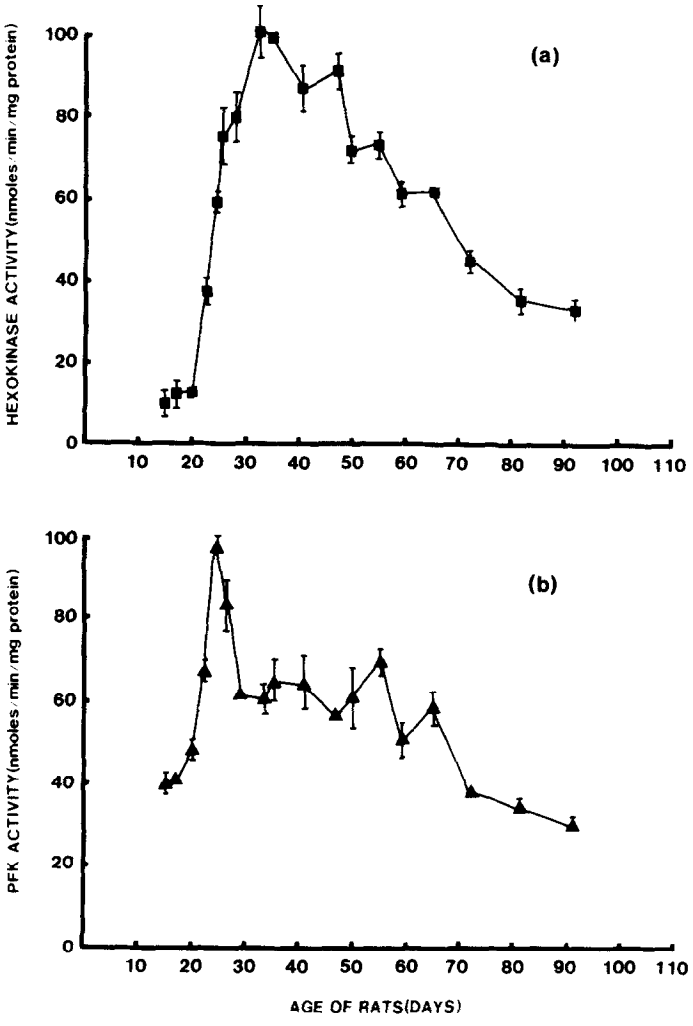

FIGURE 3

Changes in the activity per mg protein of hexokinase and 6-phosphofructokinase in interscapular BAT of the rat with age.

Assays were carried out as described in the Methods section. Each point represents the mean ± S.E.M. for 4-5 separate animals, ■ hexokinase; ▲ 6-phosphofructokinase.

lipogenesis 3 fold in BAT, but the same dose of insulin increased lipogenesis 10 fold in BAT from weaned rats. These results indicate a greater capacity for lipogenesis in BAT of weaned rats rats and also an increased response to insulin in BAT of weaned rats.

TABLE 1

The effect of insulin on lipogenesis and [U-^{14}C] glucose incorporation into fatty acids in brown adipose tissue of suckling and weaned rats

	Treatment	Lipogenesis ug atoms ^{3}H/hr/g	%injected ^{14}C incorporated into fatty acids/g
Suckling	Control	3.78 ± 0.35	0.054 ± 0.014
	Insulin	10.37 ± 1.35***	0.17 ± 0.021**
Weaned	Control	18.8 ± 2.0	0.243 ± 0.011
	Insulin	196 ± 11.0***	3.67 ± 0.50***

For experimental detail see Materials and Methods section. Each result is the mean ± S.E.M. for 5-9 separate animals. Statistical difference between insulin treated and control animals was determined using Students t test.

** $P < .01$
*** $P < .005$

To compare the contribution of carbon from glucose to the increased lipogenic rates in weaned rats the percentage of injected [U-^{14}C] glucose incorporated into fatty acid was measured (Table 1). The initial specific radioactivity of blood glucose was the same for both suckling and weaned rats (data not shown) and so comparison of glucose contribution to lipogenesis is possible between suckling and weaned rats as well as between control and insulin injected animals of the same group. In suckling rats the incorporation of [U-^{14}C] glucose into fatty acids in BAT was stimulated 3 fold by insulin, but both control and insulin stimulated values were less than control incorporation for weaned rats. When [U-^{14}C] glucose incorporation into BAT lipid was measured in insulin injected weaned rats a 15 fold increase over the control value was observed (Table 1).

DISCUSSION

The fact that the activities of the key glycolytic enzymes hexokinase and 6-phosphofructokinase increased dramatically during the transition from suckling to weaning in BAT of the rat suggest that this pathway becomes quantitatively important after weaning. This increased capacity for glucose utilisation in BAT coincides with a change in the diet of the animals from the high fat diet of milk to the high carbohydrate diet of laboratory chow. The marked increase in the incorporation of ^{14}C from glucose to fatty acid in response to insulin in BAT of the weaned animal suggests that one role for the glycolytic pathway in this tissue after weaning is to provide precursors for lipogenesis. Although several previous studies have shown that glucose may be an important precursor for fatty acid synthesis in BAT (2, 6, 12) the present work emphasises the metabolic transition that occurs at weaning and provides further support for the view that BAT can play a quantitatively important role in regulation of the blood glucose level (13). It must also be borne in mind that glycolysis may provide acetyl CoA for oxidation in the tricarboxylic acid cycle in order to generate heat (14).

Recently there have been several reports showing changes in BAT thermogenesis in rats in response to a single meal of various compositions. A high carbohydrate meal markedly increased blood flow and thermogenesis in BAT while a high fat meal had little effect on BAT thermogenesis (15, 16).

This acute nutritional effect on BAT thermogenesis coupled with the adaptive changes in metabolism occuring with the nutritional change from suckling to weaning suggest an important role for BAT in carbohydrate utilisation in the

rat and show the sensitivity of this tissue to the nutritional status of the animal.

In previous studies of lipogenesis in BAT during ageing in the rat (7) and mouse (17) the increase in lipid synthesis at weaning is followed by a gradual decrease in lipogenesis in this tissue. In the present study the specific activity of the glycolytic enzymes also decreased gradually with age and so it appears that the ability of BAT to utilise glucose is involved with the ability of the tissue to synthesise lipid. A decrease in lipid synthesis and thermogenesis in BAT could be an important factor in diversion of excess nutrients to lipid synthesis in other tissues such as white adipose tissue and liver and to increased adiposity in older animals.

REFERENCES

1. Himms-Ha en, J. (1984) New Eng. J. Med. 311 1549-1558
2. Agius, l & Williamson, D.H. (1980) Biochem. J. 166 627-630
3. Trayhurn P. (1979) FEBS Letts 104 13-16
4. Trayhurn P. (1981) Biochim. Biophys. Acta 713 663-669
5. Cooney, G.J. & Newsholme, E.A. (1982) FEBS Letts 148 198-200
6. McCormack, J.G. & Denton, R.M. (1977) Biochem. J. 190 477-480
7. Pillay, D. & Bailey, E. (1982) Biochim. Biophys. Acta 713 663-669
8. Trayhurn, P. (1981) Biochim. Biophys. Acta 664 549-560
9. Newsholme, E.A., Robinson, J. & Taylor, K. (1967) Biochim. Biophys. Acta 132 338-344
10. Opie, L.H. & Newsholme, E.A. (1967) Biochem. J. 103 391-399
11. Stansbie, D., Brownsey, R.W., Crettaz, M. & Denton, R.M. (1976) Biochem. J. 160 413-416
12. Sugden, M.C., Watts, D.F., Marshall, C.E. & McCormack, J.G. (1982) Biosci. Reps. 2 289-297
13. Cooney, G.J. & Newsholme, E.A. (1984) Trends in Biochem. Sci. 9 303-305
14. McCormack, J.G. (1982) Prog. Lipid Res. 21 195-223
15. Glick, Z., Teague, R.J., Bray, G.A. and Lee, M. (1983) Metabolism 32 1146-1150
16. Glick, Z., Wickler, S. J., Stern, J. S., Horwitz, B. A. (1984) Amer. J. Physiol. 247 R160-166
17. Mercer, S.W. & Trayhurn, P. (1983) Biochem. J. 212 393-398