Decreased rate of fatty acid synthesis in brown adipose tissue of hypothalamic obese rats

Masayuki Saito and Takashi Shimazu

Department of Medical Biochemistry, School of Medicine, Ehime University, Shigenobu, Ehime 791-02, Japan

Received 19 October 1983

Obese rats with lesions of the ventromedial hypothalamus (VMH) exhibited a 3-fold increase in the wet weight of interscapular brown adipose tissue (IBAT), due to an incresed triglyceride content, compared with lean controls. The rate of lipogenesis in IBAT was much higher than that in white adipose tissues in control rats, but decreased greatly in rats with VMH lesions and approximated the value in white adipose tissues. It was suggested that thermogenesis in BAT was impaired after VMH lesions by decreasing triglyceride turnover in BAT.

Brown adipose tissue *Fatty acid synthesis* *Hypothalamic obesity* *Lipogenesis* *Thermogenesis* *Ventromedial hypothalamus*

1. INTRODUCTION

Brown adipose tissue (BAT) has recently been recognized as the major site for diet-induced thermogenesis as well as cold-induced non-shivering thermogenesis [1,2]. It is established that thermogenesis in BAT, which is associated with activation of lipid metabolism in the tissue, is under direct control of sympathetic nervous system. A possible link between BAT thermogenesis and the ventromedial nucleus of hypothalamus (VMH) was first suggested by our findings that electrical stimulation of the VMH in rats caused a substantial increase in lipogenesis in BAT, but not in white adipose tissues or the liver [3,4]. It has subsequently been demonstrated [5] that stimulation of the VMH elicited a biphasic temperature change in BAT similar to that produced by sympathetic nerve stimulation. Thus, it is likely that BAT thermogenesis is controlled by the VMH-sympathetic nervous system through activation of lipid metabolism in this tissue. Accordingly, it might be expected that some dysfunction of the VMH results in impaired thermogenesis in BAT. In support of this, it was recently reported [6,7] that changes in the NAD(P)H/NADP redox state of BAT in response to norepinephrine or to electrical nerve stimulation in vitro were reduced in tissue preparation from rats with VMH lesions. To obtain more direct evidence for the above view, we have examined the rate of fatty acid synthesis in vivo in BAT of rats with VMH lesions.

2. METHODS

Female Sprague-Dawley rats weighing 230–270 g were used. They were kept in plastic cages at 25 ± 1°C with a 12–12 h light–dark cycle (lights on at 06.00–18.00 h) and given laboratory chow and water ad libitum.

Under phenobarbital anesthesia (40 mg/kg) of rats, bilateral lesions of the VMH were made by applying a 1.2 mA anodal current for 15 s through an insect-pin electrode insulated except for 0.3 mm at the tip. The stereotaxic coordinates for the electrode were: 0 mm on the bregma, ± 0.6 mm lateral to the midline and 9.5 mm below the surface of the skull. Sham operations were performed in an identical manner but current was not applied.

Eight to ten weeks after the operation, the rats were starved for 24 h, and their plasma levels of insulin were determined by the double antibody

Published by Elsevier Science Publishers B.V.
00145793/84/$3.00

method (Insulin RIA kit, Dainabot, Tokyo) with blood samples obtained from the tail vein. Then, the rate of fatty acid synthesis was determined in vivo by measuring the incorporation of ^3H from ^3H_2O into fatty acids. One hour after an intraperitoneal injection of 3–5 mCi of ^3H_2O (New England Nuclear), the animals were decapitated and blood samples were collected for the determination of specific radioactivity of plasma water. The interscapular brown adipose tissue (IBAT), parametrial and retroperitoneal white adipose tissues and the liver were removed quickly and weighed. Lipids were extracted from each tissue by homogenizing with chloroform–methanol (2:1). For isolation of radioactive fatty acids, the lipid extract was washed as in [8], saponified with ethanolic KOH, acidified with sulfuric acid and then extracted with petroleum ether, as in [3]. The rate of fatty acid synthesis was calculated from the radioactivities in fatty acids and in the plasma, with correction for isotope effects of ^3H *vs* H [9].

Small pieces of IBAT were also homogenized with water, and aliquots of the homogenate were used for assays of protein [10], DNA [11] and triglyceride [12].

All values were presented as mean ± SE. Statistical significance of difference between groups was determined by the Student's *t*-test.

3. RESULTS AND DISCUSSION

It is known that VMH lesions cause hyperphagia and hyperinsulinemia and lead to obesity in rats. In fact, rats with VMH lesions exhibited marked obesity, as judged by both their greater body weight and much larger amounts of white adipose tissue in the peritoneal cavity than sham-operated control rats. The wet weight of IBAT from the obese rats was also about 3 times greater than that of controls (table 1). The contents of protein and DNA/g wet wt of IBAT were decreased, while the triglyceride content was increased significantly by VMH lesions. The contents of protein and DNA per whole tissue appeared to increase in rats with VMH lesions, but the increases were not significant. The total content of triglycerides in IBAT was much greater in rats with VMH lesions than in controls. Thus, VMH lesions produced a marked enlargement of IBAT with an increased triglyceride content, but no change in total content of DNA or protein. These results are in good agreement with recent studies [13] and [6], except that the latter study noted a significant reduction in the total amount of DNA. Although there is a slight discrepancy among studies, it can be concluded that after VMH lesions an apparent hypertrophy of IBAT occurs primarily by an increase in cell size due to excessive accumulation of triglycerides.

Table 1

Body weight and chemical composition of interscapular brown adipose tissue (IBAT) in control and VMH-lesioned rats

	Control	VMH-lesioned
Body wt (g)	298 ± 7	495 ± 20[a]
IBAT		
Wet wt (mg)	388 ± 21	1121 ± 123[a]
Protein content (mg)		
per g wet wt	65.3 ± 7.0	32.4 ± 4.3[a]
per whole tissue	26.1 ± 3.7	35.1 ± 4.4
DNA content (mg)		
per g wet wt	1.33 ± 0.09	0.63 ± 0.04[a]
per whole tissue	0.54 ± 0.04	0.64 ± 0.05
Triglyceride content (mg)		
per g wet wt	582 ± 41	695 ± 18[a]
per whole tissue	222 ± 17	729 ± 62[a]

[a] Significantly different from values for control rats ($p < 0.05$)

Values are means ± SE for 12 (control) and 15 (VMH-lesioned) rats

The abnormal accumulation of triglycerides in IBAT from the obese rats implies that severe alterations of lipid metabolism may occur in BAT or other tisues or both. To clarify this problem, we examined the effects of VMH lesions on lipogenic activities in BAT, white adipose tissue, and the liver by measuring the rate of incorporation of tritium from ^3H_2O into fatty acids in vivo (fig.1). The rate of fatty acid synthesis per g of tissue was higher in IBAT than in white adipose tissues or the liver in control rats. In rats with VMH lesions, the synthetic rate was insignificantly higher in white adipose tissues and more than 2 times higher in the liver than in controls. The increased lipogenic activity observed in these tissues after VMH lesions, which is consistent with previous reports [14,15],

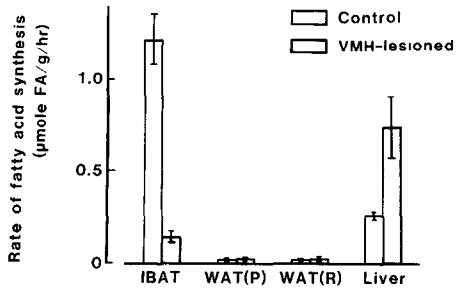

Fig.1. Rates of fatty acid synthesis per g of tissue in control and VMH-lesioned rats. Values are means ± SE for 7 (control) and 6 (VMH-lesioned) rats. IBAT, WAT(P) and WAT(R) denote the interscapular brown adipose tissue, parametrial and retroperitoneal white adipose tissues, respectively. Differences between values for control and VMH-lesioned rats are significant in IBAT and the liver ($p < 0.05$).

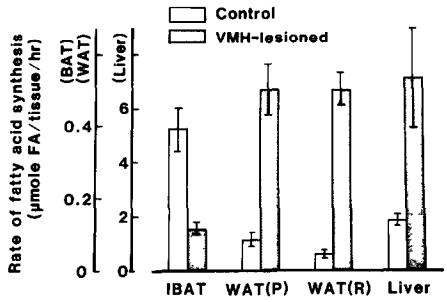

Fig.2. Rates of fatty acid synthesis per whole tissue in control and VMH-lesioned rats. Differences between values for control and VMH-lesioned rats are significant in every tissue ($p < 0.05$). Others are as in fig.1.

may be attributable to hyperinsulinemia incidental to VMH lesions [16]. In fact, even after 24 h starvation, plasma insulin level was still higher in rats with VMH lesions than in controls (69.5 ± 9.5 *vs* 24.8 ± 5.0 μunits/ml).

In contrast to the liver and white adipose tissues, the rate of fatty acid synthesis in IBAT decreased greatly in rats with VMH lesions, as compared with controls. The rate of synthesis per g of tissue may be misleading when comparing the lean controls with obese animals, since the triglyceride content of IBAT differs considerably between two groups (table 1). Therefore, the synthetic rates per whole tissue were also calculated. As shown in fig.2, the rates of fatty acid synthesis in white adipose tissues and the liver on a whole tissue basis were much higher in rats with VMH lesions than in controls, owing to both the increase in the synthetic rate and enlargement of the tissues. However, the synthetic rate of IBAT per whole tissue in VMH-lesioned rats was only about one-third of that in controls. Thus, the rate of fatty acid synthesis in IBAT was decreased after VMH lesions, either expressed as per g tissue or per whole tissue. These results suggest that triglyceride turnover in IBAT is reduced after VMH lesions, which probably reflects the accumulation of triglycerides in this tissue. In view of a close association of thermogenesis with triglyceride turnover in BAT [4], it can be concluded that thermogenesis in BAT is impaired in rats with VMH lesions. In support of this, we obtained preliminary results indicating that the temperature rise in IBAT in response to electrical stimulation of the sympathetic nerves to this tissue was markedly impaired in rats with VMH lesions: the temperature response to the nerve stimulation for 1 min (7.5 V, 2 ms 10 Hz) was 0.56 ± 0.02°C in control rats, whereas the temperature rise was only 0.07 ± 0.01°C in VMH-lesioned rats. Decreased triglyceride turnover and impaired thermogenesis in BAT were also suggested in genetically obese (ob/ob) mice [17,18].

Like in other tissues, lipogenesis in BAT has been shown to be enhanced by insulin [4,19]. This study, however, indicated that after VMH lesions the rate of fatty acid synthesis decreased markedly in BAT, but not in other tissues, despite the presence of hyperinsulinemia, and approached the rate in white adipose tissues (fig.1). A plausible explanation for this finding is that adipocytes of the interscapular adipose tissue in VMH-lesioned rats have metabolic characteristics, at least in lipogenic capacity, similar to those of white adipocytes rather than of brown adipocytes. This idea is supported by the fact that IBAT of rats with VMH lesions is composed of enlarged adipocytes with excessive accumulation of triglycerides (table 1). It was recently reported [20] that IBAT from genetically obese (fa/fa) rats was composed of uniloculated adipocytes, but had no multiloculated adipocytes that are abundant in IBAT from lean controls. In histological examinations, we also found similar morphological changes in adipocytes of IBAT from rats with VMH lesions (not shown).

We demonstrated previously that electrical stimulation of the VMH enhanced lipogenesis in BAT preferentially, without affecting lipid synthesis in white adipose tissues [3,4]. This effect of VMH stimulation is mediated by a mechanism not involving insulin but probably by activation of sympathetic innervation of BAT. On the other hand, it has been reported [21] that the surgically denervated BAT looked like white adipose tissue and had a decreased lipogenic activity. In addition, it has been shown [22] that VMH lesions in rats result in a reduction of sympathetic nerve activity in BAT, as demonstrated by the decreased norepinephrine turnover. Our present results, together with these previous observations, suggest that VMH lesions cause a chronic impairment of triglyceride turnover in consequence of a decreased sympathetic activity in BAT, which in turn would lead to an apparent transformation of BAT into white. These changes in BAT may result in a defect in its thermogenic capacity and thereby contribute, at least in part, to the development or maintenance of this type of obesity.

ACKNOWLEDGEMENTS

We wish to thank Miss Chiaki Nishiyama for her excellent technical assistance. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

REFERENCES

[1] Himms-Hagen, J. (1976) Annu. Rev. Physiol. 38, 315–351.
[2] Rothwell, N.J. and Stock, M.J. (1979) Nature 281, 31–35.
[3] Shimazu, T. and Takahashi, A. (1980) Nature 284, 62–63.
[4] Takahashi, A. and Shimazu, T. (1982) J. Auton. Nerv. Syst. 6, 225–235.
[5] Perkins, N.M., Rothwell, N.J., Stock, M.J. and Stone, T.W. (1981) Nature 289, 401–402.
[6] Seydoux, J., Rohner-Jeanrenaud, F., Assimacopoulos-Jeannet, F., Jeanrenaud, B. and Girardier, L. (1981) Pflügers Arch. 390, 1–4.
[7] Seydoux, J., Ricquier, D., Rohner-Jeanrenaud, F., Assimacopoulos-Jeannet, F., Giacobino, J.P., Jeanrenaud, B. and Girardier, L. (1982) FEBS Lett. 146, 161–164.
[8] Folch, J., Lee, M. and Sloane-Stanley, G.H. (1957) J. Biol. Chem. 226, 497–509.
[9] Windmueller, H.G. and Spaeth, A.E. (1966) J. Biol. Chem. 241, 2891–2899.
[10] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[11] Ceriotti, G. (1955) J. Biol. Chem. 214, 59–70.
[12] Soloni, F.G. (1971) Clin. Chem. 17, 529–534.
[13] Hogan, S., Coscina, D.V. and Himms-Hagen, J. (1982) Am. J. Physiol. 243, E338–E344.
[14] Frohman, L.A., Goldman, J.K. and Bernardis, L.L. (1972) Metabolism 21, 799–805.
[15] Løvø, A. and Hustvedt, B.E. (1973) Metabolism 22, 1459–1465.
[16] Bray, G.A. and York, D.A. (1979) Physiol. Rev. 59, 719–809.
[17] Seydoux, J., Assimacopoulos-Jeannet, F., Jeanrenaud, B. and Girardier, L. (1982) Endocrinology 110, 432–438.
[18] Mercer, W. and Trayhurn, P. (1983) Biochem. J. 212, 393–398.
[19] Agius, L. and Williamson, D.H. (1980) Biochem. J. 190, 477–480.
[20] Levin, B.E., Comai, K., O'Braien, R.A. and Sullivan, C. (1982) Am. J. Physiol. 243, E217–E224.
[21] Steiner, G., Loveland, M. and Schonbaum, E. (1970) Am. J. Physiol. 218, 566–570.
[22] Vander Tuig, J.G., Knehans, A.W. and Romsos, D.R. (1982) Life Sci. 30, 913–920.