CARBOXYLATION OF PYRUVATE BY HUMAN ADIPOSE TISSUE MITOCHONDRIA

M.S. Patel

Fels Research Institute and Departments of Biochemistry and Pediatrics, Temple University School of Medicine, Philadelphia, Penna. 19140 and St. Christopher's Hospital for Children, 2600 North Lawrence Street, Philadelphia, Penna. 19133

Received September 18, 1970

Summary. The carboxylation of pyruvate by human adipose tissue mitochondria was found to be very low as compared to that by rat adipose tissue mitochondria. This finding supports the previous reports of neglegible fatty acid synthesis from glucose by human adipose tissue and underlines an important role of this process in lipogenesis. It is suggested that the availability of intramitochondrial oxaloacetate limits a translocation of acetyl CoA as citrate from the mitochondria to the cytosol. In contrast to an age-dependency of mitochondrial metabolism in rat adipose tissue, pyruvate carboxylation by human adipose tissue mitochondria does not respond to aging.

Recent studies have shown that glucose is a poor precusor for the synthesis of fatty acids by human adipose tissue (1,2). In contrast lipogenesis occurs rapidly from glucose in rat adipose tissue (3). It is generally agreed that oxaloacetate required for the synthesis of citrate which translocates acetyl CoA across the mitochondrial membrane is generated by pyruvate carboxylase (4,5). Recent studies on carboxylation of pyruvate by rat adipose tissue mitochondria have suggested the importance of this process in lipogenesis (6). In view of the difference in lipogenic capacity of fat pads from these two species the carboxylation of pyruvate by human and rat adipose tissue mitochondira were compared. Also a possible effect of aging on this process in these tissues was investigated.

Experimental Procedure. Source of Tissues: About 30 g of human abdominal subcutaneous adipose tissue was obtained during surgery from

two subjects who were on a diet of 2500 calories per day. General anaesthesia was induced by halothane and nitrous oxide with oxygen. During the operation saline was given intravenously. In experiments with rats, epididymal adipose tissue was obtained from Sprague-Dawley male rats of the ages indicated. Rats were fed a commerical diet ad libitum.

Isolation of Mitochondria: Mitochondria from human and rat adipose tissue were isolated as described previously (6). Adipose tissue mitochondria isolated using this procedure have been shown to be functionally intact as judged from both the P:O and respiratory control ratios (6).

Fixation of Labeled ^{14}C Bicarbonate: The composition of the reaction mixture is described in the legend of Fig. 1. Incubations were carried out as described previously (6). Labeled organic acids were separated on a high voltage electrophoresis apparatus (6).

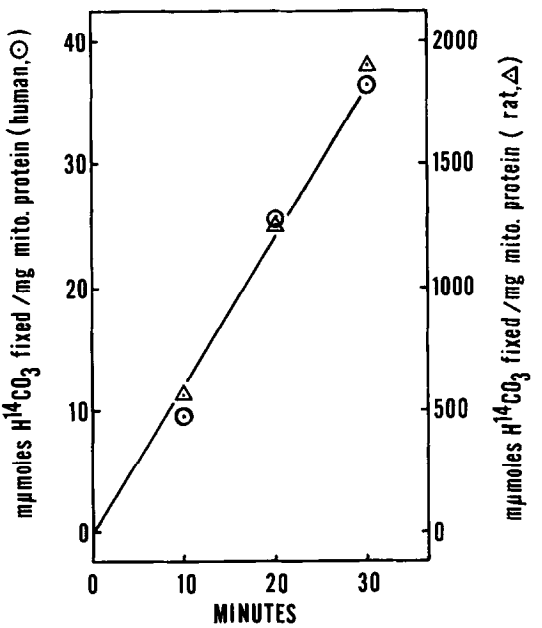

Fig. 1. The fixation of $H^{14}CO_3^-$ by human and rat adipose tissue mitochondria. The reaction mixture contained in a final volume of 1 ml, 0.25 M sucrose; 6.6 mM potassium phosphate, pH 7.4; 6.6 mM triethanolamine, pH 7.4; 10 mM potassium pyruvate; 8 mM ATP; 20 mM $MgCl_2$ and 40 mM potassium bicarbonate (10μCi). The incubation was carried out at 37° and time varied as indicated. Young rats weighing about 180 g were used in this experiment.

Results and Discussion. The incorporation of glucose into fatty acids requires the transfer of acetyl CoA from mitochondria to the cytosol. The translocation of the acetyl moiety across the mitochondrial membrane is carried out by a series of reactions invlolving the formation of oxaloacetate from pyruvate by pyruvate carboxylase and the synthesis of citrate from acetyl CoA and oxaloacetate by citrate synthase followed by its efflux to the cytosol (4,5). Citrate is cleaved by ATP-citrate lyase to acetyl CoA and oxaloacetate and the latter is further metabolized to pyruvate via NAD-and NADP-malate dehydrogenases (5,7,8). The loss of oxaloacetate in the cytosol necessiates the generation of oxaloacetate in the mitochondria by pyruvate carboxylase. The very low rate of pyruvate carboxylation by human adipose tissue mitochondria (Fig. 1) is in agreement with the low activity of pyruvate carboxylase in this tissue (0.023 unit per g tissue; Hanson, R.W., unpublished data). Pertinent to this observation is the report on low lipogenic capacity from glucose of cow and sheep adipose tissues which also have a neglegible pyruvate carboxylase activity (9).

It has been reported that very low activity of ATP-citrate lyase in human adipose tissue may limit lipogenesis from glucose (10). It should be emphasized however, that the maximum velocity of an enzyme may not represent its functional capacity in vivo. For example, carboxylation of pyruvate by rat adipose tissue mitochondria is only about one-tenth of pyruvate carboxylase activity observed in the disrupted mitochondria (0.066 µmole as compared to 0.70 µmole per mg mitochondrial protein per min (6)). As pyruvate carboxylation replenishes oxaloacetate for mitochondrial citrate formation it is possible that it limits the amount of citrate made available to ATP-citrate lyase in the cytosol.

Rat adipose tissue mitochondria have been previously shown to form 3 times more citrate than malate ((6), also see Fig. 2). In contrast human adipose tissue mitochondria form less citrate than malate (Fig. 2) suggesting a limitation in the translocation of acety CoA across the mem-

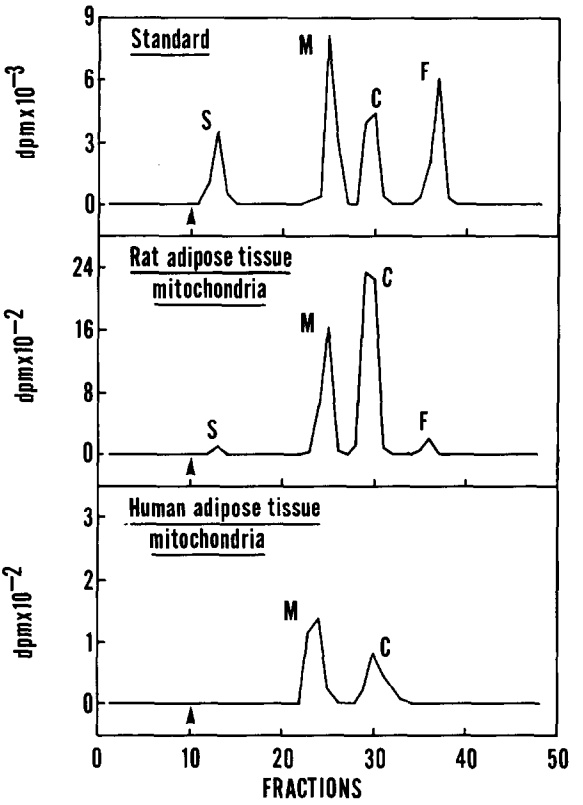

Fig. 2. Separation of the labeled intermediates formed from $H^{14}CO_3^-$ and pyruvate by human and rat adipose tissue mitochondria using high voltage electrophoresis. The migration of known ^{14}C-labeled compounds was compared with that of labeled organic acids formed by mitochondria. The sample experiments shown were for a 30-min mitochondrial incubation as described in Figure 1. S, succinate; M, malate; C, citrate; and F, fumarate. Arrow indicates the origin.

brane of these organelles. A possible role for malate in glyceroneogenesis in human adipose tissue is not well understood at present.

The rate of lipogenesis in rat adipose tissue depends upon both the nutritional status as well as the age of the animal (11-14). It is possible that the differences observed in carboxylation of pyruvate by the mitochondria from human and rat adipose tissue (Fig. 1) are due to the relative difference in the age of rat and human. Young rats (about 180 g body weight, about 6 weeks old) were used in the experiment shown in Figure 1 whereas the human subject under study was 57 years old. In

Table 1. The effect of age on carboxylation of pyruvate by human and rat adipose tissue mitochondria.

The reaction mixture was as described in Figure 1. Incubations were carried out at 37° for 30 minutes.

Source	Age	Pyruvate carboxylation
		mμmoles $H^{14}CO_3^-$ fixed/mg protein
Human	22 years	39
	54 years	37
Rat	6 weeks	1798
	6 weeks	1895
	10 weeks	350
	14 weeks	238

view of this a possible effect of aging on adipose tissue pyruvate carboxylation was investigated. As seen in Table 1 mitochondrial carboxylation of pyruvate decreases markedly in adipose tissue with increasing age of the rat. A similar magnitude of reduction in lipogenesis in rat adipose tissue from older animals has been observed (14). In contrast to the aging phenomenon observed in rat adipose tissue the carboxylation of pyruvate by human adipose tissue mitochondria (Table 1) is not affected by age.

Acknowledgement: I wish to express my sincerest appreciation to Dr. R.W. Hanson of Fels Research Institute for his support, encouragement and help in all aspects of this work. I would like to thank Dr. V.H. Auerbach and Dr. A.M. DiGeorge of St. Christopher's Hospital for Children for their support; and Dr. L.I. Goldman and Dr. O.E. Owen of Temple University School of Medicine for supplying human tissues. This work was supported in part by Grants AM011279, CA-10916, HD-2870, RR-5624 and an institutional grant IN-88 from the American Cancer Society.

References

1. Hirsch, J. and Goldrick, R.B., J. Clin. Invest., 43, 1776 (1964).
2. Galton, D.J., J. Lipid Res., 9, 19 (1968).
3. Winegard, A.I. and Renold, A.E., J. Biol. Chem., 233, 267 (1958).
4. Spencer, A.F. and Lowenstein, J.M., J. Biol. Chem., 237, 3640 (1962).
5. Leveille, G.A. and Hanson, R.W., J. Lipid Res., 7, 46 (1966).
6. Patel, M.S. and Hanson, R.W., J. Biol. Chem., 245, 1302 (1970).
7. Wise, E.M., Jr. and Ball, E.G., Proc. Nat. Acad. Sci., 52, 1255 (1964).
8. Young, J.W. Shrago, E. and Lardy, H.A., Biochemistry, 3, 1687 (1964).
9. Hanson, R.W. and Ballard, F.J., Biochem. J., 105, 529 (1967).
10. Shrago, E., Spennetta, T. and Gordon, E., J. Biol. Chem., 244, 2761 (1969).
11. Tepperman, J. and Tepperman, H.M., Am. J. Physiol., 193, 55 (1958).
12. Patel, M.S. and Mistry, S.P., J. Nutr., 98, 235 (1969).
13. Hanson, R.W., Patel, M.S., Jomain-Baum, M. and Ballard, F.J., in Symposium on Recent Research in Diabetes, Intermediary and Drug Metabolism, Mehlman, M.A. and Hanson, R.W. (Editors), in press.
14. DiGirolamo, M. and Rudman, D., Federation Proc., 25, 441 (1966).