ON THE OCCURRENCE OF ENZYMES OF KETONE-BODY METABOLISM IN HUMAN ADIPOSE TISSUE

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SUMMARY

The activities of hydroxymethylglutaryl (HMG) CoA synthetase and lyase, acetoacetyl CoA thiolase and deacylase were measured in mitochondria of human adipose tissue. All enzymes with the exception of HMG CoA synthetase, were found to be very active. Our results strongly suggest that acetoacetate can be formed in these mitochondria due to the presence of an active acetoacetyl CoA deacylase and that in this tissue, where the activity of the citrate cleavage enzyme is very low, acetyl CoA units leave mitochondria in the form of acetoacetate to participate in cytoplasmic fatty acid synthesis.

INTRODUCTION

The aim of this study was to test the occurrence of the enzymes responsible for the synthesis of ketone-bodies, particularly acetoacetate, in the mitochondria of human adipose tissue. It is well known that citrate cleavage enzyme is not very efficient in the adipose tissue of man (1-3) and mice (4) and therefore citrate is not considered a good precursor of fatty acids in this tissue. The best precursors of fatty acid synthesis are acetate (5) and acetoacetate (6-8). In this context it would be of interest to know whether adipose tissue is able to synthesize acetoacetate or that it derives this intermediate solely from the blood. The activities of the enzymes acetoacetyl CoA thiolase, HMG CoA synthetase and HMG CoA lyase which govern the synthesis of acetoacetate in liver (9-12) were measured in addition to acetoacetyl CoA deacylase. Our results indicate that human adipose tissue mitochondria are capable of forming acetoacetate and hence this compound can be considered to be an effective carrier of acetyl CoA units to the extramitochondrial compartment for fatty acid synthesis in this tissue.

MATERIALS and METHODS

Fresh samples of human adipose tissue, obtained from patients undergoing abdominal surgery at the local hospital, were homogenized with 1.5 volume of 10 mM Tris buffer, 0.25 M saccharose, 1 mM EDTA, 1 mM mercaptoethanol, pH 7.4. After elimination of nuclei obtained by centrifugation at 500 g for 5 min, the resulting supernatant was centrifuged at 10,000 g for 15 min and the mitochondrial sediment collected and suspended in 0.5 ml of Tris-buffer containing 0.5 % Triton X-100 (w/v). The supernatant fraction obtained by centrifugation of this preparation at 100,000 g for 1 hour was used for the measurement of enzyme activities. Acetoacetyl CoA thiolase was assayed by following the disappearance of absorbance of acetoacetyl CoA at 313 nm at 30° C (12). One unit of the enzyme catalyses the CoA-dependent cleavage of 1 mμM of acetoacetyl CoA per min/milligram of protein. Acetoacetyl CoA deacylase activity was determined by measuring the rate of removal of acetoacetyl CoA in the presence of iodoacetamide according to the method of Williamson (12). β-OH-β-methylglutaryl CoA lyase was determined by following the rate of reduction of NAD in the presence of malate, citrate condensing enzyme and malate dehydrogenase at 340 nm and 37° C (13). One unit corresponds to the amount of enzyme that catalyses the formation of 1 mμM of acetyl CoA (calculated from the absorbance of NADH formed) per min/milligram of mitochondrial protein. β-OH-β-methylglutaryl CoA synthetase was measured by following the acetyl CoA dependent decrease in A 300 nm due to consumption of acetoacetyl CoA (14). Protein was determined by the methods of Lowry (15) and Biuret (16).

RESULTS and DISCUSSION

It is generally admitted that the human adipose tissue (1-3), in contrast to that of mice (17) does not participate very efficiently in fatty acid synthesis due to the low activity of citrate cleavage enzyme. It has been already demonstrated that there is no correlation between the *in vivo* activity of this enzyme and lipogenesis (18-20). Since it is known that acetoacetate is a good precursor of fatty acid synthesis in mice and human adipose tissue (6, 7) it would be tempting to postulate an important physiological role for this compound in that it acts as an extramitochondrial carrier of acetyl CoA. For this it should be able to convert the acetyl CoA formed inside the mitochondria to acetoacetate. It therefore seemed of interest to us to determine whether the adipose tissue possesses an enzyme system which is capable of synthesizing acetoacetate from acetyl CoA. Since in liver the acetoacetyl CoA deacylase activity is low (11) and the synthesis of acetoacetate proceeds from the HMG CoA pathway, we first measured the activities of these enzymes. As can be seen in Table 1, acetoacetyl CoA thiolase, which largely determines the concentration of acetoacetyl CoA in the liver, and HMG CoA lyase are very active. The values obtained for these enzymes are very similar to those obtained for the

Table 1

Mitochondrial enzymes of ketone-body metabolism
in human adipose tissue

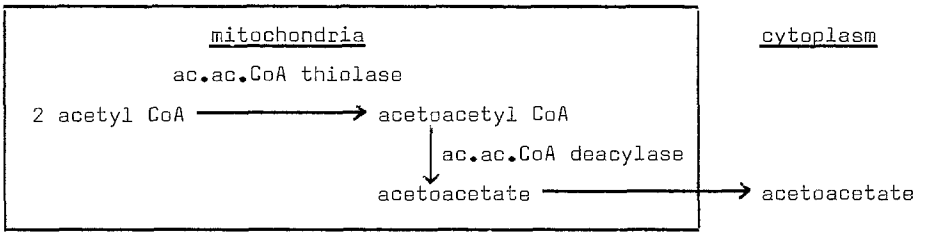
HMG CoA lyase (units)	HMG CoA synthetase (units)	Ac.Ac. CoA thiolase (units)	Ac.Ac. CoA deacylase (units)
83.7 ± 7.6	traces	172 ± 10.3	28.3 ± 2.4

- Enzymatic units are expressed as nanomoles of substrates converted/min/mg of mitochondrial protein.
- Other conditions : cf Materials and Methods.

liver enzymes when expressed per mg protein and are 172 and 83.7 nmoles of substrate converted per min respectively.

On the contrary the activity of HMG CoA synthetase, which seems to be the limiting enzyme of hepatic ketone-body synthesis, is far less active and virtually absent. This made us to wonder whether adipose tissue, in contrast to liver, possesses an active acetoacetyl CoA deacylase. We therefore measured the activity of this enzyme and found that it indeed was active and could hydrolyse 28.3 nmoles of acetoacetyl CoA/min/mg protein.

Thus, from the foregoing data it would appear that acetoacetate can be synthesized in the mitochondria of human adipose tissue despite the low level of HMG CoA synthetase. The pathway by which acetyl CoA units leave the mitochondria would probably be as follows :



It is possible that the ketone-bodies are reactivated after being transferred to the cytoplasm as evidenced by our observation that cytoplasmic acetoacetyl CoA synthetase has an activity identical to that of acetyl CoA synthetase (0.75 $\pm$ 0.14 nmoles of acetoacetate transformed into acetoacetyl CoA/min/mg of protein). This value is about one-third of that observed for the cytoplasmic acetyl CoA synthetase of rat adipose tissue (5) and hence points to the possibility of acetoacetate being a good precursor of fatty acid synthesis. Hence, according to the above scheme, human adipose tissue could play a significant role in the synthesis of fatty acids.

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