Mitochondrial acetyl-CoA carboxylase

Time course of mobilization/activation in liver of refed rats

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Fasted (48 h) rats were killed at 0, 2, 4, 6, 8, 12, 16, 20 and 24 h after they were refed on a high-carbohydrate diet. An increase in the maximal activity and quantity of cytosolic acetyl-CoA carboxylase was found in liver of refed rats after a lag time of about 8 h. The increased quantity of cytosolic enzyme was attributable primarily to mobilization of mitochondrial storage forms and not to a substantial increase in the rate of synthesis of acetyl-CoA carboxylase.

INTRODUCTION

When fasted rats are refed on a diet high in carbohydrate, liver lipogenesis and activity of lipogenic enzymes increase to supernormal values over the next 48–72 h [1]. The mechanism of this ‘over-shoot’ in hepatic lipogenesis has not been established, although it has been shown that the increased activity of a regulatory enzyme in the process [2], acetyl-CoA carboxylase, could be attributed to an increase concentration of immunologically precipitable cytosolic enzyme [3,4]. This observation has generally been considered as evidence that fasting/refeeding results in increased enzyme synthesis [5]. However, when we used a method which detects acetyl-CoA carboxylase on the basis of biotin content rather than enzyme activity [6], we found that rat liver contains substantial quantities of two relatively inactive immunologically precipitable forms of acetyl-CoA carboxylase bound to the mitochondrial outer membrane [7]. When fasted rats were refed, the concentration of active cytosolic acetyl-CoA carboxylase increased, with an apparent compensatory decrease in the mitochondrial forms of the enzyme, so that there was no change in total liver enzyme concentration [8]. These observations suggested that refeeding fasted rats resulted in mobilization/activation of mitochondrial enzyme rather than increased enzyme synthesis [7,8]. The release and activation of mitochondrial enzyme have been found to be insulin-dependent [9].

The present work was undertaken to ascertain the time course of activation/mobilization of mitochondrial acetyl-CoA carboxylase over the first 24 h period of refeeding. The results indicate that the maximal activity and the concentration of cytosolic acetyl-CoA carboxylase increased after an 8 h lag time, with a compensatory decrease in mitochondrial forms of the enzyme. Thus the total concentration of liver acetyl-CoA carboxylase did not change over the 24 h period.

MATERIALS AND METHODS

Animals

Sprague–Dawley rats (150–200 g) were maintained in

a controlled environment with a light/dark cycle of 12 h each. A high-carbohydrate/low-fat diet [9] and water were provided *ad libitum*. Rats were fasted for 48 h before they were refed on the same diet, beginning with the dark cycle. Three representative animals were killed by decapitation at each of nine time points (0, 2, 4, 6, 8, 12, 16, 20 and 24 h) after refeeding. Liver was quickly removed, cooled in ice and weighed.

Preparation of subcellular fractions of liver

Liver was homogenized in 2 vol. of cold (0–4 °C) sucrose/mannitol buffer described by Greenawalt [10]. Cytosolic and mitochondrial fractions were prepared by differential centrifugation as previously described [7]. Minimal cross-contamination was established by assay of marker enzymes. The activity [11] of a cytosolic marker enzyme, lactate dehydrogenase (EC 1.1.1.27), in the mitochondrial preparation was found, in each case, to be no more than 2 % of cytosolic activity. Activity [12] of an mitochondrial-outer-membrane marker [10], monoamine oxidase (EC 1.4.3.4), was not detectable in cytosol.

Analytical methods

Activity of acetyl-CoA carboxylase in cytosol and mitochondrial fractions was measured by fixation of [¹⁴C]bicarbonate in acid-stable form after the preparations were incubated with 10 mM-citrate in iso-osmotic homogenization buffer for 30 min at 37 °C as previously described [7–9]. This is sufficient time for heat [13] and citrate [14] activation as well as dephosphorylation to occur [15,16], so that the assay is a measure of maximal activity. The acid-stable products have been shown by t.l.c. [17] to be malonate and malonyl-CoA [8]. One unit of enzyme activity is defined as the fixation of 1 μmol of radioactive bicarbonate in acid-stable form/min.

The quantity of acetyl-CoA carboxylase was measured as previously described [6]. Blood glucose concentration [9] and liver glycogen content [18] were measured enzymically. Protein was measured by dye binding [19], with bovine serum albumin as a standard.

Statistical analysis was performed by using a fixed and mixed model least-squares and maximum-likelihood

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Fig. 1. Concentration of blood glucose and liver glycogen (a) and activity and quantity of liver acetyl-CoA carboxylase (b) as a function of refeeding time

(a) The concentrations of glucose in blood (mm) and of glycogen in liver (mg/g) were measured at various times after refeeding. (b) The maximal activity (munits/mg of protein) and the concentration of cytosolic acetyl-CoA carboxylase (pmol/mg of protein) were measured as a function of refeeding time. In each case, vertical lines represent S.E.M. for three rats per time period.

computer program (Harvey's Least-Squares Program; SAS User's Guide, SAA Institute, Cary, NC, U.S.A.).

Materials

Sources of materials have been described [7].

RESULTS AND DISCUSSION

Fig. 1 shows the temporal relationships among blood glucose concentration, liver glycogen content and maximal activity and quantity of cytosolic acetyl-CoA carboxylase in the liver of refed rats. Although the concentration of blood glucose began to rise immediately after refeeding, an increase in liver glycogen concentration was delayed for 2-4 h (Fig. 1a). The observed time-dependent increases in both of these parameters are in close agreement with results reported by Holness *et al.* [20]. The rise in maximal activity of cytosolic acetyl-CoA carboxylase was not apparent, however, until about 8 h after refeeding (Fig. 1b). Such a lag time in the increase in acetyl-CoA carboxylase activity after refeeding has also been previously observed [21].

The data in Fig. 1(b) also show that there was a similar

Fig. 2. Concentration of the different subunit M_r forms of acetyl-CoA carboxylase in cytosolic and mitochondrial fractions of liver from refed rats

The concentration (nmol/g of liver) of the M_r -280000 (□) and M_r -250000 (■) mitochondrial forms and the M_r -280000 (○) and M_r -150000 (●) cytosolic forms of acetyl-CoA carboxylase were measured as a function of refeeding time. In each case, vertical lines represent S.E.M. for three rats per time period. The subunit M_r values include that of monomeric avidin (16000). Statistical analysis showed that there was a linear change in the concentration of each form of the enzyme over time ($P < 0.01$).

lag time in the increase in the quantity of the high-subunit- M_r form [7] of cytosolic acetyl-CoA carboxylase. In fact, the rate of regain of acetyl-CoA carboxylase activity and quantity was very similar. Such a close relationship between activity and quantity of the active form of the enzyme is to be expected, since activity was measured after the preparations were preincubated with citrate at 37 °C for 30 min. This preincubation period was sufficient time for heat [13] and citrate [14] activation as well as dephosphorylation [15,16] to occur. Thus activity measurements reflected maximal values, i.e. the effects of these short-term regulatory mechanism were excluded by the experimental procedure [9].

These results confirm what has been known for some time, namely that refeeding fasted rats results in a marked increase in maximal acetyl-CoA activity in liver cytosol [1], which suggested an increased quantity of enzyme [2,5]. The observation that more [14 C]leucine was incorporated into immunoprecipitated cytosolic acetyl-CoA carboxylase after fasted rats were refed [3,4] also indicated that refeeding resulted in a greater quantity of cytosolic enzyme. Our measurement of the quantity of the active form of the cytosolic acetyl-CoA carboxylase (Fig. 1b) supports that conclusion. A remaining question is, what is the source of the cytosolic enzyme?

We have found mitochondrial storage forms of acetyl-CoA carboxylase [7] which can be mobilized and activated under lipogenic conditions. Thus the concentration of active cytosolic enzyme decreased when rats were fasted (48 h), with a compensatory increase in that of mitochondrial forms [7,8]. When fasted (48 h) rats were refed (48 h), the concentration of the active form of the cytosolic enzyme increased at the apparent expense of

Fig. 3. Total quantity of all forms of acetyl-CoA carboxylase and the percentage of enzyme in the active cytosolic form in liver of refed rats

The total quantity of all forms of acetyl-CoA carboxylase (●) (nmol/g of liver) was calculated by adding the quantity of each form of the enzyme for each rat. The percentage of the total enzyme in the active cytosolic form (○) was calculated. In each case, vertical lines represent S.E.M. for three rats per time period.

mitochondrial forms [8]. Acute alloxan-diabetes (3 days) resulted in a decreased concentration of the active cytosolic form of acetyl-CoA carboxylase, again with a compensatory increase in mitochondrial forms [9]. When all forms of acetyl-CoA carboxylase were considered, fasting, refeeding and acute alloxan-diabetes did not alter the total concentration of the enzyme in liver. These results indicated that the dietary/hormonal manipulation did not affect the rate of synthesis of the enzyme, but rather affected its subcellular distribution [7–9].

Fig. 2 shows the quantity of each of the forms of acetyl-CoA carboxylase in liver of rats as a function of refeeding time. The quantity of the active cytosolic form of the enzyme (M_r 280000) increased ($P < 0.01$) over time, but the quantity of both mitochondrial forms decreased ($P < 0.01$). The relationships are more clearly evident in Fig. 3. As time of refeeding increased, a greater percentage of the enzyme was found in the active cytosolic form, whereas, over the course of the refeeding period, the concentration of all forms of the enzyme in liver remained constant at 10.73 ± 0.20 nmol/g of liver. Since liver weight increased over time (results not shown), the total quantity of acetyl-CoA carboxylase per liver was increased by refeeding. However, since the concentration of the enzyme remained constant, our results indicate that acetyl-CoA carboxylase was not accumulated any faster than other liver constituents, and are therefore inconsistent with the conclusion of previous workers [3–5] that refeeding specifically induces the synthesis of acetyl-CoA carboxylase.

The steady-state concentration of acetyl-CoA carboxylase is a function of the relative rates of synthesis and degradation of the enzyme. The quantity of the low-subunit- M_r (150000) form of acetyl-CoA carboxylase appears to be a measure of the rate of its degradation [9]. As shown in Fig. 2, the quantity of the low-subunit- M_r form of acetyl-CoA carboxylase decreased ($P < 0.01$) by a small amount as refeeding time increased, indicating

that refeeding decreased the rate of enzyme degradation, in agreement with previous observations [3,4].

Taken together, our results suggest that the rates of synthesis and degradation of acetyl-CoA carboxylase were essentially balanced over the 24 h refeeding period. However, in the time frame of this study, neither of these processes appears to be nearly as important as changes in subcellular distribution in accounting for increased lipogenic capacity in liver of refed rats. This conclusion differs substantially from that of previous workers.

Majerus & Kilburn [3] and Nakanishi & Numa [4] concluded that fasting and fasting/refeeding altered the rate of synthesis of acetyl-CoA carboxylase. Their results were based, however, on measurement of incorporation of [14 C]leucine only into cytosolic acetyl-CoA carboxylase, because they were unaware of the substantial quantity of mitochondrial storage forms of the enzyme. Since the radioactive amino acid was incorporated, the enzyme was clearly synthesized, but the method employed could not have differentiated between dietary effects on enzyme synthesis and effects on mobilization of mitochondrial forms. Our results suggest that the apparent increase in the rate of enzyme synthesis in liver of refed rats previously observed [3,4] is attributable to increased mobilization of mitochondrial forms.

On the basis of the present evidence, the regulation of acetyl-CoA carboxylase occurs at more than one level, differing in the time frame of the effects. Covalent modification [22], metabolic effectors such as citrate [14] and feedback inhibition of enzyme activity by malonyl-CoA [23] are determinants of lipogenic capacity over a very short time frame of minutes. Evidence in the present paper, as well as our previous observations [7–9], indicate that the alteration of subcellular distribution is important over the time frame of 8–72 h. Finally, the total quantity of enzyme, dependent on the relative rates of synthesis and degradation, appears to be important over a more extended time frame, the magnitude of which has yet to be established.

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Received 19 December 1988/20 March 1989; accepted 21 April 1989