Activity and expression of hepatic mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase during the starved-to-fed transition

PATTI A. QUANT
Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, U.K.

Introduction
Refeeding starved rats for 2–3 h completely reverses the effects of starvation on hepatic ketogenic capacity [1] and decreases blood ketone body concentrations markedly [2]. However, acute depression of ketogenesis on refeeding is not accompanied by any reversal of changes (induced by starvation) in carnitine palmitoyltransferase (CPT I) during the first 6 h of refeeding [2, 3], indicating that control is exerted at other (intramitochondrial) regulatory site(s) (distal to CPT I) during the rapid reversal of starvation ketosis. (CPT I is an important locus for the control of ketogenic flux in the livers of normal fed adult rats [4, 5].)

I have used the ‘top-down’ approach [6] of Metabolic Control Theory [7–9] to establish that, in addition to CPT I, the enzymes of the 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) pathway (acetoacetyl-CoA thiolase, HMG-CoA synthase and HMG-CoA lyase) can contribute significantly to control of ketogenesis [10]. My earlier findings [11–15] and those of others [16, 17] suggest this control may be exerted at the level of mitochondrial HMG-CoA synthase (EC 4.1.3.5).

My research has shown that changes in the rate of ketone body production occur in parallel with changes in the activity of the key enzyme mitochondrial HMG-CoA synthase [11]. These changes result from glucagon-induced changes in succinylation (and inactivation) of the synthase and changes in the absolute amounts of the enzyme protein [12–15]. The pool of succinylated (inactive) enzyme allows for rapid fine control of HMG-CoA synthase (and hence ketogenesis) as metabolic requirements change (e.g. during the fetal–neonatal transition) [13]. This is an insulin-independent control. A change in the absolute amounts of enzyme appears to be a more acute long-term response to changes in nutrition (starvation or fat feeding) and hormone levels (diabetes, starvation or fat feeding) [13–15]. Elevated levels of glucagon stimulate ketogenesis. High levels of insulin depress it [18–20]. Starvation results in an increased glucagon/insulin concentration ratio and refeeding reverses this (see [3]). The aim of this present paper is to establish whether the rapid depression of ketogenesis on refeeding can be explained by rapid resuccinylation and inactivation of HMG-CoA synthase (induced by the decreasing glucagon levels).

Experimental
Methods The treatment of animals, isolation of mitochondria and the protein assay have been described previously [11]. The assay of active and total mitochondrial HMG-CoA synthase, antibody production and statistics were performed as previously described [13].

Results
Active HMG-CoA synthase in mitochondria isolated from the livers of control, 48-h starved, or refed adult rats, is shown in Table 1 (column 2). Starvation or refeeding for 1 h resulted in significantly increased enzyme activities when compared with those in mitochondria from control fed rats, whereas refeeding for 2 h did not.

Total HMG-CoA synthase in mitochondria isolated from adult rats treated as shown was calculated as follows:

\[
\text{'Total' enzyme} = \text{active' enzyme} \times \frac{\text{activity without desuccinylating agents}}{\text{activity with desuccinylating agents}}
\]

(see Methods for details). Values are means ± S.D. with the number of animals in parentheses. Active or total HMG-CoA synthase which is significantly different from that in mitochondria isolated from control rats: *P<0.02, **P<0.01.

<table>
<thead>
<tr>
<th>HMG-CoA synthase</th>
<th>Group</th>
<th>Active enzyme (m-unit/mg)</th>
<th>Total enzyme (m-unit/mg)</th>
<th>Succinylation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>15.0 ± 1.3</td>
<td>23.8 ± 1.3</td>
<td>40.7</td>
</tr>
<tr>
<td>Starved</td>
<td></td>
<td>23.5 ± 2.7**</td>
<td>27.8 ± 0.4**</td>
<td>16.5</td>
</tr>
<tr>
<td>Refed (1 h)</td>
<td></td>
<td>19.1 ± 0.8**</td>
<td>27.5 ± 0.3**</td>
<td>30.0</td>
</tr>
<tr>
<td>Refed (2 h)</td>
<td></td>
<td>16.1 ± 1.0</td>
<td>27.7 ± 0.5**</td>
<td>42.0</td>
</tr>
</tbody>
</table>

Table 1. Activity and succinylation state of HMG-CoA synthase in mitochondria isolated from control or treated rats

Discussion
HMG-CoA synthase activity appears to be controlled by two different mechanisms in adult rats.

In the normal fed adult rat the enzyme is 40% succinylated. This reservoir or inactivated enzyme allows for rapid desuccinylation and activation of HMG-CoA synthase activity as metabolic requirements of the animal change (e.g. fine control of HMG-CoA synthase activity with progressive starvation). Desuccinylation is induced by elevated plasma glucagon levels characteristic of starved rats. It is an insulin-independent effect, as mannoheptulose treatment of rats results in the same effect on the succinylation state of HMG-CoA synthase as glucagon treatment or starvation (see [12]).

Changes in absolute amounts of HMG-CoA synthase appear to be more acute long-term (48 h) responses to
Role of substrate availability on net l-lactate uptake by liver of fed and 24-h-starved rats

ANTONIO FELIPE, XAVIER REMESAR and MARÇAL PASTOR-ANGLADA*

Biochemistry and Molecular Biology Unit, Department of Biochemistry and Physiology, University of Barcelona, Av. Diagonal 645, 08071 Barcelona, Spain

L-lactate transport into liver parenchymal cells is carrier mediated and selectively inhibited by other metabolites such as pyruvate and ketone bodies [1–5]. This monocarboxylate carrier can respond to nutritional and hormonal stimuli such as starvation and diabetes by increasing its activity [4, 5]. Its K_M for l-lactate is in the physiological range of l-lactate concentrations in blood (around 3 mM, [5]) which suggests that substrate availability might modulate l-lactate influx into the hepatocytes. During fasting, l-lactate availability decreases, while net hepatic balance is markedly increased. Nonetheless, when livers of 48-h-starved rats were perfused with 0.4–1.5 mM-l-lactate, it was found that l-lactate uptake was a linear function of substrate concentration in the perfusate [6]. Thus, it is somewhat controversial to what extent substrate availability should be modulating lactate uptake by liver. This is the goal of the present work. To do so, we used a more physiological approach than those used in previous studies. We were able to modify l-lactate concentrations in the portal vein just by infusing l-lactate in the mesenteric vein of anesthetized intact animals. Fed and 24-h-starved Wistar rats (about 200 g body wt.) were laparatomized after pentobarbital anaesthesia and an indwelling catheter was placed into the mesenteric vein. A buffered solution of l-lactate was infused at the rate equivalent to 0.125, 0.25 and 0.5 times the basal l-lactate turnover rate (19 mg/min per kg body wt.). Control rats were infused with an l-lactate-free buffer. After a 5 min infusion at a rate of 90 µl/min, blood was sampled from the afferent and efferent vessels of the liver as previously described [7]. No changes in portal pH were found at any of the l-lactate infusion rates used. Blood samples were then deproteinized for l-lactate determination by using a standard fluorimetric method. l-lactate balances were calculated as reported before [7]. Basal lactate levels in portal 1 were 1.66 ± 0.02 µM (n = 6) and 1.15 ± 0.1 µM (n = 8) for fed and 24-h-starved rats respectively (P < 0.001). Infusion of l-lactate resulted in huge similar increases in portal l-lactate concentrations in both experimental groups (Fig. 1). When net l-lactate uptake by liver was plotted against l-lactate concentration in portal (Fig. 1), no saturability was observed and a linear correlation was found in 24-h-starved rats (r = 0.99). However, livers of fed rats did not show a net l-lactate balance unless portal concentrations had reached 5.5 mM. Then, a significant uptake was found, but it remained clearly below those values of fasted animals. The fractional extraction rate for the livers of 24-h-starved rats was about 45%, which means that the removal efficiency is high during starvation and does not depend on substrate availability, because it remained constant for all the l-lactate infusion rates used. The fractional extraction rate for l-lactate in fed animals was about 20% at the highest l-lactate portal concentration tested (about 5.5 mM). These results were not masked by any changes of pH values induced by l-lactate infusion, because they remained identical in all the animals used. It is known that l-lactate transport across the plasma membrane of the hepatocyte might be accelerated when a pH gradient (pH out < pH in) is present [1, 3]. These observations suggest that, after a 24 h fast, substrate availability limits l-lactate net uptake by liver. This finding is in agreement with previous reports using partial hepatectomy and suggest that the hepatic capacity for l-lactate disposal greatly exceeds its rate of provision to the liver [8]. Furthermore, net hepatic balance seems to be dependent on liver metabolism rather than on the activity of the l-lactate carrier. Indeed, it has been recently suggested that l-lactate transport into rat hepatocytes is unlikely to limit the rate of its metabolism [3].

*To whom correspondence should be addressed.