Mitochondrial enzymes responsible for oxidizing medium-chain fatty acids in developing rat skeletal muscle, heart, and liver

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Prior to weaning, medium-chain fatty acids constitute an important energy source in the developing rat. Fatty acid oxidation rates increase with age in most developing tissues, but the pattern of this increase may vary according to the role of the particular organ. In skeletal muscle, heart, and liver of developing rats, we measured mitochondrial activities of long- and short-chain enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase, and long- and short-chain acyl-CoA thiolase. In skeletal muscle, the pattern of development in fatty acid oxidation enzymes favored utilization of long-chain rather than medium-chain fatty acids. In liver, enzyme activities for medium-chain fatty acids were highest prior to weaning. Heart occupied a position intermediate between skeletal muscle and liver.

Keywords: enoyl CoA hydratase; 3-hydroxyacyl-CoA dehydrogenase; thiolase; rat; liver; heart; skeletal muscle

Introduction

In rat skeletal muscle, fatty acid oxidation increases from birth until adulthood.[1,2] In heart, data are less clear-cut; some investigators have shown that the fatty acid oxidation rate increases,[2,3] while others have documented a decrease in fatty acid oxidation during the same period.[4] In contrast to heart and skeletal muscle, fatty acid oxidation rates in liver are high soon after birth and consequently do not show as much increase as the other two organs.[1,4,5] Thus, the overall patterns of increase in fatty acid oxidation rates reflect the metabolic role of the organ in early development. Liver is responsible for fatty acid catabolism and gluconeogenesis in the period immediately after birth, while heart and skeletal muscle lag behind in development of fatty acid oxidation.

The lipid content of rat milk consists mainly of triacylglycerols of saturated fatty acids from 8 to 16 carbons and unsaturated 18-carbon fatty acids. Thirty-five percent of these fatty acids are from 8 to 12 carbons in length.[6] Additionally, these medium-chain fatty acids are preferentially absorbed and released into the portal blood during digestion.[6] Therefore, those enzymes acting on medium-chain-length CoA derivatives in liver should be of particular importance for regulating fatty acid oxidation in the suckling rat. The intramitochondrial enzymes that initially oxidize medium-chain-length fatty acids include medium-chain acyl-CoA dehydrogenase,[7] both long- and short-chain enoyl-CoA hydratase,[8] 3-hydroxyacyl-CoA dehydrogenase, and long-chain acyl-CoA thiolase.[9] Short-chain acyl-CoA dehydrogenase and short-chain acyl-CoA thiolase participate subsequent to the shortening of the chain length by the first step in oxidation. Only long-chain acyl-CoA dehydrogenase does not participate.

If substrate availability and organ utilization are major factors in the control of enzyme activity, we would expect the enzymes responsible for the oxidation of medium-chain fatty acyl-CoA derivatives to demonstrate prominent changes early in liver development. The changes would be less in early development

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in heart and skeletal muscle, and in these organs might not involve the enzymes for medium-chain fatty acid catabolism to as great a degree.

We previously examined the first step of fatty acid β-oxidation, dehydrogenation, and showed that activities of the three fatty acyl-CoA dehydrogenases increased with age in skeletal muscle and to a somewhat lesser degree in heart and liver.[10] To assess the other enzymes responsible for the breakdown of the medium-chain fatty acids, we examined skeletal muscle, heart, and liver for activities of long- and short-chain enoyl CoA hydratase,[8] 3-hydroxyacyl-CoA dehydrogenase and long-and short-chain mitochondrial acyl-CoA thiolase.[9] Since they were not commerically available, we synthesized the substrates for long-chain enoyl-CoA hydratase and long-chain acyl-CoA thiolase.

Materials and methods

Eight-week old male and midterm pregnant Sprague–Dawley rats were obtained from Harlan Sprague–Dawley Co. (Indianapolis, IN, USA). The animals were housed in solid-bottom plastic cages (males two per cage and pregnant females one per cage) in a 12-h light/dark controlled room at 23 ± 2°C. Animals had free access to tap water and were fed standard laboratory chow (Purina Rat Chow, Ralston Purina, St. Louis, MO, USA). Litters were culled to eight pups. The pups were sacrificed at approximately 10 AM at 2, 6, 11, 17, and 21 days of age for removal of heart, liver, and both gastrocnemius muscles. Adult animals were sacrificed similarly at 10 weeks of age.

The muscle, heart, and liver tissues were immediately suspended in cold 10 mM Tris–HCL (pH 7.4), 250 mM sucrose, and 2 mM EDTA (Seth's buffer) at a 1:9 w/v ratio. Each tissue preparation was minced into fine particles with scissors and hand-homogenized using a glass Duall homogenizer. Tissue homogenates were centrifuged at 600*g* for 20 min to remove cellular debris. Supernatants were centrifuged at 30,000*g* for 20 min to obtain a mitochondrial pellet. The 30,000*g* pellets were dissolved in a minimal volume of Seth's buffer with 0.1% Triton X-100, freeze-thawed twice and centrifuged at 15,000*g* for 10 min. All supernatants were measured for protein concentration as described by Bradford.[11]

Enzyme assays were performed at 25°C with a Bausch and Lomb Spectronic 1001 spectrophotometer equipped with a thermal printer to record changes in optical density values. Chemicals were purchased from Sigma Chemical Co., with the exception of the 2,3-hexadecenoyl-CoA and 3-ketohexadecanoyl-CoA.

2,3-Hexadecenoyl-CoA was synthesized by conversion of the free acid to the acid chloride with oxalyl chloride and subsequent reaction with the lithium salt of CoA in aqueous tetrahydrofuran at pH 8.[12]

3-Ketohexadecanoyl-CoA was synthesized from the ketal by the mixed anhydride method followed by removal of the ketal function with *p*-toluenesulfonic acid.[13]

Enoyl-CoA hydratase activities were measured by following the change in optical density of NAD.[14,15] Rates for short-chain activity were measured using crotonyl-CoA as substrate and for long-chain activity using 2,3-hexadecenoyl-CoA. Then 50–100 μg of mitochondrial protein from each tissue was assayed in a 1.0-mL reaction mixture containing 50 mM Tris–HCl, pH 8.9, 1.5 μM rotenone, 0.3 mM KCN, 0.2 units hydroxyacyl-CoA dehydrogenase, 2 mM glutathione, 2 mM NAD, 1 mM EDTA, and the short-chain substrate at 0.2 mM or the long-chain substrate at 0.05 mM.

3-Hydroxyacyl-CoA dehydrogenase activity was measured by following the change in optical density of NADH.[16] Twenty-five micrograms of mitochondrial protein was assayed in a 1.0-mL reaction mixture containing 50 mM potassium phosphate, pH 7.0, 0.06% Triton X-100, 0.12 mM NADH, and 60 μM *S*-acetoacetyl-CoA as the substrate.

3-Ketoacyl-CoA thiolase activity was measured at 303 nm by following the disappearance of the magnesium complex formed in the reaction mixture.[9] Using 0.33 μM *S*-acetoacetyl-CoA as the substrate, activities were measured with 15–50 μg of mitochondrial protein in a 1.0-mL, reaction mixture containing 100 mM Tris–HCl, pH 8.0, 0.13 mM CoA, 25 mM magnesium chloride, 30 mM potassium chloride, 0.06% Triton X-100, and 0.13% bovine and serum albumin. When using 0.33 μM 3-ketohexadecanoyl-CoA as the long-chain substrate, the reaction mixture was the same except that 30 mM sodium chloride was substituted for the potassium chloride.

Results

In skeletal muscle, short-chain enoyl-CoA activity rose from day 2 through adulthood except for a fall on day 11 (*Figure 1*). In heart, activity rose over the same period but to a greater degree. In liver, activity rose to a peak at 21 days and then fell in adulthood.

In skeletal muscle, long-chain enoyl-CoA hydratase activity rose from day 2 until adulthood (*Figure 2*). In heart, activity increased similarly but to greater degree, except for a fall on day 11. Activity in liver was at a very low level on day 2 and increased to a peak at day 17 after a fall on day 11.

Skeletal muscle activity of 3-hydroxyacyl-CoA dehydrogenase increased from day 2 until adulthood (*Figure 3*). In heart, activity increased over the same period with a peak on day 17. Activity in liver reached a peak on day 17.

Skeletal muscle activity of short-chain acyl-CoA thiolase increased throughout the developmental period studied (*Figure 4*). In heart, activity showed a marked increase from day 2 until adulthood. In liver, peak activity was reached at 17 days of age with little change overall.

Activity of long-chain acyl-CoA thiolase in skeletal muscle showed an increase over the period of development studied (*Figure 5*). In heart, activity increased from day 2 until adulthood with a decrease on day 21.

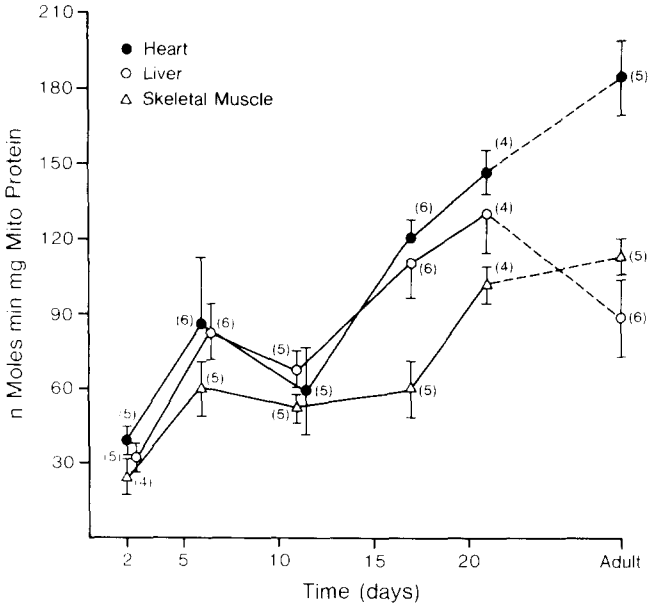

Figure 1 Activities of short-chain enoyl-CoA hydratase were measured in mitochondrial extracts of rat tissues during development. *N*, number of animals at each point with means ± SEM.

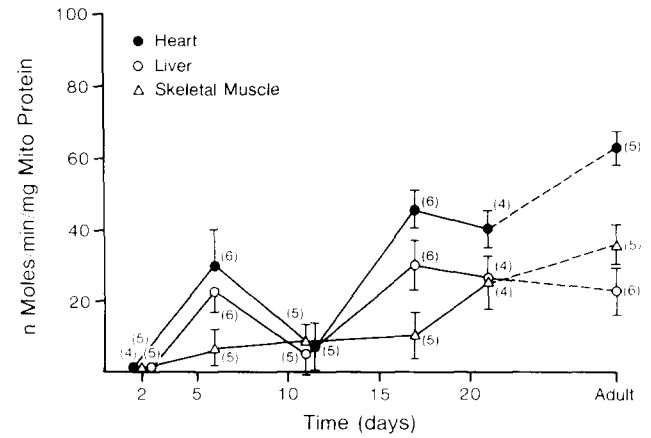

Figure 2 Long-chain enoyl-CoA hydratase activities.

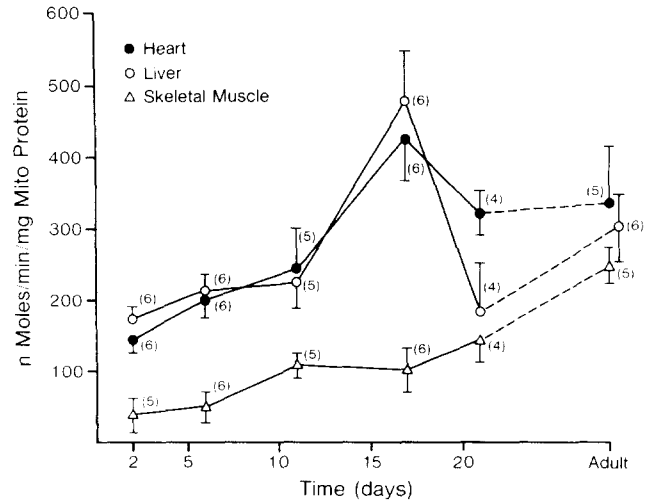

Figure 3 3-Hydroxyacyl-CoA dehydrogenase activities.

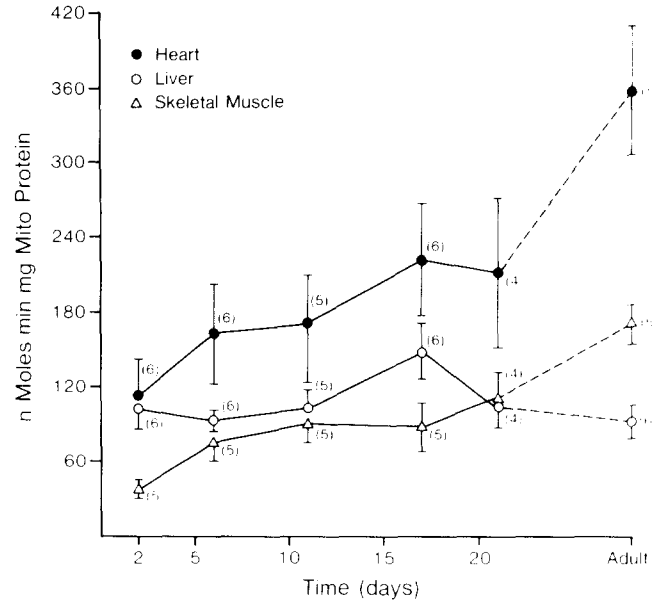

Figure 4 Short-chain acyl-CoA thiolase activities.

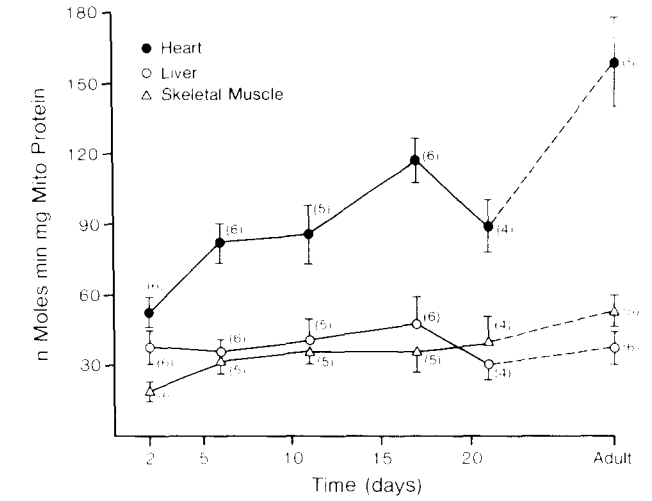

Figure 5 Long-chain acyl-CoA thiolase activities.

Activity in liver showed little change, with a modest peak at day 17.

Discussion

Skeletal muscle demonstrated an upward trend in activities of the fatty acid oxidation enzymes studied from birth until adulthood. In our previous paper dealing with the acyl-CoA dehydrogenases,[10] all three of the dehydrogenases also increased from birth until adulthood. Medium-chain fatty acids are most available early in the suckling period[6] and decline as the animal weans. The pattern of development in fatty acid oxidation enzymes in skeletal muscle, therefore, did not correspond with the period of highest medium-chain fatty acid availability. Rather, skeletal muscle development followed a pattern more suited to the

utilization of long-chain fatty acids in the mature animal.

In general, the activities of short- and long-chain hydratase, 3-hydroxyl-CoA dehydrogenase, and short- and long-chain thiolase in heart increased over the period studied from 2 days until adulthood. Exceptions to this trend were seen in peaks in long- and short-chain hydratase activity on day 7, a peak in activity of 3-hydroxyacyl-CoA dehydrogenase at 17 days, and a decrease in activity of long- and short-chain thiolase at 21 days. Long-chain acyl-CoA dehydrogenase increased from birth to adulthood, while short- and medium-chain acyl-CoA dehydrogenase demonstrated little change.[10] These results suggest that heart is reliant on medium-chain fatty acids to a greater degree than skeletal muscle, but not to the degree of liver. In contrast to long-chain acyl-CoA dehydrogenase, the enzymes responsible for medium-chain fatty acid catabolism tended to show peaks of activity during the period of greater medium-chain fatty acid availability. These phenomena may explain the discrepancies in heart fatty acid oxidation found by earlier workers.

Liver activities of these same enzymes showed only modest increases in activity over the period of development, with peaks usually occurring at 17 days. In our previous paper on the acyl-CoA dehydrogenases, liver activities of medium- and short-chain acyl-CoA dehydrogenases were relatively high during the suckling period, while long-chain acyl-CoA dehydrogenase did not reach its highest level until near the end of suckling and into adulthood.[10] Chalk et al. found a similar pattern in liver for the acyl-CoA dehydrogenases.[17] Foster and Bailey found that liver enoyl-CoA hydratase activity increased from birth into early adulthood, but they used only crotonyl-CoA as substrate.[18] For 3-hydroxyacyl-CoA dehydrogenase activity, they found a peak in activity at 17 days, just as we did. For mitochondrial acetoacetyl-CoA thiolase, they found a peak in activity at 13 days, while the peak for our data occurred on day 17 for both acetoacetyl-CoA and 3-ketohexadecanoyl-CoA. Taken together, these observations are consistent with a high degree of liver utilization of the available medium-chain fatty acids prior to weaning. Medium-chain fatty acids are oxidized in liver more rapidly than long-chain fatty acids during this period.[19]

Overall, activities of these intramitochondrial enzymes of fatty acid oxidation increased in activity in skeletal muscle and to a lesser degree in heart from birth until adulthood, while liver activities tended to reach a maximum prior to the time of weaning. Concurrent with the high medium-chain fatty acid intake during the suckling period, the liver rapidly adapted with high enzyme activities for medium-chain fatty acids throughout the period. Liver activities for enzymes metabolizing medium-chain fatty acids peaked earlier than long-chain acyl-CoA dehydrogenase. In contrast, the activities of the enzymes in skeletal muscle did not respond to the high intake of medium-chain fatty acids occurring immediately after birth. The pattern shown in heart muscle was intermediate between skeletal muscle and liver. This adaptive process may spare medium-chain fatty acids for liver metabolism and gluconeogenesis.

The mechanism of the early adaptation in liver for medium-chain fatty acid oxidation is unknown. Changes in mitochondrial mass are not responsible for the increase in oxidative rate. Escriva et al. previously demonstrated that the changes in hepatic enzyme activity shortly after birth are not due to increases in mitochondrial mass.[20] Our data were expressed with mitochondrial protein as the denominator, and therefore we are in agreement with the observations of Escriva et al. However, induction in activity may occur due to exposure of the liver to higher levels of circulating substrate. Medium-chain fatty acids are absorbed directly into the portal system and are first carried to the liver.[6] Factors other than substrate concentration, such as concentrations of metabolic intermediates, may also play important regulatory roles in fatty acid oxidation. McGarry and Foster have delineated the central role in liver of the removal of malonyl-CoA-mediated inhibition of fatty acid oxidation and the subsequent increase in oxidation rate.[21] Finally, the genetic regulation of the enzymes of medium-chain fatty acid oxidation in liver may differ from that in heart and skeletal muscle.

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