Volume 232, number 1, 156–158 FEB 05844 May 1988

Intramitochondrial factors controlling hepatic fatty acid oxidation at weaning in the rat

Jean-François Decaux, Danielle Robin, Pierre Robin, Pascal Ferré and Jean Girard

Centre de Recherches sur la Nutrition du CNRS, 9 rue Jules Hetzel, 92190 Meudon-Bellevue, France

Received 12 March 1988

Fatty acid oxidation was studied in isolated liver mitochondria of rats during the suckling-weaning transition. The oxidation rate of oleyl-CoA and palmitoylcarnitine was reduced 2.5-fold in rats weaned on a high-carbohydrate diet compared to suckling rats, when acetyl-CoA produced by β-oxidation was directed towards ketone-body synthesis. Weaning on a high-fat diet minimized this change. Channeling of acetyl-CoA towards citrate synthesis doubled the oxidation rate of both substrates in HC-weaned rats. Thus, in addition to changes in carnitine palmitoyltransferase I activity, the β-hydroxymethylglutaryl-CoA synthase pathway is also involved in the decreased fatty acid oxidation at weaning. This was confirmed by measurement of β-hydroxymethylglutaryl-CoA synthase pathway activity.

Fatty acid oxidation; Weaning; (Rat liver mitochondria)

1. INTRODUCTION

Weaning in the rat is concomitant with a large decrease in the capacity for hepatic fatty acid oxidation [1–3]. This is not linked to a mere variation in malonyl-CoA concentration but also involves decreased activity of carnitine palmitoyltransferase I (CPT I) and lower sensitivity of CPT I to inhibition by malonyl-CoA [3]. It does not preclude the possibility that, in addition, intramitochondrial steps such as the activity of specific enzymes of β-oxidation and ketone-body synthesis may become limiting in weaned rats.

In order to document whether the decreased fatty acid oxidation observed at weaning in rats is linked to coordinated alterations in various mitochondrial steps, experiments were performed on isolated mitochondria, i.e. in the absence of external effectors, using a polarographic technique. In addition, we examined whether the transition from milk to the adult diet was the major signal for the changes observed.

Correspondence address: P. Ferré, Centre de Recherches sur la Nutrition du CNRS, 9 rue Jules Hetzel, 92190 Meudon-Bellevue, France

2. MATERIALS AND METHODS

2.1. *Animals*

Rats of the Wistar strain bred in the laboratory were used. Animals were studied either 15 days after birth, i.e. during the suckling period or on day 28 after birth, i.e. 10 days after weaning on a high-carbohydrate low-fat diet (HC) or a high-fat carbohydrate-free (HF) diet. The exact composition of these diets is described elsewhere [4].

2.2. *Preparation of liver mitochondria*

Mitochondria were isolated according to [5]. Protein concentration was estimated by the method of Lowry et al. [6], using crystalline bovine serum albumin as standard.

2.3. *Polarographic measurements*

Measurements were carried out with an oxygraph (Gilson, model 5/6H) equipped with a 2 ml water-jacketed chamber maintained at 30°C and using a Clark electrode. Each preparation of mitochondria was tested for intactness by the respiratory control ratio method. The respiratory control ratio from glutamate + malate was measured as in [7]. The respiratory medium for oxidation of long-chain fatty acid CoA and carnitine esters was that described by Osmundsen and Sherratt [8]. After addition of mitochondria (2–3 mg protein), oxidation of the substrate was monitored in the presence of either malonate (10 mM) or malate (2.5 mM), both in the presence of 2,4-dinitrophenol (0.1 mM) (uncoupling conditions). Addition of oleyl-CoA (40 μM) or palmitoylcarnitine (10 μM) initiated the reaction. The rate of oxygen consumption due to oxidation of substrate was taken as the linear rate of oxygen consumption in the presence of this substrate and 2,4-dinitrophenol minus

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

that consumption in the presence of 2,4-dinitrophenol alone. Rates of fatty acid utilization were calculated by dividing the rate of oxygen consumption by the ratio oxygen consumed (nmol·min^{-1}·mg protein^{-1})/fatty acid utilized (nmol·min^{-1}·mg protein^{-1}). For oleyl-CoA and palmitoylcarnitine in the presence of malonate, the theoretical value of this ratio is 15 and 14, respectively, since acetyl-CoA is channeled exclusively towards acetoacetate formation [7–9]. In the presence of malate and dinitrophenol, citrate is the main end-product and the theoretical ratio is 24 and 22, respectively [7,10–12]. These values have been validated previously [8].

2.4. *Measurement of β-hydroxymethylglutaryl-CoA synthase pathway activity*

Assay of the β-hydroxymethylglutaryl-CoA synthase pathway was carried out by the method of Williamson et al. [13].

2.5. *Chemicals*

Substrates and cofactors of the best available grade were obtained from Boehringer (Meylan, France) and bovine serum albumin fraction V (fatty acid-free) was purchased from Sigma (St. Louis, MO) and dialysed before use.

2.6. *Statistics*

Results are presented as means ± SE. Statistical significance of differences was assessed by Student's *t*-test.

3. RESULTS AND DISCUSSION

Measurement of oxygen consumption and respiratory control ratio from glutamate + malate shows (table 1) that oxygen utilization was not different among the three groups of rats and that the respiratory control ratio was always greater than 6. Similar results (not shown) were obtained with succinate as respiratory substrate. This indicates that the respiratory chain capacity did not differ between the three experimental groups and that isolated mitochondria were intact.

The oxidation of oleyl-CoA reflects the activity of CPT I and II and of the intramitochondrial β-oxidation pathway. Acetyl-CoA formed from β-oxidation might enter either the β-hydroxymethylglutaryl-CoA pathway and form ketone bodies or the citric acid cycle and form CO_2.

Since the removal of acetyl-CoA from either pathway might influence the rate of fatty acid oxidation, oxidation of oleyl-CoA was measured under conditions where the end-products were well-known. The first series of experiments was performed in the presence of malonate, an inhibitor of succinate dehydrogenase which also exchanges with intramitochondrial malate. Thus, oxaloacetate is not available for citrate formation and the end-product of β-oxidation is acetoacetate [7,9].

Table 1

Respiratory rates and respiratory control ratio from glutamate + malate in isolated mitochondria from suckling, HC-weaned and HF-weaned rats

Group	Rate of oxygen consumption (nmol O·min^{-1}·mg protein^{-1})		Respiratory control ratio
	State 4	State 3	
15-day-old suckling	17 ± 2	120 ± 10	7.2 ± 0.3
28-day-old HC-weaned	19 ± 1	117 ± 9	6.4 ± 0.6
28-day-old HF-weaned	18 ± 2	128 ± 8	7.0 ± 0.2

Results are means for 10–12 determinations

Oxidation of oleyl-CoA by isolated mitochondria of rats weaned on a HC diet is halved when compared to that obtained in suckling rats. This suggests that, in addition to the potential regulation of CPT I by malonyl-CoA [3], intramitochondrial factors can contribute to the low rate of fatty acid oxidation in HC-weaned rats. When palmitoylcarnitine is used as substrate, the difference persists (fig.1). This indicates that the 2-fold lower activity of CPT I observed in HC-weaned rats when compared to suckling rats [7,14] is not solely responsible for the difference in oxidation rate of oleyl-CoA.

Next, we investigated whether a limited capacity for the utilization of acetyl-CoA in the β-hydroxymethylglutaryl-CoA synthase pathway might exist in HC-weaned rats when compared to suckling rats. This might lead to the accumulation of acetyl-CoA and subsequent inhibition of fatty acid oxidation. Acetyl-CoA was thus directed towards citrate synthesis by incubating mitochondria in the presence of malate, a donor of oxaloacetate, and 2,4-dinitrophenol. Under these experimental conditions, the oxidation rate of both oleyl-CoA and palmitoyl carnitine was increased by 80 and 85%, respectively, in mitochondria of HC-weaned rats (fig.1) as compared to incubation in the presence of malonate, whereas it was increased by 9 and 25%, respectively, in mitochondria of suckling rats.

This strongly suggests that in isolated mitochondria of HC-weaned rats, the low capacity for ketone-body synthesis from acetyl-CoA limits the

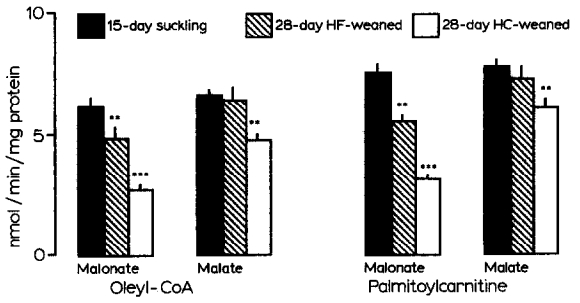

Fig.1. Oxidation of oleyl-CoA and palmitoylcarnitine in mitochondria isolated from 15-day old suckling, 28-day-old HF-weaned and 28-day-old HC-weaned rats. Measurements were performed in the presence of malonate or malate. ** $p < 0.01$, *** $p < 0.001$ when compared to suckling rats. Results are means for 6–8 determinations.

overall oxidation rate of oleyl-CoA. Measurement of the activity of the β-hydroxymethylglutaryl-CoA pathway (table 2 and [15]) confirms this assumption.

Nevertheless, even in the presence of malate, when ketone-body synthesis from acetyl-CoA is not rate-limiting, a small difference persisted between the oxidation rates of oleyl-CoA and palmitoylcarnitine in mitochondria of suckling and HC-weaned rats, suggesting that other steps of the β-oxidation process (CPT II, acyl-CoA dehydrogenases and enolases) might be affected by weaning, although to a smaller extent.

In order to determine whether the low ketogenic capacity observed in isolated mitochondria of HC-weaned rats was due to a developmental stage or to consumption of the HC-diet, similar studies were performed on rats weaned on a high-fat (HF) diet. In the presence of malonate, the oxidative capacity from both oleyl-CoA and palmitoylcarnitine was nearly 2-fold higher than in mitochondria from HF-weaned rats than in mitochondria from HC-weaned rats (fig.1) but was still lower (25–30%) than in mitochondria from suckling rats. In the presence of malate, the capacity for oleyl-CoA and palmitoylcarnitine oxidation was similar in mitochondria from suckling and HF-weaned rats. This suggested that the β-hydroxymethylglutaryl-CoA pathway was somehow rate-limiting in HF-weaned rats but to a smaller extent than in HC-weaned animals. This trend was confirmed by direct measurement of the enzyme activity (table 2).

It has been shown previously that both the activity of CPT I and its sensitivity to malonyl-CoA are greatly decreased at weaning by the consumption of a HC diet [3]. The present study shows that CPT I is not the only step which is affected and which can participate in limitation of fatty acid oxidation at weaning in the rat. Whether these changes are due to similar effectors (substrates, hormones) and mechanisms (transcriptional or post-transcriptional events) remains to be determined.

Table 2

β-Hydroxymethylglutaryl-CoA (HMG-CoA) synthase pathway activity in suckling, HC-weaned and HF-weaned rats

Group	HMG-CoA pathway activity (nmol acetoacetate formed · min^{-1} · mg $protein^{-1}$)
15-day-old suckling	48 ± 3
28-day-old HF-weaned	38 ± 2[a]
28-day-old HC-weaned	20 ± 1[b]

[a] $p < 0.05$ and [b] $p < 0.001$ when compared to suckling rats
Results are means for 6 determinations

REFERENCES

[1] Sly, M.R. and Walker, D.G. (1978) Comp. Biochem. Physiol. 61, 501–506.
[2] Benito, M., Whitelaw, E. and Williamson, D.H. (1979) Biochem. J. 180, 137–144.
[3] Decaux, J.-F., Ferré, P., Robin, D., Robin, P. and Girard, J. (1988) J. Biol. Chem., in press.
[4] Decaux, J.-F., Ferré, P. and Girard, J. (1986) Biol. Neonate 50, 331–336.
[5] Mersmann, H.J., Goodman, J., Houk, J.M. and Anderson, S. (1972) J. Cell Biol. 53, 335–347.
[6] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[7] Escriva, F., Ferré, P., Robin, D., Robin, P., Decaux, J.-F. and Girard, J. (1986) Eur. J. Biochem. 156, 603–607.
[8] Osmundsen, H. and Sherratt, H.S.A. (1975) FEBS Lett. 55, 38–41.
[9] Shepherd, D., Yates, D.W. and Garland, P.B. (1965) Biochem. J. 97, 38c.
[10] Di Marco, J.P. and Hoppel, C.L. (1975) J. Clin. Invest. 55, 1237–1244.
[11] Brady, L.J. and Hoppel, C.L. (1983) Am. J. Physiol. 245, E239–E245.
[12] Lopes-Cardozo, M. and Van Den Bergh, S.G. (1972) Biochim. Biophys. Acta 283, 1–15.
[13] Williamson, D.H., Bates, M.W. and Krebs, H.A. (1968) Biochem. J. 108, 353–361.
[14] Foster, P.C. and Bailey, E. (1976) Biochem. J. 154, 49–56.
[15] Hipolito-Reis, C., Bailey, E. and Bartley, W. (1974) Int. J. Biochem. 5, 31–39.