0031-3998/88/2303-0262$02.00/0
PEDIATRIC RESEARCH

Vol. 23, No. 3, 1988
Printed in U.S.A.

Control of Fatty Acid Oxidation by Intramitochondrial [NADH]/[NAD$^+$] in Developing Rat Small Intestine

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ABSTRACT. The oxidation of palmityl-coenzyme A and acetate to CO_2 by mitochondria isolated from rat small intestine increases 10-fold at the time of weaning (18–21 days of age). Carnitine palmitoyltransferase (EC 2.3.1.21) activity is 2-fold greater in mitochondria of suckling rat intestine compared to postweaned intestine. These data indicate that carnitine palmitoyltransferase does not control the increase in intestinal fatty acid oxidation during weaning. We have previously reported that the estimated intramitochondrial [NADH]/[NAD$^+$] as determined by the ratio of tissue levels of 3-hydroxybutyrate and acetoacetate is fivefold greater in suckling rat intestine compared to postwean animals. High intramitochondrial [NADH]/[NAD$^+$] which is present in suckling rat small intestine is associated with a decrease in citric acid cycle activity and β oxidation. The addition of acetoacetate causes a decrease in intramitochondrial [NADH]/[NAD$^+$]. The oxidation of acetate and glucose to CO_2 by suckling rat intestine mitochondria was stimulated by the addition of 1 mM acetoacetate. These data suggest that the lower rate of fatty acid oxidation by suckling rat small intestine is controlled by elevated intramitochondrial [NADH]/[NAD$^+$]. (*Pediatr Res* 23: 262–265, 1988)

Abbreviations

KRP, Krebs Ringer phosphate
CoA, coenzyme A

During the suckling period, rat pups absorb 75% of their caloric intake in the form of fatty acids (1). This high fatty acid intake by neonatal rats is associated with serum lipids that are twice that of the adult. Neonatal gut mucosa has higher levels of intracellular esterified fatty acids when compared to adult intestinal mucosal cells (2, 3).

At the time of weaning there is a 4- to 10-fold decrease in the concentration of medium chain fatty acids in the triglycerides of small intestine. Iemhoff and Hulsmann (4) and Hulsmann (5) reported that mitochondria isolated from adult rat small intestinal epithelium oxidized sodium octonaote to CO_2 at high rates. These authors reported that the rate of octonaote oxidation was approximately 15-fold more than that of palmitate. Hulsmann (5) further reported that palmitate was oxidized in preference to glucose adult rat small intestine. Windmueller and Spaeth (6), using a jejunal segment preparation, reported that unesterified fatty acids contributed to less than 4% of all CO_2 production.

During the suckling period, fatty acid oxidation by liver is more than that seen postwean. In association with the high fatty acid concentrations seen prior to weaning, liver carnitine palmitoyltransferase levels are elevated, thus enhancing fatty acid oxidation. Herein, we determined the capacity of small intestinal mitochondria of developing rat to oxidize fatty acids. We also determined the possible control mechanism of intestinal fatty acid oxidation.

MATERIALS AND METHODS

Isolation of small intestinal mitochondria. Fed sprague-Dawley rat pups (Charles River Labs, Cambridge, MA) were killed by decapitation in the morning and the proximal half of the small intestine was removed and homogenized using a Brinkman Polytron (Cincinnati, OH) (rheostat level 3.75 for two 10-s bursts). In order to isolate mitochondria of whole small intestine, homogenization was performed in a buffer containing 0.25 M sucrose, 20 mM Tris-Cl, pH 7.4, 1 mM EDTA, 5 mM $MgCl_2$, 1 mM ATP, and fat-free bovine serum albumin (4 mg/ml) with a final concentration of 1 g of tissue/10 ml of buffer. Cellular debris and nuclei were removed by centrifugation at 750 × *g* for 10 min at 4° C. The resulting supernatant was then centrifuged at 27,000 × *g* for 10 min at 4° C and the mitochondrial pellet was resuspended in the original homogenizing buffer (5–10 mg protein/ml).

Oxidation of palmityl Co-A and acetate. Oxidation of 0.2 mM [1-^{14}C] palmityl-CoA (0.1 Ci/mol) and 0.2 mM [1-^{14}C] acetate (0.15 Ci/mol) to CO_2 was determined by placing approximately 1 mg of mitochondria into Erlenmeyer flasks containing 1 ml of assay medium reported by Iemhoff and Hulsmann (4), containing 0.13 M sucrose, 33 mM Tris-Cl, pH 7.4, 15 mM K^+-phosphate, pH 7.4, 0.2 mM EDTA, 1.5 mM $MgCl_2$, 1.0 mM NAD, 0.03 mM cytochrome C, 2.0 mM *dl*-malate, 0.5 mM *l*-carnitine, 20 mM KCl, 10 mg bovine serum albumin (fat free). The metabolic flasks were sealed with rubber caps with polypropylene center wells (Kontes Glass Company, Vineland, NJ). The flasks were removed from ice and the reaction was started by placing the flask in a shaking water bath at 37° C. The reactions were terminated after a 60-min incubation by cooling on ice and adding 0.5 ml of 6 N perchloric acid. Hyamine hydroxide (0.3 ml) was injected through the rubber caps into the polypropylene center well to absorb CO_2 and the flasks were incubated 45 min at 37° C. The hyamine hydroxide was removed to scintillation vials containing 5 ml of Econofluor (New England Nuclear, Boston, MA) for determining radioactivity in a Beckmann LS 1800 liquid scintillation counter (Palo Alto, CA).

Changes in oxygen consumption by isolated intestinal mitochondria in the presence of acetate and acetoacetate were deter-

Received July 27, 1987; accepted October 27, 1987.
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Supported by NIH Grant 1-RD23 HD 16908-01.

mined using polarographic methods. Isolated adult intestinal mitochondria were incubated in a 3 ml polarographic chamber (Fisher model 5301 and 53) with the incubation mixture that was used in determination of fatty acid oxidation described earlier. Five mM Na-acetate and/or 10 mM acetoacetate were added to the incubation mixture. Approximately 1 mg of isolated mitochondria was added to 3 ml of incubation mixture. Change in oxygen saturation were determined using a Clark electrode. The rate of mitochondrial oxygen consumption was determined without substrate, with acetate, with acetoacetate, and with acetate and acetoacetate. Results were calculated as changes in percent oxygen saturation/min/mg protein.

In order to determine the utilization of palmityl-CoA by mitochondria during a 60-min incubation, the following procedure was used: after mitochondria were incubated in the assay solution described above, we saponified the assay mixture by adding potassium hydroxide to a final concentration of 50% (v/v) and then by heating the reaction mixture at 70° C for 30 min. After acidification with HCl the long chain fatty acids were extracted with pentane and evaporated to dryness in a liquid scintillation vial. The material was then counted in the Beckman LS 1800 liquid scintillation counter (Palo Alto, CA).

Oxidation of [UL-^{14}C] glucose. To determine [UL-^{14}C] glucose to CO_2 by intestinal tissue slices, rat pups were killed by decapitation. After the intestines were removed, they were flushed with ice cold KRP pH 7.4, slit length-wise, and placed in KRP. Intestinal slices of 0.5 mm thickness were prepared with McIlwain tissue chopper (Brinkman Instruments, Westbury, NJ). Slices were blotted dry, weighed, and placed into 10-ml Erlenmeyer flasks containing 1 ml of KRP and substrate (ice cold). Twenty-five to 50 mg of tissue was used unless otherwise designated in the text. Five mM glucose containing [UL-^{14}C]glucose with a final specific activity of 0.16 Ci/mol was used. The metabolic flasks were sealed in the presence of room air with rubber caps which were fitted with polypropylene center wells (Kontes Glass Co.). After incubating the flasks at 37° C for 30 min, the reaction was stopped by adding 0.5 ml of 6 N $HClO_4$ to the reaction mixture. $^{14}CO_2$ was collected and counted using the procedure described for fatty acid oxidation.

Carnitine palmitoyltransferase was determined by the isotope exchange method of Bremer and Norum (7). The protein concentration was determined using the Biuret method (8).

The radioisotopes were obtained from New England Nuclear (Boston, MA) and the biochemicals and enzymes were obtained from Sigma Chemical Co. (St. Louis, MO).

RESULTS

Fatty acid oxidation by developing rat intestine. The rate of oxidation of palmityl-CoA to CO_2 by isolated intestinal mitochondria of developing rat pups increases 10-fold at the time of weaning (Fig. 1). One possible explanation for this low rate of fatty acid oxidation during the suckling period is the dilution of the palmityl-CoA isotope by increased endogenous pools of fatty acids. The increase in fatty acid oxidation at the time of weaning could then be secondary to a decrease in endogenous pools of fatty acids when the rat pup changes from a high fat milk diet to a predominately carbohydrate diet. In order to rule out this possibility we have determined that the rate of CO_2 production from fatty acids is linear with respect to time during the assay period (30–60 min). The presence of large endogenous pools of fatty acids would cause greater dilution during short incubation periods and result in a lower calculated rate of oxidation. With our incubation period there is no change in the calculated rate of oxidation indicating a minimal dilution effect. In addition, preincubation of mitochondria of suckling and adult rat small intestine for 5, 10, and 15 min in the assay mixture without substrate did not alter the oxidation rate of C_{16} CoA. This suggests that endogenous pools of substrates that would dilute the [1-^{14}C] C_{16} CoA isotope do not exist in significant amounts.

We determined the activity of carnitine palmitoyltransferase system. As shown in Figure 2, carnitine palmitoyltransferase activity of rat intestine mitochondria is elevated during the suckling period and decreases after the time of weaning. The low rates of palmityl-CoA oxidation by intestinal mitochondria of suckling rats is seen despite high levels of carnitine palmitoyltransferase activity and therefore suggests that palmityl-CoA oxidation in mitochondria is not controlled by carnitine palmitoyltransferase.

Citric Acid Cycle Activity in Developing Rat Small Intestine. The rate of acetate oxidation by mitochondria isolated from rat intestine (Table 2) is low during the suckling period and increases abruptly during the time of weaning. This increase in oxidation of acetate is similar to that observed for fatty acids. While these

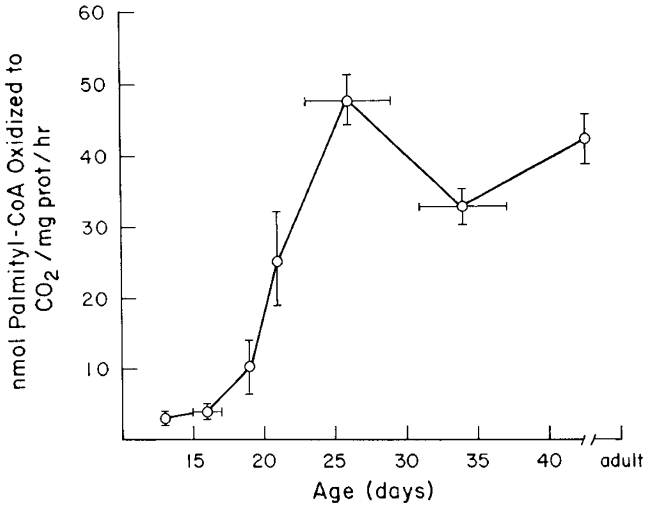

Fig. 1. Developmental profile of oxidation of [1-^{14}C] palmityl-CoA to $^{14}CO_2$ by isolated mitochondria of rat small intestine. The assay for palmityl-CoA oxidation is described in the experimental section. Values are expressed as mean ± SEM (*vertical symbols*) of at least four observations (*horizontal symbols* define the range of age for the values).

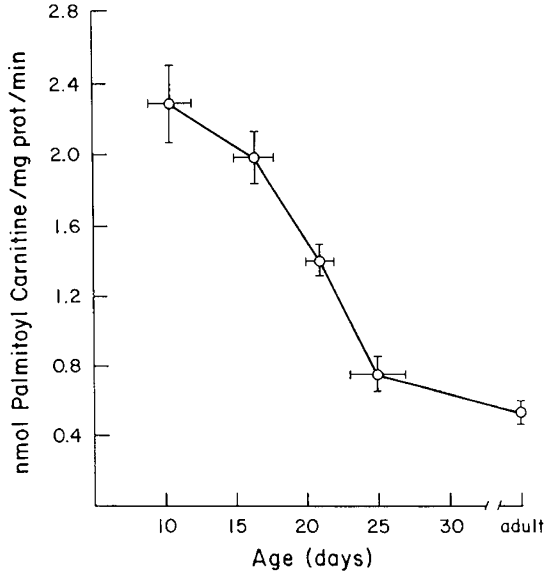

Fig. 2. Developmental profile of carnitine palmitoyltransferase activity in isolated mitochondria of rat small intestine. Carnitine palmitoyltransferase was determined by the method described in the experimental section. Values are expressed as mean ± SEM (*vertical symbols*) of at least four observations. Horizontal symbols define the range of age for the values.

Table 1. *Pentane extract of fatty acid incubation mixture**

Age (days)	Experiments	Fate of radioactivity (% of total [^{14}C]) Pentane extract	CO_2	Total accountable [^{14}C]
12–18	(37)	27.4 ± 1.7	9.6 ± 1.0	37.0 ± 1.3
Adult	(18)	24.1 ± 2.1	29.7 ± 1.8†	53.8 ± 2.2†

* Mitochondrial oxidation of palmityl-CoA to CO_2 was determined. A pentane extraction of the resulting assay mixture was performed and the [^{14}C] was determine in the pentane extract. Values are means ± SEM.

† Values significantly different from (12–18) $p < 0.001$, Student's t test.

Table 2. *Effect of acetoacetate on acetate and glucose oxidation by intestine of developing rat**

	Acetate oxidation to CO_2 isolated mitochondria		
	Control	+ 1 mM acetoacetate	
Age (days)	nmol acetate to CO_2/mg protein/h)		% of control
12–17	9.4 ± 2.7 (6)	24.5 ± 8.4 (6)	266 ± 39
Adult	27.6 ± 4.9 (3)	14.0 ± 3.7 (3)	52 ± 14
	Glucose oxidation to CO_2 by intestinal tissue slices		
	Control	+ 1 mM acetoacetate	
	(nmol glucose oxidized/mg wet wt tissue/h)		% of control
15	0.49 ± 0.02 (6)	0.70 ± 0.09 (6)	145 ± 19
Adult	2.49 ± 0.23 (3)	1.96 ± 0.25 (3)	74 ± 2

* Values are means ± SEM (no. of experiments).

Table 3. *Effect of acetic acid and acetoacetate on adult intestinal mitochondrial oxygen consumption**

Assay mixture	+ Acetic acid	+ Acetoacetate	+ Acetic Acid + Acetoacetate
2.31 ± 0.35	3.03 ± 0.43†	2.39 ± 0.30	2.32 ± 0.30

* Rates of oxygen consumption by adult intestinal mitochondria were determined with the assay mixture described in the text. Oxygen consumption rates were determined with the addition of acetic acid, acetoacetate, and both acetic acid and acetoacetate. Values represent change in percent oxygen saturation/mg prot/min (mean ± SE of six experiments).

† Value different from assay mixture alone ($p < 0.005$, paired Student's t test).

data suggest that citric cycle activity is suppressed during the suckling period and increases abruptly at the time of weaning, a decrease in β oxidation of long-chain fatty acids cannot be excluded. To determine if β oxidation in intestine is decreased during the suckling period, the presence of [^{14}C] in long-chain fatty acids in the assay medium after 1 h of incubation was determined by saponifying and extracting long-chain fatty acids with pentane. Approximately 27% of initial radioactivity was in the pentane extract of the assay medium in which isolated mitochondria of suckling rat intestine was used (Table 1). Only 10% of total [^{14}C] isotope was found in the form of CO_2. In contrast, in the postwean intestine 24% of the total radioactivity was in the pentane extract and 30% in the form of CO_2. There was no significant difference in the percent of [^{14}C] in the pentane extract between suckling and postweaned rat intestine, suggesting similar rates of palmityl-CoA utilization. However, there was a 3-fold increase in CO_2 in adult studies. These data indicate metabolism of palmityl-CoA to shorter chain fatty acids and water soluble metabolites by intestinal mitochondria of both suckling and postweaned rats.

Stimulation of Acetate and Glucose Oxidation by Acetoacetate During the suckling period. In the presence of 1 mM acetoacetate, the rate of acetate oxidation to CO_2 by mitochondria from suckling rat intestine increases 3-fold (Table 2). In the postwean animal the addition of acetoacetate decreased [1-^{14}C] acetate oxidation to [$^{14}CO_2$] by isolated mitochondria. In adult intestinal mitochondria, oxygen consumption rate increased by 32% with the addition of acetate ($p < 0.005$) (Table 3). The addition of acetoacetate did not cause a significant increase in oxygen consumption rate. The addition of both acetate and acetoacetate resulted no increase in intestinal mitochondrial oxygen consumption.

Glucose oxidation to CO_2 by tissue slices of suckling and postweaned rat intestine was also measured in the presence and absence of 1 mM acetoacetate. Glucose oxidation by intestine of suckling pups increased approximately 50% with the addition of acetoacetate, whereas in the postweaned intestine the addition of acetoacetate decreased glucose oxidation by 25% (Table 2). These data suggest that in whole intestine the intramitochondrial [NADH]/[NAD$^+$] regulates glucose oxidation in developing rat.

DISCUSSION

Medium and long chain fatty acid oxidation by isolated adult rat villus and crypt cells has been reported by Iemhoff and Hulsmann (4). Gangl and Ockner (10) using a perfused intestinal rat model reported that 42% of total radioactivity injected in the form of [^{14}C] palmitate can be recovered in a water soluble metabolite pool which includes CO_2 and keto acids. Only 28 and 16% of the radioactivity was recovered as phospholipids and triglycerides, respectively. We have shown that intestinal mitochondria isolated from adult rats oxidizes palmityl-CoA to CO_2. However, fatty acid oxidation by intestinal mitochondria from suckling rats appears to be suppressed. This was surprising in view of the high fat content of milk.

Possible mechanisms of suppression of fatty acid oxidation during the suckling period. During the suckling period the estimated intramitochondrial [NADH]/[NAD$^+$] is 5-fold more than the ratio determined after weaning. We have previously reported that the percent of intestinal pyruvate dehydrogenase in the active form is low during the suckling period (13), which is consistent with an increased intramitochondrial [NADH]/[NAD$^+$] (9). It has been reported that the rate of β oxidation of fatty acids is regulated by the intramitochondrial [NADH]/[NAD$^+$] (14, 15). Suppression of small intestinal fatty acid oxidation by an increase in intramitochondrial redox state has been previously suggested by Gangl and Ockner (10). Because the percent of [^{14}C] in the pentane extract of the assay medium of mitochondria from both suckling and postweaned rats were equal (27 and 24%, respectively), this indicates that use of palmityl-CoA is not decreased in intestinal mitochondria of suckling rat pups. This suggests that β oxidation is not decreased in intestinal mitochondria of suckling rat pups.

Increases in intramitochondrial [NADH]/[NAD$^+$] have been reported to decrease citric acid cycle activity primarily at citrate synthetase (EC 4.1.3.7) and at isocitrate dehydrogenase (EC 1.1.1.41) (16). Palmitoyl-CoA use is not decreased in mitochondria of suckling rat intestine, suggesting a decrease in citric acid cycle activity as cause of decreased in fatty acid oxidation to CO_2. In the studies in which [^{14}C] was measured in the form of CO_2 and long-chain fatty acids, the total accountable [^{14}C] in adult intestinal mitochondria experiments was 53.8% and in suckling intestinal mitochondria experiments was 37%. The unaccountable [^{14}C] must be in a water soluable fraction or short-chain fatty acids. This increase in the unaccountable [^{14}C] in suckling rat small intestinal mitochondria is consistant with a

decrease in citric acid cycle activity. Acetate oxidation to CO_2 is suppressed in mitochondria of suckling rat intestine supporting this conclusion.

Activation of fatty acid and glucose oxidation in suckling rat intestine by acetoacetate. Acetate and glucose oxidation by suckling rat intestine increased significantly when incubated with 1 mM acetoacetate. It has previously been reported that the addition of acetoacetate and 3-hydroxybutyrate can shift the intramitochondrial [NADH]/[NAD^+] through the activity of 3-hydroxybutyrate dehydrogenase. Hansford (9) has used changes in acetoacetate and 3-hydroxybutyrate concentrations in isolated heart mitochondria to determine the effect of changing intramitochondrial redox state on pyruvate dehydrogenase activity. In these studies, rotenone was added to the assay mixture in order to prevent the oxidation of acetoacetate. A 100-fold increase in the 3-hydroxybutyrate to acetoacetate ratio resulted in an increase in the intramitochondrial [NADH]/[NAD^+] by 12-fold. We were not able to use rotenone because it would decrease oxidation. However, we utilized short assay periods (15 min) and assumed that insignificant acetoacetate was oxidized. Under these conditions the addition of acetoacetate resulted in decreasing the ratio of tissue levels of 3-hydroxybutyrate and acetoacetate which would cause a decrease in intramitochondrial [NADH]/[NAD^+]. If acetoacetate is oxidized to acetyl-CoA, the resulting dilution of the acetate isotope at the intermediate acetyl-CoA would lower our calculated rate of oxidation. The stimulation of acetate oxidation may be greater than the calculated rates that we presented. The activation of acetate and glucose oxidation by the addition of acetoacetate in suckling rat intestine suggests a key regulatory role of intramitochondrial [NADH]/[NAD^+] during the suckling period.

In the postweaned animal, where the intestinal intramitochondrial [NADH]/[NAD^+] ratio is low, the addition of acetoacetate decreased acetate oxidation (Table 2). We have determined that the rate of oxygen consumption by adult intestinal mitochondria is stimulated by the addition of acetate. This increase in oxygen consumption is inhibited by the addition of acetoacetate. This suggests that the decrease in [1-^{14}C] acetate oxidation to [$^{14}CO_2$] is secondary to inhibition of mitochondrial oxidation.

We have previously reported (13) that the addition of 3-hydroxybutyrate, which increases intramitochondrial [NADH]/[NAD^+], inhibits glucose oxidation by postweaned rat intestine and does not effect glucose oxidation by intestine of suckling pups. These data indicate that in postweaned rat intestine, glucose oxidation can be inhibited by high intramitochondrial [NADH]/[NAD^+].

During the suckling period, oxidation of fatty acids by liver is elevated and appears to be regulated by an increase in carnitine palmitoyltransferase (11). Our data show that carnitine palmitoyltransferase is elevated in rat intestine during the suckling period, but is not associated with an increase in fatty acid oxidation (Fig. 2). Aas and Daae (12) have shown an increase in carnitine palmitoyltransferase activity in the liver of rats provided a high fat diet. However, unlike intestine, the increase in carnitine palmitoyltransferase activity in liver results in an increase in fatty acid oxidation (12). Our studies indicate that intestinal fatty acid oxidation to CO_2 is not controlled by carnitine palmitoyl transferase but by other factors such as intramitochondrial [NADH]/[NAD^+].

Acknowledgments. The authors thank Jasminka Ilich and Kris Snow for their technical assistance and Jeanne Ferretti for help with the manuscript.

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