ELSEVIER

Journal of Nutritional Biochemistry 12 (2001) 242–250

THE JOURNAL OF Nutritional Biochemistry

Effect of various levels of supplementation with sodium pivalate on tissue carnitine concentrations and urinary excretion of carnitine in the rat

Heather K. Nelson, Renee P. Lauber, Nancy F. Sheard*

Departments of Nutrition and Food Sciences and Medicine, University of Vermont, Burlington, VT 05405 USA

Received 10 January 2000; received in revised form 22 August 2000; accepted 6 December 2000

Abstract

In previous studies, sodium pivalate has been administered to rats in their drinking water (20 mmoles/L; equivalent to 0.3% of the diet) as a way to lower the concentration of carnitine in tissues and to produce a model of secondary carnitine deficiency. Although this level of supplementation results in a marked decrease in carnitine concentration in a variety of tissues, it does not produce the classical signs of carnitine deficiency (i.e., decreased fatty acid oxidation and ketogenesis). The present study was designed (1) to determine if increasing the level of pivalate supplementation (0.6, 1.0% of the diet) would further reduce the concentrations of total and free carnitine in rat tissues without altering growth or food intake, and (2) to examine the effect of length of feeding (4 vs. 8 weeks) on these variables. Male, Sprague-Dawley rats were randomly assigned to either a control (0.2% sodium bicarbonate) or experimental diet (0.3, 0.6, 1.0% sodium pivalate) for either four or eight weeks. Animals ($n = 6$/group) were housed in metabolic cages; food and water were provided ad libitum throughout the study. Supplementation with sodium pivalate did not alter water intake or urine output. Ingestion of a diet containing 1.0% pivalic acid decreased food intake (g/day; $P < 0.05$), final body weight ($P < 0.007$), and growth rate ($P < 0.001$) after four weeks. The concentration of total carnitine in plasma, heart, liver, muscle, and kidney was reduced in all experimental groups ($P < 0.001$), regardless of level of supplementation or length of feeding. The concentration of free carnitine in heart, muscle, and kidney was also reduced ($P < 0.001$) in rats treated with pivalate for either four or eight weeks. The concentration of free carnitine in liver was reduced in animals supplemented with pivalate for eight weeks ($P < 0.05$), but no effect was observed in livers from rats treated for four weeks. Excretion of total carnitine and short chain acylcarnitine in urine was increased in pivalate supplemented rats throughout the entire feeding period ($P < 0.001$). Free carnitine excretion was increased during Weeks 1 and 2 ($P < 0.01$), but began to decline during Week 3 in experimental groups. During Weeks 6 and 8, free carnitine excretion in pivalate supplemented rats was less than that of control animals ($P < 0.01$). In summary, no further reduction in tissue carnitine concentration was observed when rats were supplemented with sodium pivalate at levels greater than 0.3% of the diet. Food intake (g/day) and growth were decreased in rats fed a diet containing 1.0% sodium pivalate. These data indicate that maximal lowering of tissue carnitine concentrations is achieved by feeding diets containing 0.3% sodium pivalate or less.

Keywords: Carnitine; Pivalic acid; Carnitine deficiency; Rat

1. Introduction

Carnitine is a water soluble compound that is required for the transport of long chain fatty acids into the mitochondria [1,2]. It also participates in modulating the ratio of bound coenzyme A/free coenzyme A inside the mitochondria, and in the selective elimination of acyl residues [3]. Carnitine can be synthesized from the amino acids lysine and methionine in the liver and epidydimus of rats, and in the liver, kidney, and brain of humans [2]. Tissues, such as heart and muscle, take up carnitine from the plasma via specific transport proteins [1,4–7]. Carnitine deficiency has been described in humans [8] and may be classified as primary (genetic or myopathic) or secondary [9]. Secondary deficiencies are believed to be largely the result of increased losses of carnitine due to hemodialysis, Fanconi syndrome, and organic acidurias [9]. Specific drug treatments (i.e., pivampicillin, pivmecillinam) have also been associated with diminished concentration of carnitine in plasma and skeletal muscle in humans [10–13].

* Corresponding author. Tel.: +1-802-656-5485; fax: +1-802-656-0407.

E-mail address: nsheard@zoo.uvm.edu (N.F. Sheard).

0955-2863/01/$ – see front matter
PII: S0955-2863(00)00160-1

Pivalic acid (trimethylacetic acid) is commonly attached to drugs to increase their intestinal absorption [14]. It can be metabolized in a variety of tissues to form pivaloylcarnitine or pivaloylglucuronate and is excreted in the urine as these metabolites or as free pivalic acid [11,15–20]. In humans, treatment with pivalate-containing medications (i.e., pivampicillin, pivmecillinam) decreases the concentrations of total carnitine in plasma and muscle, and free carnitine in urine [10–13]. Broderick et al. [21] have suggested that if pivalate intake is sufficiently high, the excretion of these esters will exceed the sum of dietary carnitine intake and de novo synthesis of carnitine resulting in carnitine deficiency.

Bianchi and Davis [17] examined the feasibility of using pivalate ingestion as a means of developing an animal model of secondary carnitine deficiency in rats by supplementing drinking water with 20 mmol/L sodium pivalate for varying time periods (four days, two weeks, eight weeks). This level of supplementation, which approximates 0.3% (300 mg/g) of the diet, is effective in reducing carnitine concentrations in plasma, heart, liver, muscle, and kidney in the rat [17,21–24]. This effect on tissue carnitine concentrations in the rat was rapid and consistent, resulting in a 50% reduction in plasma, heart, and muscle concentrations within two weeks [17]. Although a reduction in carnitine concentration of this magnitude is significant, metabolic sequelae (e.g., decreased fatty acid oxidation and ketogenesis) associated with a deficiency of carnitine are usually not observed until tissue concentrations are reduced to approximately 30% of normal [24–29]. Fat deposition in the liver and muscle, however, may be an early sign of inadequate tissue carnitine [30,31].

Our objective was to develop an animal model that resulted in a greater reduction in tissue carnitine concentration than previously described. Finding no published reports using higher doses of sodium pivalate, we conducted the studies described below. The aims of the investigations were (1) to determine the effect of increased sodium pivalate ingestion (greater than 0.3% of the diet) on growth, food intake, and carnitine concentration in rat tissues and (2) to examine the effect of length of feeding (four vs. eight weeks) on these variables.

2. Methods and materials

2.1. Animals

In each study described, male, Sprague-Dawley rats (125–150 g; Charles River, Canada) were housed in metabolic cages and provided food and water ad libitum. The light cycle was 12 hours and the ambient room temperature was held constant at 20°C. Animals were randomly assigned to a control (0.2% sodium bicarbonate) or experimental diet (0.3, 0.6, 1.0% sodium pivalate) for either four or eight weeks. Sodium bicarbonate was added to the control diet at a level that was similar to that described by Bianchi and Davis [17]. In their study, control animals received an equimolar amount of sodium bicarbonate in order to maintain a constant sodium intake between groups. Supplementation with this amount of sodium bicarbonate results in tissue carnitine concentrations that are not different from those of rats that have been fed a control diet without sodium bicarbonate (results not shown).

In the four-week study, rats ($n = 6$/group) were randomly assigned to each of the following groups: control (0.2% sodium bicarbonate), 0.3% pivalate, 0.6% pivalate, 1.0% pivalate. In the eight week study, the 1.0% pivalate supplemented group was omitted. Food and water intake, urine output, and body weight were measured three times during each week of the study. Urine was collected for 72 hours weekly. This study was approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Vermont.

At the end of the experimental period (i.e., four or eight weeks), animals were anesthetized by an intraperitoneal injection of sodium pentobarbital (12–15 mg/100 g body weight). A midline incision was made, and a blood sample was obtained by cardiac puncture. Heparinized blood samples were kept on ice until centrifugation and separation of plasma from red blood cells could be performed. The heart was removed, rinsed in ice-cold saline, and frozen between Wollenberger clamps pre-cooled in liquid nitrogen. The liver, kidneys, and portion of femoral bicep muscle were removed, trimmed of fat, rinsed in ice cold saline, weighed, and frozen in liquid nitrogen. Tissues were kept at −70°C until carnitine concentration could be analyzed. Results are expressed as μmol/g wet weight.

2.2. Assay of liver triglyceride

Samples of frozen liver were homogenized in water and triglyceride content was determined using Sigma Kit #336-10. Results are expressed as mg/g wet weight.

2.3. Carnitine assay

The concentration of total carnitine was analyzed in plasma, tissues, and urine. Tissue samples were homogenized in ice-cold 7% perchloric acid with a Tekmar homogenizer. An aliquot of the homogenate was removed and dried under vacuum. Dried samples were resuspended in distilled water prior to base hydrolysis. The concentration of free carnitine in each sample was assayed using a radioenzymatic assay as described by Borum et al. [32].

The concentration of free carnitine was also analyzed in urine. Perchloric acid (7%) was added to the urine and the samples were centrifuged at 10,000 g. The supernatant was removed and the pellet was rinsed twice with 7% perchloric acid. The supernatant was dried under vacuum and resuspended in 1 M potassium phosphate buffer (pH = 7.0) prior to carnitine analysis. Shortchain acylcarnitine (SCAC) in

Table 1
Pivalate intake

Group	μmol/100 g Body Weight/ 24 hours
4 week	
0.3% Pivalate	239
(n = 6)	(8)
0.6% Pivalate	486
(n = 6)	(18)
1.0% Pivalate	749
(n = 6)	(28)
8 week	
0.3% Pivalate	200
(n = 6)	(18)
0.6% Pivalate	397
(n = 6)	(31)

Values represent average daily intake (SEM). Sodium pivalate was added to the synthetic diet (AIN 76B-65) and rats were fed for either 4 or 8 weeks.

urine was estimated by subtracting the concentration of free carnitine from that of total carnitine.

2.4. Statistical analysis

Results are expressed as the mean ± SEM. Comparison between groups for body weight, food and water intake, and urine output were made by two-way, repeated measures ANOVA. All other comparisons between groups were carried out by one-way ANOVA. If the F-value was significant ($P \leq 0.05$), Dunnett's test was performed to compare experimental groups to controls. Statistical significance is defined as $P \leq 0.05$. Prior to the initiation of the study, we calculated that 5–6 animals per group were needed to detect a difference between groups in the primary outcome measures (total and free carnitine concentration) with a power of 0.8 and an alpha of $p < 0.05$.

3. Results

The average daily intake of sodium pivalate for each supplemented group is shown in Table 1.

Food intake, expressed as g/day, was reduced in the pivalate supplemented rats in the 4 week trial ($P < 0.05$). The decline in food intake was most marked in the 1.0% pivalate supplemented animals (25% reduction compared to control animals; $P < 0.05$); intakes averaged 93% and 88% of control in the 0.3% and 0.6% pivalate groups, respectively, in the four week study. Average food intake in rats fed the 0.3% or 0.6% pivalate supplemented diets did not differ from control values in the 8 week study. In both the four and eight week experiments, no effect of pivalate supplementation was observed when food intake was expressed per unit of body mass. Neither water intake (ml/100 g body weight/24 hours) nor urine output (ml/100 g body weight/day) were altered by dietary pivalate supplementation at any level, regardless of length of feeding (data not shown).

The rate of growth (grams/day) in animals supplemented with pivalate for four weeks was reduced ($P \leq 0.001$). In particular, growth was markedly decreased in the 1.0% pivalate treated rats ($P < 0.001$; Fig. 1). The growth rate in the 0.6% pivalate animals was also lower ($P < 0.05$), although this finding was not replicated when the experiment was repeated for eight weeks (Fig. 1). Final body weight was also decreased in the 1.0% pivalate supplemented group ($P < 0.007$) (Table 2).

Organ weights (g wet weight/100 g body weight) of heart and liver were not different from control values in either study (i.e., four or eight weeks) (Table 2). At the end of four weeks, the wet weight of the kidney was increased in all pivalate supplemented groups compared to kidney wet weight in control animals ($P = 0.027$). This finding, however, was not observed after eight weeks of feeding. Dry weight of kidney at the end of four and eight weeks of pivalate feeding was not different from control values (data not shown).

The triglyceride content of liver was increased in pivalate supplemented rats following eight weeks of dietary treatment (control = 73.4 mg/g wet wt; 0.3% pivalate = 83.7 mg/g wet wt, 0.6% pivalate = 85.6 mg/g wet wt; $P = 0.02$).

The concentration of total carnitine in plasma, heart, liver, muscle and kidney was markedly reduced in all pivalate-treated rats when compared to controls in both four and eight week studies ($P < 0.001$; Table 3). The reduction in the concentration of total carnitine ranged from approximately 75–85% in plasma to 45–60% in tissues. Tissue carnitine concentration was not further reduced by the addition of sodium pivalate to the diet in amounts greater than 0.3%. The concentration of free carnitine in heart, kidney and muscle declined to a similar extent ($P < 0.001$) in rats supplemented with pivalate for either four or eight weeks (Table 4). In the liver, however, the concentration of free carnitine was reduced in animals treated with pivalate for eight weeks ($P < 0.05$), but not in those who were supplemented with this compound for four weeks (Table 4).

Total carnitine excretion (μmol carnitine/100 g body weight/24 hours) in urine was increased in all experimental groups when compared to control animals in both four and eight week studies ($P < 0.001$; Fig. 2). Free carnitine excretion was initially increased in all experimental groups during the first two weeks of feeding ($P < 0.01$), but by the fourth week, free carnitine excretion was below that of control animals ($P < 0.01$; Fig. 3). SCAC excretion was increased in all pivalate supplemented groups throughout both studies ($P < 0.001$; Fig. 4). Urinary creatinine was not affected by pivalate treatment (data not shown).

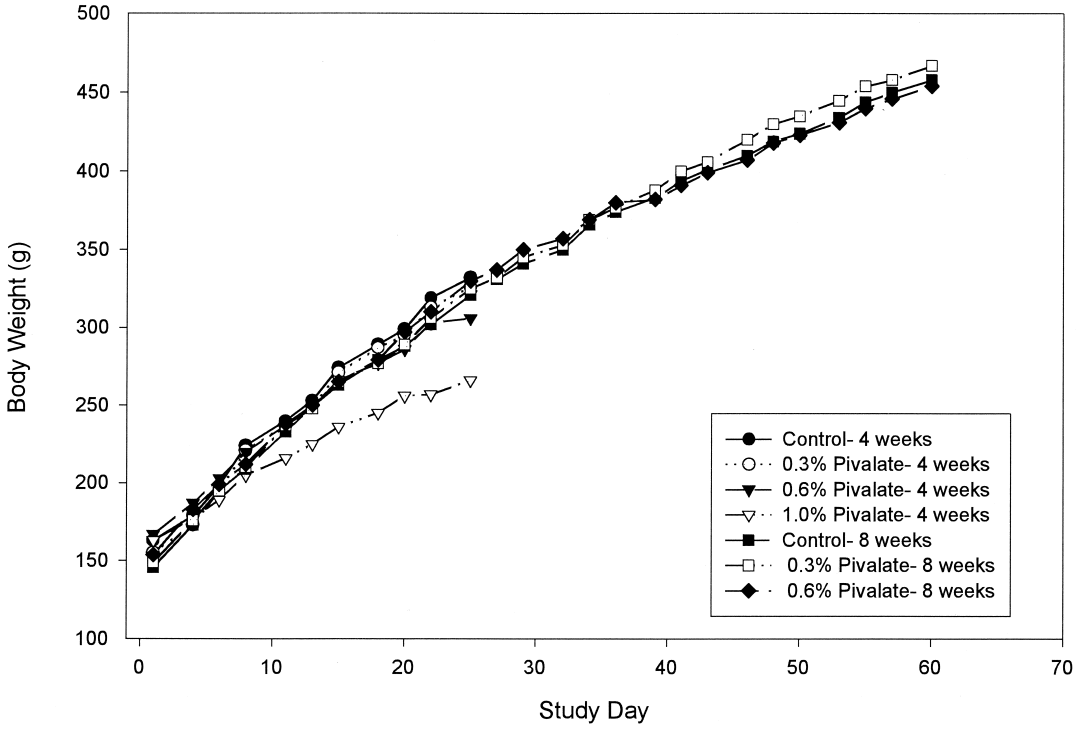

Fig. 1. Body weights of rats fed a control (0.2% sodium bicarbonate) or pivalate supplemented diet (0.3, 0.6, 1.0%) for either 4 or 8 weeks. Values represent group means; $n = 6$ per group.

4. Discussion

Pivalate is conjugated to specific pharmacologic agents in order to enhance their intestinal absorption [14]. Following uptake, the prodrug undergoes hydrolysis in the intestinal cell releasing pivalate and the parent drug [33]. The released pivalate is activated to the CoA thioester, pivaloyl-CoA [14,18]. The acyl portion of pivaloyl-CoA can be transferred to an acyl-acceptor such as carnitine, forming pivaloylcarnitine, which is excreted in the urine [14,18,34]. Alternatively free pivalic acid can be conjugated to glucuronic acid [15–17,20]. The transfer of pivalate from CoA to carnitine is catalyzed by carnitine acyltransferases [18,34]. Diep et al. [19] have reported that heart and brown adipose

Table 2
Final body weight and organ wet weight

Group	Body weight (g)	Heart (g/100 g body weight)	Liver	Kidney
4 week				
Control	359	0.310	3.99	0.653
($n = 6$)	(15.3)	(0.007)	(0.094)	(0.022)
0.3% Pivalate	335	0.362	4.41	0.826[a]
($n = 6$)	(17.7)	(0.032)	(0.329)	(0.066)
0.6% Pivalate	329	0.355	4.29	0.808[a]
($n = 6$)	(14.6)	(0.028)	(0.238)	(0.041)
1.0% Pivalate	278[a]	0.356	4.16	0.816[a]
($n = 6$)	(10.1)	(0.020)	(0.162)	(0.023)
8 week				
Control	464	0.306	3.40	0.644
($n = 6$)	(16.1)	(0.013)	(0.187)	(0.017)
0.3% Pivalate	477	0.302	3.70	0.648
($n = 6$)	(19.5)	(0.011)	(0.146)	(0.016)
0.6% Pivalate	456	0.311	3.54	0.610
($n = 6$)	(9.09)	(0.006)	(0.226)	(0.019)

Values are mean (SEM). Male, Sprague-Dawley rats were fed control or pivalate supplemented diets for either 4 or 8 weeks. Comparisons made by one-way ANOVA followed by Dunnett's test. Values with superscripts are different from controls. [a] ($P \leq 0.05$).

Table 3
Total carnitine concentrations in plasma and tissues

Group	Plasma (μmol/L)	Heart (μmol/g wet weight)	Liver	Kidney	Muscle
4 week					
Control	46.5	0.934	0.177	0.699	1.01
(n = 6)	(5.20)	(0.056)	(0.023)	(0.108)	(0.095)
0.3% Pivalate	11.8[a]	0.539[a]	0.0637[a]	0.372[a]	0.462[a]
(n = 6)	(0.784)	(0.029)	(0.013)	(0.050)	(0.072)
0.6% Pivalate	8.32[a]	0.467[a]	0.0721[a]	0.322[a]	0.424[a]
(n = 6)	(0.772)	(0.028)	(0.007)	(0.033)	(0.078)
1.0% Pivalate	11.7[a]	0.504[a]	0.0862[a]	0.355[a]	0.383[a]
(n = 6)	(0.693)	(0.049)	(0.008)	(0.034)	(0.050)
8 week					
Control	80.0	1.04	0.211	0.723	0.883
(n = 6)	(3.09)	(0.073)	(0.027)	(0.073)	(0.088)
0.3% Pivalate	12.0[a]	0.490[a]	0.098[a]	0.391[a]	0.294[a]
(n = 6)	(0.398)	(0.031)	(0.012)	(0.023)	(0.017)
0.6% Pivalate	12.6[a]	0.417[a]	0.090[a]	0.414[a]	0.368[a]
(n = 6)	(0.787)	(0.054)	(0.006)	(0.025)	(0.025)

Values are means (SEM). Male, Sprague-Dawley rats were fed control or pivalate supplemented diets for either 4 or 8 weeks. Comparisons made by one-way ANOVA followed by Dunnett's test. Values with superscripts are different from controls. [a] ($P \leq 0.001$).

tissue, not liver, play a greater role in the formation of pivaloylcarnitine in the rat.

The ingestion of pivalate (in an amount equivalent to the lowest dose ingested in this study) and its excretion as pivaloylcarnitine results in a reduction in carnitine concentration in a variety of tissues in the rat [17,21–23,28]. Despite this marked reduction (50%) in carnitine concentration, however, clinical symptoms of carnitine deficiency (e.g., decreased fatty acid oxidation and ketogenesis) have not been observed [17,26–29]. In the present study, our objective was to develop an animal model in which tissue carnitine concentrations were reduced to a greater extent than previously reported [17,21–23,28]. We hypothesized that increasing the dose of pivalate would increase the amount of carnitine required for its excretion, thereby reducing tissue concentrations further. We found no published reports of the effect of increased amounts of pivalate on tissue carnitine concentrations, although in a personal communication with the authors, Dr. A.T. Davis, reported that 50 mM sodium pivalate in drinking water (equivalent to 0.6% of the diet) resulted in decreased water and food intake and significant weight loss in rats. Previous studies in our laboratory suggested that adding the pivalate to the diet instead of to the water supply would not alter water or food

Table 4
Free carnitine concentrations in tissues

Group	Heart (μmol/g wet weight)	Liver	Kidney	Muscle
4 week				
Control	0.652	0.050	0.483	0.647
(n = 6)	(0.059)	(0.013)	(0.057)	(0.085)
0.3% Pivalate	0.322[a]	0.029	0.241[a]	0.234[a]
(n = 6)	(0.024)	(0.012)	(0.014)	(0.035)
0.6% Pivalate	0.287[a]	0.030	0.207[a]	0.204[a]
(n = 6)	(0.020)	(0.006)	(0.016)	(0.045)
1.0% Pivalate	0.291[a]	0.037	0.257[a]	0.178[a]
(n = 6)	(0.020)	(0.006)	(0.030)	(0.038)
8 week				
Control	0.724	0.055	0.696	0.671
(n = 6)	(0.022)	(0.011)	(0.056)	(0.040)
0.3% Pivalate	0.345[a]	0.031[b]	0.305[a]	0.200[a]
(n = 6)	(0.023)	(0.005)	(0.021)	(0.008)
0.6% Pivalate	0.328[a]	0.028[b]	0.332[a]	0.219[a]
(n = 6)	(0.049)	(0.006)	(0.026)	(0.022)

Values are means (SEM). Male, Sprague-Dawley rats were fed control or pivalate supplemented diets for either 4 or 8 weeks. Comparisons made by one-way ANOVA followed by Dunnett's test. Values with superscripts are different from controls. [a] ($P \leq 0.001$), [b] ($P < 0.05$).

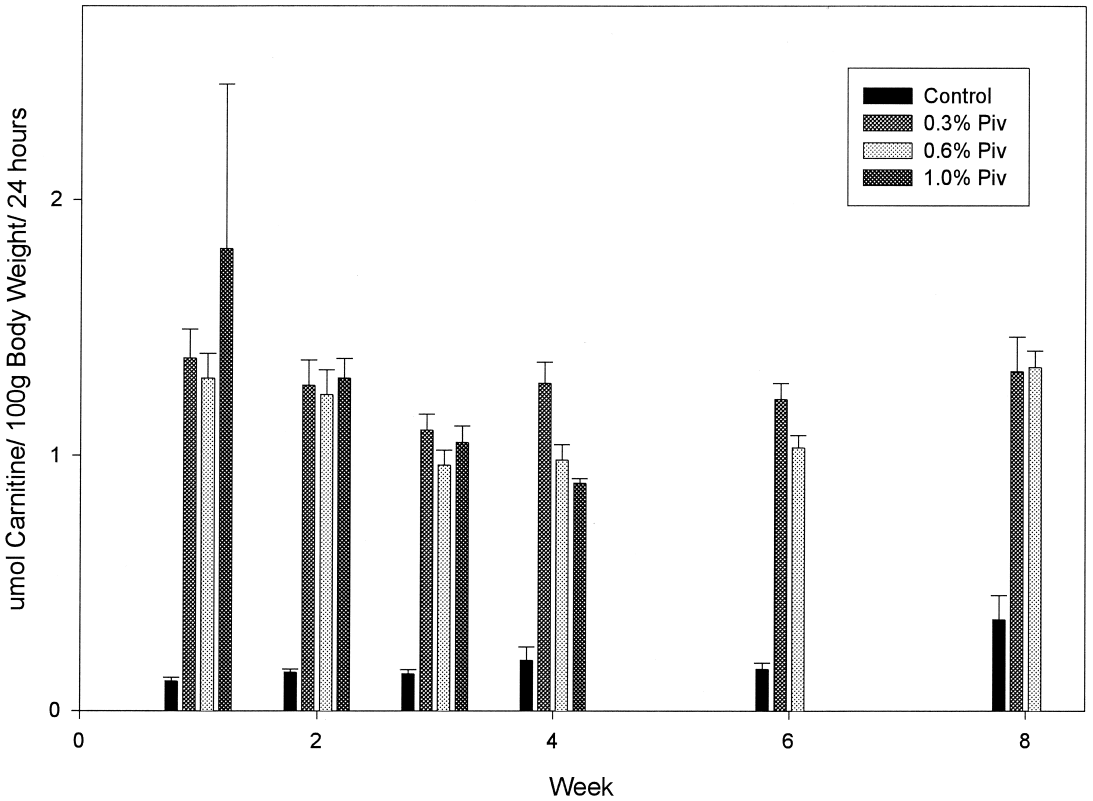

Fig. 2. Concentration of total carnitine in urine of rats fed a control (0.2% sodium bicarbonate) or pivalate supplemented diet (0.3, 0.6, 1.0%) for either 4 or 8 weeks. Values are group means (SEM); $n = 6–9$/group. Data were analyzed using 1-way ANOVA followed by Dunnett's test. Carnitine excretion in pivalate supplemented rats was significantly less than in control rats ($P < 0.001$) for all weeks.

intake, thereby resulting in an overall increase in pivalate ingestion. Indeed, water intake was not altered in pivalate supplemented animals in the present study, and food intake was only reduced at the highest level of pivalate supplementation (1.0%).

In the present report, we describe two feeding studies conducted several weeks apart. In the four week feeding trial, a marked reduction in growth was observed in pivalate supplemented rats. The rate of weight gain was decreased in both 0.6% and 1.0% pivalate supplemented animals, although final body weight at the end of four weeks was only different in the 1.0% rats. In the eight week study, neither the rate of growth nor final body weight in the 0.6% group were different from controls. No effect on growth was observed in rats fed a diet containing 0.3% pivalic acid for either four or eight weeks, a finding that is in agreement with others [17,21]. Morris et al. [23] reported a reduction in final body weight following 11–12 weeks of pivalate supplementation (equivalent to 0.3% of diet), although Broderick et al. [21] observed no effect on body weight after 26 weeks of supplementation at this level. Our findings suggest that pivalate ingestion reaches toxic levels, as evidenced by a decrease in body weight, when supplementation nears 1.0% of the diet.

With the exception of the kidney, final tissue weights (g wet weight/100 g body weight) were not altered by pivalate supplementation. In the kidney, an increase in wet weight was observed in pivalate supplemented rats, most likely as a result of the accumulation of pivalic acid and the metabolites of pivalate, pivaloylglucuronate and pivaloylcarnitine (SCAC) [17,20]. By eight weeks, however, this effect of pivalate supplementation had disappeared, suggesting an adaptative response of the kidney to chronic pivalate ingestion.

Triglyceride content of liver was increased in pivalate supplemented animals, a finding consistent with a decrease in carnitine concentration in this tissue [9] and one that is in agreement with previous studies that have examined the effects of pivalate administration [30,31]. The magnitude of the increase in our study was smaller than in previous reports, however, most likely due to differences in metabolic state. In our study, animals were not fasted prior to sacrifice; fasting increases liver triglyceride concentrations several-fold when carnitine concentration is reduced [35].

The increased excretion of SCAC in the urine of pivalate supplemented rats reported here agrees with the observation of Bianchi and Davis [17]. In contrast to their findings, however, both total carnitine and SCAC excretion in urine remained elevated throughout our studies. Free carnitine excretion did decline over time in pivalate supplemented rats, reflecting, perhaps, the reduction in total body carnitine and a slowing of carnitine turnover from tissues. Although

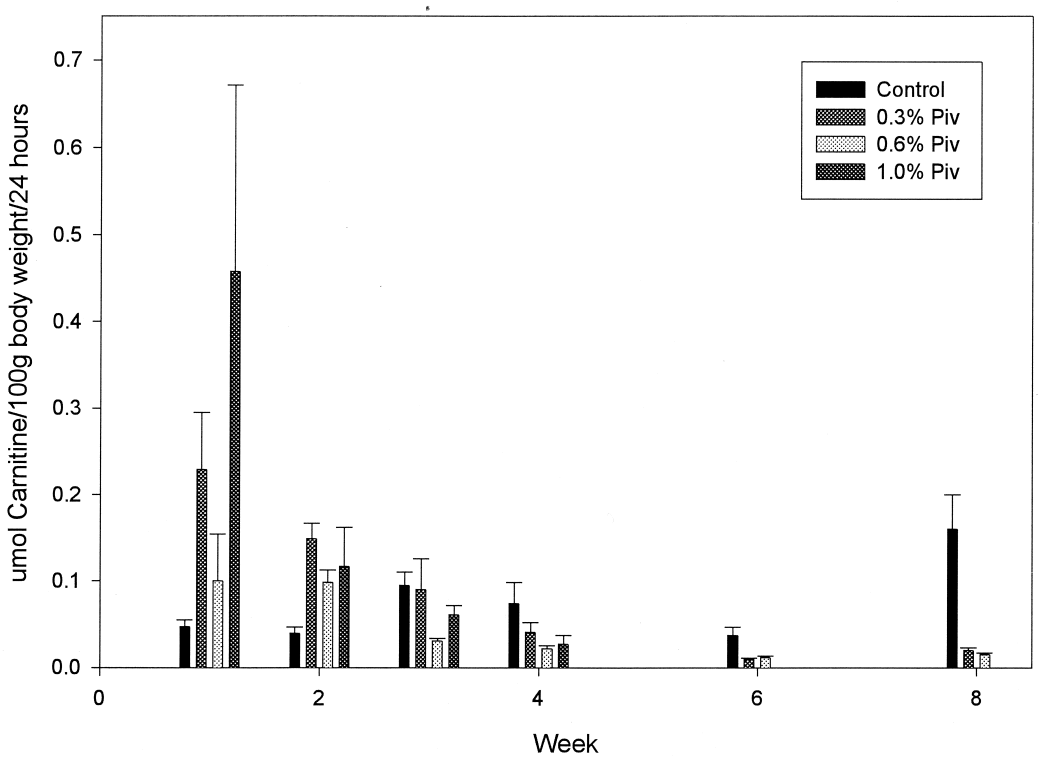

Fig. 3. Concentration of free carnitine in urine of rats fed a control (0.2% sodium bicarbonate) or pivalate supplemented diet (0.3, 0.6, 1.0%) for either 4 or 8 weeks. Values are group means; $n = 6–9$/group. Data were analyzed using 1-way ANOVA followed by Dunnett's test. In weeks 1 and 2, free carnitine excretion was greater in all pivalate supplemented groups ($P < 0.01$); in weeks 6 and 8 free carnitine excretion was lower in all pivalate supplemented groups ($P < 0.01$).

pivaloylcarnitine (SCAC) excretion was markedly elevated in supplemented animals, our findings, as well as those of other investigators [17,20], indicate that most of the ingested pivalic acid was excreted in some other form (i.e., 200–750 μmol of pivalic acid ingested/100 g body wt/day and only 1–1.5 μmol/100 g body wt/day of pivaloylcarnitine (SCAC) recovered in urine). In addition, while the amount of pivalic acid ingested increased with the level of supplementation, the excretion of pivaloylcarnitine did not, suggesting that this pathway for the metabolism and excretion of pivalic acid reaches its maximum level of activity at a dietary concentration of 0.3% or less.

As previously reported, total carnitine concentration was markedly reduced in plasma, heart, liver, kidney, and muscle of rats supplemented with 0.3% sodium pivalate when compared to control animals [17,21–23,28]. No further reduction in tissue or plasma carnitine concentration was observed when the level of supplementation was increased and/or continued for eight weeks. The concentration of free carnitine in heart, muscle, and kidney was similarly altered in rats treated with pivalate. In liver, however, free carnitine concentration was reduced only in animals treated with pivalate for eight weeks. This finding suggests that the liver, the primary site of de novo carnitine synthesis in the rat, has a greater potential to resist depletion of this nutrient and/or that, other tissues are more involved in the metabolism of pivalate [19].

Cederblad and Lindstedt [36] estimated that the rat synthesizes 20 μmol carnitine/kg of body weight/24 hours (2 μmol carnitine/100 g body weight/24 hours) and that the total amount of carnitine in the rat is approximately 35 μmol/100 g body weight. Our findings in urine indicate that the amount of carnitine (approximately 1.5 μmoles SCAC/100 g body weight/day) used to conjugate/detoxify pivalic acid was similar to the amount of carnitine that a normal rat would synthesize on a daily basis. In the present study, however, the amount of pivalate ingested (200–750 μmol/100 g/day) greatly exceeded the sum of carnitine synthesized over twenty-four hours and the amount of carnitine found in the whole body. This finding supports the role of alternative pathways for the metabolism of pivalate [17,20], but also indicate that in the rat there are protective mechanisms that prevent excessive depletion of tissue carnitine levels.

The lack of effect of increasing levels of sodium pivalate supplementation on tissue carnitine concentration and urinary SCAC excretion was somewhat surprising to us. One possible explanation is that the efficiency of absorption of pivalate declined as the dose increased. This is not likely, however, because pivalic acid is a relatively small, lipophilic molecule that is very well absorbed by the intestinal tract of the rat. Indeed, Shindo et al. [15] observed that 85% of an orally administered dose of pivalic acid was recovered in the urine within 24 hours. In addition, the

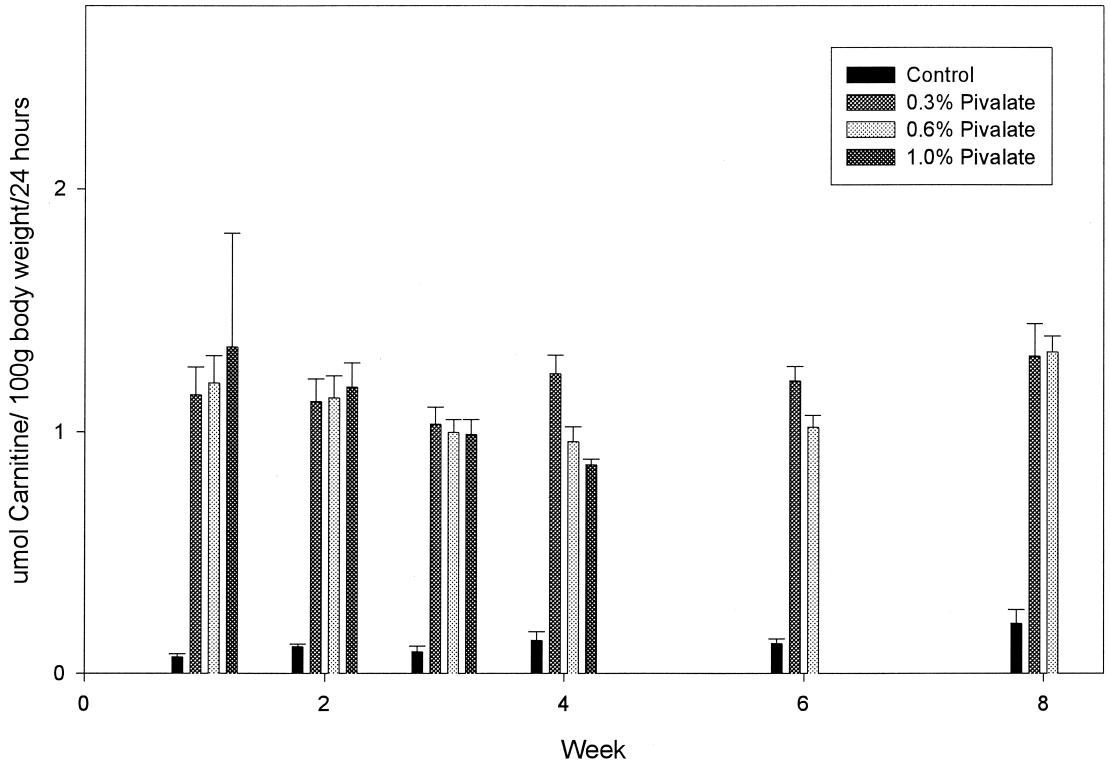

Fig. 4. Concentration of shortchain acylcarnitine (SCAC) in urine of rats fed a control (0.2% sodium bicarbonate) or pivalate supplemented diet (0.3, 0.6, 1.0%) for either 4 or 8 weeks. Values are group means (SEM); $n = 6–9$/group. Data were analyzed using 1-way ANOVA followed by Dunnett's test. Carnitine excretion in pivalate supplemented rats was significantly less than in control rats ($P < 0.001$) for all weeks.

percent of absorbed pivalate was likely maximized in the present study because the compound was mixed into the diet which was eaten over a 12 hour period rather than administered as a single, bolus dose.

A more likely explanation for our findings is that the alternative pathways for the metabolism of pivalic acid are more active in the rat, especially at higher intakes of the compound. Unesterified pivalic acid can be conjugated to several intracellular compounds, including carnitine and glucuronic acid [15–17,20]. Pivaloylcarnitine is the predominate byproduct of pivalate metabolism in humans, accounting for 96% of urinary pivalate [10,16]. In the cynomologus monkey, however, 70% of a dose of pivalate is excreted as pivaloylglucuronate with only 10% as pivaloylcarnitine [16]. Dziewaitkowski and Lewis [20] reported that pivalate is excreted both as the free acid and as pivaloylglucuronate in the rabbit and the rat. Bianchi and Davis [17] also observed that although pivalate supplemented rats excrete large amounts of pivaloylcarnitine, the majority of the administered pivalate was not excreted as this metabolite. Thus, in agreement with previous reports [17,20], our findings suggest that, in the rat, supplementation with pivalic acid likely results in the excretion of pivalate in several forms, including pivaloylcarnitine. In addition, our results clearly indicate that increasing the level of pivalate supplementation above 0.3% does not increase the amount of pivalate that is metabolized and excreted as pivaloylcarnitine.

In summary, high levels of sodium pivalate supplementation (1.0% of the diet) decrease food intake and alter the growth rate in rats. Increasing the level of supplementation above 0.3% of the diet does not lead to a further reduction in the concentration of carnitine in tissues. The lack of additional lowering of tissue carnitine concentration with increasing levels of pivalate ingestion suggests that other mechanisms of detoxifying pivalate, such as formation of pivaloylglucuronate or excretion of free pivalic acid, are more active in the rat, allowing for the preservation of total body carnitine.

Acknowledgments

This work was supported, in part, by grants from the American Heart Association, the Vermont Agricultural Experiment Station, and the University of Vermont-University Committee for Research and Scholarship. We also wish to thank Cristopher Amanti and Corey Nichols who assisted in the care and feeding of the animals used in these studies.

References

[1] J. Bremer, Carnitine metabolism and function. Ann. Rev. Physiol. 63 (1983) 1420–80.
[2] P.R. Borum, Carnitine. Ann. Rev. Nutr. 3 (1983) 233–59.

[3] L.L. Bieber, Carnitine. Ann. Rev. Biochem. 57 (1988) 261–83.
[4] J. Bahl, T. Navin, A. Manian, R. Bressler, Carnitine transport in isolated adult rat heart myocytes and the effect of 7,8-diOH chlorpromazine. Circ. Res. 48 (1981) 378–85.
[5] C. Cantrell, P.R. Borum, Identification of a cardiac binding protein. J. Biol. Chem. 257 (1982) 10599–604.
[6] C.J. Rebouche, Carnitine movement across muscle cell membranes. Studies in isolated rat muscle. Biochim. Biophys. Acta. 471 (1977) 145–55.
[7] J.H. Willner, S. Ginsburg, S. Dimauro, Active transport of carnitine into skeletal muscle. Neurology 28 (1978) 721–4.
[8] A.G. Engel, C. Angelini, Carnitine deficiency of human skeletal muscle with associated lipid storage myopathy: a new syndrome. Science 179 (1973) 899–902.
[9] C.J. Rebouche, D.J. Paulson, Carnitine metabolism and function in humans. Ann. Rev. Nutr. 6 (1986) 41–66.
[10] B. Melegh, J. Kerner, L.L. Bieber, Pivampicillin-promoted excretion of pivaloylcarnitine in humans. Biochem. Pharm. 36 (1987) 3405–9.
[11] B. Melegh, J. Kerner, V. Jaszai, L.L. Bieber, Differential excretion of xenobiotic acyl-esters of carnitine due to administration of pivampicillin and valproate. Biochem. Med. Met. Biol. 43 (1990) 30–8.
[12] K. Abrahamsson, B.O. Eriksson, E. Holme, U. Jodal, A. Jonsson, S. Lindstedt, Pivalic acid-induced carnitine deficiency and physical exercise in humans. Metabolism 45 (1996) 1501–7.
[13] E. Holme, C.E. Jacobson, I. Nordin, J. Greter, S. Lindstedt, B. Kristiansson, U. Jodal, Carnitine deficiency induced by pivampicillin and pivmecillinam therapy. Lancet 2 (1989) 469–72.
[14] A.A. Sinkula, S.H. Yalkowsky, Rationale for design of biologically reversible drug derivatives: prodrugs. J. Pharm. Sci. 64 (1975) 181–210.
[15] H. Shindo, E. Shigehara, K. Kawai, Whole-body autoradiographic study on the distribution of pivampicillin-^{14}C labeled at the ampicillin and ester moieties in mice, as compared with those of ampicillin-^{14}C, formaldehyde-^{14}C, and pivalic acid-^{14}C. Oyo Yakuri 17 (1979) 923–33.
[16] S. Vickers, C.A.H. Duncan, S.D. White, H.G. Ramjit, J.L. Smith, R.W. Walker, H. Flynn, B.H. Arison, Carnitine and glucuronic acid conjugates of pivalic acid. Xenobiotica 15 (1985) 453–8.
[17] P.B. Bianchi, A.T. Davis, Sodium pivalate treatment reduces tissue carnitines and enhances ketosis in rats. J. Nutr. 121 (1991) 2029–36.
[18] L.J. Ruff, E.P. Brass, Metabolic effects of pivalate in isolated rat hepatocytes. Toxicol. Appl. Pharm. 110 (1991) 295–302.
[19] Q.N. Diep, T. Bohmer, S. Skrede, Formation of pivaloylcarnitine in heart and brown adipose tissue in the rat. Biochim. Biophys. Acta. 1234 (1995) 65–70.
[20] D.D. Dziewiatkowski, H.B. Lewis, The metabolism of trimethylacetic (pivalic) and tertiary butylacetic acid. New examples of conjugation with glucuronic acid. J. Biol. Chem. 158 (1945) 77–87.
[21] T.L. Broderick, S. Christos, B.A. Wolf, D. DiDomenico, A. Shug, D. Paulson, Fatty acid oxidation and cardiac function in the sodium pivalate model of secondary carnitine deficiency. Metabolism 44 (1995) 499–505.
[22] Q.N. Diep, T. Bohmer, L. Schjerven, Reduced carnitine and ketogenesis in the pivampicillin treated rat. Bioch. Pharm. 44 (1992) 1226–8.
[23] G.S. Morris, Q. Zhou, B.A. Wolf, S.C. Christos, D.F. DiDomenica, A.L. Shug, D.J. Paulson, Sodium pivalate reduces cardiac carnitine content and increases glucose oxidation without affecting cardiac functional capacity. Life Sci. 57 (1995) 2237–44.
[24] B. Wittels, R. Bressler, Biochemical lesion of diphtheria toxin in the heart. J. Clin. Invest. 43 (1964) 630–7.
[25] B.Z. Simkhovich, Z. Shutenko, D.V. Meirena, K.B. Khagi, et al., 3-(2,2,2-Trimethylhydrazinium) proprionate (THP)—A novel γ-butyrobetaine hydorxylase inhibitor with cardioprotective properties. Bioch. Pharm. 37 (1988) 195–202.
[26] O.J. Heinonen, J. Takala, Moderate carnitine depletion and long-chain fatty acid oxidation, exercise capacity, and nitrogen balance in the rat. Pediatr. Res. 36 (1994) 288–92.
[27] N. Asaka, Y. Muranaka, T. Kirimoto, H. Miyake, Cardioprotective profile of MET-88, an inhibitor of carnitine synthesis, and insulin during hypoxia in isolated perfused rat hearts. Fundam. Clin. Pharmacol. 12 (1998) 158–63.
[28] H. Nakajima, N. Kodo, F. Inoue, Z. Kizaki, S. Nukina, A. Kinugasa, T. Sawada, Pivalate affects carnitine status but causes no severe metabolic changes in rat liver. J. Nutr. 126 (1996) 1683–7.
[29] P.B. Bianchi, A.T. Davis, β-hydroxybutyrate oxidation is reduced and hepatic balance of ketone bodies and free fatty acids is unaltered in carnitine-depleted, pivalate-greated rats. J. Nutr. 126 (1996) 2867–72.
[30] M. Tsoko, F. Beauseigneur, J. Gresti, I. Niot, et al. Enhancement of activities relative to fatty acid oxidation in the liver of rats depleted of L-carnitine by D-carnitine and a γ-butyrobetaine hydroxylase inhibitor. Bioch. Pharm. 49 (1995) 1403–10.
[31] P. Bianchi, D. Lehotay, A.T. Davis, Carnitine supplementation ameliorates the steatosis and ketosis induced by pivalate in rats. J. Nutr. 126 (1996) 2873–9.
[32] P.R. Borum, C. York, S. Bennett, Carnitine concentration of red blood cells. Am. J. Clin. Nutr. 41 (1985) 653–6.
[33] B. Melegh, B. Sumegi, A.D. Sherry, Preferential elimination of pivalate with supplemental carnitine via formation of pivaloylcarnitine in man. Xenobiotica 23 (1993) 1255–61.
[34] H.S. Sheratt, Acyl-CoA esters of xenobiotic carboxylic acids as biochemically active intermediates. Biochem. Soc. Trans. 13 (1985) 856–8.
[35] M. Tomomura, A. Tomomura, M.A. Dewan, M. Horiuchi, et al., Suppressed expression of the urea cycle enzyme genes in the liver of carnitine-deficient juvenile visceral steatosis (JVS) mice in infancy and during starvation in adulthood. J. Biochem. 121 (1997) 172–7.
[36] G. Cederblad, S. Lindstedt, Metabolism of labeled carnitine in the rat. Arch. Biochem. Biophys. 175 (1976) 173–80.