Dietary L-carnitine suppresses mitochondrial branched-chain keto acid dehydrogenase activity and enhances protein accretion and carcass characteristics of swine[1,2]

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ABSTRACT: A trial was conducted to biochemically explain the decreased lipid deposition and increased protein accretion observed in pigs fed carnitine. Our hypothesis was that an increase in the ratio of acetyl CoA:CoA-SH produced by stimulation of fatty acid oxidation by supplemental L-carnitine may decrease branched-chain α-keto acid dehydrogenase activity and increase pyruvate carboxylase activity. Such changes could reduce oxidative loss of branched-chain amino acids and provide more carbons for amino acid biosynthesis. Yorkshire gilts (n = 36; 12 per treatment) were fed a control diet or diets containing either 50 or 125 ppm of added L-carnitine during growth from 56 to 120 kg. After slaughter, the semitendinosus muscle and liver were collected for isolation of mitochondria and hepatocytes. Increasing dietary L-carnitine did not influence growth performance ($P > 0.10$) but linearly decreased ($P < 0.05$) 10th rib backfat thickness and increased (linear, $P < 0.05$) percentages of lean and muscle. The rates of [1-^{14}C]palmitate oxidation in isolated hepatocytes and isolated mitochondria, and incorporation of [^{35}S]methionine into the acid insoluble fraction of isolated hepatocytes were increased (linear, $P < 0.01$) in pigs fed L-carnitine. Flux through branched-chain α-keto acid dehydrogenase linearly decreased ($P < 0.01$) in isolated liver and muscle mitochondria with increasing dietary carnitine. Flux through pyruvate carboxylase was increased (linear, $P < 0.01$) in isolated mitochondria from liver of pigs fed carnitine, and assays with particle-free extracts indicated that the amount of mitochondrial pyruvate carboxylase was tripled by feeding carnitine (linear, $P < 0.01$). The association of increased protein accretion and reduced backfat thickness with greater rates of palmitate oxidation, more rapid flux through pyruvate carboxylase, and reduced flux through branched-chain α-keto acid dehydrogenase suggests pigs fed carnitine are more able to use fat for energy, divert carbon toward synthesis of amino acids, and spare branched-chain amino acids for protein synthesis.

Key Words: Carnitine, Growth, Pigs, Protein Synthesis

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Introduction

It has long been established that the primary role of carnitine in intermediary metabolism is to transport fatty acyl groups across the mitochondrial membrane into the mitochondrial matrix where β-oxidation occurs (Fritz, 1955). However, recent research suggests that carnitine also has other functions. Ji et al. (1996) reported dietary carnitine decreased lipid content by 73 and 43%, and increased protein content by 29 and 35% in Atlantic salmon filet and visceral organs, respectively. Also, palmitate oxidation, lactate-dependent gluconeogenesis, and protein synthesis in hepatocytes from adult

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salmon were increased 2.5-, 3-, and 2-fold, respectively, when carnitine was added to the diet.

Previous research conducted with pigs has shown that feeding L-carnitine during the growing-finishing phase resulted in increases in longissimus muscle area and decreases in backfat thickness and lipid accretion rates (Newton and Haydon, 1989; Owen et al., 1993). Our recent study (Owen et al., 2001) showed that feeding 50 ppm L-carnitine reduced average (2.9 mm) and 10th rib (5.1 mm) backfat thickness and increased longissimus muscle area (3.94 cm^2), percentage of lean (7.6%) and muscle (4.6%), as well as daily protein accretion rates (20 g/d) for growing-finishing pigs (34 to 103 kg). These effects of dietary carnitine on body composition in swine are similar to those reported for Atlantic salmon. However, the influence of dietary carnitine on intermediary metabolism in swine has not been investigated. Therefore, the objective of this study was to evaluate the influence of dietary carnitine on growth performance, carcass characteristics, and intermediary metabolism rates in finishing swine.

Table 1. Diet composition (as-fed basis)[a]

Item	Percentage
Corn[b]	73.80
Soybean meal (48% CP)	21.32
Soybean oil	2.50
Dicalcium phosphate (18% P)	0.85
Limestone	0.78
Salt	0.35
Copper sulfate	0.10
Antibiotic[c]	0.05
Vitamin and mineral premix[d]	0.25

[a]Formulated to contain 0.85% lysine, 0.60% Ca, and 0.50% P per kilogram of diet.
[b]L-Carnitine replaced corn to provide dietary carnitine levels of 50 and 125 ppm. Analyzed values were 9, 49, and 123 mg/kg for basal diet and two added levels, respectively.
[c]Provided tylosin at 18 mg/kg of complete diet.
[d]Premix provided per kilogram of complete diet: Mn, 12 mg; Fe, 165 mg; Zn, 165 mg; Cu, 16 mg; I, 0.3 mg; Se, 0.3 mg; vitamin A, 11,025 IU; vitamin D_3, 1,103 IU; vitamin E, 44 IU; menadione (menadione sodium bisulfate complex), 4.4 mg; riboflavin, 8.3 mg; D-pantothenic acid, 29 mg; niacin, 50 mg; choline, 166 mg; and vitamin B_{12}, 33 μg.

Materials and Methods

Animal Care. The experimental protocols used in the present study were approved by the Oklahoma State University Institutional Animal Care and Use Committee.

Animals, Diets, and Housing. Yorkshire gilts (n = 36; initially 56 kg) were blocked by weight and sire group in a randomized complete block design. Three pigs were housed per pen (2.13 m × 2.44 m) in an environmentally regulated finishing barn with total slatted concrete flooring. There were three dietary treatments, and each treatment had four replicate pens. Each pen contained a single-hole self-feeder and a nipple waterer to provide free access to feed and water. Pig weights and feed disappearance were recorded every 14 d to determine ADG, ADFI, and feed efficiency (**G/F**).

A basal diet based on corn and soybean meal (Table 1) was formulated to contain 0.85% lysine and 2.5% soy oil, and was fed in meal form. L-carnitine replaced corn in the basal diet to provide added dietary carnitine levels of 50 and 125 ppm. Chemical composition (CP, lipid, moisture, ash) of the diets was determined by AOAC (1990) procedures. Dietary carnitine was assayed by the method of Parvin and Pande (1977). Amino acids were analyzed by ion exchange chromatography following acid hydrolysis (Knabe et al., 1989). All nutrients either met or exceeded NRC (1988) requirement estimates for pigs between 50 and 109 kg.

Carcass Characteristics. After 18 to 24 h without feed, pigs were slaughtered, and standard carcass measurements were recorded. Carcasses were weighed immediately following slaughter and reweighed 24 h postmortem to record hot and chilled carcass weights, respectively. The heart, liver, kidney, and kidney fat were removed from each carcass following slaughter and weighed. After the liver was weighed, three 35- to 50-g samples were taken for proximate analysis and measurements of tissue carnitine and free amino acid concentrations. Samples for each analysis were taken from the same anatomical location. Additionally, three samples per muscle (25 to 50 g) were taken from the longissimus muscle (a three-rib sample taken between the 9th and 11th rib), the biceps femoris, and semitendinosus muscles of the ham. After the muscles were dissected, they were ground and subsampled for proximate analysis, and measurement of tissue carnitine (Parvin and Pande, 1977) and free amino acid concentrations (Borum, 1988). Total tissue carnitine was analyzed in a tissue extract that was subjected to heat and alkaline pH in order to hydrolyze carnitine from acyl carnitine. Proximate analysis (CP, lipid, moisture, and ash) of tissues was determined by AOAC (1990) procedures. Dressing percentage was determined as the ratio of hot carcass weight to live weight. Backfat thickness was measured at the first and last ribs, and last lumbar vertebrae from both the right and left sides of the carcass. All six measurements were used to calculate the average backfat thickness. Tenth-rib fat depth was measured at 3/4 the length from the midline of the longissimus muscle. The longissimus muscle area at the 10th rib was traced and measured with a grid. Carcass color, firmness, and marbling were measured according to NPPC (1991) guidelines. Percentage lean and muscle were calculated using NPPC (1991) equations for percentage lean with 5% fat and percentage muscle with 10% fat.

Blood Samples. Blood samples were collected via vena cava puncture between 2 and 3 h after feeders were removed from pens. Within 1 h of collection, samples were centrifuged at 2,500 × *g* for 25 min at 5°C. Plasma was harvested and stored at –20°C until analysis for plasma carnitine (Parvin and Pande, 1977).

Chemicals. Radiolabeled chemicals and liquid scintillation cocktail (Aquasol) were purchased from Du Pont

NEN Products (Boston, MA). L-carnitine was supplied by Lonza Inc. (Fair Lawn, NJ). Other chemicals (unless specified otherwise) and reagent enzymes were purchased from Sigma Chemical Co. (St. Louis, MO).

Animals for Assays. Because of time constraints for each assay performed for the intermediary metabolism portion of the experiment, a total of 24 gilts (eight pigs per treatment) were randomly selected from the original 36 gilts. Also, because of the number of assays conducted on each pig, only two pigs were slaughtered per day. The heaviest pig in each treatment based on weekly pig weights was selected for slaughter. For example, if the heaviest two pigs on test were from the 0 and 125 mg/kg L-carnitine treatment, then these pigs were selected for slaughter. The next day, the heaviest pig on the 50 mg/kg L-carnitine treatment was selected with one additional pig. This ensured that one pig per treatment was slaughtered every 2 d. All blocks (replications/treatment) were represented. All pigs removed from test had final BW between 118 and 121 kg.

Isolation of Liver Mitochondria. Immediately after the gilts were slaughtered, a 5- to 10-g sample of liver was excised and placed in 10 vol of ice-cold homogenization buffer (pH 7.4) containing KH_2PO_4, 5 m*M*; mannitol, 280 m*M*; and EDTA, 1 μ*M*. Mitochondria were isolated according to the method of Suarez and Hochachka (1981). Each sample was taken from the same anatomical location in the liver. The liver was minced into small pieces with scissors and homogenized using eight strokes in a glass homogenizer with a Teflon pestle driven by a motor. The homogenate was transferred to a centrifuge tube and centrifuged at 4°C and 2,000 × *g* for 5 min. The supernate was decanted into another tube and centrifuged at 9,500 × *g* for 10 min. The supernate was discarded, and the pellet was resuspended in 30 mL of the buffer and centrifuged at 9,500 × *g* for 10 min. The pellet was washed by resuspension and centrifugation two more times. After the final wash, mitochondria were resuspended in a small volume of homogenization buffer to contain 40 to 60 mg protein/mL. Protein content was determined by the biuret method using bovine albumin as the standard (Gornall et al., 1949).

Isolation of Muscle Mitochondria. A 20- to 30-g sample of the semitendinosus muscle from the ham was excised and placed in 10 vol of ice-cold homogenization buffer (pH 7.4) containing KH_2PO_4, 5 m*M*; mannitol, 280 m*M*; and EDTA, 1 μ*M*. All other procedures for isolation of mitochondria were the same as described for liver mitochondria.

Isolation of Hepatocytes. As the gilts were slaughtered, a 20- to 30-g sample of the liver was excised and used to isolate hepatocytes by collagenase digestion according to a modification of the method of Reese and Byard (1981). Liver was excised with only one exposed cut surface and immediately placed in ice-cold modified Hank's balanced salt solution (Hanks and Wallace, 1949). Each sample was taken from the same anatomical location in the liver. The liver sample then was placed on a gauze sponge (Johnson & Johnson Consumer Products, Inc., New Brunswick, NJ) that was secured over a funnel by an elastic band, and a blunt 22-gauge needle was inserted into a vein on the exposed cut surface. A piece of siliconized tubing (T5717-8; Baxter Healthcare Corporation, McGaw Park, IL) connected a 22-gauge needle to a peristaltic pump (Simon Varistaltic pump; 10-Manostat Corp.) The peristaltic pump was used to perfuse the liver with modified Hank's balanced salt solution (Hanks and Wallace, 1949) at a flow rate of 10 mL/min per gram of liver, until the liver was free of blood. The solution was kept at 40°C in a water bath before and during the perfusion. As soon as blood was cleared from the liver, as indicated by a change in color from a deep red to tan, the inlet of the pump was switched to a 125-mL Erlenmeyer flask containing 50 mL of a collagenase solution (modified Hank's balanced salt solution made in 1 m*M* $CaCl_2$ and 276 unit/mL collagenase, Sigma, C-5138) warmed to 40°C. The flask was placed under the funnel to allow recycling of the perfusion medium. After the liver became soft and swollen (15 to 20 min), perfusion was discontinued and the liver was transferred to a Petri dish and minced with scissors. The minces were put back into the flask containing the perfusion medium. From this point, all solutions used were kept on ice. The contents of the flask were filtered through two layers of gauze sponge. The liver minces on the gauze were stirred gently to facilitate separation of hepatocytes from the tissue residue. The filtrate, containing hepatocytes, was centrifuged at 4°C at 100 × *g* for 1.5 min. The resultant supernate was decanted, and the pellet of hepatocytes was resuspended in 40 mL of the washing medium (modified Hank's balanced salt solution made in 1 m*M* $CaCl_2$) and centrifuged again. Hepatocytes were washed two more times and then resuspended at a concentration of 20 to 30 mg protein/mL in washing medium. Viability of the hepatocytes, assessed by exclusion of trypan blue, was routinely higher than 95%. Protein content of the hepatocyte suspension was determined by the biuret method, using bovine serum albumin as the standard (Gornall et al., 1949).

Palmitate Oxidation. Oxidation of palmitate by isolated mitochondria and isolated hepatocytes was assayed by measuring the generation of radiolabeled CO_2 and acid-soluble products upon incubation with [1-^{14}C]Na palmitate, as described by Ji et al. (1996).

Pyruvate Carboxylase Flux in Isolated Mitochondria. Pyruvate carboxylase (**PC**) flux in isolated intact mitochondria was assayed by measuring pyruvate-dependent incorporation of [^{14}C] $KHCO_3$ into acid-stable radiolabeled products during 10 min of incubation at 37°C as described previously (Cyr et al., 1991; Ji et al., 1993).

Branched-Chain α-Keto Acid Dehydrogenase Flux in Isolated Mitochondria. Branched-chain α-keto acid dehydrogenase (**BCKDH**) flux was assayed in the same reaction mixture as that for measuring pyruvate carboxylase flux, except [1-^{14}C]Na isocaproate (50 m*M*, 0.2 mL) was used in place of pyruvate, and $KHCO_3$ (154 m*M*) was used instead of [^{14}C]$KHCO_3$. The vial was incubated at 37°C for 10 min; the $^{14}CO_2$ collected at the end of the

incubation period was taken as the measure of flux through the BCDKH (Ji et al., 1996).

Pyruvate Carboxylase Activity in Particle-Free Extracts of Liver Mitochondria. Particle-free extracts of mitochondria were prepared by homogenizing mitochondria (~20 mg protein) in 1 mL of detergent solution containing: deoxycholate (0.1%); Tris buffer (0.1 *M*, pH 7.2); and glutathione (1 m*M*). Measurement of PC activity was based on NADH oxidation upon coupling with excess malate dehydrogenase (Ji et al., 1996).

Protein Synthesis in Isolated Hepatocytes. Protein synthesis was assayed by measuring incorporation of [^{35}S]methionine into the TCA insoluble fraction of the reaction mixture, as described by Bhattacharya et al. (1985).

Statistical Analysis. The pen was the experimental unit for analyses of performance and carcass data, and the pig was the experimental unit for analyses of metabolic activities in liver and muscle. Data were analyzed as a randomized complete block design. Pigs were blocked on the basis of initial weight and sire. Analysis of variance was performed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Means were separated using linear and quadratic polynomials (Peterson, 1985). Carcass data and organ weights were analyzed using cold carcass weight as a covariate. A level of statistical significance was predetermined to be $P < 0.10$.

Results

Growth Performance and Carcass Characteristics. Dietary carnitine content in the basal and two carnitine diets was analyzed to be 9, 49, and 123 mg/kg, respectively. Dietary L-carnitine supplementation did not influence growth performance or dressing percentage ($P > 0.10$; Table 2). However, increasing dietary L-carnitine reduced average backfat thickness (linear, $P < 0.10$) and 10th rib backfat depth (linear, $P < 0.05$), and improved percentages of lean and muscle (linear, $P < 0.05$; Table 2). Increasing dietary L-carnitine had no effect on weights of liver, heart, kidney, or kidney fat ($P > 0.10$; Table 2). Visual scores for carcass muscling, longissimus marbling, and firmness were not affected ($P > 0.10$) by dietary treatment (Table 2). However, increasing dietary L-carnitine increased visual scores for longissimus color (quadratic, $P < 0.05$). Carnitine concentrations in liver, longissimus muscle, biceps femoris muscle, and plasma increased (linear, $P < 0.01$) with increasing dietary L-carnitine (Table 3).

Palmitate Oxidation. Liver mitochondria and hepatocytes isolated from pigs fed L-carnitine oxidized palmitate more rapidly (linear, $P < 0.01$) than did liver mitochondria and hepatocytes from pigs without added L-carnitine (Tables 4 and 5).

Pyruvate Carboxylase. Pyruvate carboxylase flux was higher in liver mitochondria isolated from pigs fed supplemental L-carnitine (linear, $P < 0.01$; Table 4). Assays of particle-free extracts of liver mitochondria showed that PC activity in lysed mitochondria rose with increasing dietary L-carnitine (linear, $P < 0.01$; Table 4).

Branched-Chain α-Keto Acid Dehydrogenase. Increasing dietary L-carnitine decreased ($P < 0.01$) BCKDH flux in liver mitochondria (Table 4). Flux through BCKDH in isolated mitochondria of muscle also was decreased (quadratic, $P < 0.01$; Table 4) with increasing dietary L-carnitine.

Protein Synthesis and Amino Acid Profile of Liver and Muscle Tissue. Incorporation of [^{35}S]methionine into TCA-precipitable material was higher (linear, $P < 0.01$) in hepatocytes isolated from pigs fed increasing dietary L-carnitine (Table 5). In longissimus muscle tissue (Table 6), significant increases or tendencies were found in aspartic acid (linear, $P < 0.05$); threonine (linear, $P < 0.10$); glutamic acid (linear, $P < 0.10$); proline (linear, $P < 0.10$); glycine (linear, $P < 0.10$); alanine (linear, $P < 0.05$); cysteine (linear, $P < 0.10$); methionine (linear, $P < 0.10$); leucine (linear, $P < 0.05$); tyrosine (linear, $P < 0.05$); phenylalanine (linear, $P < 0.05$); histidine (quadratic, $P < 0.10$); lysine (linear, $P < 0.10$); arginine (linear, $P < 0.10$); and tryptophan (linear, $P < 0.10$) by increasing dietary L-carnitine. In the bicep femoris muscle of the ham, concentrations of valine (linear, $P < 0.05$), isoleucine (linear, $P < 0.01$), leucine (linear, $P < 0.05$), phenylalanine (linear, $P < 0.10$), and lysine (linear, $P < 0.10$) were increased in pigs fed increasing levels of dietary L-carnitine (Table 7). Increasing dietary L-carnitine also increased the concentrations of aspartic acid (linear, $P < 0.05$), threonine (linear, $P < 0.01$), serine (linear, $P < 0.01$,) glutamic acid (linear, $P < 0.01$), proline (linear, $P < 0.01$), alanine (linear, $P < 0.10$), cysteine (linear, $P < 0.05$), methionine (linear, $P < 0.01$), and leucine (linear, $P < 0.05$) in the liver (Table 8).

Discussion

Our objectives were to examine the influence of supplemental carnitine on growth and carcass characteristics of pigs, using conditions similar to those in our previous study (Owen et al., 2001) and to identify possible metabolic alterations that might explain differences detected. Although supplemental L-carnitine increased carnitine concentrations in plasma, liver, and muscle tissues, adding L-carnitine at levels up to 125 ppm had no effect on growth performance. This indicates that either finishing pigs synthesized enough endogenous carnitine to support normal growth or the amount of carnitine in the basal diet was sufficient. However, supplemental L-carnitine significantly improved carcass characteristics and altered intermediary metabolism.

In the present trial, greater than 50 ppm added L-carnitine apparently was required to obtain optimal carcass characteristics and metabolic differences, whereas 50 ppm was sufficient in our previous dose titration study (Owen et al., 2001). This inconsistency could have been due to differences in leanness (49 vs 52% lean) or end weights (103 vs 120 kg) of pigs used in the two studies. As pigs get heavier and lean tissue deposition

Table 2. Performance and carcass characteristics of pigs fed L-carnitine from 56 to 121 kg[a]

Item	Added dietary L-carnitine, ppm 0	50	125	SEM
Growth performance				
ADG, kg	0.89	0.91	0.88	0.02
ADFI, kg	2.84	2.93	2.80	0.06
G/F, kg/kg	0.32	0.31	0.32	0.007
Carcass characteristics				
Live wt, kg	121.00	120.00	118.00	1.35
Dressing percentage	75.51	74.53	74.62	0.79
Average BF, cm[b]	3.05	2.97	2.92	0.10
10th-rib BF, cm[c]	2.43	2.24	2.16	0.13
Longissimus muscle, cm^2	37.35	37.87	40.77	1.64
Percentage lean[cd]	50.03	50.91	52.08	0.76
Percentage muscle[ce]	54.19	54.86	55.48	0.89
Organ weights, g				
Liver	1,680	1,666	1,710	75.7
Heart	411	419	388	23.5
Kidney	378	370	353	15.7
Kidney fat	1,160	1,114	1,044	90.6
Quality				
Muscle score[f]	2.40	2.64	2.68	0.16
Color[gh]	2.64	3.00	2.75	0.16
Firmness[f]	3.18	3.07	2.92	0.21
Marbling[f]	2.59	2.63	2.50	0.43

[a]A total of 36 pigs, three pigs/pen, four replicate pens/treatment.
[b]Linear effect of dietary L-carnitine ($P < 0.10$).
[c]Linear effect of dietary L-carnitine ($P < 0.05$).
[d]Percentage lean was calculated from NPPC (1991) equation for percentage lean with 5% fat.
[e]Percentage muscle was calculated from NPPC (1991) equation for percentage muscle with 10% fat.
[f]Carcasses were evaluated on a 3-point scale ranging from thin muscling (1) to extremely thick muscling (3).
[g]Quadratic effect of dietary L-carnitine ($P < 0.05$).
[h]Loins were evaluated on a 5-point scale according to NPPC (1991) procedures with 1 = light and 5 = dark.

decreases, higher concentrations of L-carnitine may be required to compensate for the higher lipid accretion rates.

The primary objective of the present study was to find metabolic evidence to explain the improvement in carcass characteristics observed in our previous work (Owen et al., 2001). To investigate this, we applied a metabolic model used by Ji et al. (1996) in their work with Atlantic salmon. This metabolic model anticipated that, by promoting fatty acid oxidation, added dietary L-carnitine could increase mitochondrial ratios of acetyl CoA/HS-CoA and ATP/ADP, thereby altering flux through BCKDH and PC. Accelerated β-oxidation should stimulate flux through PC, which is activated by acetyl

Table 3. Tissue carnitine concentrations in liver, longissimus muscle, biceps femoris, and plasma[a]

Item	Added dietary L-carnitine, ppm 0	50	125	SEM
Whole tissue, nmol/g				
Liver[b]	93.4	123.9	155.1	12.5
Longissimus muscle[b]	864.6	1,316.5	1,569.4	59.7
Biceps femoris[b]	838.7	1,239.8	1,640.2	42.9
Plasma carnitine, μM				
Total[b]	6.2	10.7	14.3	0.96
Free[b]	5.0	8.4	12.0	0.84
Esters[c]	1.2	2.3	2.2	0.41

[a]A total of 24 pigs, eight pigs/treatment.
[b]Linear effect of dietary L-carnitine ($P < 0.01$).
[c]Linear effect of dietary L-carnitine ($P < 0.10$).

Table 4. Effect of dietary L-carnitine on palmitate oxidation, pyruvate carboxylase, and branched-chain α-keto acid dehydrogenase in liver mitochondria; branched-chain α-keto acid dehydrogenase in muscle mitochondria; and pyruvate carboxylase activity in particle-free liver extracts of pigs[a]

	Added dietary L-carnitine, ppm			
Item	0	50	125	SEM
Liver mitochondria				
Palmitate oxidation, nmol·mg protein^{-1}·h^{-1} [bc]	10.6	11.9	15.3	0.62
Pyruvate carboxylase flux, nmol·mg protein^{-1}·h^{-1} [cd]	20.4	30.9	44.2	1.86
Branched-chain α-keto acid dehydrogenase flux, nmol·mg protein^{-1}·h^{-1} [cef]	82.2	60.4	54.1	3.06
Muscle mitochondria				
Branched-chain α-keto acid dehydrogenase flux, nmol·mg protein^{-1}·h^{-1} [ceg]	108.8	110.1	86.5	3.31
Liver mitochondrial extracts				
Pyruvate carboxylase activity, nmol·mg protein^{-1}·h^{-1} [cfh]	0.09	0.15	0.26	0.01

[a]A total of 24 pigs, eight pigs/treatment.
[b]Palmitate oxidation was measured as generation of radiolabeled CO_2 and acid-soluble products from [1-^{14}C]palmitate.
[c]Linear effect of dietary L-carnitine ($P < 0.01$).
[d]Pyruvate carboxylase flux was assayed as pyruvate-dependent incorporation of [^{14}C]$KHCO_3$ into acid-stable products.
[e]Branched-chain α-keto acid dehydrogenase flux was assayed as generation of $^{14}CO_2$ from [1-^{14}C]isocaproate.
[f]Quadratic effect of dietary L-carnitine ($P < 0.10$).
[g]Quadratic effect of dietary L-carnitine ($P < 0.01$).
[h]Pyruvate carboxylase activity was assayed by coupling with malic dehydrogenase and measuring the resultant pyruvate-dependent oxidation of NADH as the decrease in absorbance at 339 nm.

CoA. Activation of PC favors gluconeogensis and the use of carbon chains of pyruvate for the production of amino acids such as alanine, aspartate, and glutamate. Also, enhanced fatty acid oxidation should inhibit BCKDH activity by elevating concentrations of acetyl CoA, NADH, and ATP and thereby reduce the oxidation of branched-chain amino acids. These changes favor amino acid synthesis over degradation, which could promote protein synthesis, as observed by Owen et al. (2001) and Heo et al. (2000). The precise mechanisms underlying the effects of L-carnitine in modulating mitochondrial acetyl CoA concentrations, BCKDH and PC activities, and amino acid metabolism remain to be elucidated. Also, the effect of supplemental L-carnitine on protein turnover needs to be addressed in future studies.

First, we determined whether changes noted in backfat reduction might be associated with carnitine's role in accelerating fatty acid oxidation. Indeed, an increase in the rate of fatty acid oxidation was demonstrated in both liver mitochondria (1.4x) and hepatocytes (2.5x). The higher rates of palmitate oxidation in vitro were associated with 1.6- and 2.3-fold increases in the concentration of carnitine in liver and plasma isolated from L-carnitine-fed pigs, respectively. Kempen and Odle (1995), using newborn pigs, also demonstrated that dietary carnitine is responsible for increased fatty acid oxidation rates in hepatocytes, which, in turn, increased the rate of acetyl CoA production. These results suggest a mechanism underlying the reduction in backfat thickness in pigs fed L-carnitine.

Table 5. Effect of dietary L-carnitine on palmitate oxidation and protein synthesis in isolated hepatocytes of pigs[a]

	Added dietary L-carnitine, ppm			
Item	0	50	125	SEM
Palmitate oxidation, nmol·mg protein^{-1}·h^{-1} [bc]	0.94	1.56	2.38	0.14
Protein synthesis, nmol·mg protein^{-1}·h^{-1} [bc]	0.95	1.25	1.75	0.05

[a]A total of 24 pigs, eight pigs/treatment.
[b]Palmitate oxidation was measured as generation of radiolabeled CO_2 and acid-soluble products from [1-^{14}C]palmitate.
[c]Linear effect of dietary L-carnitine ($P < 0.01$).
[d]Protein synthesis was determined as incorporation of [^{35}S]methionine into TCA precipitate.

Table 6. Amino acid concentrations in longissimus muscle (sample from 9th, 10th, and 11th rib) of pigs[a]

	Added dietary L-carnitine, ppm			
Item, μmol/g	0	50	125	SEM
Aspartic acid[b]	7.09	7.49	7.70	0.18
Threonine[c]	3.44	3.66	3.77	0.10
Serine	2.65	2.88	2.96	0.14
Glutamic acid[c]	10.99	11.89	12.12	0.37
Proline[c]	2.82	2.99	3.16	0.11
Glycine[c]	3.49	3.52	3.69	0.07
Alanine[b]	4.38	4.60	4.73	0.10
Cysteine[c]	0.90	0.98	0.97	0.02
Valine	3.90	4.11	4.10	0.11
Methionine[c]	2.15	2.35	2.36	0.06
Isoleucine	3.59	3.82	3.79	0.10
Leucine[b]	6.26	6.66	6.80	0.14
Tyrosine[b]	2.74	2.94	2.98	0.07
Phenylalanine[b]	3.15	3.37	3.42	0.07
Histidine[cd]	3.37	3.69	3.66	0.11
Ornithine	0.06	0.07	0.07	0.01
Lysine[c]	6.89	7.31	7.49	0.17
Arginine[c]	5.01	5.31	5.42	0.13
Tryptophan[c]	0.95	1.07	1.05	0.03

[a]A total of 24 pigs, eight pigs/treatment.
[b]Linear effect of dietary L-carnitine ($P < 0.05$).
[c]Linear effect of dietary L-carnitine ($P < 0.10$).
[d]Quadratic effect of dietary L-carnitine ($P < 0.10$).

Whether the increased rate of fatty acid oxidation was directly attributable to an increase in mitochondrial carnitine cannot be verified by our data. However, we observed dose-dependent increases in both liver and muscle tissues. The rates of fatty acid oxidation in both liver mitochondria and isolated hepatocytes were significantly correlated with the increased concentration of carnitine in the liver. The possibility exists that supplemental carnitine may alter mitochondrial carnitine palmitoyltransferase I (**CPT-I**) activity and, hence, fatty acid oxidation. A recent study of Heo et al. (2000) using young pigs demonstrated that the CPT-I activities of liver and

Table 7. Amino acid concentrations found in biceps femoris muscle of pigs[a]

	Added dietary L-carnitine, ppm			
Item, μmol/g	0	50	125	SEM
Aspartic acid	5.86	6.13	6.13	0.17
Threonine	2.69	2.93	2.83	0.10
Serine	2.13	2.37	2.27	0.14
Glutamic acid	8.27	9.76	9.67	0.61
Proline	2.63	2.62	2.69	0.43
Glycine	2.97	3.25	3.17	0.29
Alanine	3.64	3.86	3.93	0.12
Cysteine	1.17	0.79	0.77	0.23
Valine[b]	2.86	3.26	3.21	0.19
Methionine	1.96	1.85	1.85	0.14
Isoleucine[c]	2.72	3.13	3.19	0.11
Leucine[b]	4.82	5.42	5.45	0.20
Tyrosine	2.65	2.35	2.37	0.26
Phenylalanine[d]	2.55	2.72	2.78	0.07
Histidine	2.29	2.81	2.76	0.20
Ornithine	0.45	0.05	0.06	0.22
Lysine[d]	4.83	5.95	6.02	0.43
Arginine	4.48	4.39	4.48	0.18
Tryptophan	1.28	0.81	0.86	0.27

[a]A total of 24 pigs, eight pigs/treatment.
[b]Linear effect of dietary L-carnitine ($P < 0.05$).
[c]Linear effect of dietary L-carnitine ($P < 0.01$).
[d]Linear effect of dietary L-carnitine ($P < 0.10$).

Table 8. Amino acid concentrations found in liver tissue of pigs[a]

Item, μmol/g	Added dietary L-carnitine, ppm 0	50	125	SEM
Aspartic acid[b]	5.73	5.99	6.08	0.10
Threonine[c]	2.83	2.92	3.01	0.05
Serine[c]	2.49	2.68	2.88	0.08
Glutamic acid[c]	6.99	7.42	7.88	0.16
Proline[c]	2.78	2.92	3.10	0.07
Glycine	3.74	3.83	3.89	0.09
Alanine[d]	3.66	4.03	4.08	0.09
Cysteine[b]	1.24	1.29	1.29	0.04
Valine	4.06	4.22	4.12	0.10
Methionine[c]	1.46	1.51	1.56	0.02
Isoleucine	3.01	3.06	3.08	0.07
Leucine[b]	6.08	6.46	6.52	0.13
Tyrosine	2.56	2.61	2.64	0.04
Phenylalanine	3.40	3.54	3.54	0.07
Histidine	1.86	1.95	1.90	0.07
Ornithine	0.39	0.37	0.39	0.01
Lysine	5.14	5.30	5.32	0.09
Arginine	3.92	4.02	4.06	0.07
Tryptophan	0.89	0.90	0.93	0.03

[a]A total of 24 pigs, eight pigs/treatment.
[b]Linear effect of dietary L-carnitine ($P < 0.05$).
[c]Linear effect of dietary L-carnitine ($P < 0.01$).
[d]Linear effect of dietary L-carnitine ($P < 0.10$).

muscle mitochondria isolated from control and carnitine-supplemented groups increased in a curvilinear fashion. However, the mitochondria from both groups responded similarly to a wide range (0 to 3,000 μ*M*) of carnitine added to the assay media.

Next, we examined the possibility that carnitine might stimulate PC activity, thereby providing more carbon for the synthesis of nonessential amino acids. Ferre et al. (1979; 1981) have demonstrated that in vivo, accelerated hepatic fatty acid oxidation can increase the gluconeogenic activity by providing the acetyl CoA necessary for activation of PC. Cyr et al. (1991) observed changes in acetyl CoA concentrations in isolated hepatocytes to correlate with PC flux and gluconeogenesis from lactate. In addition, accelerated fatty acid oxidation can also increase the availability of reducing equivalents (NADH) to shift the reversible reaction catalyzed by glyceraldehyde-3-phosphate dehydrogenase in the direction of gluconeogenesis. In the present study, we observed 1.5 and 2.2 times greater PC flux in liver mitochondria isolated from pigs receiving 50 and 125 ppm of added dietary L-carnitine, respectively. In addition, assays with particle free extracts indicated that added dietary L-carnitine induced 1.6 to 2.8 times greater concentrations of PC in liver mitochondria. These findings are similar to those of Ji et al. (1996), who also reported an increase in PC flux and induction of PC in liver mitochondria from Atlantic salmon fed supplemental carnitine. The extent to which accelerated PC flux is provoked by increases in the mitochondrial concentration of the enzyme and changes in the concentrations of its metabolic regulators remains to be examined.

As hypothesized, for conditions of accelerated fatty acid oxidation and stimulated generation of NADH, Acetyl CoA, and ATP, the BCKDH flux in isolated mitochondria from liver and muscle was reduced in pigs fed increasing L-carnitine. This expectation is based on evidence by Pettit et al. (1978), Williamson et al. (1979), Spydevold and Hokland (1981), and Harris and Paxton (1985) who demonstrated BCKDH to be inhibited by NADH, acetyl CoA, and ATP.

Our metabolic model predicts that if dietary L-carnitine increases palmitate oxidation, with a resultant increase in flux through PC and decrease in flux through BCKDH, then nitrogen retention should be improved. This was evaluated by examining the concentration of free amino acids in liver and muscle tissues. Compared with pigs fed the basal diet, pigs fed supplemental L-carnitine had consistently higher levels of some free amino acids including leucine, one of the branched-chain amino acids, as anticipated from the observed reduction in flux through the mitochondrial BCKDH. Other amino acids that were increased included: glutamine, proline, aspartic acid, threonine, cysteine, methionine, tyrosine, phenylalanine, histidine, and lysine. Most of these are essential amino acids that might be spared by the influence of carnitine on intermediary metabolism.

Failure to detect elevated aspartate and glutamate in carnitine-fed salmon was regarded by Ji et al. (1996) as an inconsistency with the metabolic model. Therefore, it is significant to emphasize that, in the present study, both of these amino acids were elevated in the liver and longissimus muscle of carntitine-fed pigs.

Bohles and Lehnert (1984) also reported the concentrations of branched-chain amino acids to be increased

in skeletal muscle of 6-kg pigs fed carnitine. They hypothesized that this increase would favor protein synthesis. Our observations of increased protein synthesis in the isolated hepatocytes and an increase in percentage muscle of pigs fed L-carnitine are consistent with the effect of branched-chain amino acids on protein synthesis observed in 6-kg pigs (Bohles and Lenhert, 1984), rats (Fulks et al., 1975), and humans (Alverstrand et al., 1990).

In conclusion, these results provide evidence for a role for carnitine in fostering protein accretion, and provide insight into the biochemical mechanisms.

Implications

In the present study, finishing pigs fed diets containing added L-carnitine were leaner and had decreased backfat thickness. The increased protein accretion and reduced backfat thickness were associated with greater rates of palmitate oxidation, more rapid flux through pyruvate carboxylase, and reduced flux through branched-chain α-keto acid dehydrogenase. These changes favor re-utilization of waste nitrogen for protein synthesis, and accelerated incorporation of amino acids into protein was observed in hepatocytes of L-carnitine-fed pigs. Our data suggest that pigs fed added L-carnitine are more able to use fat for energy, divert carbon toward synthesis of amino acids, and spare branched-chain amino acids for protein synthesis.

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