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A moderate excess of dietary lysine lowers plasma and tissue carnitine concentrations in pigs

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This study was performed to investigate whether dietary lysine concentration influences the carnitine status of pigs. Therefore, an experiment with twenty young pigs with an average body weight of 21 kg was performed which were fed either a control diet (9·7 g lysine/kg) or a diet with a moderate excess of lysine (16·8 g lysine/kg). Concentrations of all the other amino acids did not differ between the diets. Pigs fed the high-lysine diet had lower concentrations of free and total carnitine in plasma, liver, kidney and skeletal muscle than control pigs ($P<0·05$). Pigs fed the high-lysine diet moreover had an increased concentration of trimethyllysine (TML), a reduced mRNA abundance of TML dioxygenase and reduced concentrations of γ-butyrobetaine (BB) in muscle, indicating that the conversion of TML into BB in muscle was impaired. Concentrations of BB, the metabolic precursor of carnitine, in plasma, liver and kidney were also reduced in pigs fed the high-lysine diet while the activity of BB dioxygenase in kidney was not different and that in liver was even increased compared to control pigs ($P<0·05$). In conclusion, this study shows that a moderate dietary excess of lysine lowers plasma and tissue carnitine concentrations in pigs. Reduced concentrations of BB in liver and kidney suggest that the depressed carnitine status was likely caused by a decreased rate of carnitine synthesis due to a diminished availability of carnitine precursor, probably mainly as a result of an impaired BB formation in muscle.

Lysine: Carnitine: Pigs: γ-Butyrobetaine: Trimethyllysine

Carnitine (L-3-hydroxy-4-*N*-*N*-*N*-trimethylaminobutyrate) is an essential metabolite, which has a number of indispensable functions in intermediary metabolism. The most prominent function lies in its role in the transport of activated long-chain fatty acids from the cytosol to the mitochondrial matrix where β-oxidation takes place[1–3]. All tissues that use fatty acids as a fuel source require carnitine for normal function. Carnitine is derived from dietary sources and endogenous biosynthesis[4,5]. Carnitine biosynthesis involves a complex series of reactions. Lysine provides the carbon backbone of carnitine. Lysine in protein peptide linkages undergoes methylation of the ε-amino group to yield trimethyllysine (TML), which is released upon protein degradation. Muscle is the major source of TML. The released TML is further oxidised to γ-butyrobetaine (BB) by the action of trimethyllysine dioxygenase (TMLD), 3-hydroxy-*N*-trimethyllysine aldolase and 4-*N*-trimethylaminobutyraldehyde dehydrogenase. BB is hydroxylated by γ-butyrobetaine dioxygenase (BBD) to form carnitine[6]. Carnitine produced in liver and kidney is secreted into the blood and taken up into tissues by novel organic cation transporters (OCTN), particularly OCTN2 which is the most important carnitine transporter[7–10]. OCTN2 not only plays a major role in tissue distribution of carnitine but also operates in the reabsorption of carnitine from the urine and is the key transporter involved in intestinal absorption of carnitine[9]. The fact that inborn or acquired defects of OCTN2 lead to primary or secondary systemic carnitine deficiency demonstrates the essential role of these transporters in carnitine homeostasis[8].

Due to its involvement in β-oxidation of fatty acids, it has been hypothesized that carnitine is able to promote energy production from fatty acids in farm animals. Therefore, several studies have been performed which investigated the effects of dietary supplementation of carnitine on growth performance and body composition in various farm animal species. In pigs, some experiments revealed an improvement of the gain:feed ratio, higher rates of protein accretion and lower rates of fat accretion compared to untreated control pigs[11–16]. These effects have been attributed to an increased β-oxidation of fatty acids which favour the formation of protein[13]. However, other studies have reported no effects of added L-carnitine on growth of pigs[17,18]. Owen *et al.* [11], who summarized these conflicting results in their paper, pointed out that L-carnitine exerted its strongest effect on the growth performance of pigs if the concentration of lysine in the diet was relatively low. A relationship between dietary lysine concentration and the efficacy of carnitine supplementation on growth performance in pigs indicates that the dietary

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Abbreviations: BB, γ-butyrobetaine; BBD, γ-butyrobetaine dioxygenase; OCTN, novel organic cation transporter; TML, trimethyllysine; TMLD, trimethyllysine dioxygenase.

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lysine concentration has an influence on their carnitine status. In pigs, however, the effect of dietary lysine concentration on carnitine status has not yet been investigated. In man, few studies have been conducted which investigated the role of lysine as a nutrient that could influence carnitine biosynthesis[19–21]. The effects of dietary lysine on carnitine concentrations in these studies, however, were variable. In one of them, a rise in plasma carnitine concentration was noticed in response to a lysine supplement[19]. In another one, lysine supplementation did not change plasma carnitine concentration but increased plasma trimethyllysine concentration[20]. In the third study, supplementation of lysine plus methionine had no effect on plasma and muscle carnitine concentrations but increased renal carnitine excretion, suggesting that carnitine synthesis was enhanced by supplementation of these amino acids[21]. In rats, an excess of dietary lysine increased TML concentration in plasma and muscle and lowered the concentration of carnitine in plasma[22]. The reason for this phenomenon, however, has not yet been clarified.

The present study was performed to investigate whether dietary lysine concentration has an effect on carnitine status in growing pigs. For this purpose, growing pigs were fed a diet with a lysine concentration according to the recommendations for growing pigs in the respective body weight range (according to the National Research Council[23]) or the same diet supplemented with L-carnitine to yield a moderate excess of lysine. To assess the carnitine status of the animals, carnitine concentrations in plasma and tissues were determined. To find out the reasons for alterations of the carnitine status in response to an excess of dietary lysine, concentrations of carnitine precursors in plasma and tissues and enzymes involved in carnitine synthesis in liver and kidney were considered.

Materials and methods

All experimental procedures described followed established guidelines for the care and use of laboratory animals according to law on animal welfare and were approved by the local veterinary office (Halle/Saale, Germany).

Animals and diets

Twenty male 10-week-old crossbred pigs [(German Landrace × Large White) × Pietrain] with an average body weight of 21 (SD 1) kg were used. The animals were kept in flat-decks, in an environmentally controlled facility with a temperature of 23°C, relative humidity between 55 and 60 %, and light from 06.00 to 18.00 hours. The building and flat-deck pens were disinfected before commencement of the trial. The day before the start of the experimental feeding period the pigs were weighed and randomly assigned to two groups of ten animals each.

One basal diet was prepared which was formulated to yield a lysine concentration of 9·5 g/kg diet which agrees with the recommendation for pigs in a body weight range between 20 and 50 kg according to the National Research Council[23] (Table 1). Concentrations of all other indispensable amino acids were in slight excess to National Research Council recommendations[23]. One group of pigs received the basal diet without further supplementation (control diet). The analysed lysine concentration of that diet was 9·7 g/kg. The second group received the same basal diet supplemented

Table 1. Composition and nutrient concentrations of the control diet and the high-lysine diet

	Control diet (g/kg)	High-lysine diet (g/kg)
Composition		
Wheat	490	490
Barley	220·9	213·9
Extracted soyabean meal	230	230
Soya oil	30	30
Premix*	25	25
Mono calcium phosphate	2	2
L-Lysine	0·4	7·4
DL-Methionine	0·7	0·7
L-Threonine	1·0	1·0
Concentration of nutrients		
Metabolisable energy (MJ/kg)†	13·7	13·7
Crude protein‡	181	188
Lysine‡	9·74	16·8
Methionine + cysteine‡	6·61	6·58
Threonine‡	7·42	7·39
Tryptophan‡	2·16	2·15

* The premix provided the following per kg feed: 3·75 mg all-*trans* retinol; 30 μg cholecalciferol; 25 mg all-rac-α-tocopheryl acetate; 1·8 mg menadione sodium bisulphate; 2 mg thiamine-HCl; 5 mg riboflavine; 3 mg pyridoxine-HCl; 10 mg Ca pantothenate; 15 mg nicotinic acid; 0·25 mg folic acid; 0·025 mg cyanocobalamin; 180 mg choline chloride; 100 mg iron; 100 mg copper; 100 mg zinc; 75 mg manganese; 0·77 mg iodine; 0·25 mg selenium; 5·5 g calcium; 1·5 g phosphorus; 1·75 g sodium.
† Calculated according to recommendations of the German Nutrition Society[25].
‡ Analysed (mean values of three analyses per diet).

with 7 g L-lysine hydrochloride (obtained from Lohmann Animal Health, Cuxhaven, Germany) at the expense of barley (high-lysine diet). The analysed lysine concentration of that diet was 16·8 g/kg. Concentrations of all the other amino acids did not differ between the diets. The diets had a low concentration of L-carnitine (5 mg/kg). Animals were given free access to diet. To control the feed intake, unconsumed feed was weighed weekly. Water was available *ad libitum* from a nipple drinker system. Diets were administered for 2 weeks.

Sample collection

The animals were anaesthetised and exsanguinated after overnight fasting. Blood samples were collected into heparinised polyethylene tubes. Liver, kidneys and samples from *musculus longissimus dorsi* and *m. semimembranosus* were excised. Plasma was obtained by centrifugation of the blood samples (1100 ***g***, 10 min, 4°C). Plasma and tissue samples were stored at −20°C pending further analysis.

Determination of nutrients in the diets

Concentrations of crude protein in the diets were analysed according to the official German VDLUFA methodology[24]. The metabolisable energy of the diet was calculated as recommended by the German Nutrition Society[25]. The amino acid concentrations of the diet were also determined according to the official VDLUFA method[24]. Samples were oxidised and subsequently hydrolysed with 6 M-HCl. Separation and quantification of the amino acids was performed by ion exchange chromatography following post-column derivatisation in an amino acid analyser (Biotronic LC 3000; Eppendorf,

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Hamburg, Germany). For the determination of tryptophan, the diet was digested with barium hydroxide[26]. The tryptophan concentration was determined by reversed-phase HPLC[27].

Analysis of carnitine, γ-butyrobetaine and trimethyllysine

Concentrations of free carnitine, acetyl carnitine, TML and BB in plasma and tissues as well as concentration of carnitine in the diet were determined by tandem mass spectrometry using deuterated carnitine-d_3 (Larodane Fine Chemicals, Malmö, Sweden) as internal standard[28]. Freeze-dried tissues (50 mg) were extracted with 0·5 ml methanol–water (2:1, v/v) by homogenisation (Tissue Lyzer, Qiagen, Hilden, Germany), followed by sonification for 20 min and incubation at 50°C for 30 min in a shaker. After centrifugation (13 000 ***g***, 10 min) 20 μl of the supernatant were added to 100 μl methanol containing the internal standard, mixed, incubated for 10 min and centrifuged (13 000 ***g***, 10 min). Plasma samples were handled at 4°C in the same manner as the supernatant after tissue extraction. The final supernatants were used for quantification of the compounds by a 1100-er series HPLC (Agilent Technologies, Waldbronn, Germany) equipped with a Kromasil 100 column (125 mm × 2 mm, 5 μm particle size; CS-Chromatographie Service Langerwehe, Germany) and an API 2000 LC-MS/MS-System (Applied Biosystems, Darmstadt, Germany). The analytes were ionised by positive ion (5500 V) electrospray. As eluents, methanol and a methanol–water–acetonitrile–acetic acid mixture (100:90:9:1, by vol.) were used.

Activity of γ-butyrobetaine dioxygenase

Activity of BBD in liver, kidney and muscles was determined as described previously in detail by Van Vlies *et al.*[29]. Homogenates from liver, kidney and muscles were prepared by homogenising tissue in 10 mM-3-morpholinepropansulphonic acid buffer (pH 7·4) containing 0·9 % (w/v) sodium chloride, 10 % (w/v) glycerol and 5 mM-dithiothreitol.

RNA isolation and real-time detection PCR analysis

For the determination of mRNA expression levels of TMLD and OCTN2 and glyceraldehyde-3-phosphate dehydrogenase for normalisation, total RNA was isolated from liver, kidney and muscles using Trizol™ reagent (Invitrogen, Karlsruhe, Germany) according to the manufacturer's protocol. Glyceraldehyde-3-phosphate dehydrogenase served as an appropriate reference gene in this experiment since the cycle threshold values of glyceraldehyde-3-phosphate dehydrogenase did not differ between the groups. RNA concentration and purity were estimated from the optical density at 260 and 280 nm, respectively. Total RNA (1·2 μg) was subjected to cDNA synthesis using M-MuLV reverse transcriptase (MBI Fermentas, St. Leon-Rot, Germany). For determination of mRNA expression levels real-time detection RT–PCR using a MJ Research Opticon System (Biozym Diagnostik, Oldendorf, Germany) was applied[30]. cDNA templates (2 μl) were amplified in 100 μl Rotorgene PCR tubes in a final volume of 20 μl containing 500 μmol/l dNTP (Roth, Karlsruhe, Germany), 3·5 mmol/l $MgCl_2$, 1·25 U GoTaq® Flexi DNA polymerase, 4 μl 5 × buffer (all from Promega, Mannheim, Germany), 0·5 μl 10 × Sybr Green I (Sigma-Aldrich, Taufkirchen, Germany) and 26·7 pmol of each primer pair.

The primer sequences used for RT–PCR were as follows: 5′-AGG-GGC-TCT-CCA-GAA-CAT-CAT-CC-3′ (forward) and 5′-TCG-CGT-GCT-CTT-GCT-GGG-GTT-GG-3′ (reverse) for pig glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12); 5′-GCA-CCA-TAC-AGC-CTC-CAA-GT-3′ (forward) and 5′-TGG-TCT-CAT-CCA-GAC-GAA-CA-3′ (reverse) for pig TMLD; 5′-TGA-CCA-TAT-CAG-TGG-GCT-A-3′ (forward) and 5′-AGT-AGG-GAG-ACA-GGA-TGC-T-3′ (reverse) for pig OCTN2. The PCR protocol comprised an initial denaturation at 95°C for 3 min and twenty to thirty-five cycles of amplification comprising denaturation at 95°C for 25 s, annealing at primer-specific temperatures (60°C) for 30 s and elongation at 72°C for 55 s. Subsequently, melting curve analysis was performed from 50 to 99°C with continuous fluorescence measurement. The amplification of a single product of the expected size was confirmed using 1 % agarose gel electrophoresis. Amplification efficiencies for all primers were determined by template dilution series. Calculation of the relative mRNA concentration was made using the amplification efficiencies and the cycle threshold values[31]. Relative expression ratios are expressed as fold changes of mRNA concentration in the high-lysine group compared to the control group.

Statistical analysis

Student's *t* test was used to compare means of the high-lysine group and the control group. Differences with $P < 0·05$ were considered to be significant. Data are given as means and standard deviations.

Results

Growth performance of pigs

Initial body weights were similar in piglets of both groups (control group 20·9 (SD 0·9) kg, high-lysine group 20·7 (SD 1·2) kg). The daily feed intake during the experimental period was similar in both groups (control group 1·23 (SD 0·09) kg/d, high-lysine group 1·26 (SD 0·03) kg/d). Body weight gain during the experiment and the final body weight were, however, higher in pigs fed the high-lysine diet than those fed the control diet (daily body weight gain: control group 723 (SD 70) g/d, high-lysine group 938 (SD 65) g/d, $P < 0·05$; final body weight: control group 30·3 (SD 1·1) kg, high-lysine group 32·9 (SD 1·3) kg, $P < 0·05$). The gain:feed ratio was also lower in pigs fed the control diet than in those fed the high-lysine diet (control group 0·59 (SD 0·05) g gain/g feed, high-lysine group 0·75 (SD 0·04) g gain/g feed, $P < 0·05$).

Plasma and tissue concentrations of carnitine, γ-butyrobetaine and trimethyllysine

Concentrations of carnitine and its metabolic precursors (BB, TML) were determined in plasma, liver, kidney, *m. longissimus dorsi* and *m. semimembranosus*. Pigs fed the high-lysine diet had lower concentrations of free carnitine, acetyl carnitine, total carnitine and BB in plasma, liver, kidney and both muscles than pigs fed the control diet

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($P<0·05$; Table 2). There was, moreover, an increased concentration of TML in muscles of pigs fed the high-lysine diet compared to those fed the control diet ($P<0·05$; Table 2). Concentrations of TML in plasma, liver and kidney did not differ between both groups of pigs (Table 2).

Activity of γ-butyrobetaine dioxygenase in tissues

In order to find out whether reduced plasma and tissue carnitine concentrations could be due to a reduced activity of BBD, we determined the activity of that enzyme in liver, kidney and muscles. The activity of BBD in the liver was higher in pigs fed the high-lysine diet than in those fed the control diet ($P<0·05$); the activity of that enzyme in kidney did not differ between the groups (Fig. 1). In *m. longissimus dorsi* and *m. semimembranosus*, the activity of BBD was very low (<0·02 nmol/mg protein per min) and did not differ between the groups of pigs.

Relative mRNA abundance of trimethyllysine dioxygenase in tissues

In order to find out whether increased TML concentrations in muscles of pigs fed the high-lysine diet could be due to impaired degradation, we determined relative mRNA abundance of TMLD, a mitochondrial enzyme that converts TML to (3-hydroxy-)N^6-trimethyllysine. Indeed, pigs fed the high-lysine diet had a reduced mRNA abundance of that enzyme in *m. longissimus dorsi* and *m. semimembranosus* compared to pigs fed the control diet ($P<0·05$; Fig. 2). In contrast, relative abundance of TMLD in liver and kidney was not different between the groups of pigs (Fig. 2).

Relative mRNA concentration of novel organic cation transporter 2 in tissues

To investigate whether changes in tissue carnitine or BB concentrations could be due to alteration in the rate of the delivery of these metabolites from plasma into tissues or reabsorption in the kidney, we determined relative mRNA abundance of OCTN2 in tissues. Relative mRNA abundance of OCTN2 in liver, kidney and muscles, however, did not differ between pigs fed the high-lysine diet and those fed the control diet (liver: control group 1·00 (SD 0·60), high-lysine group 0·86 (SD 0·30); kidney: control group 1·00 (SD 0·59), high-lysine group 0·74 (SD 0·26); *m. longissimus dorsi*: control group 1·00 (SD 0·32), high-lysine group 1·03 (SD 0·43); *m. semimembranosus*: control group 1·00 (SD 0·38), high-lysine group 0·82 (SD 0·13)).

Discussion

The present study was performed to investigate whether dietary lysine concentration influences carnitine status in pigs. For this purpose, we determined an experiment in which pigs were fed identical diets which differed, due to supplementation with L-lysine hydrochloride, only in the concentration of lysine. The lysine concentration of the control diet (9·7 g/kg) agreed well with the recommendation of the National Research Council[23] for pigs in a body weight range between 20 and 50 kg, being 9·5 g/kg diet. The diet of the treatment group contained a moderate excess of lysine (16·8 g/kg diet). The finding that body weight gain and gain:feed ratio were lower in pigs fed the control diet than in pigs fed a similar amount of the same diet supplemented with 7 g lysine/kg shows that the dietary lysine concentration of 9·7 g/kg was insufficient to yield maximum growth in pigs of an initial weight of 20 kg. This shows that the recommendations given by the National Research Council[23] for lysine are below the lysine requirement of modern, high lean growth genotypes with a greater capacity for body growth and protein accretion. There are also several other studies which show that the lysine requirement of 20 kg pigs with a high growth capacity is in the range between 11 and 14 g/kg diet[32–36]. Due to the importance of lysine for growth as the first-limiting amino acid, lysine concentrations

Table 2. Concentrations of carnitine (free, acetyl, total), γ-butyrobetaine (BB) and trimetyllysine (TML) in plasma and tissues of pigs fed a control diet (9·7 g lysine/kg) or a high-lysine diet (16·8 g lysine/kg)†

(Mean values and standard deviations for ten determinations)

	Free carnitine		Acetyl carnitine		Total carnitine		BB		TML	
Treatment	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Plasma (μM)										
Control	6·45	0·69	1·08	0·32	7·66	0·71	1·32	0·22	1·35	0·16
High lysine	5·17*	1·07	0·79*	0·19	6·02*	1·24	1·03*	0·24	1·36	0·17
Liver (nmol/g)										
Control	42·5	6·7	2·27	0·67	46·1	7·5	5·75	1·20	2·45	0·56
High lysine	35·1*	7·6	1·39*	0·26	37·2*	7·7	4·69*	0·98	2·86	1·24
Kidney (nmol/g)										
Control	80·7	9·2	13·2	1·6	98·1	9·5	9·67	1·11	4·53	0·86
High lysine	59·6*	14·4	9·2*	1·2	71·1*	15·0	6·35*	1·57	3·92	1·04
m. longissimus dorsi (nmol/g)										
Control	309	72	241	40	554	108	98·2	16·9	2·07	0·30
High lysine	256*	38	186*	29	446*	62	83·7*	9·54	3·78*	1·00
m. semimembranosus (nmol/g)										
Control	289	53	237	21	528	49	72·1	8·1	2·94	0·48
High lysine	225*	34	180*	37	408*	67	61·8*	11·7	4·99*	1·63

Mean value was significantly different from that of the control group: *$P<0·05$.
† For details of procedures and diets, see the Materials and methods section and Table 1.

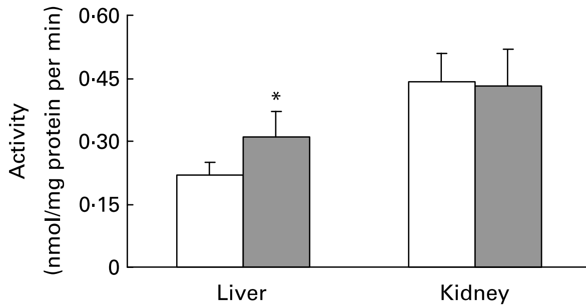

Fig. 1. Activity of γ-butyrobetaine dioxygenase in liver and kidney of pigs fed a control diet (9·7 g lysine/kg; □) or a high-lysine diet (16·8 g/kg; ■). Values are means with standard deviations depicted by vertical bars (*n* 10). Mean value was significantly different from that of the control group: *$P < 0·05$.

adjusted in practical feed mixtures by supplementation of synthetic lysine are commonly in excess of these levels and can ever reach 17 g/kg. Therefore, even the high-lysine diet is of relevance for practical pig nutrition with respect to its lysine concentration.

The present study shows for the first time that a moderate excess of lysine in the diet lowers carnitine concentrations in plasma and tissues (liver, kidney and skeletal muscle) of pigs. To find out whether reduced plasma and tissue carnitine concentrations in pigs fed the high-lysine diet could be due to an impairment of carnitine biosynthesis, we determined the activity of BBD in liver, kidney and muscle. To our knowledge, the sites of carnitine synthesis in pigs have not yet been investigated. However, it has been suggested that liver and kidney are the main tissues in which carnitine synthesis occurs in mammals[6,29]. We detected a considerable activity of BBD in liver and kidney while the activity of that enzyme in skeletal muscle was negligible. This suggests that liver and kidney may be the major sites of carnitine synthesis in the pig, as in other mammals. It is shown that pigs fed the high-lysine diet do not have a reduced activity of this enzyme in liver and kidney, but in fact have an even higher activity in the liver than pigs fed the control diet. This means that the activity of BBD can be ruled out as a reason for the depressed carnitine concentrations in pigs fed the high-lysine diet. In man, it has been shown that it is not the activity of BBD but the availability of carnitine precursors, particularly BB, that is rate limiting for carnitine synthesis[21]. In pigs, the rate-limiting factor of carnitine synthesis, either activity of BBD or availability of BB, has not yet been elucidated. Assuming that the availability of BB is rate-limiting for carnitine synthesis in pigs as in man, reduced concentrations of BB in liver and kidney in pigs fed the high-lysine diet indicate that these pigs had a lower carnitine synthesis in these tissues than those fed the control diet due to a decreased availability of the metabolic precursor of carnitine. A depression of carnitine synthesis may therefore be the major reason for diminished carnitine concentrations in plasma and tissues of pigs fed the high-lysine diet.

All tissues are able to produce BB from TML[6]. Most of the BB excreted into the blood, available for carnitine synthesis in liver and kidney, however, originates from skeletal muscle, the greatest reservoir of protein-bound TML[6]. To our knowledge, carnitine synthesis and homeostasis in pigs has not yet been studied. It should be noted, however, that BB concentrations in muscle of pigs observed in the present study are 10–20-fold higher than those reported for rats or mice[29,37]. This suggests that pigs have generally a high capacity to produce and store BB in muscle. The present study shows that pigs fed the high-lysine diet have an increased concentration of TML, a reduced concentration of BB and a reduced expression of TMLD, a mitochondrial enzyme which hydroxylates TML to hydroxy TML[38], in skeletal muscle. The present findings suggest that the enzymatic conversion of TML into BB in muscle was impaired in pigs fed the high-lysine diet. An impaired formation of BB in muscle may be the major reason for the reduced plasma BB concentration found in pigs fed the high-lysine diet.

Diminished BB concentrations in liver and kidney of pigs fed the high-lysine diet could have two different reasons. Either, the amount of BB delivered from blood into these tissues was reduced or the production of BB in these tissues was depressed. Uptake of BB from plasma into cells is mediated by OCTN2[10]. The finding that gene expression of OCTN2 in liver and kidney was unaffected suggests that the transport activity for BB was not lowered in pigs fed the

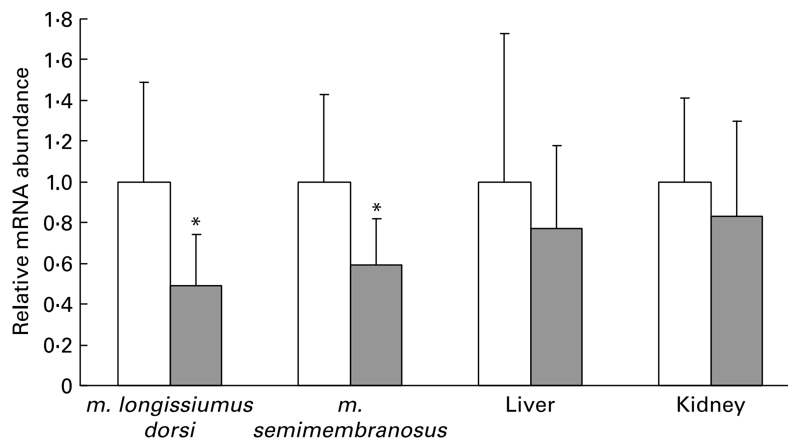

Fig. 2. Relative mRNA abundance of trimethyllysine dioxygenase in *m. longissimus dorsi* and *m. semimembranosus*, liver and kidney of pigs fed a control diet (9·7 g lysine/kg; □) or a high-lysine diet (16·8 g/kg; ■). Total RNA was extracted from the samples and relative mRNA levels of the genes were determined by real-time detection RT–PCR analysis using glyceraldehyde-3-phosphate dehydrogenase for normalisation. Data are expressed relative to mRNA concentrations of the control group (= 1·0). Values are means with standard deviations depicted by vertical bars (*n* 10). Mean value was significantly different from that of the control group: *$P < 0·05$.

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high-lysine diet. However, it is likely that less BB was delivered from blood into liver and kidney due to the lower plasma BB concentration in these pigs. The finding that TML concentrations and gene expression of TMLD in liver and kidney did not differ between both groups of pigs suggests that the conversion of TML into BB was probably not impaired in these tissues. Therefore, it is likely that decreased BB concentrations in liver and kidney in pigs fed the high-lysine diet were mainly due to the reduced plasma BB concentration which was probably caused mainly by an impaired formation of that metabolite in skeletal muscle.

Concentrations of carnitine in tissues that are incapable of carnitine synthesis such as skeletal muscle are influenced by the uptake of carnitine from plasma into cells by carnitine transporters[39]. The present study shows that mRNA abundance of OCTN2, the most important carnitine transporter[9,10], in muscle was not different between the groups of pigs. This suggests that alterations in carnitine concentrations in skeletal muscle between the two groups of pigs are probably not due to changes in transport capacity. However, uptake of carnitine from plasma into muscle may have been diminished in pigs fed the high-lysine diet due to the reduced plasma carnitine concentration. Diminished carnitine concentrations in muscle, moreover, could be due to mobilisation of carnitine from that tissue. Muscle, which contains more than 95 % of whole body carnitine, serves as a carnitine storage[2]. When plasma carnitine concentrations are lowered such as by treatment with pivalate, carnitine can be mobilised from the muscle in order to normalise plasma carnitine concentrations[40].

Noteworthy, similar findings with respect to changes of carnitine and TML concentrations in plasma and tissues were previously made in rats fed a diet with an excess of lysine[22]. These rats had, like the pigs fed the high-lysine diet, an increased concentration of TML in muscle and a reduced concentration of carnitine in plasma compared to control rats fed a diet with a nutritionally adequate lysine concentration. The reason for the reduced plasma carnitine concentration in that study could not be clarified. BB concentration and activity of BBD were not measured in that study. According to the similarities of rats and pigs with respect to alterations of carnitine and TML concentrations, it seems however likely that the reduction of plasma carnitine concentration in rats could have been also due to a decreased availability of BB for carnitine synthesis such as in pigs. The present findings in pigs are, however, in contrast to findings in man in which supplementation of lysine did either not change[19,21] or even increase[20] plasma carnitine concentration. In one of those human studies, supplementation of lysine and methionine stimulated carnitine synthesis in the body which is in contrast to the present study in pigs[21]. Apparently, there are differences in the response of the carnitine metabolism on supplemental lysine between man and pigs or rats.

From a practical point of view, the present study is relevant with respect to the relationship between dietary lysine concentration and the efficacy of carnitine supplementation on growth performance in pigs. Owen *et al.*[11] suggested that carnitine supplementation exerts its greatest effects on growth performance of pigs if the dietary lysine concentration is low. The present study, however, shows that piglets indeed have not a lower but an even higher carnitine status if the dietary lysine concentration is low. This also means that, assuming that an elevated carnitine status can have beneficial effects on the growth performance of pigs, supplementation of surplus lysine is not a means to improve the carnitine status of pigs. Due to the lower carnitine status, it is expected that carnitine supplementation should exert even greater effects on growth performance when the dietary lysine is high. Whether this expectation comes true should be investigated in further experiments.

In the present study, we varied the lysine concentration of the diet exclusively without changing the concentrations of the other indispensable amino acids. This design was chosen to study the isolated effect of the dietary lysine concentration on the carnitine status of the pigs. In practical feeding of pigs, however, indispensable amino acids are commonly supplemented in such amounts that fixed ratios between lysine and the other amino acids are adjusted based on the concept of the ideal amino acid profile[41,42]. Therefore, it would be interesting to find out whether an increase of the dietary lysine concentration would have similar effects on carnitine metabolism if the concentrations of the indispensable amino acids were also increased.

In conclusion, the present study shows for the first time that pigs fed a high-lysine diet have lower plasma and tissue carnitine concentrations than pigs fed a control diet. The finding that BB concentrations in liver and kidney were diminished in pigs fed the high-lysine diet suggests that these animals had a reduced carnitine synthesis in these tissues which may provide an explanation for their depressed carnitine status. Reduced BB concentrations are probably due to an impaired production of BB in muscle. These findings may be of practical relevance for pig nutrition and moreover are helpful in identifying nutrients which influence carnitine homeostasis in mammals.

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