Aus dem Institut für Agrar- und Ernährungswissenschaften
(Geschäftsführender Direktor: Prof. Dr. R. Jahn)
der
Naturwissenschaftlichen Fakultät III
(Dekan: Prof. Dr. P. Wycisk)
der
Martin-Luther-Universität Halle-Wittenberg

„Experimentelle Untersuchungen zum Carnitin-Metabolismus beim Schwein“

Dissertation

zur Erlangung des akademischen Grades
Doktor der Trophologie (Dr. troph.)

von

Diplom-Ernährungswissenschaftlerin
Maren Fischer

Halle/Saale 2009

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vorgelegt von

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Abkürzungsverzeichnis

ATB^{0+}	*amino acid transporter* B^{0+}
γ-BBD	γ-Butyrobetaindioxygenase
CPT	Carnitin-Palmitoyltransferase
CT	*novel carnitine transporter*
CoA	Coenzym A
IGF	*insulin-like growth factor*
mRNA	*messenger ribonucleic acid*
OAT	*organic anion transporter*
OCT	*organic cation transporter*
OCTN	*novel organic cation transporter*
PPAR	*peroxisome proliferator-activated receptor*
TMLD	Trimethyllysindioxygenase

1. Einleitung

Carnitin gehört zu den Vertretern der Betaine. Physiologisch wirksam ist es in der Form des L-Isomers von γ-Trimethylamino-β-Hydroxybutyrat, welches aufgrund der kationischen Trimethylammonium-Gruppe und der anionischen Carboxylgruppe amphoteren Charakter besitzt. Nach der Entdeckung der Substanz (Gulewitsch und Krimberg, 1905; Kutscher, 1905) wurde seine chemische Struktur von Tomita und Sendju (1927) aufgeklärt. Später wurden große Mengen von Carnitin in Milch, Hefe und tierischen Geweben nachgewiesen, während pflanzliche Produkte wie Getreide und Weizenkeime nur geringe Mengen von Carnitin enthielten (Fraenkel, 1951).
In der vorliegenden Arbeit ist mit dem Begriff Carnitin stets das stoffwechselaktive L-Carnitin gemeint.

1.1 Biosynthese des Carnitins im Organismus

Neben der Carnitin-Aufnahme über die Nahrung sind Säuger in der Lage, Carnitin endogen zu synthetisieren (Hoppel und Davis, 1986; Rebouche und Seim, 1998; Vaz und Wanders, 2002). Erster Schritt der Synthese ist die Methylierung von Lysin, wobei diese Aminosäure das Kohlenstoffgerüst liefert und Methionin vier Methylgruppen zur Verfügung stellt. Aufgrund der Bedeutung von Lysin in der Carnitin-Synthese ist anzunehmen, dass man durch variable Lysin-Konzentrationen in der Nahrung den Carnitin-Status beeinflussen kann. In Humanstudien wurde diese mögliche Interaktion untersucht (Khan-Siddiqui und Bamji, 1983; Rebouche *et al*., 1989; Vijayasarathy *et al*., 1987). Dabei gab es allerdings unterschiedliche Effekte zu verzeichnen. Khan-Siddiqui und Bamji (1983) beobachteten nach einer Lysin-Supplementation einen Anstieg der Carnitin-Konzentration im Plasma. In einer weiteren Humanstudie erhöhte sich nach Lysin-Zufuhr lediglich die Trimethyllysin-Konzentration im Plasma (Vijayasarathy *et al*., 1987). Nach Supplementation von Lysin und Methionin waren bei Rebouche *et al*. (1989) keine Konzentrationsänderungen von Carnitin im Plasma und Muskel zu verzeichnen. Allerdings war in dieser Studie die renale Ausscheidung von Carnitin erhöht. Durch die Lysin- und Methionin-Zufuhr ist hier vermutlich die Synthese des Carnitins angestiegen. Es ist jedoch nicht erforscht, inwieweit eine gesteigerte Lysin-Aufnahme den Carnitin-Status des Schweines beeinflussen kann. Owen *et al*. (1996) hoben lediglich hervor, dass die deutlichsten Auswirkungen von Carnitin-Zugaben auf das Wachstum des Schweines bei geringer Lysin-Konzentration in der Diät zu beobachten sind.
Im weiteren Verlauf der Synthese erfolgt nach der Methylierung des Lysins die Freisetzung von Trimethyllysin, welches durch die Trimethyllysindioxygenase (TMLD), 3-Hydroxy-N-

Trimethyllysin-Aldolase und 4-N-Trimethylaminobutyraldehyd-Dehydrogenase über mehrere Schritte zu Butyrobetain oxidiert wird (Vaz und Wanders, 2002). In der letzten Reaktion der Carnitin-Synthese wird Butyrobetain durch die γ-Butyrobetaindioxygenase (γ-BBD) zu Carnitin hydroxyliert (Abb.1). Katalysiert wird dieser Vorgang mittels 2-Oxoglutarat, molekularem Sauerstoff, zweiwertigem Eisen und Ascorbat (Lindstedt und Lindstedt, 1970; Vaz und Wanders, 2002). Durch seine bedeutende Funktion in der Umsetzung von Butyrobetain zum Carnitin ist das Enzym γ-BBD interessanter Gegenstand der aktuellen Forschung. Studien zur γ-BBD sind im Rahmen der Untersuchung der Carnitin-Synthese und der Carnitin-Homöostase beim Schwein unabdingbar. Allerdings gibt es in der Literatur bislang keine Aussagen zu Vorkommen und Aktivität dieses Enzyms im Schwein.

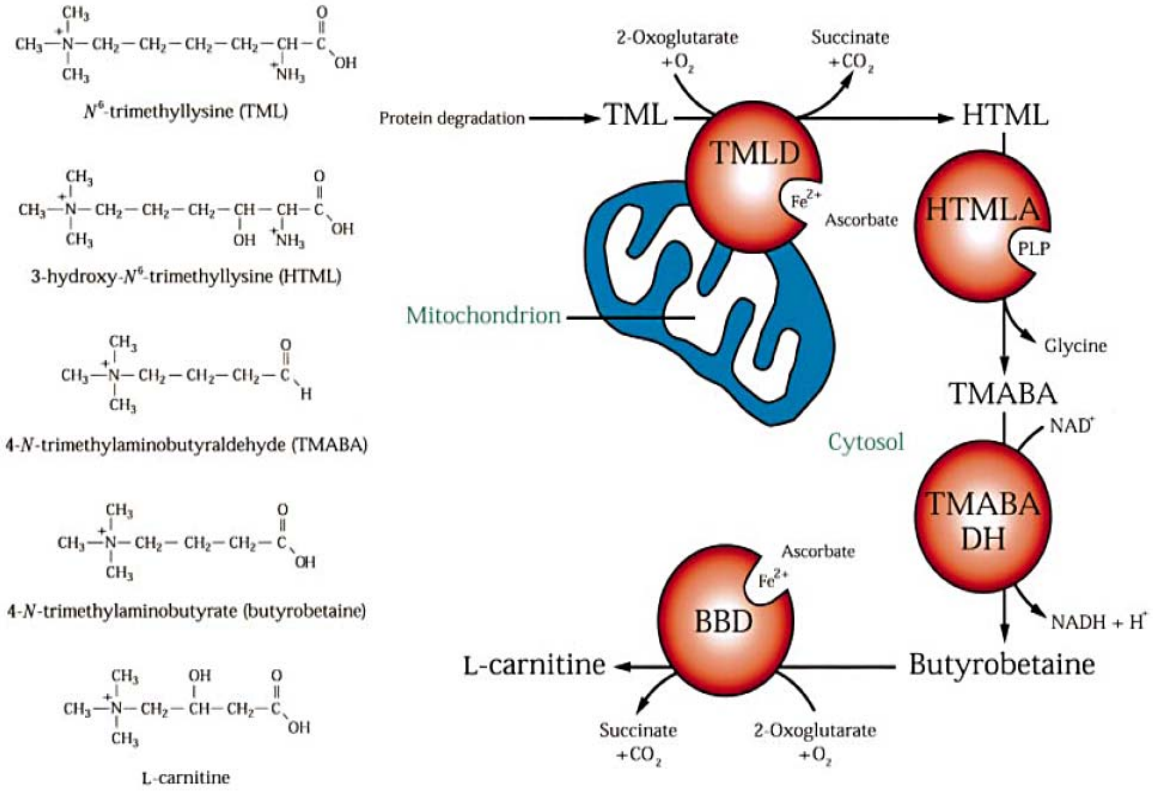

Abb.1: Metabolite und Verlauf der Carnitin-Biosynthese. (aus Vaz und Wanders, 2002)

Der Mensch ist aufgrund des Vorkommens der γ-BBD in Leber, Niere und geringfügig im Gehirn in der Lage, in diesen drei Geweben Carnitin zu synthetisieren (Rebouche und Engel, 1980). Dabei ist die Aktivität des Enzyms in der Niere weitaus höher als in der Leber (Vaz und Wanders, 2002). Während in der Niere von Katze, Rind, Hamster, Kaninchen und Rhesusaffe ebenso eine Aktivität gemessen wurde, ist das Enzym γ-BBD in der Niere von Cebusaffe, Schaf, Hund, Meerschwein, Maus und Ratte nicht oder nur sehr gering aktiv (Vaz

und Wanders, 2002). Des Weiteren wurde eine γ-BBD-Aktivität in Hoden und Nebenhoden von Ratten nachgewiesen (Carter *et al*., 1987), welche durch Galland *et al.* (1999) allerdings nicht bestätigt werden konnte. In Schafen wurde eine Aktivität des Enzyms auch im Muskel gemessen (Erfle, 1975).

In früheren Studien wurde vermutet, dass die Aktivität von γ-BBD limitierender Schritt der Carnitinsynthese des Menschen ist (Cederblad *et al*., 1986; Rebouche und Engel, 1980). Dies wurde jedoch von Olsen und Rebouche (1987) widerlegt. Rebouche *et al*. (1989) zeigten, dass vorrangig Butyrobetain limitierender Faktor in der Carnitinsynthese des Menschen ist. Es ist bislang nicht erforscht, ob dies auch für die Carnitin-Synthese des Schweines zutrifft.

Die Aktivität der γ-BBD korreliert in der menschlichen Leber mit dem Alter, während die Aktivität in der Niere des Menschen nicht altersabhängig ist (Olson und Rebouche, 1987; Rebouche und Engel, 1980). In diesem Zusammenhang stellt sich bei Forschungen zur γ-BBD im Schwein die Frage, ob auch in dieser Spezie eine Altersabhängigkeit des Enzyms vorliegt. Mögliche Hinweise dazu liefern zahlreiche Studien, die vermuten, dass Schweine am Tag der Geburt einen unzureichenden Carnitin-Status besitzen (Heo *et al*., 2000; Penn *et al*., 1997; Van Kempen und Odle, 1995).

1.2. Aufnahme und Transport von Carnitin

Carnitin wird im Dünndarm durch Carrier-vermittelten aktiven Transport und passive Diffusion absorbiert (Li *et al*., 1992). Die Absorption im Kolon scheint allein passiv zu sein (Hamilton *et al*., 1986). Im Menschen wird Carnitin bei normalen Verzehrsgewohnheiten zu 54-87% absorbiert (Rebouche und Chenard, 1991), allerdings beträgt die Absorption bei oraler Carnitin-Gabe von 1-6 g nur 5-18% (Evans und Fornasini, 2003). Bei einer Vielzahl von Spezies ist der Ort der Carnitin-Aufnahme im Dünndarm weitestgehend unbekannt. Lediglich bei Ratten ist erwiesen, dass die Absorption von Carnitin hauptsächlich im oberen jejunalen und teilweise im duodenalen Segment stattfindet (Gross und Henderson, 1984; Gudjonsson *et al.,* 1985; Shaw *et al*., 1983). Bislang sind die Kapazität und der Ort der Carnitin-Absorption im Dünndarm des Schweines nicht erforscht. Ebenso gibt es keine Aussagen darüber, inwieweit eine Supplementation von Carnitin Einfluss auf die Bioverfügbarkeit dieser Substanz besitzt. In Anbetracht des zunehmenden Einsatzes von Carnitin als Futtermittelzusatzstoff sind Kenntnisse über die Absorption von supplementiertem Carnitin in der Fütterungspraxis des Schweines von großer Bedeutung. In diesem Zusammenhang ist zu klären, wie hoch die Carnitin-Konzentrationen in relevanten Geweben des Schweines sind und in welchem Umfang diese durch Carnitin-

Supplementationen beeinflusst werden können. Bis zum jetzigen Zeitpunkt gibt es dazu nur wenige Aussagen. Im Menschen ist die höchste Konzentration von Carnitin im Skelettmuskel zu verzeichnen, wobei die hier vorliegenden Konzentrationen von 2000-4000 nmol Carnitin/g Originalmasse um den Faktor 100 höher sind als im Plasma (Angelini *et al*., 1992; Bertoli *et al*., 1981; Moorthy *et al*., 1983). Niere, Leber und Gehirn des Menschen beinhalten 300-1000 nmol Carnitin/g Originalmasse (Angelini *et al*., 1992; Moorthy *et al*., 1983). Es ist nicht geklärt, ob solche Konzentrationen auch in den Geweben des Schweines vorliegen.

Carnitin gelangt nach exogener Aufnahme oder endogener Synthese durch einen aktiven natrium-abhängigen Transporter, dem *novel organic cation transporter* 2 (OCTN2), aus dem Blutkreislauf in die Gewebe (Tamai *et al*., 1998). OCTN2 gehört wie OCTN1 und OCTN3 zur Familie der OCTN, einer Unterfamilie der *organic cation transporter* (OCT), die hauptsächlich Xenobiotika in Geweben wie Niere, Dünndarm, Leber und Placenta eliminieren und in der Plasmamembran von Zellen lokalisiert sind. OCTN2 ist essentiell für die Aufrechterhaltung der Carnitin-Konzentration in den Zellen. Der Transporter ist involviert in renaler Reabsorption und wird gehemmt durch Substanzen wie Acetyl-D-, L-Carnitin, dem D-Isomer des Carnitins, Butyrobetain sowie durch Pharmazeutika wie Emetin, Quinidin und Verapamil. Daher können diese Substanzen einen sekundären Carnitin-Mangel verursachen (Gross und Henderson, 1984; Wagner *et al*., 2000). OCTN2 transportiert mit hoher Affinität Carnitin bzw. seine kurzkettigen Acyl-Ester und wird im Menschen in Herz- und Skelettmuskel, im proximalen und distalen renalen Tubulus, in Dünndarm, Leber, Placenta und einigen Gehirn-Arealen exprimiert (Grube *et al*., 2005; Tamai *et al.*, 1998; Wu *et al.*, 1999). In der Ratte sind hohe mRNA-Konzentrationen von OCTN2 in Niere und Darm sowie geringe Konzentrationen in Herz, Leber, Hoden, Muskel, Thymus, Milz und Großhirnrinde nachgewiesen worden (Slitt *et al*., 2002). OCTN2-Protein wurde des Weiteren bei ausgewachsenen Ratten in bestimmten Regionen des Nebenhodens nachgewiesen (Rodriguez *et al*., 2002). OCTN3 transportiert im Gegensatz zu OCTN1 und OCTN2 natriumunabhängig und ist als Membrantransporter in Peroxisomen lokalisiert (Lamhonwah *et al*., 2005). OCTN1 transportiert Carnitin im Menschen pH-abhängig (Tamai *et al*., 1997). Es zeigte sich, dass sowohl die mRNA-Konzentrationen von OCTN1 als auch die Konzentrationen von OCTN2 mit zunehmendem Alter ansteigen (Slitt *et al*., 2002). Ein weiterer Transporter des Carnitins ist der *amino acid transporter* B^{0+} (ATB^{0+}), welcher in Lunge, Milchdrüse und im Intestinaltrakt exprimiert ist und eine deutlich geringere Affinität zum Carnitin zeigt als OCTN2. Allerdings wird vermutet, dass der ATB^{0+} im Rahmen der passiven Diffusion von Carnitin, bei genetischen oder pharmakologischen Beeinträchtigungen des OCTN2 sowie bei

therapeutischer Supplementation von Carnitin eine große Bedeutung besitzt (Taylor, 2001). Des Weiteren wurde im menschlichen Hoden der *novel carnitine transporter* (CT2) entdeckt, der phylogenetisch zwischen OCT und *organic anion transporter* (OAT) einzuordnen ist (Enomoto *et al*., 2002).

Die mögliche Regulation des OCTN2 durch den Transkriptionsfaktor *peroxisome proliferator-activated receptor* α (PPARα) wurde in zahlreichen Studien bei Maus und Ratte untersucht. Bei Behandlungen von Wildtyp-Mäusen und Ratten mit den PPARα-Liganden WY14643 oder Clofibrat wurden im Vergleich zu PPARα $^{-/-}$-Mäusen oder unbehandelten Nagern höhere mRNA-Konzentrationen von OCTN2 in Leber, Muskel, Niere und Dünndarm sowie höhere Carnitin-Konzentrationen in Leber und Muskel nachgewiesen (Luci *et al*., 2006; Koch *et al*., 2008; Maeda *et al*., 2008; Ringseis *et al*., 2008a; Van Vlies *et al*., 2007). Wildtyp-Mäuse, die mit WY14643 behandelt wurden, zeigten im Vergleich zu unbehandelten Wildtyp-Mäusen eine erhöhte mRNA-Expression von γ-BBD in Leber und Muskel und eine höhere mRNA-Expression von OCTN3 in Niere und Dünndarm. In Studien, in denen der PPARα durch einen Fastenzustand aktiviert wurde, erfolgte ein Anstieg der Genexpression von OCTN2 in Leber, Niere und Herz (Luci *et al*., 2008; Van Vlies *et al*., 2007). Bei restriktiver Fütterung und im Fastenzustand waren die Acyl-CoA-Oxidase und Carnitin-Palmitoyltransferase 1 (CPT1) als Marker der Fettsäure-Oxidation in Leber, Niere und Herz deutlich erhöht. Makowski *et al*. (2009) zeigten ebenso eine Erhöhung der mRNA-Expression der γ-BBD im Fastenzustand. Des Weiteren war die Expression des OCTN2 bei PPARα $^{-/-}$-Mäusen im Vergleich zu PPARα$^{+/+}$-Mäusen um 40 % reduziert. Die Tatsache, dass eine PPARα-Aktivierung durch Clofibrat die mRNA-Konzentrationen von OCTN2 in Leber und Dünndarm von Ratten erhöht, lässt vermuten, dass diese Aktivierung die Absorption von Carnitin begünstigt (Luci *et al*., 2006; Ringseis *et al*., 2007). Bei der nicht-proliferierenden Spezie Schwein ist die Expression von PPARα allerdings weitaus geringer als bei Nagern (Holden und Tugwood, 1999), was das Ausmaß der Regulation von OCTN2 durch PPARα vermutlich limitiert und möglicherweise die geringeren Carnitin-Konzentrationen in Leber, Niere, Herz und Plasma bei Schweinen im Vergleich zu Ratten begründet. Es stellt sich die Frage, ob auch beim Schwein ein Anstieg der mRNA-Expression des OCTN2 durch PPARα-Aktivierung zu erwarten ist, was wiederum die Carnitin-Homöostase im Schwein beeinflussen würde. Durch Klärung dieser Fragestellung kann man auch Rückschlüsse auf die Wirkung einer PPARα-Aktivierung beim Menschen ziehen, da dieser vergleichbare Konzentrationen von PPARα in der Leber besitzt.

1.3 Funktion des Carnitins

Fettsäuren werden in allen Geweben außer dem Gehirn als Energiesubstrat umgesetzt. Fritz *et al*. (1963) sowie Bremer (1963) beschrieben erstmals die Bedeutung des Carnitins als Transportcarrier bei der Fettsäureoxidation von mittel- und langkettigen Fettsäuren im Organismus. In darauf folgenden Publikationen wurde der Mechanismus des Carnitins als Transportcarrier entschlüsselt und erläutert (Bremer, 1983; McGarry und Brown, 1997; Ramsay et al., 2001; Vaz und Wanders, 2002). Hierbei werden Acylgruppen langkettiger Fettsäuren mit der Hydroxylgruppe des Carnitins an der äußeren Mitochondrienmembran verestert, um für weitere Stoffwechselreaktionen in das Mitochondrium gelangen zu können. Als Katalysator fungiert das Enzym CPT1. Acyl-Carnitin gelangt im Anschluss mit Hilfe der Carnitin-Acylcarnitintranslokase über die innere Mitochondrienmembran in die mitochondriale Matrix. Hier erfolgt, katalysiert durch CPT2, eine Reveresterung von Acylcarnitin zu Acyl-CoA und Carnitin. Carnitin ist in der Lage, das Mitochondrium entweder eigenständig zum weiteren Fettsäure-Transport oder in Form von Acetyl-Carnitin zu verlassen, wobei die zugehörige Acetylgruppe nach der ß-Oxidation von kurz- oder mittelkettigen Acyl-CoA abgespalten werden kann. Katalysiert wird diese Umwandlung durch die Carnitin-Acetyltransferase (Abb.2).

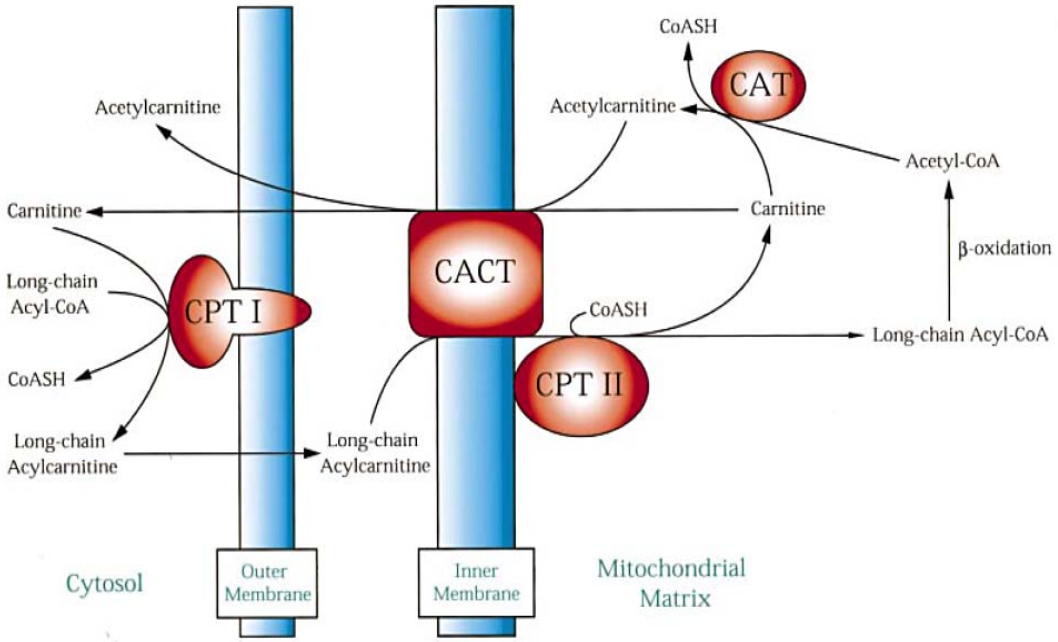

Abb.2: Funktion des Carnitins als Transporter von langkettigen Fettsäuren vom Cytosol zur mitochondrialen Matrix. (aus Vaz und Wanders, 2002)

Durch die Möglichkeit der Reveresterung zum Acyl-Carnitin bzw. Acyl-CoA zeigt sich eine weitere Funktion des Carnitins in der Aufrechterhaltung des Verhältnisses von Acyl CoA/CoA (Carter *et al*., 1995). Dabei werden die in Höchstdosen toxisch wirkenden Acyl-Gruppen als Carnitinester ausgeschieden, die den höchsten Anteil der Derivate im Körper darstellen (Duran *et al*., 1990; Rebouche, 1996). Carnitin trägt des Weiteren bei der peroxisomalen Fettsäureoxidation zur intrazellulären Kommunikation bei (Jakobs und Wanders, 1995), indem es kurzkettige Produkte der ß-Oxidation aus den Peroxisomen in die Peripherie transportiert. Es dient in Form von Acetyl-Carnitin als Energiespeicher (Bremer, 1983), zur Membranstabilisierung und ist am verzweigtkettigen Aminosäure-Metabolismus beteiligt (Sewell und Böhles, 1995).

1.4 Carnitin in der Fütterungspraxis des Schweines

Zahlreiche Untersuchungen zu Biosynthese, Absorption und Funktion des Carnitins beziehen sich auf das Säugetier im Allgemeinen. Aufgrund der potentiellen Möglichkeit durch Carnitin-Supplementierungen den Energiestoffwechsel beeinflussen zu können, sind Forschungen zur Synthese und Homöostase des Carnitins im Schwein als landwirtschaftliches Nutztier von besonderer Bedeutung. Dabei liegt das agrarwissenschaftliche Interesse im Muskelaufbau und der folglichen Reduzierung des Fettansatzes im Schwein. Weiterhin erfordert eine ökonomische Schweineproduktion eine hohe Anzahl lebend geborener Ferkel mit maximalen Wachstumsraten, die u.a. durch die Fütterung gewährleistet werden muss. In diesem Rahmen ist eine mögliche Anreicherung des Futters mit Carnitin bedeutsam und vielversprechend. Da Sauenmilch beachtliche Mengen an Carnitin enthält (Kerner *et al*., 1984), liegt es nahe, dass frühzeitig entwöhnte Ferkel nicht in der Lage sind, Carnitin ausreichend zu synthetisieren (Owen *et al*., 1996). Ebenso ist die Kapazität zur Fettsäureoxidation im neugeborenen Ferkel von einer exogenen Carnitin-Zufuhr abhängig (Coffey *et al*., 1991; Van Kempen und Odle, 1993, 1995; Wolfe *et al*., 1978). Es wurde gezeigt, dass die Fütterung hoher Mengen Carnitins das Wachstum von abgesetzten Ferkeln positiv beeinflusst (Newton und Haydon 1988, 1989; Owen *et al.*, 1993).

Weiterhin wird die Stickstoffbilanz beim Schwein durch Supplementierung von Carnitin beeinflusst (Bohles *et al.,* 1984; Heo *et al*., 2000). Während sich der Proteinaufbau erhöht, verringert sich der Umfang des Körperfetts des Schweines (Birkenfeld *et al*., 2005; Owen *et al*., 1996, 2001a,b; Penn *et al.*, 1997; Rincker *et al*., 2003). Diese Ergebnisse sind auf die erhöhte ß-Oxidation von Fettsäuren zurück zu führen, welche den Proteinaufbau unterstützt.

In anderen Untersuchungen sind dagegen keine positiven Effekte einer Carnitin-Supplementierung beim Schwein zu verzeichnen (Cho *et al.*, 1999; Hoffman *et al.*, 1993).
Bei trächtigen Sauen resultierte ein Carnitin-Einsatz in erhöhten Körpergewichtszunahmen (Musser *et al*., 1999; Ramanau *et al*., 2002) sowie erhöhten Konzentrationen des *insulin-like growth factor-I* (IGF-I) (Musser *et al.*, 1999; Doberenz *et al.*, 2006) und IGF-II (Doberenz *et al.*, 2006) im Plasma. Dabei war die Chorionmasse sowie die Protein-Konzentration des Glukose Transporters-1 in den mit L-Carnitin supplementierten Sauen signifikant erhöht, was auf eine bessere Nährstoffversorgung der Ferkel schließen lässt (Doberenz *et al*., 2006). Es konnte gezeigt werden, dass eine Supplementierung von Carnitin in der Trächtigkeitsphase der Sau die Anzahl der lebend geborenen Ferkel erhöht (Ramanau *et al.*, 2004) sowie die Anzahl der totgeborenen Ferkel vermindert (Musser *et al.*, 1999). Weiterer Effekt der Carnitin-Zufuhr während der Trächtigkeit ist das erhöhte Wurfgewicht am Tag der Geburt (Eder *et al*., 2001; Musser *et al*., 1999; Ramanau *et al.*, 2002) sowie eine höhere Körpergewichtszunahme der Ferkel in der Säugeperiode (Eder *et al*., 2001; Ramanau *et al*., 2002, 2004). Ursache für diese größere Gewichtszunahme ist die angestiegene Milchproduktion der mit L-Carnitin behandelten Sau (Ramanau *et al*., 2004), bedingt durch die längere Säugezeit der Ferkel von Carnitin supplementierten Sauen (Birkenfeld *et al*., 2006).
Diese verschiedenen positiven Wirkungen von Carnitin im Metabolismus verstärken die Bedeutung weiterer Forschungen zum Carnitin-Stoffwechsel und Carnitin-Status beim Schwein als landwirtschaftliches Nutztier.

2. Zielstellung

In den letzten Jahrzehnten wuchs das Interesse an der Erforschung des Carnitins beträchtlich. Schwerpunkte waren dabei hauptsächlich Fragen zur Synthese, Absorption, Regulation und Supplementierung von Carnitin. In Humanstudien und Studien am Versuchstier Ratte konnten dazu bisher zahlreiche Kenntnisse gewonnen werden. Während beim Schwein hauptsächlich Untersuchungen zum Einfluss des Carnitins auf Leistungsparameter durchgeführt wurden, fehlen Forschungsergebnisse zur Carnitin-Synthese und zum Carnitin-Status bislang gänzlich. Diese sind jedoch essentiell zur Optimierung der Energieeffizienz und zur Beurteilung von Carnitin-Supplementationen beim Schwein als landwirtschaftliches Nutztier. Ziel der vorliegenden Arbeit war es daher, den Carnitin-Status in verschiedenen Lebensstadien des Schweines sowie dessen Beeinflussung durch Carnitin- und Lysin-Supplementierungen zu erforschen. Des Weiteren sollten grundlegende Kenntnisse zur Carnitin-Synthese im Schwein gewonnen werden. Dazu wurde in Abhängigkeit des Alters und der Carnitin-Zufuhr des Schweines die Aktivität des Enzyms γ-BBD bestimmt, welches eine große Bedeutung in der Carnitin-Synthese besitzt. Die Gewebe-Konzentrationen von Carnitin und seiner Derivate wurden erfasst, um Aussagen zum Carnitin-Status treffen zu können. Durch Verabreichung von Carnitin-Supplementen wurde erstmals die Absorption von Carnitin im Schwein bestimmt. Weiterhin wurden Untersuchungen zur möglichen Regulation der Carnitin-Aufnahme durch den Transkriptionsfaktor PPARα durchgeführt.

2.1 Untersuchungen zur Wirkung von Lysin auf den Carnitin-Status

Owen *et al*. (1996) wiesen darauf hin, dass bei geringer Lysin-Konzentration in der Diät die deutlichsten Effekte einer Carnitin-Zufuhr auf das Wachstum des Schweines zu verzeichnen sind. Dies ist ein Indiz dafür, dass eine Interaktion zwischen Lysin und Carnitin im Stoffwechsel besteht. Bislang wurde beim Schwein nicht erforscht, inwieweit die Lysin-Zufuhr den Status des Carnitins beeinflussen kann. Ziel der ersten Studie war es daher, mögliche Effekte von Lysin auf die Carnitin-Homöostase beim wachsenden Schwein zu untersuchen. Dazu wurden die Versuchstiere in zwei Gruppen gegliedert. Der Kontrollgruppe wurde eine Basisdiät mit einer Lysin-Konzentration gemäß den Bedarfsempfehlungen für wachsende Schweine verabreicht (National Research Council, 1998), während die Behandlungsgruppe eine Diät mit moderatem Lysin-Überschuß erhielt. Zur Ermittlung des Carnitin-Status wurden die Konzentrationen von Carnitin in Plasma, Leber, Niere sowie Skelettmuskel bestimmt. Um Aussagen zu möglichen Änderungen in der Carnitin-Synthese durch moderaten Lysin-Überschuß treffen zu können, wurden die Konzentrationen der

Carnitin-Vorstufen Trimethyllysin und Butyrobetain gemessen. Des Weiteren wurde die Aktivität des Enzyms γ-BBD, welches in die Carnitin-Synthese involviert ist, in Leber, Niere und Skelettmuskel erfasst. Um zu untersuchen, ob erhöhte Konzentrationen an Trimethyllysin im Skelettmuskel das Resultat einer verminderten Genexpression der TMLD sind, wurde diese in Leber, Niere und Skelettmuskel bestimmt. Ebenso wurde die Genexpression des OCTN2 in Leber, Niere und Skelettmuskel gemessen, um auszuschließen, dass Änderungen in der Genexpression dieses Transporters Ursache für unterschiedliche Konzentrationen des Carnitins in den jeweiligen Versuchsgruppen sind.
Versuchsdesign, Ergebnisse und Schlussfolgerungen dieser Untersuchungen sind ersichtlich in:

Studie 1:
Fischer M, Hirche F, Kluge H, Eder K (2009a) A moderate excess of dietary lysine lowers plasma and tissue carnitine concentrations in pigs. ***Br J Nutr*** *101(2), 190-196*

2.2 Untersuchungen zur Absorption von Carnitin-Supplementen

Da Carnitin maßgeblich an der ß-Oxidation von Fettsäuren beteiligt ist, wird vermutet, dass eine Verabreichung von Carnitin und die dadurch erhöhten Carnitin-Konzentrationen in den Geweben die Energiegewinnung bei Nutztieren fördert. Bei wachsenden Schweinen wurden durch eine Carnitin-Supplementation die Futterverwertung sowie der Proteinansatz positiv beeinflusst, während die Fettanlagerung vermindert war (Owen *et al.*, 1996; Heo *et al.*, 2000; Owen *et al*., 2001a, 2001b; Rincker *et al.*, 2003; Birkenfeld *et al.*, 2005). Diese Effekte resultieren aus einer erhöhten ß-Oxidation, welche den Proteinaufbau begünstigt (Owen *et al.*, 2001b). Im Gegensatz dazu zeigten weitere Studien keine oder nur geringe Effekte einer Carnitin-Supplementation auf Wachstum und Körperzusammensetzung von Schweinen (Hoffman *et al.*, 1993; Cho *et al.*, 1999). Zu möglichen Auswirkungen von Carnitin-Supplementationen auf den Carnitin-Status beim Schwein gibt es bislang nur geringe Erkenntnisse. Weiterhin ist nicht bekannt, in welchem Ausmaß Carnitin beim Schwein absorbiert wird. Diese Fragestellungen sollten in der zweiten Studie geklärt werden. Dazu wurde Schweinen Carnitin in verschieden Dosen verabreicht. Nach einer Versuchsdauer von 3 Wochen wurden die Konzentrationen von Carnitin und Acetyl-Carnitin in Plasma und Gewebe bestimmt. Zusätzlich erfolgte die Messung der Absorption des Carnitins im Dünndarm des Schweines mittels Indikatormethode. Um zu untersuchen, ob eine Verabreichung von Carnitin die Biosynthese dieses Stoffes im Schwein beeinflusst, wurden

die Konzentrationen der Carnitin-Vorläufer Trimethyllysin und Butyrobetain erfasst, sowie die Aktivität der γ-BBD gemessen. Versuchsdesign, Ergebnisse und Schlussfolgerungen dieser Untersuchungen sind ersichtlich in:

Studie 2:

Fischer M, Varady J, Hirche F, Kluge H, Eder K (2009b) Supplementation of L-carnitine in pigs: Absorption of carnitine and effect on plasma and tissue carnitine concentrations. ***Arch Anim Nutr*** *63(1), 1-15*

2.3 Untersuchungen zur Regulation der Carnitin-Aufnahme durch PPARα-Aktivierung

Es ist bekannt, dass der Zustand des Fastens oder die Behandlung mit Clofibrat die Konzentration von Carnitin in der Leber von Ratten erhöht (McGarry et al., 1975; Brass und Hoppel, 1978). Ursache hierfür ist vermutlich die erhöhte mRNA-Expression von OCTN2, welche durch PPARα-Aktivierung vermittelt wird (Luci et al., 2006; Ringseis et al., 2007). Daraus kann man schlussfolgern, dass die Carnitin-Absorption im Dünndarm sowie der Carnitin-Transport vom Plasma zur Leber mutmaßlich durch PPARα-Aktivierung stimuliert werden. In den einzelnen Spezies zeigen sich allerdings beträchtliche Unterschiede hinsichtlich der Expression von PPARα und der Wirkung dieses Transkriptionsfaktors auf mutmaßliche Zielgene. Im Gegensatz zum Nager, wird in der Leber von Schweinen und Menschen durch PPARα-Agonisten keine Peroxisomen-Proliferation hervorgerufen. Diese nicht-proliferierenden Spezies besitzen geringere Expressionen von PPARα in der Leber. Ebenso ist die Reaktion von vermutlichen Zielgenen des PPARα auf Aktivierung dieses Transkriptionsfaktors weitaus geringer als bei Vertretern der proliferierenden Spezie (Holden und Tugwood, 1999). In der dritten Studie stellte sich die Frage, inwieweit eine PPARα-Aktivierung die Carnitin-Homöostase im Schwein als nicht-proliferierende Spezie beeinflusst. Die PPARα-Aktivierung erfolgte dabei mittels Clofibrat als PPARα-Agonist. Es wurden die mRNA-Konzentrationen des OCTN2 in Dünndarm, Leber und Skelettmuskel sowie die Konzentrationen an Carnitin und Butyrobetain in Plasma, Leber und Skelettmuskel bestimmt. Um einen möglichen Einfluss von Clofibrat auf die Carnitin-Synthese in der Leber zu untersuchen, wurde in diesem Gewebe die Aktivität der γ-BBD gemessen.

Versuchsdesign, Ergebnisse und Schlussfolgerungen dieser Untersuchungen sind ersichtlich in:

Studie 3:

Ringseis R, Luci S, Spielmann J, Kluge H, Fischer M, Geissler S, Wen G, Hirche F, Eder K (2008) Clofibrate treatment up-regulates novel organic cation transporter (OCTN)-2 in tissues of pig as a model of non-proliferating species. ***Eur J Pharmacol*** *583(1), 11-7*

2.4 Untersuchungen zur γ-BBD-Aktivität und Carnitin-Konzentration in Geweben

Die Aktivität der γ-BBD wurde in der Leber von vielen Säugetieren nachgewiesen (Vaz und Wanders, 2002). In weiteren Geweben ist die Aktivität des Enzyms jedoch speziesabhängig. Während in der Niere von Mensch, Katze, Rind, Hamster, Kaninchen und Rhesusaffe ebenso eine Aktivität gemessen wurde, ist das Enzym γ-BBD in der Niere von Cebusaffe, Schaf, Hund, Meerschwein, Maus und Ratte nicht oder nur sehr gering aktiv (Vaz und Wanders, 2002). Beim Menschen wurde zusätzlich eine γ-BBD-Aktivität im Gehirn nachgewiesen (Rebouche und Engel, 1980). Im Gegensatz dazu gibt es beim Schwein wenige Erkenntnisse zu Vorkommen und Aktivität der γ-BBD, zu Gewebekonzentrationen von Carnitin und zum Carnitin-Metabolismus im Schwein. Daher wurden in der vierten Studie die Konzentrationen von Carnitin in verschiedenen Geweben des Schweines gemessen. Außerdem wurden die mRNA- und Protein-Konzentrationen sowie die Aktivitäten der γ-BBD in Geweben des Schweines bestimmt. In der Literatur gibt es Hinweise, dass der Carnitin-Status bei Schweinen am Tag der Geburt vermutlich unzureichend ist (Heo *et al.*, 2000; Penn *et al.*, 1997; Van Kempen und Odle, 1995). Da Sauenmilch beachtliche Mengen an Carnitin enthält (Kerner *et al*., 1984), liegt es nahe, dass frühzeitig entwöhnte Ferkel nicht in der Lage sind, Carnitin ausreichend zu synthetisieren (Owen *et al*., 1996). Um zu untersuchen, ob der Carnitin-Status des Ferkels am Tag der Geburt gering ist, wurden die Gewebe-Konzentrationen von Carnitin und Acetyl-Carnitin im Ferkel vom Tag der Geburt bis zur 7. Lebenswoche ermittelt. Weiterhin wurden die Aktivitäten der γ-BBD in Leber und Niere sowie die Gewebe-Konzentrationen von Butyrobetain erfasst, um festzustellen, ob die Syntheserate des Carnitins in den ersten Lebenswochen gering ist.

Versuchsdesign, Ergebnisse und Schlussfolgerungen dieser Untersuchungen sind ersichtlich in:

Studie 4:

Fischer M, Keller J, Hirche F, Kluge H, Ringseis R, Eder K (2009c) Activities of γ-butyrobetaine dioxygenase and concentrations of carnitine in tissues of pigs. ***Comp Biochem Physiol A Mol Integr Physiol*** *(online published)*

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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

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 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.

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).

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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($P<0{\cdot}05$; 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 ($P<0{\cdot}05$; 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 ($P<0{\cdot}05$); 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 ($P<0{\cdot}05$; 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: *$P<0{\cdot}05$.

† For details of procedures and diets, see the Materials and methods section and Table 1.

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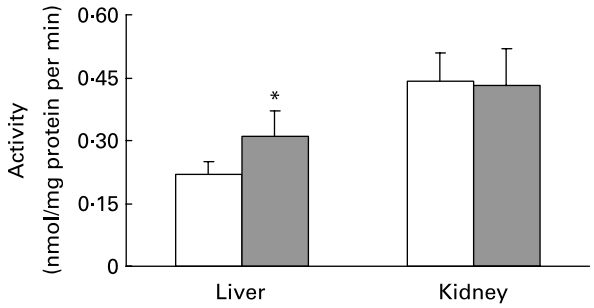

Fig. 1. Activity of γ-butyrobetaine dioxygenase in liver and kidney of pigs fed a control diet (9·7 g lysine/kg; □) or a high-lysine diet (16·8 g/kg; ■). Values are means with standard deviations depicted by vertical bars (*n* 10). Mean value was significantly different from that of the control group: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

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

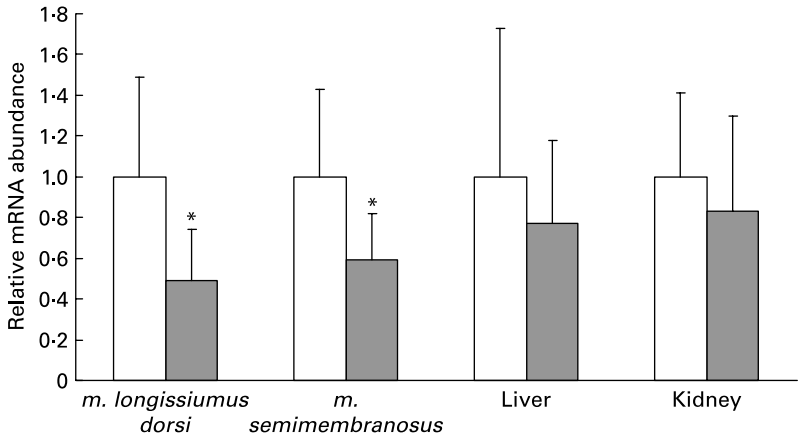

Fig. 2. Relative mRNA abundance of trimethyllysine dioxygenase in *m. longissimus dorsi* and *m. semimembranosus*, liver and kidney of pigs fed a control diet (9·7 g lysine/kg; □) or a high-lysine diet (16·8 g/kg; ■). Total RNA was extracted from the samples and relative mRNA levels of the genes were 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: *$P<0·05$.

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.

Acknowledgements

M. F. conducted the animal experiments and gene expression analyses and prepared the manuscript; F. H. determined concentrations of carnitine, γ-butyrobetaine and trimethyllysine and activity of γ-butyrobetaine dioxygenase; H. K. planned and supervised animal experiments; K. E. conceived of the study and its design, coordinated work, participated in the interpretation of the results and helped to draft the manuscript. The study was in part supported by LONZA, Basel, Switzerland. There is no potential conflict of interest.

References

1. McGarry JD & Brown NF (1997) The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. *Eur J Biochem* **244**, 1–14.
2. Brass EP (2002) Pivalate-generating prodrugs and carnitine homeostasis in man. *Pharmacol Rev* **54**, 589–598.
3. Steiber A, Kerner J & Hoppel CL (2004) Carnitine: a nutritional, biosynthetic, and functional perspective. *Mol Aspects Med* **25**, 455–473.
4. Rebouche CJ & Seim H (1998) Carnitine metabolism and its regulation in microorganisms and mammals. *Annu Rev Nutr* **18**, 39–61.
5. Hoppel CL & Davis AT (1986) Inter-tissue relationships in the synthesis and distribution of carnitine. *Biochem Soc Trans* **14**, 673–674.

6. Vaz FM & Wanders RJ (2002) Carnitine biosynthesis in mammals. *Biochem J* **361**, 417–429.
7. Lahjouji K, Mitchell GA & Qureshi IA (2001) Carnitine transport by organic cation transporters and systemic carnitine deficiency. *Mol Genet Metab* **73**, 287–297.
8. Tein I (2003) Carnitine transport: pathophysiology and metabolism of known defects. *J Inherit Metab Dis* **26**, 147–169.
9. Tamai I, Ohashi R, Nezu JI, Sai Y, Kobayashi D, Oku A, Shimane M & Tsuji A (2000) Molecular and functional characterization of organic cation/carnitine transporter family in mice. *J Biol Chem* **275**, 40064–40072.
10. Tamai I, Ohashi R, Nezu J, Yabuuchi H, Oku A, Shimane M, Sai Y & Tsuji A (1998) Molecular and functional identification of sodium ion-dependent, high affinity human carnitine transporter OCTN2. *J Biol Chem* **273**, 20378–20382.
11. Owen KQ, Nelssen JL, Goodband RD, Weeden TL & Blum SA (1996) Effect of L-carnitine and soybean oil on growth performance and body composition of early-weaned pigs. *J Anim Sci* **74**, 1612–1619.
12. Heo K, Odle J, Han IK, Cho W, Seo S, van Heugten E & Pilkington DH (2000) Dietary L-carnitine improves nitrogen utilization in growing pigs fed low energy, fat-containing diets. *J Nutr* **130**, 1809–1814.
13. Owen KQ, Jit H, Maxwell CV, Nelssen JL, Goodband RD, Tokach MD, Tremblay GC & Koo SI (2001) Dietary L-carnitine suppresses mitochondrial branched-chain keto acid dehydrogenase activity and enhances protein accretion and carcass characteristics of swine. *J Anim Sci* **79**, 3104–3112.
14. Owen KQ, Nelssen JL, Goodband RD, Tokach MD & Friesen KG (2001) Effect of dietary L-carnitine on growth performance and body composition in nursery and growing-finishing pigs. *J Anim Sci* **79**, 1509–1515.
15. Rincker MJ, Carter SD, Real DE, *et al.* (2003) Effects of increasing dietary L-carnitine on growth performance of weanling pigs. *J Anim Sci* **81**, 2259–2269.
16. Birkenfeld C, Ramanau A, Kluge H, Spilke J & Eder K (2005) Effect of dietary L-carnitine supplementation on growth performance of piglets from control sows or sows treated with L-carnitine during pregnancy and lactation. *J Anim Physiol Anim Nutr* **89**, 277–283.
17. Hoffman LA, Ivers DJ, Ellersieck MR & Veum TL (1993) The effect of L-carnitine and soybean oil on performance and nitrogen and energy utilization by neonatal and young pigs. *J Anim Sci* **71**, 132–138.
18. Cho WT, Kim JH, Bae SH, Han IK, Han YK, Heo KN & Odle J (1999) Effects of L-carnitine with different lysine levels on growth and nutrient digestibility in pigs weaned at 21 days of age. *Asian-Aust J Anim Sci* **12**, 799–805.
19. Khan-Siddiqui L & Bamji MS (1983) Lysine–carnitine conversion in normal and undernourished adult men – suggestion of a nonpeptidyl pathway. *Am J Clin Nutr* **37**, 93–98.
20. Vijayasarathy C, Khan-Siddiqui L, Murthy SN & Bamji MS (1987) Rise in plasma trimethyllysine levels in humans after oral lysine load. *Am J Clin Nutr* **46**, 772–777.
21. Rebouche CJ, Bosch EP, Chenard CA, Schabold KJ & Nelson SE (1989) Utilization of dietary precursors for carnitine synthesis in human adults. *J Nutr* **119**, 1907–1913.
22. Davis AT, Kruggel EM & Randall S (1993) Excess dietary lysine increases skeletal muscle and plasma trimethyllysine in rats. *J Nutr* **123**, 1109–1116.
23. National Research Council (1998) *Nutrient Requirement of Swine*, 10th rev. ed. Washington, DC: National Academy of Sciences.
24. Bassler R & Buchholz H (1993) Methodenbuch Band III. In *Die chemische Untersuchung von Futtermitteln, 3. Ergänzungslieferung*. Darmstadt: VDLUFA-Verlag.
25. Gesellschaft für Ernährungsphysiologie (2006) *Empfehlungen zur Energie- und Nährstoffversorgung von Schweinen*. Frankfurt/Main: DLG-Verlag.
26. Fontaine J, Bech-Andersen S, Bütikofer U & De Froidmont-Görtz I (1998) Determination of tryptophan in feed by HPLC – development of an optimal hydrolysis and extraction procedure by the EU Commission DG XII in three international collaborative studies. *Agribiol Res* **51**, 97–108.
27. Eder K, Peganova S & Kluge H (2001) Studies on the tryptophan requirement of piglets. *Arch Anim Nutr* **55**, 281–297.
28. Ringseis R, Pösel S, Hirche F & Eder K (2007) Treatment with pharmacological peroxisome proliferator-activated receptor α agonist clofibrate causes upregulation of organic cation transporter 2 in liver and small intestine of rats. *Pharmacol Res* **56**, 175–183.
29. Van Vlies N, Wanders RJ & Vaz FM (2006) Measurement of carnitine biosynthesis enzyme activities by tandem mass spectrometry: differences between the mouse and the rat. *Anal Biochem* **354**, 132–139.
30. König B & Eder K (2006) Differential action of 13-HPODE on PPARα downstream genes in rat Fao and human HepG2 hepatoma cell lines. *J Nutr Biochem* **17**, 410–418.
31. Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* **29**, e45.
32. Roth FX, Eder K & Kirchgessner M (1999) The effect of energy density and the lysine to energy ratio of diets on the performance of piglets. *J Anim Physiol Anim Nutr* **82**, 1–7.
33. Kendall DC, Gaines AM, Allee GL & Usry JL (2008) Commercial validation of the true ileal digestible lysine requirement for eleven- to twenty-seven-kilogram pigs. *J Anim Sci* **86**, 324–332.
34. van Lunen TA & Cole DJA (1998) The effect of dietary energy concentration and lysine/digestible energy ratio on growth performance and nitrogen deposition of young hybrid pigs. *Anim Sci* **67**, 117–129.
35. Smith JW II, Tokach MD, Nelssen JL & Goodband RD (1999) Effects of lysine:calorie ratio on growth performance of 10- to 25-kilogram pigs. *J Anim Sci* **77**, 3000–3006.
36. Urynek W & Buraczewska L (2003) Effect of dietary energy concentration and apparent ileal digestible lysine:metabolizable energy ratio on nitrogen balance and growth performance of young pigs. *J Anim Sci* **81**, 1227–1236.
37. Davis AT & Monroe TJ (2005) Carnitine deficiency and supplementation do not affect the gene expression of carnitine biosynthetic enzymes in rats. *J Nutr* **135**, 761–764.
38. van Vlies N, Ofman R, Wanders RJ & Vaz FM (2007) Submitochondrial localization of 6-*N*-trimethyllysine dioxygenase – implications for carnitine biosynthesis. *FEBS J* **274**, 5845–5851.
39. Rebouche CJ (2004) Kinetics, pharmacokinetics, and regulation of L-carnitine and acetyl-L-carnitine metabolism. *Ann N Y Acad Sci* **1033**, 30–41.
40. Nakajima H, Kodo N, Inoue F, Kizaki Z, Nukina S, Kinugasa A & Sawada T (1996) Pivalate affects carnitine status but causes no severe metabolic changes in rat liver. *J Nutr* **126**, 1683–1687.
41. Wang TC & Fuller MF (1989) The optimum dietary amino acid pattern for growing pigs. 1. Experiments by amino acid deletion. *Br J Nutr* **62**, 77–89.
42. Fuller MF, McWilliam R, Wang TC & Giles LR (1989) The optimum dietary amino acid pattern for growing pigs. 2. Requirements for maintenance and for tissue protein accretion. *Br J Nutr* **62**, 255–267.

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Supplementation of L-carnitine in pigs: Absorption of carnitine and effect on plasma and tissue carnitine concentrations

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This study was performed to investigate the bioavailability of carnitine supplements and their effects on the carnitine status of pigs. Seven groups of young pigs with an average body weight of 10 kg were fed a basal diet or the same diets supplemented with 25, 50, 100, 200, 500 or 1000 mg of L-carnitine per kg for 20 days. Absorption rate of the supplemented carnitine in the small intestine, assessed by the use of titanium dioxide as an indigestible indicator, was greater than 95% for the lower doses (25, 50, 100 mg/kg) and greater than 90% for the higher doses (200, 500, 1000 mg/kg). Supplementation of carnitine caused a dose-dependent increase of free carnitine, acetyl and total carnitine concentrations in plasma, liver, kidney, heart and skeletal muscle. At the highest dose of 1000 mg/kg, plasma and tissue total carnitine concentrations were 3- to 6-fold higher than in the unsupplemented control group. In conclusion, the present study shows that young pigs have a high capacity to absorb carnitine from the diet. It is also shown that plasma and tissue carnitine concentrations in young pigs can be markedly increased by supplementation of carnitine.

Keywords: pigs; carnitine; absorption; gamma-butyrobetaine; trimethyllysine

1. Introduction

Carnitine (L-3-hydroxy-4-N-N-N-trimethylaminobutyrate) is an essential metabolite, which has a number of indispensable functions in intermediary metabolism (Steiber et al. 2004). All tissues that use fatty acids as a fuel source require carnitine for normal function. Carnitine is derived from dietary sources and endogenous biosynthesis (Hoppel and Davis 1986; Rebouche and Seim 1998). Carnitine biosynthesis involves a complex series of reactions. Lysine in protein peptide linkage provides the carbon backbone of carnitine. It undergoes methylation of the ε-amino group to yield trimethyllysine, which is released upon protein degradation. The released trimethyllysine is further oxidised to γ-butyrobetaine which is then hydroxylated in liver and kidney by γ-butyrobetaine dioxygenase to form carnitine (Vaz and Wanders 2002). 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 (Tamai et al. 1998; Tein 2003).

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Due to its involvement in β-oxidation of fatty acids, it has been hypothesised that increasing tissue carnitine concentrations by dietary supplementation of carnitine could promote energy production from fatty acids in farm animals. In growing pigs, some experiments indeed showed an improvement of the gain:feed ratio, higher rates of protein accretion and lower rates of fat accretion compared to untreated control pigs (Owen et al. 1996; Heo et al. 2000; Owen et al. 2001a, 2001b; Rincker et al. 2003; Birkenfeld et al. 2005). These effects have been attributed to an increased β-oxidation of fatty acids which favour the formation of protein (Owen et al. 2001b). There are, however, some other studies in which carnitine supplementation had less or even no effect on growth performance and body composition of pigs (Hoffman et al. 1993; Cho et al. 1999). The reason for these contradictory results is largely unknown. However, there is actually little information about the effect of carnitine supplementation on the carnitine status of pigs. Moreover, the bioavailability of oral applied carnitine supplements in the small intestine has not yet been investigated. Therefore, we performed an experiment with growing pigs fed diets supplemented with various concentrations of carnitine. To assess the carnitine status and the intestinal bioavailability of the carnitine supplements, we determined concentrations of carnitine in plasma and various tissues of these pigs and intestinal absorption rate using the indicator method. To determine whether carnitine supplementation could influence carnitine biosynthesis in the body, we also determined the activity of γ-butyrobetaine dioxygenase and concentrations of the carnitine precursors trimethyllysine and γ-butyrobetaine.

2. 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).

2.1. Animals and diets

Fifty-six male crossbred pigs [(German Landrace × Large White) × Pietrain] with an average body weight of 10 ($\pm$1) kg at four weeks of age were used. The animals were used for an experiment which lasted 21 days. They were kept in an environmentally controlled facility with a temperature of 23°C, a relative humidity between 55 and 60%, and light from 06:00 to 19:00 h. One day before the start of the experimental feeding period the pigs were weighed and randomly assigned to seven groups of eight animals each. One group received a basal diet which was nutritionally adequate for growing pigs in a body weight range between 10 and 20 kg (Table 1). The six other groups received the same diets supplemented with 25, 50, 100, 200, 500 or 1000 mg L-carnitine (obtained from Lohmann Animal Health, Cuxhaven, Germany) per kg. For the first 20 days, all pigs were housed in pairs in flat-deck pens. During this time, the animals were given free access to the diets. To control the feed intake, unconsumed feed was weighed weekly. At the evening of day 20, feed was removed and five animals per group were moved into single cages. At the morning of day 21, body weights of all pigs were recorded. The five animals per group which were separated into single cages received an amount of 300 g of their diet and were thereafter killed 2.5 h later for the determination of the absorption of carnitine. The other three pigs remained in their original pens; they also received 300 g of feed and were killed 2.5 h thereafter. Water was available *ad libitum* from a nipple drinker system during the whole experiment.

Table 1. Composition and nutrients of the basal diet.

	Contents [g/kg]
Ingredients	
Wheat	490
Barley	201.4
Soy bean meal (44% CP)	240
Soy oil	30
Calcium carbonate	1.5
Sodium chloride	0.3
Mineral feed[#]	30
Lysine HCl	0.5
DL-methionine	0.8
L-threonine	0.3
L-tryptophan	0.2
Titanium dioxide	5
Contents of metabolisable energy and nutrients	
Metabolisable energy [MJ/kg]	13.6
Crude protein [g/kg]	179
Lysine [g/kg]	11.7
Methionine + cysteine [g/kg]	6.8
Threonine [g/kg]	7.6
Tryptophan [g/kg]	2.6

[#]Mineralfutter 8581 für Ferkel (BASU, Bad Sulza, Germany) supplied the following per kg diet: 5.4 g calcium, 2.0 g phosphorus, 1.2 g sodium, 12000 IU vitamin A, 1500 IU vitamin D, 75 mg vitamin E, 3 mg vitamin K, 3 mg thiamine, 6 mg riboflavine, 3 mg pyridoxine, 30 mg nicotinic acid, 12 mg pantothenic acid, 0.39 mg folic acid, 30 μg vitamin B_{12}, 150 mg choline chloride, 180 mg iron, 72 mg zinc, 60 mg manganese, 120 mg copper, 0.3 mg selenium, 0.45 mg cobalt, 2.4 g L lysine, 0.6 g DL methionine, 0.75 g threonine, 0.15 g tryptophan, 210 FXU NSP Enzyme, 555 FYT Phytase.

2.2. *Sample collection*

The animals were anaesthesised and exsanguinated 2.5 h after their last meal. Blood samples were collected into heparinised polyethylene tubes. Liver, kidneys, heart and samples from m. *longissimus dorsi* and m. *semimembranosus* were excised. Plasma was obtained by centrifugation of the blood samples (1,100 *g*, 10 min, 4°C). Plasma and tissue samples were stored at −20°C pending further analysis. In the five pigs selected for determination of carnitine absorption, the small intestine was rapidly excised, and divided into three segments of equal length (proximal, middle, distal). The chyme from these intestinal segments was pressed out manually and collected into separate tubes for subsequent freeze-drying and determination of titanium dioxide and carnitine concentrations.

2.3. *Calculation of absorption rate of carnitine supplements in small intestine*

The absorption rate of the carnitine supplements in small intestine was determined using the ''indicator method'' (Kirchgeßner 2004). This method facilitates the calculation of the absorption rate of a nutrient in small intestine from changes in the concentration ratio of a reference substance (indicator) between diet and chyme, therefore, making quantitative collection of faeces unnecessary. The reference substance or indicator has to be indigestible and non-absorbable, and must not interact with the digestive process. Based on the concentration ratio of indicator to carnitine supplemented to the diet and chyme

from the three segments of the small intestine, the absorption rate of the supplemented carnitine could be calculated according to the following equation, in which carnitine concentrations in the chyme of the pigs fed the control diet without added carnitine were used to correct for endogenously secreted carnitine plus the native carnitine in the diet which was not absorbed. The chyme of the proximal segment was examined to calculate the absorption rate of carnitine in the first third (proximal part) of the small intestine; the chyme of the middle segment was examined to calculate the absorption rate of carnitine in the first and second third (proximal + middle part) of the small intestine; the chyme of the distal segment was examined to calculate the absorption rate of carnitine in the whole small intestine.

$$\text{Absorption rate}\,[\%] = 100 - \left[\frac{\text{TiO}_{2\,\text{diet}} \cdot (\text{Carnitine}_{\text{chyme}} - \text{Carnitine}_{\text{correction}})}{\text{TiO}_{2\,\text{chyme}} \cdot \text{Carnitine}_{\text{diet}}}\right] \cdot 100$$

where $\text{TiO}_{2\text{diet}}$ = concentration of TiO_2 in the diet [mg TiO_2/g diet], $\text{TiO}_{2\text{chyme}}$ = concentration of TiO_2 in the chyme [mg TiO_2/g chyme], $\text{Carnitine}_{\text{chyme}}$ = concentration of carnitine in the chyme [mg carnitine/g chyme], $\text{Carnitine}_{\text{diet}}$ = concentration of carnitine supplemented in the diet [mg carnitine/g diet], $\text{Carnitine}_{\text{correction}}$ = concentration of carnitine in the chyme of the control group (correction for endogenous carnitine and non-absorbed native carnitine from the diet).

2.4. Determination of titanium dioxide

Concentration of the indigestible marker titanium dioxide in chyme samples was determined in modification to the method from Brandt and Allam (1987). In brief, 0.2 g freeze dried milled chyme were transferred with 3 g of Wieninger's reagent (Merck, Darmstadt, Germany) into glass tubes. Concentrated sulphuric acid (15 ml) was added and the mixture was incubated for 45 min in a heating block at 375°C (Tecator™ Digestor 20 Auto Lift System, Foss GmbH, Rellingen, Germany). After cooling with an ejector pump water was added. The mixture was transferred into volumetric flask and filled up with water to 100 ml. Five millilitres of the solution were mixed with 0.5 ml hydrogen peroxide (30%) and 0.5 ml water as blank value, respectively. After incubation for 1 h at 4°C the absorption was measured at 405 nm (SpectraFluor Plus, Tecan, Crailsheim, Germany). TiO_2 was used as standard solution with initial weights of 10–50 mg, which were treated in a similar way as the experimental samples.

2.5. Determination of nutrients in the diets

Concentrations of crude protein in the diets were analysed according to the official German VDLUFA methodology (Bassler and Buchholz 1993). The metabolisable energy of the diet was calculated as recommended by the German Nutrition Society (Gesellschaft für Ernährungsphysiologie 2006). The amino acid concentrations of the diet were also determined according to the official VDLUFA method (Bassler and Buchholz 1993). 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, Hamburg, Germany). For the determination of tryptophan, the diet was digested with barium hydroxide. The tryptophan concentration was determined by reversed-phase high performance liquid chromatography (Eder et al. 2001a).

2.6. Analysis of carnitine, and trimethyllysine and γ-butyrobetaine

Concentrations of free carnitine, acetyl carnitine, trimethyllysine and γ-butyrobetaine 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 (Ringseis et al. 2007). Fifty milligrams of freeze dried tissues 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 with 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 × 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, v/v/v/v) were used.

2.7. Determination of the activity of γ-butyrobetaine dioxygenase

Activity of γ-butyrobetaine dioxygenase in liver and kidney was determined as described previously in detail by Van Vlies et al. (2006). Homogenates from liver and kidney were prepared by homogenising tissue in 10 mM 3-N-Morpholinopropanesulfuric acid buffer (pH 7.4) containing 0.9% (w/v) sodium chloride, 10% (w/v) glycerol, and 5 mM dithiothreitol.

2.8. Statistical analysis

Data were treated by one factorial analysis of variance with dietary carnitine concentration as factor using the Minitab statistical software (Release 13, Minitab Inc., State College, PA, USA). For statistical significant F values ($p < 0.05$) means of the seven groups were compared by Fisher's multiple range test. In the case of large differences in the variances, data were transferred to their logarithms prior to analysis. Means were considered significantly different for $p < 0.05$. Results in the text are given as means ± SD.

3. Results

3.1. Carnitine concentrations of the diets, food intake and body weight gain of the pigs

The analysed concentration of carnitine in the basal diet was 11 ± 0.2 mg/kg. Carnitine concentrations of the diets supplemented with 25, 50, 100, 200, 500 and 1000 mg carnitin/kg were 29 ± 0.4; 57 ± 3; 114 ± 12; 222 ± 9; 596 ± 30 and 1073 ± 58 mg/kg, respectively (four determinations per diet).

Initial body weights, food intake during the experimental period and final body weights of the pigs did not differ between the seven groups. Initial body weight in average of the seven groups was 9.8 ± 1.7 kg, final body weight at day 21 was 17.0 ± 3.2 kg, daily feed intake was 521 ± 119 g, feed conversion ratio (g feed/g BWG) was 1.41 ± 0.29 ($n = 56$).

3.2. Bioavailability of supplemented carnitine in various segments of the small intestine

The rate of absorption of the supplemented carnitine was generally very high, even for the high supplementation levels (Table 2). Absorption rate of carnitine in the proximal (first third) and the proximal plus middle (first plus second third) was similar for all supplementation levels. In average of the six supplementation levels, 88.5% of the supplemented carnitine was absorbed in the first segment and 90.1% was absorbed in the first plus second segment. The absorption rate over the whole small intestine was slightly higher for the lower (25, 50, 100 mg/kg) than for the higher (200, 500, 100 mg/kg) supplementary levels. For the three lower supplementary levels, average absorption rate was 96.4%; for the three higher supplementary levels, average absorption rate was 91.8%.

3.3. Activity of γ-butyrobetaine dioxygenase in liver and kidney

In pigs fed the basal diet without carnitine supplementation, kidney showed an approximately 5-fold higher activity, expressed per mg protein, of γ-butyrobetaine dioxygenase than liver (Table 3). Activity of γ-butyrobetaine dioxygenase in the liver was

Table 2. Net absorption of L-carnitine at various concentrations in various segments of the small intestine of growing pigs ($n = 5$ per treatment)[†].

Carnitine supplementation [mg/kg]	Absorption in the proximal segment of small intestine [%]	Absorption in the proximal + middle segment of small intestine [%]	Absorption in the whole small intestine [%]
25	87.4 ± 9.8	92.5 ± 4.6	97.2 ± 2.0[a]
50	92.0 ± 2.0	91.2 ± 3.5	96.5 ± 1.5[ab]
100	84.5 ± 2.0	89.2 ± 6.8	95.5 ± 1.2[ab]
200	86.4 ± 6.2	88.5 ± 5.9	92.8 ± 2.9[bc]
500	91.0 ± 3.6	89.2 ± 4.8	89.9 ± 4.4[c]
1000	89.5 ± 3.8	90.0 ± 3.8	92.7 ± 2.7[bc]

[†]Means and standard deviations. Means without the same superscript letters (a, b, c) within a column are significantly different ($p < 0.05$).

Table 3. Activity of γ-butyrobetaine dioxygenase in liver and kidney of growing pigs fed diets with various amounts of carnitine ($n = 8$ per treatment)[†].

Carnitine supplementation [mg/kg]	Dioxygenase activity [pmol · mg protein^{-1} · min^{-1}]	
	Liver	Kidney
0	139 ± 59	640 ± 249[a]
25	123 ± 42	380 ± 59[b]
50	109 ± 59	326 ± 63[b]
100	124 ± 58	340 ± 77[b]
200	118 ± 15	371 ± 54[b]
500	141 ± 17	281 ± 135[b]
1000	98 ± 23	388 ± 126[b]

[†]Means and standard deviations. Means with different superscript letters (a, b) within a column are significantly different ($p < 0.05$).

not changed by supplementation of carnitine. In contrast, activity of γ-butyrobetaine dioxygenase in the kidney was lower in pigs supplemented with carnitine than in pigs fed the basal diet without supplemented carnitine. There was, however, no difference in the activity of this enzyme between pigs fed diets with various levels (25–1000 mg/kg) of supplemented carnitine.

3.4. Concentrations of free carnitine, acetyl carnitine and total carnitine in plasma and tissues

Among the five tissues examined, skeletal muscle (m. *logissimus dorsi*, m. *semimembranosus*) had the highest concentrations of total carnitine (Table 4). In pigs fed the basal diet without carnitine supplementation, total carnitine concentration in heart was approximately 30% lower than in skeletal muscle. Liver and kidney showed the lowest total carnitine concentrations which were approximately 8- and 4-fold, respectively, lower than those of skeletal muscle. In skeletal muscle, acetyl carnitine made up approximately 40% of the total carnitine in the pigs fed the basal diet. In contrast, in plasma, heart, kidney and liver, acetyl carnitine accounted only for 14%, 6%, 4%, 1%, respectively, of the total carnitine (Table 4).

Supplementation with increasing amounts of carnitine led to a continuous increase of the concentrations of free carnitine, acetyl carnitine and total carnitine in plasma and all tissues examined (Table 4). In pigs fed the diet supplemented with 100 mg carnitine/kg, concentrations of free carnitine in plasma, liver, kidney, heart, m. *semimembranosus* and m. *longissimus dorsi* were 62, 59, 62, 49, 74 and 106%, respectively, higher than in those fed the control diet (Table 4). Concentrations of acetyl carnitine in plasma, liver, kidney, heart, m. *semimembranosus* and m. *longissimus dorsi* of pigs fed the diet supplemented with 100 mg carnitine/kg were 77, 129, 90, 164, 37 and 69% higher than in those fed the control diet. In pigs fed the diet supplemented with 1000 mg carnitine/kg, concentrations of free carnitine in plasma, liver, kidney, heart, m. *semimembranosus* and m. *longissimus dorsi* were 5.1- 5.7-, 4.3-, 3.2-, 4.9-, and 4.8-fold higher than in those fed the control diet. Concentrations of acetyl carnitine in plasma, liver, kidney, heart, m. *semimembranosus* and m. *longissimus dorsi* of pigs fed the diet supplemented with 1000 mg carnitine/kg were 4.9-, 2.8-, 2.9-, 4.1-, 1.7-, and 2.3-fold higher than in those fed the control diet (Table 4).

3.5. Concentrations of trimethyllysine and γ-butyrobetaine in plasma and tissues

Concentrations of trimethyllysine in liver, heart and m. *semimembranosus* did not differ between the seven groups of pigs (Table 5). With respect to the concentration of trimethyllysine in plasma, kidney and m. *longissimus dorsi*, there were some differences among groups. However, there was no clear trend towards a lower or a higher concentration of trimethyllysine in pigs with increasing dietary carnitine concentrations. Therefore, it is assumed that carnitine supplementation did not have an effect on trimethyllysine concentrations in these tissues.

Concentrations of γ-butyrobetaine in plasma, liver, kidney, and m. *semimembranosus* were not different in pigs fed diets supplemented with 25, 50 or 100 mg carnitine per kg compared to those fed the control diet (Table 5). Concentrations of γ-butyrobetaine in heart and m. *longissimus dorsi* remained unchanged until carnitine levels of 200 mg/kg. Carnitine levels in excess of 100 mg/kg led to a dose-dependent increase of γ-butyrobetaine concentrations in plasma, liver, kidney, and m. *semimembranosus*; carnitine levels in excess

Table 4. Concentrations of free carnitine, acetyl carnitine and total carnitine in plasma and various tissues of growing pigs fed diets with various amounts of carnitine ($n = 8$ per treatment)[†].

Carnitine supplementation [mg/kg]	Plasma [μmol/l]	Liver [nmol/g]	Kidney [nmol/g]	Heart [nmol/g]	*M. semimembranosus* [nmol/g]	*M. longissimus dorsi* [nmol/g]
Free carnitine						
0	8.88 ± 1.15^{f}	51.1 ± 12.1^{f}	108 ± 19^{e}	290 ± 58.0^{e}	277 ± 44^{e}	273 ± 94^{e}
25	10.7 ± 1.8^{f}	61.8 ± 16.8^{ef}	115 ± 21^{e}	354 ± 38.6^{d}	363 ± 100^{d}	386 ± 91^{d}
50	12.1 ± 1.8^{e}	70.2 ± 9.2^{de}	139 ± 22^{d}	396 ± 84.1^{cd}	402 ± 104^{cd}	471 ± 125^{cd}
100	14.4 ± 2.1^{d}	81.4 ± 9.4^{d}	175 ± 27^{c}	433 ± 57.1^{c}	484 ± 103^{c}	565 ± 96^{c}
200	22.8 ± 2.8^{c}	150 ± 12^{c}	282 ± 61^{b}	584 ± 99.0^{b}	851 ± 146^{b}	814 ± 257^{b}
500	35.5 ± 6.1^{b}	238 ± 83^{b}	385 ± 71^{a}	755 ± 65.4^{a}	1148 ± 325^{a}	1242 ± 294^{a}
1000	45.3 ± 7.1^{a}	291 ± 58^{a}	464 ± 91^{a}	918 ± 174^{a}	1369 ± 168^{a}	1321 ± 176^{a}
Acetyl carnitine						
0	1.42 ± 0.46^{d}	0.88 ± 0.22^{b}	5.13 ± 2.78^{d}	19.8 ± 9.0b^{e}	186 ± 44^{d}	168 ± 46^{d}
25	1.94 ± 0.47^{c}	1.20 ± 0.55^{b}	6.00 ± 3.96^{cd}	31.8 ± 20.5b^{de}	222 ± 44^{cd}	243 ± 40^{c}
50	1.99 ± 0.63^{c}	1.39 ± 0.86^{b}	9.52 ± 4.19^{b}	39.3 ± 18.2b^{cd}	224 ± 52^{cd}	252 ± 67^{b}
100	2.51 ± 0.81^{c}	2.02 ± 0.64^{a}	9.76 ± 3.83^{b}	52.4 ± 23.0^{bc}	255 ± 42^{c}	284 ± 57^{b}
200	3.75 ± 1.04^{b}	2.25 ± 1.01^{a}	8.10 ± 2.67^{bc}	33.2 ± 10.5^{cd}	254 ± 51^{bc}	275 ± 68^{b}
500	6.01 ± 1.70^{a}	2.93 ± 1.80^{a}	11.7 ± 3.61^{ab}	85.5 ± 23.1^{a}	316 ± 54^{a}	365 ± 60^{a}
1000	7.00 ± 1.57^{a}	2.44 ± 1.05^{a}	15.0 ± 3.86^{a}	81.7 ± 40.4^{a}	311 ± 58^{a}	390 ± 40^{a}
Total carnitine						
0	10.5 ± 1.2^{f}	52.9 ± 12.0^{f}	115 ± 20^{e}	310 ± 61^{f}	466 ± 68^{e}	443 ± 132^{d}
25	12.9 ± 2.1^{e}	64.0 ± 17.2^{ef}	123 ± 21^{e}	392 ± 30^{e}	589 ± 131^{d}	633 ± 116^{c}
50	14.4 ± 2.2^{de}	73.4 ± 8.2^{de}	151 ± 18de	437 ± 77de	629 ± 137^{d}	727 ± 130^{c}
100	17.2 ± 2.8^{d}	85.6 ± 9.9^{d}	187 ± 27^{d}	487 ± 64^{d}	744 ± 122^{c}	853 ± 116^{c}
200	27.6 ± 3.2^{c}	153 ± 12^{c}	294 ± 62^{c}	618 ± 106^{c}	1115 ± 180^{b}	1096 ± 264^{b}
500	42.7 ± 7.2^{b}	245 ± 83^{b}	403 ± 73^{b}	844 ± 63^{b}	1474 ± 336^{a}	1617 ± 339^{a}
1000	53.8 ± 7.6^{a}	296 ± 56^{a}	487 ± 96^{a}	1002 ± 166^{a}	1691 ± 211^{a}	1723 ± 201^{a}

[†]Means and standard deviations. Means without the same superscript letters (a–f) within a column are significantly different ($p < 0.05$).

Table 5. Concentrations of trimethyllysine and γ-butyrobetaine in plasma and various tissues of growing pigs fed diets with various amounts of carnitine ($n = 8$ per treatment)[†].

Carnitine supplementation [mg/kg]	Plasma [μmol/l]	Liver [nmol/g]	Kidney [nmol/g]	Heart [nmol/g]	*M. semimembranosus* [nmol/g]	*M. longissimus dorsi* [nmol/g]
Trimethyllysine						
0	1.46 ± 0.21^{ab}	5.00 ± 2.04	8.48 ± 2.44^{bc}	5.65 ± 1.11	2.85 ± 0.70	3.62 ± 1.05^{abc}
25	1.20 ± 0.13^{b}	4.90 ± 1.92	7.15 ± 1.00^{c}	6.01 ± 1.32	3.34 ± 1.26	4.21 ± 1.17^{a}
50	1.44 ± 0.17^{ab}	4.08 ± 1.45	7.52 ± 1.30^{c}	5.69 ± 1.53	3.15 ± 0.88	3.29 ± 0.49^{bc}
100	1.26 ± 0.22^{b}	3.68 ± 1.32	7.06 ± 1.21^{c}	5.47 ± 1.34	3.26 ± 0.92	2.95 ± 0.87^{bc}
200	1.45 ± 0.29^{ab}	3.86 ± 0.65	10.8 ± 1.76^{a}	5.55 ± 1.06	3.61 ± 1.07	3.70 ± 1.24^{abc}
500	1.70 ± 0.26^{a}	3.80 ± 1.32	8.39 ± 2.01^{bc}	4.97 ± 0.82	2.72 ± 0.72	2.75 ± 0.86^{c}
1000	1.63 ± 0.40^{a}	3.21 ± 0.68	10.2 ± 2.88^{ab}	5.97 ± 0.91	3.63 ± 0.40	3.80 ± 0.70^{ab}
γ-butyrobetaine						
0	1.07 ± 0.39^{d}	5.92 ± 1.72^{d}	10.9 ± 2.03^{d}	89.8 ± 20.6^{c}	122 ± 25de	105 ± 24^{d}
25	1.00 ± 0.17^{d}	5.19 ± 1.74^{d}	9.55 ± 1.29^{d}	90.4 ± 16.1^{c}	125 ± 23de	125 ± 26^{cd}
50	1.01 ± 0.23^{d}	5.89 ± 1.15^{d}	11.0 ± 1.3^{d}	85.1 ± 11.9^{c}	113 ± 35^{e}	119 ± 32^{cd}
100	1.08 ± 0.19^{d}	5.64 ± 0.84^{d}	11.1 ± 1.7^{d}	92.4 ± 16.3^{c}	117 ± 29^{e}	129 ± 24^{cd}
200	1.93 ± 0.63^{c}	11.3 ± 3.5^{c}	19.2 ± 3.4^{c}	102 ± 25^{c}	155 ± 20^{cd}	139 ± 33^{c}
500	3.30 ± 0.80^{b}	22.7 ± 5.7^{b}	28.1 ± 4.1^{b}	156 ± 27^{b}	219 ± 51^{b}	221 ± 31^{b}
1000	5.27 ± 1.44^{a}	34.2 ± 7.4^{a}	46.4 ± 9.1^{a}	248 ± 42^{a}	395 ± 49^{a}	373 ± 41^{a}

[†]Means and standard deviations. Means without the same superscript letters (a–d) within a column are significantly different ($p < 0.05$).

of 200 mg/kg led to a dose-dependent increase of γ-butyrobetaine concentrations in heart and m. *longissimus dorsi*.

4. Discussion and conclusion

The use of carnitine in the nutrition of pigs is increasingly discussed. There are several studies which found beneficial effects of carnitine supplementation on sows (Musser et al. 1999; Eder et al. 2001b; Ramanau et al. 2002, 2008). A few studies observed beneficial effects of carnitine supplementation on growth performance or body composition of growing pigs (Owen et al. 1996, 2001a, 2001b; Heo et al. 2000; Rincker et al. 2003; Birkenfeld et al. 2005). Nevertheless, little information is available on the bioavailability of carnitine supplements and the effects of carnitine supplementation on the carnitine status in pigs. In this study, pigs were fed diets with various supplementary levels of carnitine. We did not observe differences in growth performance between the seven groups of pigs. It was, however, not the aim of this study to investigate the effect of carnitine on growth performance of the pigs. Accordingly, the number of pigs per group would have been much too low to detect possible effects of carnitine supplementation on growth performance.

To determine the bioavailability of the carnitine supplements, we used the "indicator method" which has been reported to be a suitable method for the determination of ileal and fecal digestibility of nutrients in various animal species including pigs (Hafez et al. 1988; Jagger et al. 1992). We found that small levels of supplemented carnitine (25–100 mg/kg) are nearly completely absorbed in young pigs (>95%) and that even high amounts of carnitine supplemented to the diet, up to 1000 mg/kg, are absorbed for more than 90%. We observed, moreover, that most of the carnitine was absorbed even in the first third (proximal) segment of the small intestine. These data show that young pigs have obviously a high capacity for the absorption of carnitine. To our knowledge, the rate of absorption of carnitine in pigs has not yet been investigated. Moreover, there is also less information about the absorption rate of carnitine in other species. We have recently found that approximately 40% of carnitine was absorbed in rats fed a diet supplemented with 5 g carnitine/kg. Rebouche and Chenard (1991) have shown that 54–87% of the carnitine from dietary sources is absorbed in humans. In contrast, the absorption rate of oral doses of 1 to 6 g in men lies only in the range between 5 and 18% (Evans and Fornasini 2003). These data suggest that young pigs may have a higher capacity for the absorption of carnitine from the small intestine than humans. Mechanisms of the absorption of carnitine in the small intestine have not yet been completely clarified. It has been shown, however, that total carnitine uptake includes a saturable and a non-saturable component (Shaw et al. 1983; Gross and Henderson 1984). The saturable carnitine uptake is mediated primarily by novel organic cation transporter (OCTN)- 2, which belongs to the solute carrier 22A family, localised on the apical membrane of cells (Tamai et al. 1998). The role of this transporter for carnitine absorption has been demonstrated in mice with a genetic defect in OCTN2 transport in which the oral bioavailability of carnitine was reduced by about 50% (Yokogawa et al. 1999). In men, pharmacological doses of carnitine are absorbed primarily by passive diffusion (Li et al. 1990). As the bioavailability of carnitine was rather high in pigs (>90%), even at the high supplementation level of 1000 mg/kg, we assume that most of the carnitine was absorbed by active transport, mediated probably by OCTN2. The present results suggest also that this transporter was not saturated even with the highest level of carnitine. In rats, highest absorption capacity was found in the jejunum and the duodenum while carnitine transport activity in the ileum

was only half of that of the two other parts (Shaw et al. 1983). The finding that most of the carnitine was absorbed already in the first segment of the small intestine suggests that pigs may have also a particular high carnitine transport activity in the proximal part of the small intestine.

In order to assess the effect of carnitine supplementation on the carnitine status we determined carnitine concentrations in plasma and various tissues of the pigs. Carnitine concentrations in tissues of pigs fed the unsupplemented diet are in well agreement with those reported recently, also for pig tissues (Ringseis et al. 2008a; Fischer et al. 2008). Our measurements show that pigs have particularly high carnitine concentrations in muscle tissue (skeletal muscle, heart) while carnitine concentrations in liver or kidney are much lower. This finding confirms the role of muscle as storage of carnitine (Steiber et al. 2004). Carnitine concentrations in skeletal muscle of pigs are in the same order of magnitude as those in rats (Van Vlies et al. 2006; Ringseis et al. 2007; Luci et al. 2008). In contrast, carnitine concentrations in plasma, liver, kidney and heart of pigs are much lower than those in the respective tissues of rats. A possible reason for these different carnitine concentrations may be the different expression of peroxisome proliferator-activated receptor (PPAR)-α between pigs and rats. PPARα is a transcription factor that regulates among others the transcription of genes involved in fatty acid oxidation. It is highly expressed in tissues with a high rate of β-oxidation such as liver, kidney and heart (Desvergne and Wahli 1999). We have recently found that PPARα also regulates the carnitine status of tissues by controlling the uptake of carnitine into tissues via OCTN2, in rats, mice and pigs (Luci et al. 2006a; Ringseis et al. 2007, 2008a, 2008c; Koch et al. 2008). It is well known that expression of PPARα is generally much lower in pigs than in rodents (Holden and Tugwood 1999); i.e. in the liver, expression of PPARα in pigs is 10-fold lower than in rats (Luci et al. 2006b). Although we have no direct proof for this, it is likely that lower tissue carnitine concentrations in pigs are related to their lower expression of PPARα compared to rats.

In tissues, a part of the carnitine is converted into carnitine esters by the action of carnitine acyltransferases. Among these esters, acetyl carnitine – formed by carnitine acetyltransferase – is the quantitatively most important one. It has been shown that esterification of carnitine with acetate by carnitine acetyltransferase and release of acetate from the ester in the mitochondrion play an important role in the regulation of the concentration of acetyl-CoA (Zammit et al. 2005). Our data show that a relatively high percentage of the carnitine is esterified with acetate in pig muscle while in all the other pig tissues acetyl carnitine makes up only a low part of the whole carnitine. A similar pattern with respect to the ratio of acetyl carnitine to total carnitine has been observed in rats (Luci et al. 2008).

The finding that carnitine supplementation increases concentrations of free and acetyl carnitine in plasma and tissue was expected with respect to earlier studies in pigs (Ramanau et al. 2004; Doberenz et al. 2006). It should be noted, however, that the extent of the increase of plasma and tissue carnitine concentrations in pigs due to carnitine supplementation was relatively large compared to other species. In rats, for instance, feeding a diet supplemented with 5000 mg carnitine/kg increased the concentration of total carnitine in liver, heart, kidney, and skeletal muscle during a 14 day period only by 160%, 21%, 69% and 50%, respectively, compared to a basal diet without supplemented carnitine (Ringseis et al. 2008b). In contrast, in the present study, feeding the diet supplemented with 1000 mg/kg increased the concentration of total carnitine in all these tissues 3- to 6-fold. Probably, the high bioavailability of the supplemented carnitine contributed to the relatively strong response of plasma and tissue carnitine concentrations to supplementation in pigs.

Increasing the carnitine concentration in pig muscle by supplementation of carnitine could be also relevant for human nutrition. Carnitine is not an essential element in human nutrition due to the ability of the human body to synthesise carnitine. Nevertheless, because the carnitine synthesis depends on the availability of different vitamins (C, B_6) and the micronutrient iron, a deficiency of these compounds and physiological stress situations can require an exogenous L-carnitine supplementation (Angelini et al. 1985; Millington and Chace 1992). Previous reports indicate also that physical exercise, pregnancy or diseases such as ischemia, neuropathy, aids or hemodialysis develop a need for additional L-carnitine (Sima et al. 1996; Di Marzio et al. 1999; Evans et al. 2000; Lohninger et al. 2005). Under these conditions, consumption of meat enriched with carnitine could be helpful to supply additional carnitine.

In order to assess whether dietary carnitine supplementation could have an effect on carnitine biosynthesis, we determined the activity of γ-butyrobetaine dioxygenase, the enzyme which catalyses the last step of carnitine biosynthesis, and concentrations of precursors for carnitine synthesis, trimethyllysine and γ-butyrobetaine. Our study shows that supplementation with carnitine, even at the low dose of 25 mg/kg, causes a depression of the activity of γ-butyrobetaine dioxygenase in kidney whereas the activity of that enzyme in the liver remains unaffected by carnitine supplementation. This effect, particularly the difference between both tissues, is hard to explain. The finding that carnitine supplementation lowers the activity of γ-butyrobetaine dioxygenase in kidney contrasts with results from a recent study in rats (Davis and Monroe 2005). In that study, neither carnitine deficiency induced by treatment with pivalate nor carnitine supplementation had an influence on gene expression of γ-butyrobetaine dioxygenase in liver and kidney. The depression of γ-butyrobetaine dioxygenase activity in kidney, observed in the present study, however, seems to have less impact on the carnitine status of the pigs. Although γ-butyrobetaine dioxygenase activity in the kidney of pigs fed the diet supplemented with 25 mg carnitine/kg was reduced by approximately 40% compared to those fed the unsupplemented diet, they had slightly higher carnitine concentrations in plasma and all the tissues examined than pigs fed the unsupplemented diet. This means that the small amount of 25 mg carnitine/kg was sufficient to compensate for the strongly depressed activity of γ-butyrobetaine dioxygenase. This finding gives an indication that γ-butyrobetaine dioxygenase activity in kidney was not rate-limiting for carnitine synthesis in pigs. In humans, it has been shown that the capacity of the carnitine biosynthetic pathway far exceeds the amount of carnitine generated, and that the availability of the carnitine precursor γ-butyrobetaine is rather rate-limiting for carnitine synthesis than the activity of the enzymes (Rebouche et al. 1989). In pigs, the rate-limiting factor of carnitine synthesis, either activity of γ-butyrobetaine dioxygenase or availability of γ-butyrobetaine, has not yet been elucidated. A recent study by us indeed yielded some indication that the availability of γ-butyrobetaine could be rate-limiting for carnitine synthesis in pigs rather than the activity of γ-butyrobetaine dioxygenase (Fischer et al. 2008). All tissues are able to produce γ-butyrobetaine from trimethyllysine. Most of the γ-butyrobetaine excreted into the blood, available for carnitine synthesis in liver and kidney, however, originates from skeletal muscle, the greatest reservoir of protein bound trimethyllysine (Vaz and Wanders 2002). Carnitine synthesis and homeostasis in pigs has not yet been studied. It should be noted, however, that γ-butyrobetaine concentrations in muscle of pigs observed in this study are 10- to 20-fold higher than those reported for rats or mice (Davis and Monroe 2005; Van Vlies et al. 2006; Koch et al. 2008; Luci et al. 2008). This suggests that pigs have generally a high capacity to produce and store γ-butyrobetaine in muscle. An interesting finding of this study was that supplementation of the diet with 1 mg or more

per kg diet caused a dose-dependent increase in plasma and tissue concentrations of γ-butyrobetaine. This finding agrees with a recent study in which rats were supplemented with carnitine (Davis and Monroe 2005). Those rats had also markedly increased concentrations of γ-butyrobetaine in plasma, liver and skeletal muscle compared to rats fed a control diet without supplemented carnitine. Under the assumption that the availability of γ-butyrobetaine in liver and kidney is rate-limiting for carnitine synthesis, this would indicate that carnitine synthesis in these tissues would have been increased in pigs fed the diet supplemented with 100 mg or more carnitine per kg. The reason for increased γ-butyrobetaine concentrations in plasma and tissues in these pigs, however, remains unknown. γ-butyrobetaine is produced from trimethyllysine (Vaz and Wanders 2002). As trimethyllysine concentrations in tissues were not influenced by carnitine supplementation, it is unlikely that increased γ-butyrobetaine concentrations were due to an increased availability of trimethyllysine in tissues. It is possible that an increased formation of γ-butyrobetaine was due an increased activity of enzymes involved in the formation of γ-butyrobetaine from trimethyllysine. As we did not determine the activities of these enzymes, this, however, remains speculative and should be investigated in future studies. Overall, we observed in this study that carnitine supplementation, even at low doses, lowers the activity of γ-butyrobetaine dioxygenase in the kidney, and on the other side, at higher doses, increases the concentrations of γ-butyrobetaine in tissues. As we did not directly quantify carnitine biosynthesis, we can however not answer the question how these alterations influence carnitine synthesis in liver and kidney of the pigs.

In conclusion, the present study shows that young pigs have a high capacity to absorb supplemented carnitine from the diet in the small intestine. It is moreover shown that supplementation of carnitine strongly increases plasma and tissue carnitine concentrations in young pigs. These findings may be useful for assessing physiologic effects of supplemental carnitine in pigs.

References

Angelini C, Vergani L, Costa L, Martinuzzi A, Dunner E, Marescotti C, Nosadini R. 1985. Use of carnitine in exercise physiology. In: Moss DW, editor. Advances in clinical enzymology. London: Karger. pp 103–110.

Bassler R, Buchholz H. 1993. Methodenbuch Band III. . Die chemische Untersuchung von Futtermitteln, 3. Ergänzungslieferung. VDLUFA-Verlag, Darmstadt, Germany.

Birkenfeld C, Ramanau A, Kluge H, Spilke J, Eder K. 2005. Effect of dietary L-carnitine supplementation on growth performance of piglets from control sows or sows treated with L-carnitine during pregnancy and lactation. J Anim Physiol Anim Nutr. 89:277–283.

Brandt M, Allam SM. 1987. Analytik von TiO_2 in Darminhalt und Kot nach Kjehldalaufschluß. Arch Anim Nutr. 37:453–454.

Cho WT, Kim JH, Bae SH, Han IK, Han YK, Heo KN, Odle J. 1999. Effects of L-carnitine with different lysine levels on growth and nutrient digestibility in pigs weaned at 21 days of age. Asian-Austral J Anim Sci. 12:799–805.

Davis AT, Monroe TJ. 2005. Carnitine deficiency and supplementation do not affect the gene expression of carnitine biosynthetic enzymes in rats. J Nutr. 135:761–764.

Desvergne B, Wahli W. 1999. Peroxisome proliferator-activated receptors: Nuclear control of metabolism. Endocr Rev. 20:649–688.

Di Marzio L, Moretti S, DÀlò S, Zazzeroni F, Marcellini S, Smacchia C, Alesse E, Cifone MG, De Simone C. 1999. Acetyl-L-carnitine administration increases insulin-like growth factor 1 levels in asymptomatic HIV-1-infected subjects: Correlation with its suppressive effect on lymphocyte apoptosis and ceramide generation. Clin Immunol. 92:103–110.

Doberenz J, Birkenfeld C, Kluge H, Eder K. 2006. Effects of L-carnitine supplementation in pregnant sows on plasma concentrations of insulin-like growth factors, various hormones and metabolites and chorion characteristics. J Anim Physiol Anim Nutr. 90:487–499.

Eder K, Peganova S, Kluge H. 2001a. Studies on the tryptophan requirement of piglets. Arch Anim Nutr. 55:281–297.
Eder K, Ramanau A, Kluge H. 2001b. Effect of L-carnitine supplementation on performance parameters in gilts and sows. J Anim Physiol Anim Nutr. 85:73–80.
Evans AM, Fornasini G. 2003. Pharmacokinetics of L-carnitine. Clin Pharmacokinet. 42:941–967.
Evans AM, Faull R, Fornasini G, Lemanowicz EF, Longo A, Pace S, Nation RL. 2000. Pharmacokinetics of L-carnitine in patients with end-stage renal disease undergoing long-term hemodialysis. Clin Pharmacol Therap. 68:238–249.
Fischer M, Hirche F, Kluge H, Eder K. 2008. A moderate excess of dietary lysine lowers plasma and tissue carnitine concentrations in pigs. Brit J Nutr. (Epub ahead of print).
Gesellschaft für Ernährungsphysiologie. 2006. Empfehlungen zur Energie- und Nährstoffversorgung von Schweinen, DLG-Verlag, Frankfurt/Main, Germany.
Gross CJ, Henderson LM. 1984. Absorption of D- and L-carnitine by the intestine and kidney tubule in the rat. Biochim Biophys Acta. 772:209–219.
Hafez S, Junge W, Kalm E. 1988. Estimate of digestibility in dairy cows using an indicator method in comparison to the Hohenheimer feed value test. Arch Anim Nutr. 38:929–945.
Heo K, Odle J, Han IK, Cho W, Seo S, van Heugten E, Pilkington DH. 2000. Dietary L-carnitine improves nitrogen utilization in growing pigs fed low energy, fat-containing diets. J Nutr. 130:1809–1814.
Hoffman LA, Ivers DJ, Ellersieck MR, Veum TL. 1993. The effect of L-carnitine and soybean oil on performance and nitrogen and energy utilization by neonatal and young pigs. J Anim Sci. 71:132–138.
Holden PR, Tugwood JD. 1999. Peroxisome proliferator-activated receptor α: Role in rodent liver cancer and species differences. J Mol Endocrinol. 22:1–8.
Hoppel CL, Davis AT. 1986. Inter-tissue relationships in the synthesis and distribution of carnitine. Biochem Soc Trans. 14:673–674.
Jagger S, Wiseman J, Cole DJA, Craigon J. 1992. Evaluation of inert markers for the determination of ileal faecal apparent digestibility values in the pig. Brit J Nutr. 68:729–739.
Kirchgeßner M. 2004. Tierernährung. 11th ed. Frankfurt/Main: DLG-Verlag.
Koch A, König B, Stangl GI, Eder K. 2008. PPAR alpha mediates transcriptional upregulation of novel organic cation transporters-2 and -3 and enzymes involved in hepatic carnitine synthesis. Exp Biol Med. 233:356–365.
Li BU, Bummer PM, Hamilton JW, Gudjonsson H, Zografi G, Olsen WA. 1990. Uptake of L-carnitine by rat jejunal brush border microvillous membrane vesicles. Evidence of passive diffusion. Dig Dis Sci. 35:333–339.
Lohninger A, Karlic H, Lohninger S, Tammaa A, Jinniate S, Mascher H, Mascher D, Salzer H. 2005. Carnitine in pregnancy. Chem Monthly. 136:1523–1536.
Luci S, Geissler S, König B, Koch A, Stangl GI, Hirche F, Eder K. 2006a. PPARalpha agonists up-regulate organic cation transporters in rat liver cells. Biochem Biophys Res Commun. 350:704–708.
Luci S, Giemsa B, Kluge H, Eder K. 2006b. Clofibrate causes an upregulation of PPARa target genes but does not alter expression of SREBP target genes in liver and adipose tissue of pigs. Am J Physiol Regul Integr Comp Physiol. 293:R70–R7077.
Luci S, Hirche F, Eder K. 2008. Fasting and caloric restriction increases mRNA concentrations of novel organic cation transporter-2 and carnitine concentrations in rat tissues. Ann Nutr Metab. 52:58–67.
Millington DS, Chace DH. 1992. Carnitine and acylcarnitines in metabolic disease diagnosis and management. In: Desiderio DM, editor. Clinical and biochemical applications. New York: Plenum Press. pp 299–319.
Musser RE, Goodband RD, Tokach MD, Owen KQ, Nelssen JL, Blum SA, Dritz SS, Civis CA. 1999. Effects of L-carnitine fed during gestation and lactation on sow and litter performance. J Anim Sci. 77:3289–3295.
Owen KQ, Jit H, Maxwell CV, Nelssen JL, Goodband RD, Tokach MD, Tremblay GC, Koo SI. 2001a. Dietary L-carnitine suppresses mitochondrial branched-chain keto acid dehydrogenase activity and enhances protein accretion and carcass characteristics of swine. J Anim Sci. 79:3104–3112.
Owen KQ, Nelssen JL, Goodband RD, Tokach MD, Friesen KG. 2001b. Effect of dietary L-carnitine on growth performance and body composition in nursery and growing-finishing pigs. J Anim Sci. 79:1509–1515.

Owen KQ, Nelssen JL, Goodband RD, Weeden TL, Blum SA. 1996. Effect of L-carnitine and soybean oil on growth performance and body composition of early-weaned pigs. J Anim Sci. 74:1612–1619.

Ramanau A, Kluge H, Spilke J, Eder K. 2008. Effects of supplementation of L-carnitine on the reproductive performance of sows in stock production. Livest Sci. 113:34–42.

Ramanau A, Kluge H, Spilke J, Eder K. 2002. Reproductive performance of sows supplemented with dietary L-carnitine over three reproductive cycles. Arch Anim Nutr. 56:287–296.

Ramanau A, Kluge H, Spilke J, Eder K. 2004. Supplementation of sows with L-carnitine during pregnancy and lactation improves growth of the piglets during the suckling period through increased milk production. J Nutr. 134:86–92.

Rebouche CJ, Bosch EP, Chenard CA, Schabold KJ, Nelson SE. 1989. Utilization of dietary precursors for carnitine synthesis in human adults. J Nutr. 119:1907–1913.

Rebouche CJ, Chenard CA. 1991. Metabolic fate of dietary carnitine in human adults: identification and quantification of urinary and fecal metabolites. J Nutr. 121:539–546.

Rebouche CJ, Seim H. 1998. Carnitine metabolism and its regulation in microorganisms and mammals. Ann Rev Nutr. 18:39–61.

Rincker MJ, Carter SD, Real DE, Nelssen JL, Tokach MD, Goodband RD, Dritz SS, Senne BW, Fent RW, Pettey LA, Owen KQ. 2003. Effects of increasing dietary L-carnitine on growth performance of weanling pigs. J Anim Sci. 81:2259–2269.

Ringseis R, Pösel S, Hirche F, Eder K. 2007. Treatment with pharmacological peroxisome proliferator-activated receptor α agonist clofibrate causes upregulation of organic cation transporter 2 in liver and small intestine of rats. Pharmacol Res. 56:175–183.

Ringseis R, Luci S, Spielmann J, Kluge H, Fischer M, Geissler S, Wen G, Hirche F, Eder K. 2008a. Clofibrate treatment up-regulates novel organic cation transporter (OCTN)- 2 in tissues of pigs as a model of non-proliferating species. Eur J Pharmacol. 583:11–17.

Ringseis R, Lüdi S, Hirche F, Eder K. 2008b. Treatment with pharmacological peroxisome proliferator-activated receptor alpha agonist clofibrate increases intestinal carnitine absorption in rats. Pharmacol Res. 58:58–64.

Ringseis R, Wege N, Wen G, Rauer C, Hirche F, Kluge H, Eder K. 2008c. Carnitine synthesis and uptake into cells are stimulated by fasting in pigs as a model of non-proliferating species. J Nutr Biochem (Epub ahead of print).

Shaw RD, Li BU, Hamilton JW, Shug AL, Olsen WA. 1983. Carnitine transport in rat small intestine. Am J Physiol. 245:G376–381.

Sima AF, Ristic H, Merry A, Kamijo M, Lattimer S, Stevens MJ. 1996. Primary preventive and secondary interventionary effects of acetyl-L-carnitine on diabetic neuropathy in the bio-breeding worchester rat. J Clin Invest. 97:1900–1907.

Steiber A, Kerner J, Hoppel CL. 2004. Carnitine: A nutritional, biosynthetic, and functional perspective. Mol Asp Med. 25:455–473.

Tamai I, Ohashi R, Nezu J, Yabuuchi H, Oku A, Shimane M, Sai Y, Tsuji A. 1998. Molecular and functional identification of sodium ion-dependent, high affinity human carnitine transporter OCTN2. J Biol Chem. 273:20378–20382.

Tein I. 2003. Carnitine transport: Pathophysiology and metabolism of known molecular defects. J Inherit Metab Dis. 26:147–169.

Van Vlies N, Wanders RJ, Vaz FM. 2006. Measurement of carnitine biosynthesis enzyme activities by tandem mass spectrometry: Differences between the mouse and the rat. Anal Biochem. 354:132–139.

Vaz FM, Wanders RJ. 2002. Carnitine biosynthesis in mammals. Biochem J. 361:417–429.

Yokogawa K, Higashi Y, Tamai I, Nomura M, Hashimoto N, Nikaido H, Hayakawa J, Miyamoto K, Tsuji A. 1999. Decreased tissue distribution of L-carnitine in juvenile visceral steatosis mice. J Pharmacol Exp Ther. 289:224–230.

Zammit VA, Price NT, Jackson VN, Park B-S. 2005. The role of carnitine acyltransferases in the maintenance of cell function. Chemical Monthly. 136:1299–1309.

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Clofibrate treatment up-regulates novel organic cation transporter (OCTN)-2 in tissues of pigs as a model of non-proliferating species

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Abstract

Recent studies have shown that treatment of rodents with agonists of peroxisome proliferator-activated receptor (PPAR)-α causes an up-regulation of novel organic cation transporter (OCTN)-2, a carnitine transporter, and increases carnitine concentration in the liver. This study was performed to investigate whether such effects occur also in pigs which like humans have a lower expression of PPARα and are less responsive to treatment with PPARα agonists than rodents. An experiment with 18 pigs was performed which were fed a control diet or the same diet supplemented with 5 g clofibrate/kg for 28 days. Pigs treated with clofibrate had higher relative mRNA concentrations of OCTN2 in liver (3.1-fold), skeletal muscle (1.5-fold) and epithelial cells from small intestine (1.8-fold) than control pigs ($P<0.05$). Pigs treated with clofibrate had also higher concentrations of free and total carnitine in the liver and a higher concentration of free carnitine in skeletal muscle than control pigs ($P<0.05$). Concentrations of γ-butyrobetaine, the precursor of endogenous formation of carnitine, in liver, muscle and plasma did not differ between both groups; the activity of γ-butyrobetaine dioxygenase, the rate limiting enzyme of carnitine synthesis, in the liver was lower in pigs treated with clofibrate than in control pigs ($P<0.05$). This study shows for the first time that treatment with a PPARα agonist causes an up-regulation of OCTN2 in liver, muscle and enterocytes from small intestine of pigs. This in turn increases carnitine concentrations in liver and muscle probably by enhancing carnitine uptake into cells.

Keywords: Clofibrate; Novel organic cation transporter; Carnitine; Peroxisome proliferator-activated receptor-α; (Pig)

1. Introduction

Carnitine 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 (McGarry and Brown, 1997; Brass, 2002; Steiber et al., 2004). Carnitine is derived from dietary sources and endogenous biosynthesis (Rebouche and Seim, 1998). Carnitine biosynthesis involves a complex series of reactions involving several tissues. Lysine provides the carbon backbone of carnitine. Lysine in protein peptide linkages undergoes methylation of the ε-amino group to yield trimethyllysine, which is released upon protein degradation. The released trimethyllysine is further oxidised to γ-butyrobetaine by the action of trimethyllysine dioxygenase, 3-hydroxy-*N*-trimethyllysine aldolase and 4-*N*-trimethylaminobutyraldehyde dehydrogenase. γ-Butyrobetaine is hydroxylated by γ-butyrobetaine dioxygenase to form carnitine. In humans, this last reaction occurs primarily in liver and kidney (Vaz and Wanders, 2002).

Distribution of carnitine within the body and intracellular homeostasis of carnitine are controlled by novel organic cation transporters (OCTN) which belong to the solute carrier 22A family (Lahjouji et al., 2001; Tein, 2003). Three OCTN have been identified so far, OCTN1, OCTN2 and OCTN3, localised in the plasma membrane of cells (Tamai et al., 1997; Tamai et al., 1998; Tamai et al., 2000). OCTN are polyspecific; they transport several cations and L-carnitine (Ohashi et al., 1999; Ohashi et al., 2001). Carnitine transport by OCTN1 and OCTN2

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is sodium dependent whereas that by OCTN3 is sodium independent (Tamai et al., 2000). OCTN1 and OCTN2 are expressed in several tissues such as kidney, intestine, skeletal muscle, heart, liver and brain (Wu et al., 1999; Tamai et al., 2000; Slitt et al., 2002). In contrast, OCTN3 is expressed exclusively in testes and kidney (Tamai et al., 2000). Among the three OCTN, OCTN3 has the highest specificity for carnitine, OCTN1 has the lowest one (Tamai et al., 2000). OCTN operate on the intestinal absorption and renal reabsorption of carnitine and play a major role in tissue distribution by catalysing the uptake of carnitine into body cells. Due to its high binding affinity for carnitine and its wide expression, OCTN2 is the physiologically most important carnitine transporter, operating for the reabsorption of carnitine from the urine as well as playing a major role in tissue distribution. OCTN1 contributes less to carnitine transport than OCTN2 due to its low carnitine transport activity. OCTN3 may be important for carnitine uptake into testis, and may contribute to reabsorption of carnitine in kidney (Tamai et al., 2000).

Many years ago it has been shown that starvation or treatment of rats with clofibrate increases the concentration of carnitine in the liver (McGarry et al., 1975; Brass and Hoppel, 1978; Paul and Adibi, 1979). As both starvation and clofibrate treatment lead to an activation of peroxisome proliferator-activated receptor (PPAR)-α, a transcription factor belonging to the nuclear hormone receptor superfamily (Schoonjans et al., 1996), we have recently tested the hypothesis that activation of this nuclear receptor is responsible for the increased liver carnitine concentrations observed in those studies. We found that activation of PPARα by clofibrate increases mRNA concentrations of OCTN2 in liver and small intestine of rats (Luci et al., 2006; Ringseis et al., 2007). These data suggest that PPARα activation stimulates intestinal absorption of carnitine and the delivery of carnitine from blood into the liver which provides an explanation for the increased carnitine concentration in the liver of rats starved or treated with clofibrate. More recently, Van Vlies et al. (2007) have shown that treatment with WY-14,643, another synthetic PPARα agonist, increases gene expression of OCTN2 and the activity of γ-butyrobetaine dioxygenase in the liver in wild-type mice but not in PPARα-deficient mice. These findings clearly show that up-regulation of OCTN2 and hepatic carnitine synthesis are directly mediated by PPARα activation.

Regarding the expression of PPARα in tissues and the effects of PPARα activation on transcription of its target genes, there are great differences between various species. In rodents, PPARα is highly expressed, and activation of PPARα not only induces many genes involved in various metabolic pathways but also causes severe peroxisome proliferation in the liver (Peters et al., 2005). In contrast to rodents, PPARα agonists do not induce peroxisome proliferation in the liver of many other species, such as guinea pigs, swine, monkeys and humans. These non-proliferating species have a lower expression of PPARα in the liver and the response of many genes to PPARα activation is much weaker than in proliferating species (Holden and Tugwood, 1999). For that reason, effects related to PPARα activation observed in rodents cannot be directly applied for non-proliferating species such as humans.

We have recently shown that pigs have a similar mRNA concentration of PPARα in the liver as humans, which is approximately ten-fold lower than in rats. Therefore, we proposed that the pig may be a useful model to study biochemical effects induced by treatment with PPARα agonists (Luci et al., 2007). The aim of the present study was to find out whether treatment with PPARα activators influences carnitine homeostasis in the pig as a non-proliferating species. Therefore, we determined gene expression of OCTN2 in enterocytes of small intestine, liver and muscle and carnitine concentrations in plasma, liver and muscle of these pigs treated with clofibrate. We also determined concentrations of γ-butyrobetaine in these tissues and examined mRNA concentration and activity of γ-butyrobetaine dioxygenase in the liver in order to explore whether clofibrate treatment enhances carnitine biosynthesis in the liver.

2. Materials and methods

2.1. Animals and treatments

Eighteen male 8 weeks old crossbred pigs [(German Landrace × Large White) × Pietrain] were kept in a room under controlled temperature at 23 ± 2 °C and 55 ± 5% relative humidity with light from 0600 to 1800 h. One day before the beginning of the experimental feeding period, the pigs were weighted and randomly allocated to two groups with body weights of 12.0 ± 1.1 kg in control group and 11.9 ± 0.6 kg in treatment group. Both groups of pigs received a nutritionally adequate diet (National Research Council, 1998) for growing pigs containing (in g/kg) wheat (400), soybean meal (230), wheat bran (150), barley (100), sunflower oil (90) and mineral premix including L-lysine, DL-methionine and L-threonine (30). This diet contained 14.4 MJ metabolisable energy and 185 g crude protein per kg. The native carnitine concentration of the diet was low (<5 mg/kg as determined by tandem mass spectrometry, see Section 2.3). The diet of the treatment group was supplemented with 5 g clofibrate per kg. Diet intake was controlled, and each animal in the experiment was offered an identical amount of diet per day. The amount of diet administered was about 15% below that consumed ad-libitum by pigs of a similar weight (as assessed in a previous study). Therefore, the diet offered was completely taken in by all pigs in the experiment. During the feeding period, the amount of diet offered each day was increased continuously from 400 to 1200 g. The intake of metabolisable energy was in clear excess of the requirement for maintenance (as given by National Research Council, 1998). Therefore, all the pigs had a normal growth rate. The pigs had free access to water via nipple drinking systems. The experimental diets were administered for 28 days. All experimental procedures described followed established guidelines for the care and use of laboratory animals and were approved by the local veterinary office.

2.2. Sample collection

After completion of the feeding period the animals were killed under light anaesthesia. Four hours before euthanasia each pig was fed its respective diet. After killing, blood was

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collected into heparinised polyethylene tubes. Plasma was obtained by centrifugation of the blood (1100 g, 10 min, 4 °C). Samples of liver and *musculus longissimus dorsi* were taken and stored at −80 °C until analysis. For isolation of enterocytes of small intestine, the abdomen was immediately opened after killing, and a 35 cm intestinal segment was dissected starting at 30 cm distal to the pyloric sphincter, and flushed two times with ice-cold wash buffer (phosphate-buffered saline containing 0.2 mM phenylmethylsulphonyl fluoride and 0.5 mM dithiothreitol, pH 7.4). The isolation of porcine enterocytes of small intestine was performed by the modified distended intestinal sac technique according to Fan et al. (2004). After isolation, enterocytes of small intestine were washed two times with ice-cold phosphate-buffered saline, retained by centrifugation (400 g, 4 min, 4 °C), and immediately snap-frozen in liquid nitrogen.

2.3. Analysis of carnitine and γ-butyrobetaine

Concentrations of free carnitine, acetyl carnitine and γ-butyrobetaine in plasma and tissues were determined by tandem mass spectrometry with deuterated carnitine-d_3 (Larodane Fine Chemicals, Malmö, Sweden) as internal standard as described recently in detail (Ringseis et al., 2007).

2.4. Activity of γ-butyrobetaine dioxygenase

Activity of γ-butyrobetaine dioxygenase in liver homogenates was determined as described previously in detail by Van Vlies et al. (2006). Liver homogenates were prepared by homogenising hepatic tissue in 10 mM Mops buffer (pH 7.4) containing 0.9% (w/v) NaCl, 10% (w/v) glycerol, and 5 mM dithiothreitol.

2.5. RNA isolation and real-time detection PCR analysis

For the determination of mRNA expression levels of OCTN2, liver and muscle isoforms of carnitine palmitoyltransferase-1 (L-CPT-1, determined in liver and enterocytes from small intestine; M-CPT, determined in muscle), γ-butyrobetaine dioxygenase, acyl-CoA oxidase (ACO), liver and intestinal isoforms of fatty acid-binding protein (L-FABP, determined in liver and muscle; I-FABP, determined in enterocytes from small intestine), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) for normalisation total RNA were isolated from liver, skeletal muscle and enterocytes using Trizol™ reagent (Invitrogen, Karlsruhe, Germany) according to the manufacturer's protocol. GAPDH served as an appropriate reference gene in this experiment since the cycle threshold (Ct)-values of GAPDH did not differ between both groups. RNA concentration and purity were estimated from the optical density at 260 and 280 nm, respectively. 1.2 μg of total RNA 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. 2 μL cDNA templates were amplified in 200 μL 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. Characteristics of gene-specific primers obtained from Operon (Köln, Germany) are shown in Table 1. The PCR protocol comprised an initial denaturation at 95 °C for 3 min and 20–35 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 °C 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 Ct values (Pfaffl, 2001). Relative expression ratios are expressed as fold changes of mRNA concentration in the clofibrate group compared to the control group.

2.6. Statistical analysis

Student's t test was used to compare means of treatments with those of control. Differences with $P<0.05$ were considered to be significant. Data in the text are given as means±SD.

3. Results

3.1. Food intake and body weights

Due to the controlled feeding system, food intake throughout the feeding period was the same for each pig in the experiment, averaging 696±7 g/day. Body weight after 28 days of

Table 1
Characteristics of the primers used for real-time reverse transcriptase polymerase chain reaction analysis

Gene	Forward primer (from 5′ to 3′)	Reverse primer (from 5′ to 3′)	NCBI GenBank
ACO	CTCGCAGACCCAGATGAAAT	TCCAAGCCTCGAAGATGAGT	AF185048
BBD	AGTCACTGGGGGTGATTCAG	GTTTGGATTGGACGGAGAAA	Partial sequence (Ruan et al., 2007)
GAPDH	AGGGGCTCTCCAGAACATCATCC	TCGCGTGCTCTTGCTGGGGTTGG	AF017079
I-FABP	TACAGCCTCGCAGACGGAACTG	TGCTTGATGAGGAGAGGAAAACAG	AY960624
L-CPT-1	GCATTTGTCCCATCTTTCGT	GCACTGGTCCTTCTGGGATA	AF288789
L-FABP	TTCGGTGCATGTCTAAGCTG	TGAGAGGGAGAGGATGAGGA	DQ182323
M-CPT-1	ACTGTCTGGGCAAACCAAAC	CTTCTTGATGAGGCCTTTGC	NM_001007191
OCTN2	TGACCATATCAGTGGGCTA	AGTAGGGAGACAGGATGCT	Partial sequence (Ruan et al., 2007)

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experiment did not differ between control pigs and pigs treated with clofibrate (control: 26.0±1.5 kg; clofibrate: 25.2±1.2 kg, $P>0.05$).

3.2. mRNA concentrations of PPARα target genes in liver, skeletal muscle and enterocytes of small intestine

To assess activation of PPARα, mRNA concentrations of three PPARα target genes FABP, CPT-1 and ACO were determined in liver, skeletal muscle and enterocytes of small intestine. In the liver, relative mRNA concentrations of L-CPT-1, L-FABP and ACO were 2.2-, 2.4- and 1.7-fold, respectively, higher in pigs treated with clofibrate than in control pigs ($P<0.05$, Fig. 1). In muscle, none of the three genes was up-regulated in pigs treated with clofibrate compared to control pigs (Fig. 1). In enterocytes of small intestine, mRNA concentration of L-CPT-1 was 2.0-fold higher in pigs treated with clofibrate compared to control pigs ($P<0.05$); mRNA concentrations of ACO and I-FABP in enterocytes of small intestine, however, did not differ between both groups of pigs.

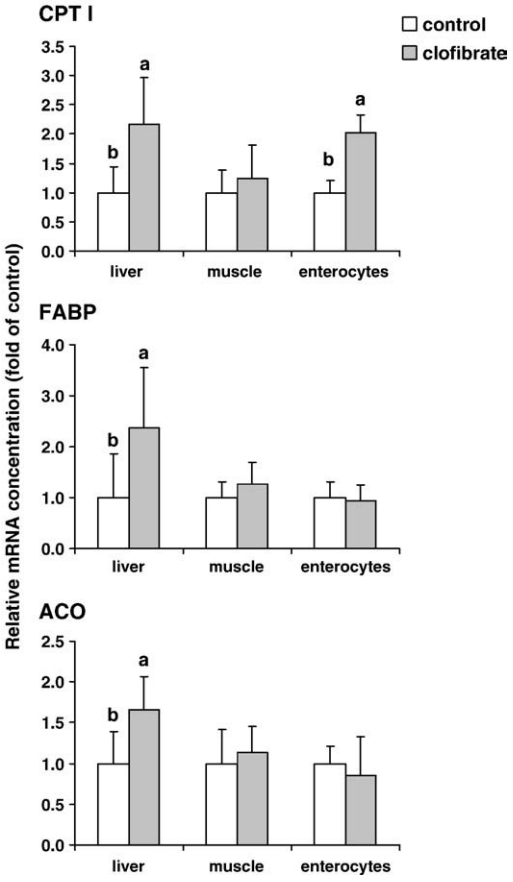

Fig. 1. Relative mRNA concentrations of ACO, CPT-1 and FABP in liver, skeletal muscle and enterocytes of small intestine in pigs fed a control diet (control) or a diet supplemented with 5 g clofibrate per kg (clofibrate) for 28 days. mRNA concentrations were determined by real-time RT-PCR and normalized to glyceraldehyde-3-phosphate dehydrogenase. Bars represent means±S.D. ($n=9$/group) and are expressed as fold increase compared to control. Bars marked without a common superscript letter differ ($P<0.05$).

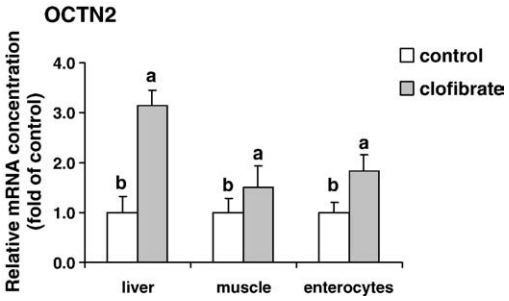

Fig. 2. Relative mRNA concentration of OCTN2 in liver, skeletal muscle and enterocytes of small intestine in pigs fed a control diet (control) or a diet supplemented with 5 g clofibrate per kg (clofibrate) for 28 days. mRNA concentrations were determined by real-time RT-PCR and normalized to glyceraldehyde-3-phosphate dehydrogenase. Bars represent means±S.D. ($n=9$/group) and are expressed as fold increase compared to control. Bars marked without a common superscript letter differ ($P<0.05$).

3.3. mRNA concentration of OCTN2 in liver, skeletal muscle and enterocytes of small intestine

Pigs treated with clofibrate had higher mRNA concentrations of OCTN2 in liver (3.1-fold), enterocytes of small intestine (1.8-fold) and skeletal muscle (1.5-fold) than control pigs ($P<0.05$, Fig. 2).

3.4. mRNA concentrations and activity of γ-butyrobetaine dioxygenase in the liver

The mRNA concentration of γ-butyrobetaine dioxygenase in the liver did not differ between both groups of pigs (clofibrate: 0.87±0.24; control: 1.00±0.33). The activity of γ-butyrobetaine dioxygenase in the liver was reduced in pigs treated with clofibrate compared to control pigs (clofibrate:

Table 2
Concentrations of carnitine (free, acetyl and total) and γ-butyrobetaine in plasma, liver and skeletal muscle of pigs fed a control diet (control) or a diet supplemented with 5 g clofibrate per kg (clofibrate) for 28 days

	Control	Clofibrate
Plasma (μmol/L)		
Free carnitine	6.34±1.33	7.52±1.68
Acetyl carnitine	1.17±0.65	1.15±0.48
Total carnitine	7.54±1.95	8.97±2.04
γ-Butyrobetaine	0.93±0.24	0.94±0.22
Liver (nmol/g)		
Free carnitine	42.6±6.8[b]	51.3±7.7[a]
Acetyl carnitine	0.80±0.22[a]	0.36±0.09[b]
Total carnitine	43.4±6.9[b]	51.7±7.8[a]
γ-Butyrobetaine	4.19±1.17	3.34±0.85
Skeletal muscle (nmol/g)		
Free carnitine	222±32[b]	273±59[a]
Acetyl carnitine	216±57	218±65
Total carnitine	441±66	493±103
γ-Butyrobetaine	61±19	55±16

Data are means±S.D. with nine animals per group. Means with different superscript letters (a, b) are significantly different ($P<0.05$).

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0.29±0.05 nmol×min^{-1}×mg protein^{-1}; control: 0.42±0.08 nmol×min^{-1}×mg protein^{-1}, $P<0.05$).

3.5. Concentrations of carnitine and γ-butyrobetaine in liver, muscle and plasma

Pigs treated with clofibrate had a higher concentration of free carnitine and a lower concentration of acetyl carnitine in the liver than control pigs ($P<0.05$, Table 2). The concentration of total carnitine in the liver was higher in pigs treated with clofibrate than in control pigs ($P<0.05$, Table 2). Pigs fed clofibrate had also higher concentration of free carnitine in the skeletal muscle than control pigs; the concentration of acetyl carnitine in skeletal muscle did not differ between both groups (Table 2). The concentration of total carnitine in skeletal muscle was 12% higher in pigs treated with clofibrate than in control pigs; this difference was however not statistically significant (Table 2). Concentrations of free, acetyl and total carnitine in plasma did not differ between the two groups of rats (Table 2). The concentrations of γ-butyrobetaine in liver, muscle and plasma did also not differ between both groups of pigs (Table 2).

4. Discussion

We have recently found that treatment of rats with clofibrate leads to an up-regulation of OCTN2 in liver and small intestine of rats (Luci et al., 2006, Ringseis et al., 2007). To find out whether clofibrate causes an up-regulation of OCTN2 also in a non-proliferating species we performed an experiment with relatively young pigs with a body weight slightly in excess of 10 kg. We used such young pigs as pigs with a similar weight have been used in other studies to characterise PPARα expression and activation in pigs (Yu et al., 2001; Cheon et al., 2005; Peffer et al., 2005). The concentration of clofibrate in the diet of 5 g per kg diet was adopted from these studies (Yu et al., 2001; Cheon et al., 2005; Peffer et al., 2005), resulting in a daily dose of 220 mg per kg body weight. This dose is relatively high compared with doses used in humans for treatment of hyperlipidaemia, which are usually in the range between 25 and 30 mg per kg body weight.

To examine activation of PPARα, we determined mRNA concentrations of three classical PPARα target genes, namely ACO, CPT-1 and FABP, in the relevant tissues. As expected, clofibrate treatment caused an up-regulation of these genes in the liver, indicative of PPARα activation in this tissue. The extent of up-regulation, was however, relatively small compared to rodents in which treatment with PPARα agonists increases hepatic mRNA concentrations of PPARα target genes typically 10 to 20-fold compared to control (He et al., 2002; Frederiksen et al., 2004; Knight et al., 2005; König et al., 2007). The reason for the comparatively low up-regulation of these enzymes in pigs by clofibrate might be the lower hepatic PPARα expression in pigs compared to rodents. Furthermore, the presence of an alternative spliced PPARα isoform, which lacks the ligand-binding domain, could contribute to the lower responsiveness of the pig to clofibrate (Sundvold et al., 2001). The finding that clofibrate causes a moderate up-regulation of PPARα target genes agrees well with recent studies conducted in piglets that were treated with clofibrate. In these studies mRNA concentrations and activities of ACO and CPT-1 were two to four times higher in livers of piglets treated with clofibrate in doses similar to that used in the present study than in untreated piglets (Yu et al., 2001; Peffer et al., 2005). The finding that PPARα target genes in muscle and enterocytes were not up-regulated by clofibrate treatment (with the only exception of CTP-1 in enterocytes) indicates that there was no or at least a very small activation of PPARα in these tissues. The reason for the lacking PPARα activation in these tissues may be the fact that expression of PPARα is much lower in muscle and small intestine than in liver (Braissant et al., 1996; Kliewer et al., 1994). In addition, a differential expression of PPARα coactivators, which are required for induction of PPARα-dependent gene transcription, between different organs, might be also responsible for the cell- and tissue-specific induction of PPARα-responsive genes (Cook et al., 2000). Recent studies reporting tissue-specific expression of coactivators of PPARα (Zoete et al., 2007) are indeed supportive of this assumption. In rats, up-regulation of PPARα target genes by clofibrate in liver was also much stronger than in small intestine or skeletal muscle (Ringseis et al., 2007).

We found for the first time that clofibrate treatment causes an up-regulation of OCTN2 in liver, muscle and epithelial cells of the small intestine of pigs. According to recent studies in rodents and in rat liver cells (Luci et al., 2006; Ringseis et al., 2007, Van Vlies et al., 2007) we propose that up-regulation of OCTN2 in pigs may be at least in part due to PPARα activation. The finding that OCTN2 was also up-regulated in muscle and in enterocytes, although there was no indication of PPARα activation in these tissues, suggests that clofibrate could stimulate expression of OCTN2 also by a mechanism independent of PPARα. Clofibrate is a relatively unspecific PPARα agonist and exerts also a PPARγ binding activity (Krey et al., 1997). Whether transcription of OCTN2 could be also influenced by PPARγ has not yet been investigated.

Van Vlies et al. (2007) have recently shown that treatment with WY-14,643 increases the activity of γ-butyrobetaine dioxygenase in wild-type mice but not in PPARα knockout mice. They proposed that PPARα activation stimulates also carnitine biosynthesis in the liver. These findings are in contrast to our studies with rats in which clofibrate treatment did not increase mRNA concentration of γ-butyrobetaine dioxygenase in the liver of rats (Luci et al., 2006; Ringseis et al., 2007). The present study shows that clofibrate treatment does not increase mRNA concentration of γ-butyrobetaine dioxygenase and even reduces its activity in the liver of pigs. Although we cannot explain the observation that activity of γ-butyrobetaine dioxygenase was even reduced by clofibrate, these findings indicate that the capacity of the liver to synthesize carnitine was obviously not increased by clofibrate treatment. It has been shown that γ-butyrobetaine which is formed in most tissues is also a substrate of OCTN2 (Tamai et al., 2000). An up-regulation of OCTN2 in the liver therefore could increase the uptake of γ-butyrobetaine from plasma into the liver. As, however, γ-butyrobetaine concentrations in liver and plasma

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were not altered by clofibrate, it is unlikely that more γ-butyrobetaine was available in the liver for carnitine biosynthesis. Although we did not directly quantify carnitine biosynthesis, we assume that increased carnitine concentrations in liver and muscle of pigs treated with clofibrate were not due to an increased carnitine biosynthesis but rather to an up-regulation of OCTN2. OCTN2 is localized in the plasma membrane of cells and delivers carnitine from the blood into cells (Lahjouji et al., 2001). An up-regulation of OCTN2 in liver and skeletal muscle indicates that more carnitine was transported from the blood into these tissues which might be a plausible explanation for the increased carnitine concentrations in these tissues of pigs treated with clofibrate. The importance of OCTN2 for the supply of cells with carnitine is evident by the fact that inborn or acquired defects of this transporter lead to primary or secondary systemic carnitine deficiency (Tein, 2003). OCTN1 is another system able to transport carnitine which was not considered in this study. In rats, hepatic OCTN1 was also up-regulated by clofibrate, half as much as OCTN2 (Luci et al., 2006; Ringseis et al., 2007). Therefore, the possibility exists that OCTN1 was also up-regulated in tissues of pigs treated with clofibrate. However, as OCTN1 plays a minor role for carnitine transport compared to OCTN2 due to its low carnitine transport activity (Tamai et al., 2000), it is likely that the effects on tissue carnitine concentrations were predominately mediated by OCTN2. OCTN3, another carnitine transporter, is not expressed in liver and muscle, and therefore does not contribute to changes in carnitine concentrations in these tissues (Tamai et al., 2000). In small intestine, OCTN2 is involved in absorption of carnitine from the diet (Sekine et al., 1998; Tamai et al., 1998). An up-regulation of OCTN2 in enterocytes of the small intestine therefore could improve carnitine absorption from the diet. As the diet used in the present study contained less carnitine, an improvement of the carnitine absorption probably had however less effect on whole body carnitine status.

The effects observed in this study are similar to those observed in our recent rat studies (Luci et al., 2006; Ringseis et al., 2007). The comparison of the present study with those recent rodent studies however shows that the up-regulation of OCTN2 in the liver and the increase of hepatic carnitine concentration are clearly stronger in rodents than in pigs. This is another indication that these effects were mediated by PPARα which is less responsive in pigs than in rats.

Activation of PPARα causes up-regulation of many genes involved in hepatic mitochondrial and peroxisomal β-oxidation. CPTs are rate limiting for β-oxidation of fatty acids (Brandt et al., 1998; Mascaro et al., 1998). The up-regulation of CPTs which is essential for the metabolic adaptations induced by activation of PPARα might increase the demand of carnitine in the respective tissue. We postulate that up-regulation of OCTN2 by PPARα activation is a means to supply cells with sufficient carnitine required for transport of excessive amounts of fatty acids into the mitochondrion, and therefore plays an important role in the adaptive response of cells to PPARα activation.

The observed up-regulation of OCTN2 in tissues due to treatment with clofibrate may be not only relevant with respect to carnitine homeostasis but also to tissue distribution and intestinal absorption of various other compounds. OCTN2 is polyspecific and is able to bind other monovalent cations and various drugs such as verapamil, spironolactone or mildronate (Wu et al., 1999; Koepsell and Endou, 2004; Lahjouji et al., 2004; Grube et al., 2006; Hirano et al., 2006; Kato et al., 2006). Therefore, it is possible that fibrates and other PPARα agonists influence intestinal absorption and tissue distribution of such compounds.

In conclusion, the present study shows for the first time that clofibrate treatment causes an up-regulation of OCTN2 in liver, muscle and enterocytes of pigs, a model of non-proliferating species, probably by activation of PPARα. Up-regulation of OCTN2 may enhance carnitine uptake from blood into tissues which may be the reason for the increased carnitine concentrations in liver and muscle of pigs treated with clofibrate. As there exists a similarity in the gene response to PPARα agonists between pigs and humans, clofibrate or other PPARα agonists could exert similar effects in humans.

References

Braissant, O., Foufelle, F., Scotto, C., Dauca, M., Wahli, W., 1996. Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-α, -β, and -δ in the adult rat. Endocrinology 137, 354–366.

Brandt, J.M., Djouadi, F., Kelly, D., 1998. Fatty acids activate transcription of the muscle carnitine palmitoyltransferase I gene in cardiac myocytes via the peroxisome proliferator-activated receptor alpha. J. Biol. Chem. 273, 23786–23792.

Brass, E.P., 2002. Pivalate-generating prodrugs and carnitine homeostasis in man. Pharmacol. Rev. 54, 589–598.

Brass, E.P., Hoppel, C.L., 1978. Carnitine metabolism in the fasting rat. J. Biol. Chem. 253, 2688–2693.

Cheon, Y., Nara, T.Y., Band, M.R., Beever, J.E., Wallig, M.A., Nakamura, M.T., 2005. Induction of overlapping genes by fasting and a peroxisome proliferator in pigs: evidence of functional PPAR alpha in nonproliferating species. Am. J. Physiol., Regul. Integr. Comp. Physiol. 288, R1525–R1535.

Cook, W.S., Yeldandi, A.V., Rao, M.S., Hashimoto, T., Reddy, J.K., 2000. Less extrahepatic induction of fatty acid β-oxidation enzymes by PPARα. Biochem. Biophys. Res. Commun. 278, 250–257.

Fan, M.Z., Matthews, J.C., Etienne, N.M., Stoll, B., Lackeyram, D., Burrin, D.G., 2004. Expression of apical membrane L-glutamate transporters in neonatal porcine epithelial cells along the small intestinal crypt–villus axis. Am. J. Physiol.: Gastrointest. Liver Physiol. 287, G385–G398.

Frederiksen, K.S., Wulff, E.M., Sauerberg, P., Mogensen, J.P., Jeppesen, L., Fleckner, J., 2004. Prediction of PPAR-alpha ligand-mediated physiological changes using gene expression profiles. J. Lipid Res. 45, 592–601.

Grube, M., Meyer zu Schwabedissen, H.E., Präger, D., Haney, J., Möritz, K.U., Meissner, K., Rosskopf, D., Eckel, L., Böhm, M., Jedlitschky, G., Kroemer, H.K., 2006. Uptake of cardiovascular drugs into the human heart: expression, regulation, and function of the carnitine transporter OCTN2 (SLC22A5). Circulation 113, 1114–1122.

He, W.S., Nara, T.Y., Nakamura, M.T., 2002. Delayed induction of delta-6 and delta-5 desaturases by a peroxisome proliferators. Biochem. Biophys. Res. Commun. 299, 832–838.

Hirano, T., Yasuda, S., Osaka, Y., Kobayashi, M., Itagaki, S., Iseki, K., 2006. Mechanism of the inhibitory effect of zwitterionic drugs (levofloxacin and grepafloxacin) on carnitine transporter (OCTN2) in Caco-2 cells. Biochim. Biophys. Acta 1758, 1743–1750.

Holden, P.R., Tugwood, J.D., 1999. Peroxisome proliferator-activated receptor alpha: role in rodent liver cancer and species differences. J. Mol. Endocrinol. 22, 1–8.

Kato, Y., Sugiura, M., Sugiura, T., Wakayama, T., Kubo, Y., Kobayashi, D., Sai, Y., Tamai, I., Iseki, S., Tsuji, A., 2006. Organic cation/carnitine transporter

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OCTN2 (Slc22a5) is responsible for carnitine transport across apical membranes of small intestinal epithelial cells in mouse. Mol. Pharmacol. 70, 829–837.

Kliewer, S.A., Forman, B.M., Blumberg, B., Ong, E.S., Borgmeyer, U., Mangelsdorf, D.J., Umesono, K., Evans, R.M., 1994. Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. Proc. Natl. Acad. Sci. U. S. A. 91, 7355–7359.

Knight, B.L., Hebbach, A., Hauton, D., Brown, A.M., Wiggins, D., Patel, D.D., 2005. A role for PPARα in the control of SREBP activity and lipid synthesis in the liver. Biochem. J. 389, 413–421.

Koepsell, H., Endou, H., 2004. The SLC22 drug transporter family. Pflugers Arch. 447, 666–676.

König, B., Koch, A., Spielmann, J., Hilgenfeld, C., Stangl, G.I., Eder, K., 2007. Activation of PPARalpha lowers synthesis and concentration of cholesterol by reduction of nuclear SREBP-2. Biochem. Pharmacol. 73, 574–585.

Krey, G., Braissant, O., L'Horset, F., Kalkhoven, E., Perroud, M., Parker, M.G., Wahli, W., 1997. Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay. Mol. Endocrinol. 11, 779–791.

Lahjouji, K., Elimrani, I., Lafond, J., Leduc, L., Qureshi, I.A., Mitchell, G.A., 2004. L-Carnitine transport in human placental brush-border membranes is mediated by the sodium-dependent organic cation transporter OCTN2. Am. J. Physiol. Cell Physiol. 287, C263–C269.

Lahjouji, K., Mitchell, G.A., Qureshi, I.A., 2001. Carnitine transport by organic cation transporters and systemic carnitine deficiency. Mol. Genet. Metab. 73, 287–297.

Luci, S., Geissler, S., König, B., Koch, A., Stangl, G.I., Hirche, F., Eder, K., 2006. PPARα agonists up-regulate organic cation transporters in rat liver cells. Biochem. Biophys. Res. Commun. 24, 704–708.

Luci, S., Giemsa, B., Kluge, H., Eder, K., 2007. Clofibrate causes an upregulation of PPARα target genes but does not alter expression of SREBP target genes in liver and adipose tissue of pigs. Am. J. Physiol., Regul. Integr. Comp. Physiol. 293, R70–R77.

Mascaro, C., Acosta, E., Ortiz, J.A., Marrero, P.F., Hegardt, F.G., Haro, D., 1998. Control of human muscle-type carnitine palmitoyltransferase I gene transcription by peroxisome proliferator-activated receptor. J. Biol. Chem. 273, 8560–8563.

McGarry, J.D., Brown, N.F., 1997. The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. Eur. J. Biochem. 244, 1–14.

McGarry, J.D., Robles-Valdes, C., Foster, D.W., 1975. Role of carnitine in hepatic ketogenesis. Proc. Natl. Acad. Sci. U. S. A. 72, 4385–4388.

National Research Council, 1998. Nutrient Requirement of Swine, Tenth Revised Edition. National Academie of Sciences, Washington DC.

Ohashi, R., Tamai, I., Nezu Ji, J., Nikaido, H., Hashimoto, N., Oku, A., Sai, Y., Shimane, M., Tsuji, A., 2001. Molecular and physiological evidence for multifunctionality of carnitine/organic cation transporter OCTN2. Mol. Pharmacol. 59, 358–366.

Ohashi, R., Tamai, I., Yabuuchi, H., Nezu, J.I., Oku, A., Sai, Y., Shimane, M., Tsuji, A., 1999. Na^+-dependent carnitine transport by organic cation transporter (OCTN2): its pharmacological and toxicological relevance. J. Pharmacol. Exp. Ther. 291, 778–784.

Paul, H.S., Adibi, S.A., 1979. Paradoxical effects of clofibrate on liver and muscle metabolism in rats. Induction of myotonia and alteration of fatty acid and glucose oxidation. J. Clin. Invest. 64, 405–412.

Peffer, P.L., Lin, X., Odle, J., 2005. Hepatic beta-oxidation and carnitine palmitoyltransferase I in neonatal pigs after dietary treatments of clofibric acid, isoproterenol, and medium-chain triglycerides. Am. J. Physiol., Regul. Integr. Comp. Physiol. 288, R1518–R1524.

Peters, J.M., Cheung, C., Gonzalez, F.J., 2005. Peroxisome proliferator-activated receptor-alpha and liver cancer: where do we stand? J. Mol. Med. 83, 774–785.

Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 29, e45.

Rebouche, C.J., Seim, H., 1998. Carnitine metabolism and its regulation in microorganisms and mammals. Annu. Rev. Nutr. 18, 39–61.

Ringseis, R., Pösel, S., Hirche, F., Eder, K., 2007. Treatment with pharmacological peroxisome proliferator-activated receptor α agonist clofibrate causes upregulation of organic cation transporter 2 in liver and small intestine of rats. Pharmacol. Res. 56, 175–183.

Ruan, J., Guo, Y., Li, H., Hu, Y., Song, F., Huang, X., Kristiensen, K., Bolund, L., Wang, J., 2007. PigGIS: Pig Genomic Informatics System. Nucleic Acids Res. 35 (Database issue), D654–D657.

Schoonjans, K., Peinado-Onsurbe, J., Lefebvre, A.M., Heyman, R.A., Briggs, M., Deeb, S., Staels, B., Auwerx, J., 1996. PPARα and PPARγ activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. Embo J. 15, 5336–5348.

Sekine, T., Kusuhara, H., Utsunomiya-Tate, N., Tsuda, M., Sugiyama, Y., Kanai, Y., Endou, H., 1998. Molecular cloning and characterization of high-affinity carnitine transporter from rat intestine. Biochem. Biophys. Res. Commun. 251, 586–591.

Slitt, A.L., Cherrington, N.J., Hartley, D.P., Leazer, M.T., Klaassen, C.D., 2002. Tissue distribution and renal developmental changes in rat organic cation transporter mRNA levels. Drug Metab. Dispos. 30, 212–219.

Steiber, A., Kerner, J., Hoppel, C.L., 2004. Carnitine: a nutritional, biosynthetic, and functional perspective. Mol. Asp. Med. 25, 455–473.

Sundvold, H., Grindflek, E., Lien, S., 2001. Tissue distribution of porcine peroxisome proliferator-activated receptor α: detection of an alternatively spliced mRNA. Gene 273, 105–113.

Tamai, I., Ohashi, R., Nezu, J., Yabuuchi, H., Oku, A., Shimane, M., Sai, Y., Tsuji, A., 1998. Molecular and functional identification of sodium ion-dependent, high affinity human carnitine transporter OCTN2. J. Biol. Chem. 273, 20378–20382.

Tamai, I., Ohashi, R., Nezu, J.I., Sai, Y., Kobayashi, D., Oku, A., Shimane, M., Tsuji, A., 2000. Molecular and functional characterization of organic cation/carnitine transporter family in mice. J. Biol. Chem. 275, 40064–40072.

Tamai, I., Yabuuchi, H., Nezu, J., Sai, Y., Oku, A., Shimane, M., Tsuji, A., 1997. Cloning and characterization of a novel human pH-dependent organic cation transporter, OCTN1. FEBS Lett. 419, 107–111.

Tein, I., 2003. Carnitine transport: pathophysiology and metabolism of known defects. J. Inherit. Metab. Dis. 26, 147–169.

Van Vlies, N., Wanders, R.J., Vaz, F.M., 2006. Measurement of carnitine biosynthesis enzyme activities by tandem mass spectrometry: differences between the mouse and the rat. Anal. Biochem. 354, 132–139.

Van Vlies, N., Ferdinandusse, S., Turkenburg, M., Wanders, R.J., Vaz, F.M., 2007. PPARα-activation results in enhanced carnitine biosynthesis and OCTN2-mediated hepatic carnitine accumulation. Biochim. Biophys. Acta 1767 (9), 1134–1142.

Vaz, F.M., Wanders, R.J., 2002. Carnitine biosynthesis in mammals. Biochem. J. 361, 417–429.

Wu, X., Huang, W., Prasad, P.D., Seth, P., Rajan, D.P., Leibach, F.H., Chen, J., Conway, S.J., Ganapathy, V., 1999. Functional characteristics and tissue distribution pattern of organic cation transporter 2 (OCTN2), an organic cation/carnitine transporter. J. Pharmacol. Exp. Ther. 290, 1482–1492.

Yu, X.X., Odle, J., Drackley, J.K., 2001. Differential induction of peroxisomal beta-oxidation enzymes by clofibric acid and aspirin in piglet tissues. Am. J. Physiol., Regul. Integr. Comp. Physiol. 281, R1553–R1561.

Zoete, V., Grosdidier, A., Michielin, O., 2007. Peroxisome proliferator-activated receptor structures: ligand specificity, molecular switch and interactions with regulators. Biochim. Biophys. Acta 1771, 915–925.

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Activities of γ-butyrobetaine dioxygenase and concentrations of carnitine in tissues of pigs

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ABSTRACT

In contrast to other species, less is known about carnitine homeostasis in the pig. This study was performed to yield information about the site of carnitine synthesis and carnitine concentrations in various tissues of pigs. We found that among several pig tissues, a considerable activity of γ-butyrobetaine dioxygenase (BBD), the last enzyme of carnitine synthesis, exists, like in humans and several other species, only in liver and kidney. Activity of that enzyme in liver and kidney was lower at birth than in the subsequent weeks of life. Highest carnitine concentrations were found in skeletal muscle and heart. Carnitine concentrations in plasma, liver and kidney at birth were higher than in the subsequent weeks of life in spite of the low BBD activity at birth. In conclusion, this study shows that liver and kidney are the major sites of carnitine synthesis and that neonatal pigs do not have an insufficient carnitine status.

Keywords: Pig, carnitine, γ-butyrobetaine, γ-butyrobetaine dioxygenase, tissue

1. Introduction

Carnitine (L-3-hydroxy-4-N-N-N-trimethylaminobutyrate) is an essential metabolite, which has a number of indispensable functions in intermediary metabolism (Steiber et al., 2004). All tissues that use fatty acids as a fuel source require carnitine for normal function. Carnitine is derived from dietary sources and endogenous biosynthesis (Hoppel and Davis, 1986; Rebouche and Seim, 1998). Carnitine biosynthesis involves a complex series of reactions. Lysine in protein peptide linkages provides the carbon backbone of carnitine. It undergoes methylation of the ε-amino group to yield trimethyllysine, which is released upon protein degradation. The released trimethyllysine is further oxidised to γ-butyrobetaine which is then hydroxylated by γ-butyrobetaine dioxygenase (BBD) to form carnitine (Vaz and Wanders, 2002). Carnitine produced in tissues expressing an active BBD 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 (Tamai et al., 2000; Lahjouji et al., 2001).

Tissue distribution of BBD is different between various mammalian species. In all mammals studied so far, BBD activity has been found in the liver (Vaz and Wanders, 2002). In some species such as in humans, cats, cows, hamsters, rabbits or Rhesus monkeys, BBD

activity has been detected also in the kidney; in these species, activity of BBD in the kidney is even higher than in the liver (Vaz and Wanders, 2002). In contrast, in several other species such as Cebus monkeys, sheep, dogs, guinea pigs, mice and rats, BBD is not, or only at very low activity, present in the kidney (Vaz and Wanders, 2002). In humans, BBD activity has been also found in the brain which is in contrast to other species (Rebouche and Engel, 1980). In rats, one study detected BBD activity in testis and epididymis (Carter et al., 1987), which could, however, not be confirmed by another study (Galland et al., 1999). In sheep, BBD activity was also observed in muscle (Erfle, 1975). There does not appear to be any evolutionary pattern with respect to the activity of BBD in tissues, since even very closely related species, like the Rhesus and Cebus monkeys, exhibit a different pattern.

In contrast to various other animal species, less is known about carnitine metabolism in the pig. For instance, the site of carnitine biosynthesis in pigs has not yet identified. Moreover, there is also less information about carnitine concentrations in tissues of pigs. In humans carnitine concentrations are highest in skeletal muscle which is regarded as a carnitine storage. Concentration of carnitine in muscle (2000-4000 nmol/g wet weight) is as much as 100times higher than that in plasma (Bertoli et al., 1981; Moorthy et al., 1983; Angelini et al., 1992). Organs such as kidney, liver and brain contain intermediate levels of 300-1000 nmol/g wet weight (Moorthy et al., 1983; Angelini et al., 1992). It is actually unclear whether a similar pattern of tissue carnitine concentrations exists also in pigs.

In humans, activity of BBD in liver is particularly low at birth (Rebouche and Engel, 1980). Moreover, it has been shown that infants fed a formula without supplemented carnitine have low plasma carnitine concentration (Olson et al., 1989). Therefore, it has been suggested that carnitine is an essential nutrient in the newborn (Borum, 1981; Penn et al., 1981). Some studies suggested that pigs have also an insufficient carnitine status at birth (van Kempen and Odle, 1995; Penn et al., 1997; Heo et al., 2000). Therefore, the neonatal pig has been suggested as model to study human neonatal carnitine metabolism (Baltzell et al., 1987; Penn et al. 1997). We are however not aware of any study which investigated the carnitine status of neonatal pigs relative to older pigs.

The aim of this study was to gain more insight into carnitine homeostasis in the pig. First, we intended to identify the sites of carnitine biosynthesis in pigs. Therefore, we determined mRNA and protein concentration and activities of BBD in various tissues of pigs. Second, to characterize the tissue carnitine concentration pattern in pigs, we determined carnitine concentrations in various tissues. Third, to find out whether newborn pigs indeed have a low carnitine status at birth, we determined concentrations of carnitine in various tissues of pigs at birth and in the subsequent weeks of life. In order to investigate the hypothesis that carnitine biosynthesis rate is particularly low at birth, we also determined the activity of BBD and concentrations of γ-butyrobetaine, the precursor of carnitine, in pig tissues of pigs at birth and in the subsequent weeks. These studies should also be helpful to characterize the carnitine homeostasis in the pig compared to humans and other mammalian species.

2. 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].

2.1 Animals and diets

In order to identify tissues containing BBD, we used four pigs (one female, two castrates, one uncastrated male of a crossbred race [(German Landrace × Large White) × Pietrain] with a body weight between 60 and 70 kg. These pigs were fed ad-libitum a

nutritionally adequate standard pig diet containing 13.4 MJ metabolizable energy and 170 g crude protein per kg. The diet had a low native carnitine concentration (< 5 mg/kg).

In order to investigate the effect of age on activity of BBD and concentrations of carnitine and precursors in plasma and tissues, we used litters of five crossbred sows [Large White × German Landrace × Hermitage) × Pietrain]. During pregnancy, these sows received a standard lactation diet for sows containing 13.4 MJ metabolizable energy and 175 g crude protein per kg. The carnitine concentration of this diet was below 5 mg/kg. Immediately after birth, the litters of those sows were standardized to eight (four male, four female) pigs per litter. From d 11 until weaning, the piglets were offered a creep feed for ad-libitum consumption which contained 15.6 MJ metabolizable energy and 200 g crude protein per kg diet; the carnitine concentration was 35 mg/kg. The piglets were weaned at day 28 and were offered the creep feed for ad libitum consumption until day 35. Thereafter, they were switched to a nutritionally adequate piglet diet which contained 13.7 MJ metabolizable energy and 185 g crude protein per kg. The native carnitine concentration of this diet was below 5 mg/kg. Immediately after birth and at the end of each following week, one piglet per litter was removed from each of the five sows and used for the collection of samples.

Concentrations of crude protein in the diets were analysed according to the official German VDLUFA methodology (Bassler and Buchholz, 1993). The metabolisable energy of the diet was calculated as recommended by the German Nutrition Society (Gesellschaft für Ernährungsphysiologie, 2006).

2.2 Sample collection

The animals were anaesthesised and exsanguinated. Blood samples were collected into heparinised polyethylene tubes. In the first experiment, liver, kidneys, heart, proximal segments of small intestine (duodenum) and colon and samples from m. *longissimus dorsi*, brain, lung and spleen were excised. In the uncastrated male animal, additionally one testis and epididymis were prepared. In the second experiment, liver, kidneys, heart and samples from m. *longissimus dorsi* and m. *semimembranosus* were excised from each animal. Plasma was obtained in each experiment by centrifugation of the blood samples (1,100 g, 10 min, 4°C). Plasma and tissue samples were stored at -20^{o}C pending further analysis.

2.3 RNA isolation and RT-PCR analysis

Total RNA was isolated from tissue samples using Trizol^{TM} reagent (Invitrogen, Karlsruhe, Germany) according to the manufacturer's protocol. Total RNA concentration and purity were estimated from the optical density at 260 and 280 nm, respectively. Synthesis of cDNA and determination of mRNA abundance by RT-PCR with real-time detection (Rotorgene 6000, Corbett Research, Australia) using Sybr Green I was performed as recently described in detail (Ringseis et al., 2007). For absolute quantification of mRNA abundance of BBD and GAPDH standard curves were generated with purified PCR products of BBD and GAPDH which were obtained by extraction of cut ethidium bromide-stained bands following 2% agarose gel electrophoresis by MinElute Gel Extraction Kit (Qiagen, Hilden, Germany). Ct values for each amplification curve were obtained using Rotorgene Series Software 1.7 (Corbett Research, Australia). Quantification of double-stranded DNA concentration of purified PCR products was performed using the PicoGreen DNA Quantitation Kit (Molecular Probes) and a spectrofluorometer (excitation: 480 nm, emission: 520 nm). For normalization purposes, the copy number of the housekeeping gene GAPDH served as an independent internal control. Sequences of gene-specific primers obtained from Operon (Köln, Germany) were as follows (forward, reverse; NCBI Genbank): BBD (5′-GTG CCG AAA GCT CAA GGA AAA A-3′, 5′-CTC TGC CGG CCG TGA AGT AAC-3′; partial sequence according to Ruan et al. (2007) and GAPDH (5′-AGG GGC TCT CCA GAA CAT CAT CC-3′, 5′-TCG CGT GCT CTT GCT GGG GTT GG-3′; AF017079).

2.4 Immunoblot analysis of BBD

For immunoblotting, homogenates of all tissues were prepared by homogenising tissue aliquots in 10 mM 3-morpholinepropansulfonic acid buffer (pH 7.4) containing 0.9% (w/v) sodium chloride, 10% (w/v) glycerol, and 5 mM dithiothreitol, and protein concentrations were determined by the Bradford method (Bradford, 1976). The brain was not considered for immunoblot analysis, because this tissue could not be completely homogenised using the above mentioned buffer. Equal amounts of protein (50 µg) were electrophoresed by 12.5% SDS-PAGE, and transferred to a nitrocellulose membrane (Pall, Pensacola, USA). The membranes were blocked overnight at 4°C in 5% skim milk in Tris-buffered saline containing 0.2% Tween (TBS-T), and then incubated with a 1:500 dilution of a mouse monoclonal anti-BBD primary antibody (ab56350; Abcam, Cambridge, UK) for 2 hours at room temperature. Membranes were washed with TBS-T, and incubated with a HRP conjugated secondary antibody anti-mouse IgG (Sigma-Aldrich, Taufkirchen, Germany) for 1.5 hour at room temperature. Afterwards blots were washed again, and developed using ECL Plus (Western Blotting Detection Reagents, GE Healthcare Europe GmbH, Freiburg, Germany). For normalisation purposes, membranes were also incubated with a mouse monoclonal anti-GAPDH primary antibody (ab8245; Abcam, Cambridge, UK). The signal intensities of specific bands (BBD and GAPDH) were detected with a Bio-Imaging system (Bio-Imaging Systems, F-ChemiBIS 3.2 M, biostep GmbH, Jahnsdorf, Germany) and quantified using TotalLab TL100-Quick Start analysis software (nonlinear dynamics). The normalised protein concentration was calculated as the ratio of the band intensities of BBD and GAPDH.

2.5 Analysis of carnitine and γ-butyrobetaine

Concentrations of free carnitine, acetyl carnitine, propionyl carnitine and γ-butyrobetaine in plasma and tissues as well as concentration of carnitine in the diet were determined by tandem mass spectrometry using deuterated analoga as internal standard (Ringseis et al., 2007). Carnitine-d_3 (N-methyl-d3) was supplied by Cambridge Isotope Laboratories (Andover, MA), acetyl carnitine-d_3 and propionyl carnitine-d_3 were products of Larodan Fine Chemicals (Malmö, Sweden). Propionyl carnitine and γ-butyrobetaine-d_3 were synthesized as described in literature (Vaz et al., 2002; Konishi and Hashimoto, 1992). Concentration of carnitine represents the sum of free carnitine, acetyl carnitine and propionyl carnitine.

2.6 Determination of the activity of BBD

Activity of BBD in tissues was determined as described previously in detail by van Vlies et al. (2006). Homogenates from tissues were prepared by homogenising tissue in 10 mM 3-morpholinepropansulfonic acid buffer (pH 7.4) containing 0.9% (w/v) sodium chloride, 10% (w/v) glycerol, and 5 mM dithiothreitol.

2.7 Statistical analysis

In both experiments, data were treated by one factorial analysis of variance (ANOVA) using the Minitab statistical software (Release 13, Minitab Inc., State College, PA, USA). In the first experiment, tissue was used as factor of ANOVA; in the second experiment age was used as factor of ANOVA. For statistically significant F values ($p < 0.05$) means were compared by Fisher´s multiple range test. In the case of large differences in the variances, data were transformed to their logarithms prior to analysis. Means were considered significantly different for $p < 0.05$. Results in the text are given as means ± SD.

3. Results

3.1 mRNA concentrations, protein concentrations and activity of BBD in various tissues of pigs

In almost all tissues examined, we detected measurable amounts of BBD mRNA, with the only exception of skeletal muscle in which BBD mRNA was almost completely absent. The highest mRNA concentration of BBD was observed in epididymis obtained from one male pig which was 14.4-fold higher than in liver (Figure 1). Relative mRNA concentration of BBD in kidney was also higher than that in liver (1.74-fold, Figure 1). mRNA concentrations of BBD in heart, colon, small intestine, lung, spleen, testes and brain were lower than those in the liver (Figure 1).

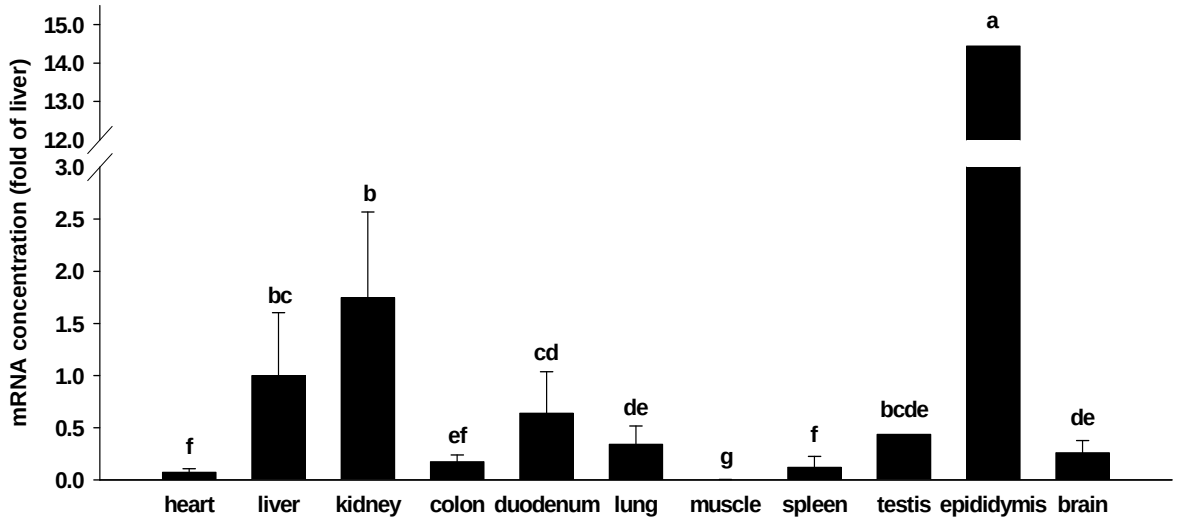

Fig. 1. Relative mRNA concentrations of BBD in various tissues of pigs. Total RNA was extracted from the samples and relative mRNA levels of γ-BBD were determined by real-time detection RT-PCR analysis using glyceraldehyde-3-phosphate dehydrogenase for normalization. Bars represent means ± standard deviations (n=4 for all tissues with the exception of testis and epididymis for which n=1), expressed relative to liver (=1.00). Bars marked without a common superscript letter differ ($P<0.05$).

BBD protein was detected in all tissues examined. However, the pattern of BBD protein concentrations in tissues reflected that of BBD mRNA concentrations only partially. Highest BBD protein concentrations were observed in epididymis, followed by kidney and testes (Figure 2). Protein concentration of BBD in testes was also higher than in liver (Figure 2). BBD protein concentrations in lung and spleen were similar to that in the liver; BBD protein concentrations in heart, muscle, colon, small intestine and skeletal muscle were lower than in the liver (Figure 2).

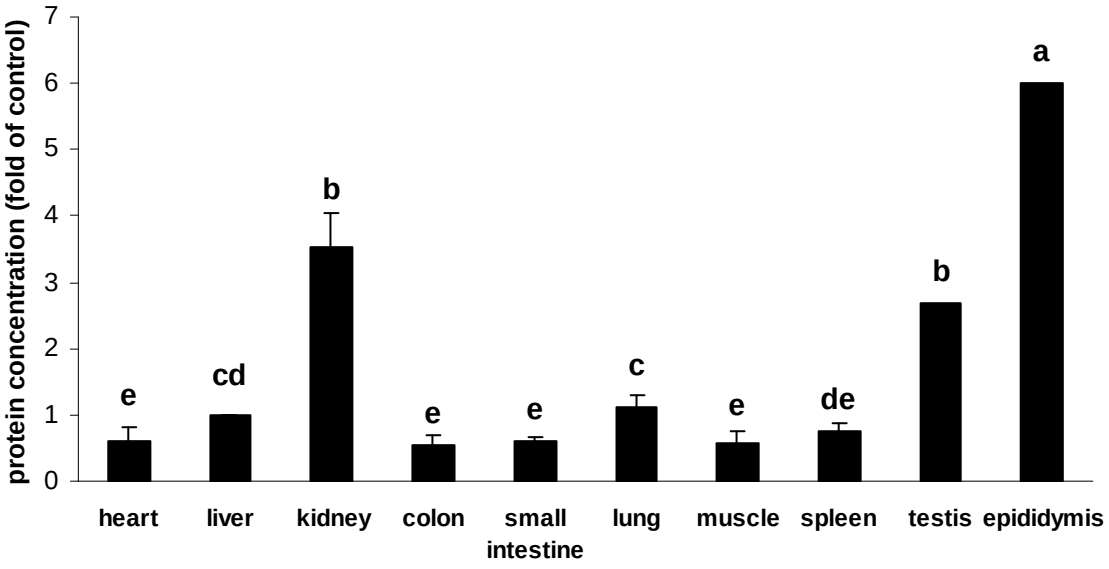

Fig. 2. Protein concentration of BBD in various tissues of pigs. Bars represent means ± standard deviations (n=4 for all tissues with the exception of testis and epididymis for which n=1), expressed relative to liver (=1.00). Bars marked without a common superscript letter differ (P<0.05).

Activities of BBD in tissues were in strong contrast to mRNA and protein concentrations of BBD. Among the tissues examined, a considerable activity of that enzyme was found only in kidney and liver (Figure 3). A small but detectable activity was also found in small intestine, epididymis, testes and brain (Figure 3). In contrast, activities of this enzyme in all other tissues (skeletal muscle, heart, colon, lung, spleen) were below the detection limit (<0.01 nmol · mg protein^{-1} · min^{-1}).

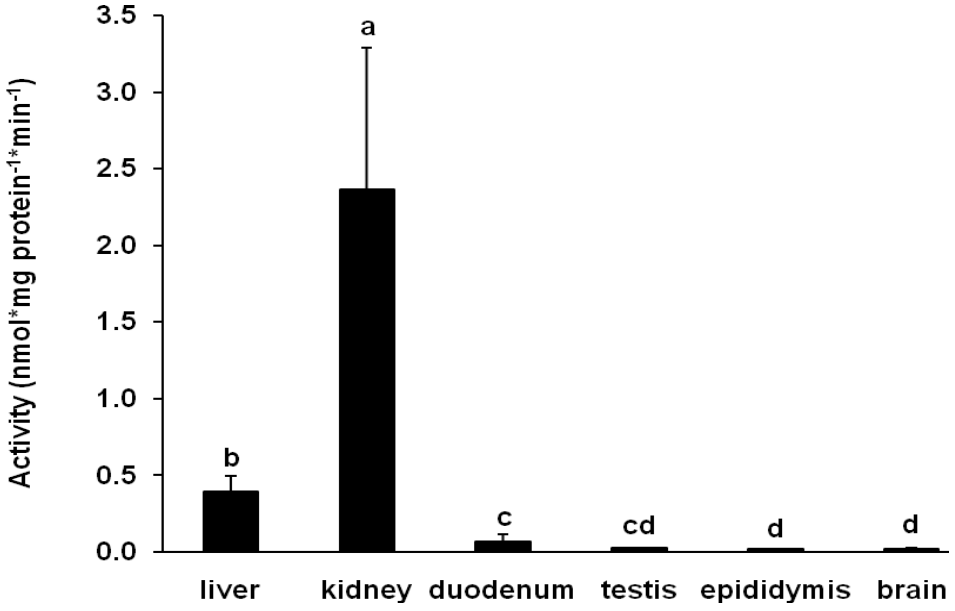

Fig. 3. Activities of BBD in various tissues of pigs. Bars represent means ± standard deviations (n=4 for all tissues with the exception of testis and epididymis for which n=1). Bars marked without a common superscript letter differ (P<0.05).

3.2 Concentrations of carnitine in various tissues of pigs

The highest concentrations of free carnitine, acetyl carnitine and total carnitine were observed in heart and skeletal muscle (Figure 4). Kidney and small intestine had the next highest concentrations of free and total carnitine which were, however, 3- to 6-fold lower than those in heart and skeletal muscle (Figure 4). Concentrations of total carnitine in colon, spleen and epididymis were similar to those in liver while carnitine concentrations in testes, brain and lung were lower than in liver ($P<0.05$, Figure 4). Concentrations of free carnitine in colon, epididymis, brain, lung and spleen did not differ from that of liver while that in testes was lower ($P<0.05$, Figure 4). Liver and brain had among all tissues examined the lowest concentrations of acetyl carnitine (Figure 4). Accordingly, these tissues had lower ratios between acetyl carnitine and free carnitine than all the other tissues (ratio of acetyl carnitine to free carnitine: heart, 0.62 ± 0.26; liver, 0.02 ± 0.01; kidney, 0.21 ± 0.01; colon, 0.20 ± 0.05; small intestine, 0.13 ± 0.02; testis, 0.27; epididymis, 0.15; brain, 0.05 ± 0.02; lung, 0.28 ± 0.06; muscle, 0.43 ± 0.08; spleen, 0.21 ± 0.04; n=4 for all tissues with exception of testis and epididymis for which n=1).

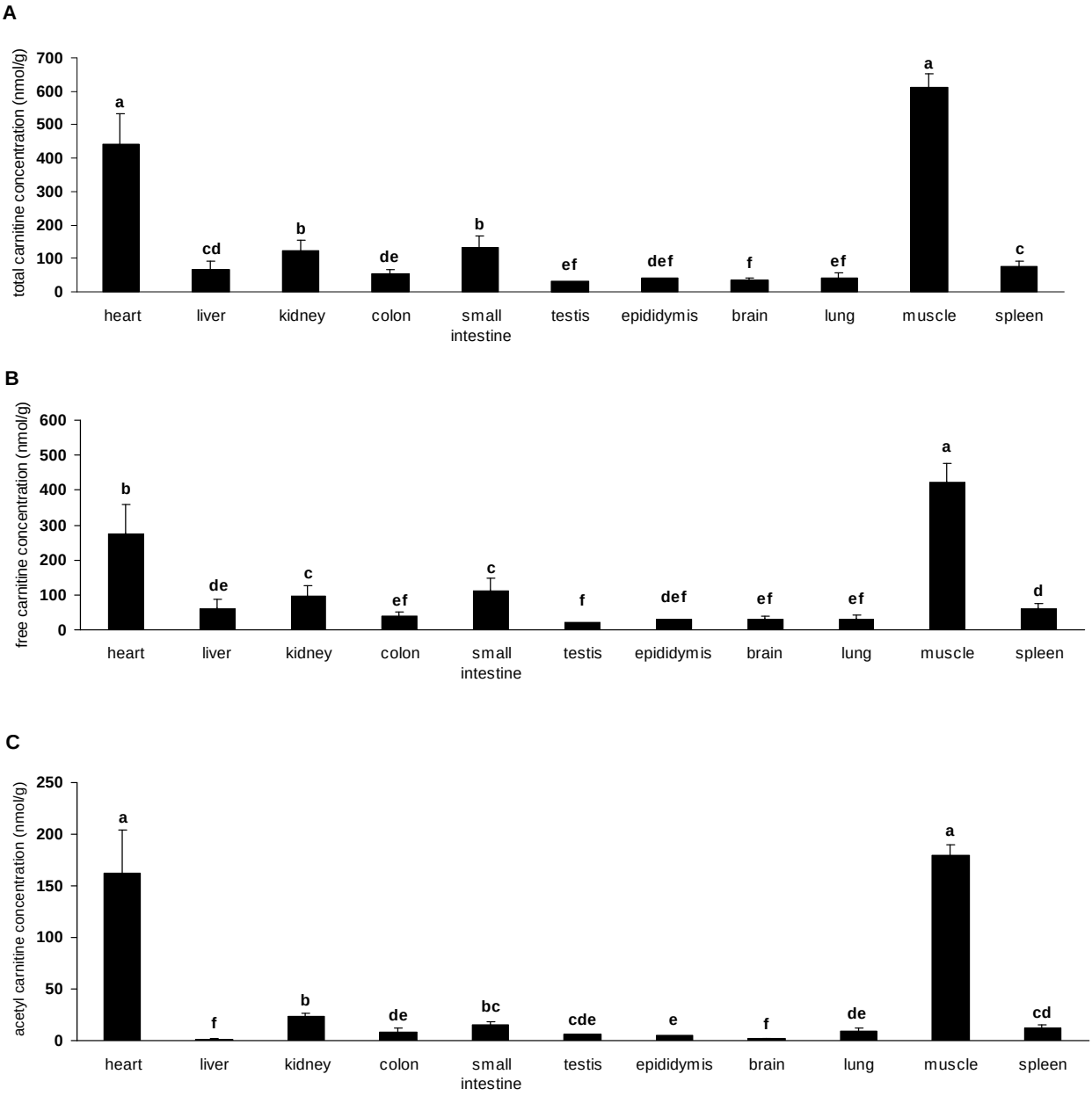

Fig. 4. Concentration of total carnitine (A), free carnitine (B) and acetyl carnitine (C) in various tissues of pigs. Bars represent means ± standard deviations (n=4 for all tissues with the exception of testis and epididymis for which n=1). Bars marked without a common superscript letter differ (P<0.05).

3.3 Concentrations of carnitine in plasma and tissues of pigs from birth to seven weeks of age

Concentrations of total carnitine in plasma, liver and kidney were highest at birth and thereafter declined until an age of 4 weeks (Table 1). Total carnitine concentrations in liver and kidney thereafter remained constant until an age of 7 weeks, whereas plasma carnitine concentration further declined until an age of 7 weeks (Table 1). In contrast, carnitine concentrations in heart and skeletal muscle rose from birth until an age of 3 to 4 weeks and thereafter declined (Table 1). The ratio of acetyl carnitine to free carnitine in plasma was falling from birth until week 4 and was thereafter, in weeks 5 to 7, increasing again. The ratio of acetyl carnitine to free carnitine in the liver was generally very low and remained unchanged during the first weeks of life (Table 1). In contrast, the ratios of acetyl carnitine to

free carnitine in kidney and heart decreased from birth to week 7 of life (Table 1). The ratio of acetyl carnitine to free carnitine in skeletal muscle was generally higher than that of the other tissues investigated (Table 1). This ratio in muscle was falling during the first two to three weeks of life and was then increasing to values in excess of those at birth (Table 1).

3.4 Activity of BBD and concentrations of BB in tissues of pigs from birth to seven weeks of age

Activity of BBD in both, liver and kidney was lowest at birth (Figure 5). It thereafter increased, reached its maximum already at an age of 2 weeks and remained on this level until 7 weeks of age (Figure 5).

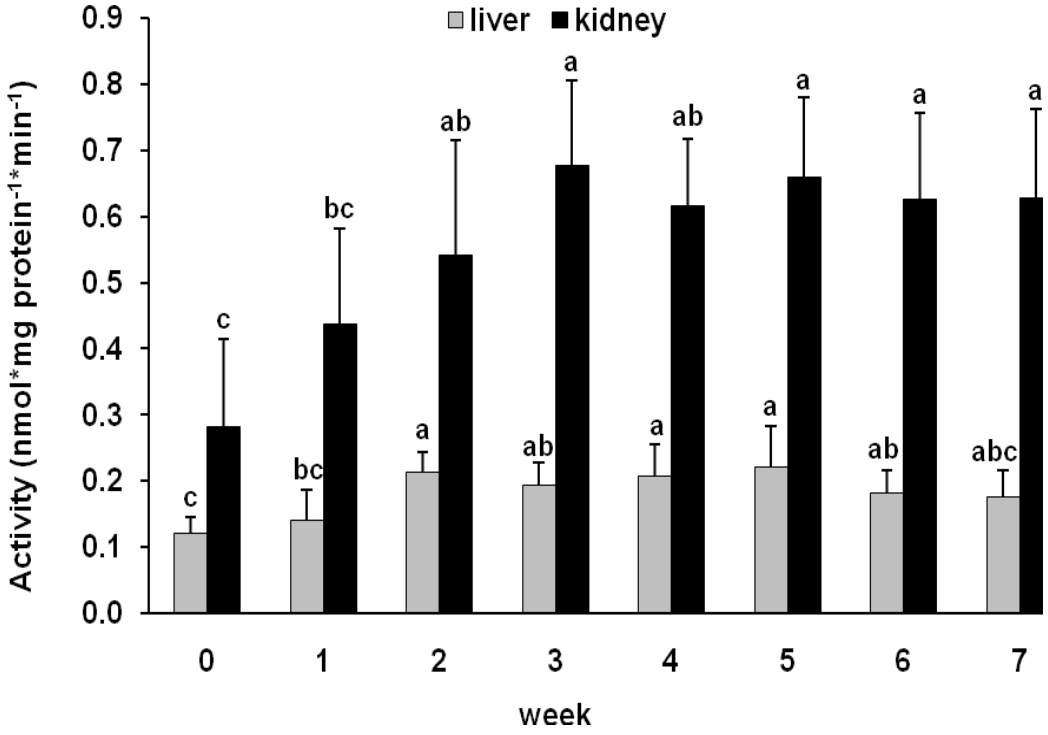

Fig. 5. Activity of BBD in liver and kidney from birth to seven weeks of age. Bars represent means ± standard deviations (n=5). Bars marked without a common superscript letter differ (P<0.05).

Among the tissues considered, γ-butyrobetaine concentrations were generally highest in skeletal muscle (m. *longissimus dorsi*, m. *semimembranosus*), followed by heart (Table 2). γ-Butyrobetaine concentrations in liver and kidney were around and 20- and 15-fold lower, respectively, than those in skeletal muscle (Table 2). γ-Butyrobetaine concentrations in liver, kidney and m. *longissimus dorsi* were highest at birth and declined during the first weeks of life (Table 2). Between an age of 1 and 7 weeks, γ-butyrobetaine concentrations in those tissues remained at a constant level (Table 2). In plasma, γ-butyrobetaine concentration was also highest at birth. Between 1 and 6 weeks of age, plasma BB concentration remained at a constant level (Table 2). At 7 weeks of age, a significant decline of plasma BB concentration was observed. γ-Butyrobetaine concentrations in heart and m. *semimembranosus* were not significantly changing from birth until an age of 7 weeks (Table 2).

Table 1. Concentrations of total carnitine and rations of acetyl carnitine:free carnitine in plasma and tissues of pigs differing in life age[†]

Age [weeks]	Plasma [µmol/L]	Liver [nmol/g]	Kidney [nmol/g]	Heart [nmol/g]	M. *longissimus dorsi* [nmol/g]	M. *semimembranosus* [nmol/g]
			Total carnitine			
Birth	21.8 ± 3.6^{a}	200 ± 22^{a}	123 ± 27^{a}	316 ± 53^{d}	284 ± 56^{d}	333 ± 74^{f}
1	17.6 ± 4.3^{b}	165 ± 58^{ab}	119 ± 35^{ab}	380 ± 58^{cd}	475 ± 58^{c}	575 ± 87^{e}
2	17.4 ± 4.0^{bc}	118 ± 37^{b}	118 ± 30^{ab}	475 ± 69^{ab}	706 ± 75^{a}	761 ± 99^{bc}
3	13.6 ± 2.0^{c}	79.1 ± 4.9^{c}	95.4 ± 11.3^{b}	571 ± 78^{a}	811 ± 32^{a}	897 ± 35^{a}
4	14.0 ± 3.1^{bc}	47.7 ± 6.1^{d}	73.6 ± 10.0^{cd}	550 ± 142^{a}	747 ± 91^{a}	818 ± 101^{ab}
5	15.8 ± 1.9^{bc}	49.3 ± 15.3^{d}	77.9 ± 5.9^{bc}	513 ± 55^{a}	596 ± 36^{b}	684 ± 44^{cd}
6	16.6 ± 1.2^{bc}	45.7 ± 9.1^{d}	72.8 ± 8.5^{cd}	408 ± 38^{bc}	586 ± 24^{b}	668 ± 95^{cde}
7	7.62 ± 0.81^{d}	41.2 ± 8.6^{d}	61.0 ± 5.1^{d}	339 ± 27^{cd}	538 ± 79^{bc}	580 ± 64de
			Acetyl carnitine:free carnitine			
Birth	0.17 ± 0.02^{b}	0.010 ± 0.003	0.031 ± 0.005^{a}	0.060 ± 0.022^{a}	0.34 ± 0.11^{c}	0.22 ± 0.05^{b}
1	0.13 ± 0.03^{cd}	0.008 ± 0.002	0.027 ± 0.006^{ab}	0.050 ± 0.021^{ab}	0.36 ± 0.02^{bc}	0.20 ± 0.11^{bc}
2	0.10 ± 0.01^{de}	0.011 ± 0.003	0.025 ± 0.010^{ab}	0.036 ± 0.008^{bc}	0.24 ± 0.04^{d}	0.18 ± 0.03^{bc}
3	0.08 ± 0.01^{e}	0.012 ± 0.002	0.022 ± 0.003^{b}	0.025 ± 0.007^{cd}	0.20 ± 0.01^{d}	0.15 ± 0.01^{c}
4	0.09 ± 0.01^{e}	0.012 ± 0.003	0.026 ± 0.003^{ab}	0.017 ± 0.003^{de}	0.33 ± 0.04^{c}	0.26 ± 0.09^{b}
5	0.13 ± 0.02^{c}	0.011 ± 0.005	0.020 ± 0.005^{bc}	0.015 ± 0.003^{e}	0.44 ± 0.05^{ab}	0.55 ± 0.06^{a}
6	0.27 ± 0.02^{a}	0.012 ± 0.003	0.016 ± 0.006^{c}	0.012 ± 0.006^{e}	0.44 ± 0.08^{ab}	0.43 ± 0.06^{a}
7	0.19 ± 0.03^{b}	0.011 ± 0.003	0.014 ± 0.003^{c}	0.012 ± 0.002^{e}	0.52 ± 0.10^{a}	0.45 ± 0.08^{a}

[†] Means and standard deviations (n=5). Means without the same superscript letters (a, b, c, d) are significantly different ($P < 0.05$).

Table 2. Concentration of γ–butyrobetaine in plasma and tissues of pigs differing in life age[†]

Age [week]	Plasma [µmol/L]	Liver [nmol/g]	Kidney [nmol/g]	Heart [nmol/g]	M. *longissimus dorsi* [nmol/g]	M. *semimembranosus* [nmol/g]
Birth	1.56 ± 0.64^{a}	7.75 ± 1.81^{a}	11.5 ± 1.4^{a}	98.1 ± 26.1	187 ± 60^{a}	144 ± 64
1	0.79 ± 0.15^{cd}	5.51 ± 1.26^{b}	7.44 ± 1.57^{c}	82.1 ± 29.9	104 ± 7^{b}	96.3 ± 21.9
2	0.84 ± 0.19^{bcd}	4.60 ± 0.52^{bc}	7.35 ± 1.74^{c}	83.0 ± 18.5	92.8 ± 11.7^{b}	93.5 ± 21.0
3	0.64 ± 0.07^{d}	3.92 ± 0.55^{c}	7.00 ± 1.21^{c}	91.8 ± 26.2	106 ± 15^{b}	129 ± 33
4	0.91 ± 0.13^{bcd}	3.80 ± 0.92^{c}	6.67 ± 0.82^{c}	70.3 ± 13.7	95.1 ± 21.0^{b}	113 ± 27
5	1.31 ± 0.27^{a}	4.25 ± 0.78^{bc}	9.38 ± 2.01^{b}	74.5 ± 14.4	102 ± 12^{b}	126 ± 19
6	1.15 ± 0.30^{bc}	4.78 ± 0.58^{bc}	8.26 ± 1.13^{bc}	71.8 ± 11.4	97.1 ± 12.8^{b}	109 ± 18
7	1.05 ± 0.19^{bc}	4.28 ± 0.29^{c}	7.73 ± 1.07^{bc}	72.7 ± 16.8	113 ± 29^{b}	129 ± 33

[†] Means and standard deviations (n=5). Means without the same superscript letters (a, b, c, d) are significantly different ($P < 0.05$).

4. Discussion

One aim of this study was to detect the site of carnitine synthesis in the pig. Therefore, we determined mRNA concentrations, protein concentrations and activities of BBD in various tissues. The study shows for the first time that mRNA and protein of BBD is expressed in several tissues, while a considerable activity of BBD occurs only in kidney and liver. With respect to the pattern of BBD activity in tissues, the pig obviously behaves like humans, cats, cows, hamsters, rabbits or Rhesus monkeys, in which liver and kidney are also the primary sites of carnitine synthesis (Vaz and Wanders, 2002; Englard and Carnicero, 1978). In all those species, activity of BBD in the kidney exceeds that in the liver as also observed for pigs in this study. In opposite, there are several other species such as rats, mice, sheep, dogs or guinea pigs which do not exhibit any measurable activity of BBD in kidney (Lindstedt et al., 1982; Erfle, 1975; Englard and Carnicero, 1978; Englard, 1979). The activity of BBD in pig liver determined in this study was two- to three fold higher than BBD activities in liver rats and mice determined with the same assay (van Vlies et al., 2006). Although enzyme activities determined in different laboratories cannot be directly compared, this finding suggests that pig liver has a relatively high capacity to convert γ-butyrobetaine into carnitine.

Interestingly, we detected mRNA and protein concentrations of BBD in nearly all tissues, also in those which did not show any activity of that enzyme such as testis, colon, lung, and spleen. From the data of this study, we cannot provide an explanation for the quite different pattern of mRNA, protein and activity of BBD in various tissues. However, investigations in rats also revealed that BBD mRNA is present in tissues such as testis and epididymis, although no significant activity of BBD has been found in these tissues (Galland et al., 1999). Noteworthy, Galland et al. (1999) observed that the size of BBD mRNA in testis (3.5 kb) and epididymis (4.5 kb) was markedly different from that in the liver (1.9 kb). This has been suggested to be the result of a tissue-specific alternative splicing of the BBD mRNA (Vaz and Wanders, 2002), which could result in either no translation or translation of the BBD mRNA into a protein with similar size as the hepatic or renal BBD but without significant BBD activity. In addition, tissue-specific posttranslational inactivation of the BBD protein, possibly mediated by phosphorylation or dephosphorylation of serine or threonine residues of the protein, could also explain the differences in BBD activities between tissues. Whether the BBD protein belongs to such interconvertible enzymes, however, is currently unknown, and, therefore, should be investigated in future studies. Furthermore, it might be also conceivable that putative inhibitors of BBD are present in specific tissues leading to a tissue-specific inhibition, either competitive, non-competitive or allosteric, of the BBD protein. This, however, is highly speculative and, thus, remains to be established. Moreover, it is known that the BBD activity is also dependent on several cofactors including molecular oxygen, Fe^{2+} and ascorbate, and that BBD activity was stimulated considerably by 2-oxoglutarate (Lindstedt, 1967; Lindstedt et al., 1968; Lindstedt and Lindstedt, 1970). Hence, differences in the availability of these cofactors between tissues might also explain tissue-specific variations in the activity of BBD.

In the present study, we determined also the concentrations of carnitine in various tissues of the pig. We found that carnitine concentrations in tissues of pigs are generally markedly lower than those reported for humans. As in humans, highest carnitine concentrations were found in skeletal muscle. This suggests that muscle serves as a carnitine storage in pigs like in humans. In humans, approximately 95% of the carnitine is localized in skeletal muscle (Evans and Fornasini, 2003). Carnitine concentrations in skeletal muscle of pigs, being around 600 nmol/g, are comparable with those of rat muscle but they are markedly lower than those of human muscle which are in the range between 2000 and 4000 nmol/g (Bertoli et al., 1981; Moorthy et al., 1983; Angelini et al., 1992). Concentrations of carnitine in other tissues such as liver, kidney or brain of pigs are also three- to five-fold lower than

those of the respective human tissues suggesting that pigs have generally a lower carnitine status than humans (Moorthy et al., 1983; Angelini et al., 1992). Total carnitine concentrations in liver, kidney and heart of pigs are moreover also much lower than in rodents (van Vlies et al., 2006; Koch et al., 2008; Ringseis et al., 2007; Luci et al., 2008).

It has been suggested that newborn infants or animals have a low carnitine status at birth which could even lead to carnitine deficiency (Borum, 1981; Penn et al., 1981). To investigate the carnitine status of piglets at birth and in the subsequent weeks of life, we considered piglets of litters adjusted to a size of eight animals each. As we removed one piglet from each litter at each of the next three subsequent weeks for analysis of tissue carnitine concentrations, litter sizes were even reduced to four piglets per litter until week 4. It is known that piglets of smaller litters receive more milk than those in larger litters (Auldist et al., 1998). Accordingly, it is likely that the piglets in this study received more carnitine through the milk than piglets of larger litters such as under practical farming conditions. The present study shows that, nevertheless, concentrations of carnitine in plasma, liver and kidney of pigs are even higher at birth than in the subsequent weeks of suckling. It is moreover shown that concentrations of carnitine in muscle are lowest at birth and are increasing thereafter. In order to achieve an indication of carnitine synthesis, we determined activities of BBD in liver and kidney and the concentrations of γ-butyrobetaine in various tissues. In humans, not the activity of BBD but the availability of γ-butyrobetaine as precursor is rate-limiting for carnitine synthesis (Olson and Rebouche, 1987; Rebouche et al., 1989). It is likely that the availability of γ-butyrobetaine in liver and kidney is the limiting factor for carnitine synthesis in pigs, too (Fischer et al., 2009a). In the present study, we found that the activity of BBD was lowest at birth. This finding agrees with reports in human neonates which have also a low activity of BBD in the liver (Rebouche and Engel, 1980). In contrast, concentrations of γ-butyrobetaine in liver and kidney were even higher at birth than in the subsequent weeks. Under the assumption that the concentration of γ-butyrobetaine is the rate-limiting factor for carnitine synthesis (Olson and Rebouche, 1987; Rebouche et al., 1989), the high γ-butyrobetaine concentrations in liver and kidney provide an explanation for the elevated carnitine concentrations at birth. It is known that γ-butyrobetaine is produced in all tissues from trimethyllysine (Vaz and Wanders, 2002). The finding that γ-butyrobetaine concentrations are highest in skeletal muscle suggests that muscle is the most important supplier of γ-butyrobetaine for carnitine synthesis in liver and kidney in the pig. Interestingly, γ-butyrobetaine concentrations in muscle of pigs are 10-20 fold higher than those reported for rat or mouse muscle (Noel et al., 1984; van Vlies et al., 2006; Ringseis et al., 2008; Koch et al., 2008). This suggests that pigs have a higher capactity to produce γ-butyrobetaine from trimethyllysine in muscle than rodents. Concentrations of γ-butyrobetaine in plasma, liver and kidney of pigs are in the same order as in rats or mice (Noël et al., 1984; van Vlies et al., 2006; Ringseis et al., 2008; Koch et al., 2008) but only one half to one fifth of those of humans (Sandor et al., 1988). This finding suggests that pigs have a lower rate of carnitine synthesis in these tissues than humans which in turn could be an explanation for the markedly lower tissues carnitine concentrations in pigs compared to humans.

The observation that γ-butyrobetaine concentrations in muscle were even higher at birth than in the subsequent weeks indicates that even the fetus has a high capacity in muscle to form γ-butyrobetaine from trimethyllysine. In humans, carnitine can be delivered in the placenta from maternal to fetal blood by OCTN2 (Lahjouji et al., 2004; Grube et al., 2007). Although such a mechanism has not yet been reported in pigs, it is likely that carnitine can cross the placenta in pregnant sows, too. Accordingly, it is possible that the high carnitine concentrations at birth could also be due to the transfer of carnitine from maternal to fetal blood during late gestation.

In humans and rats, milk intake during the early suckling period plays is important for the development of plasma and tissue carnitine concentrations. In the rat, carnitine concentrations in tissues are rising several-fold within a few days after birth due to carnitine intake via milk (Davis, 1989; Flores et al., 1996). The role of carnitine from milk for human infants is evident by the finding that infants of an age of three months fed a soy protein formula without added carnitine have markedly lower plasma carnitine concentrations than infants fed a formula supplemented with carnitine or breast-fed infants (Novak et al., 1983; Olson and Rebouche, 1987, Olson et al., 1989). Interestingly, a different picture was observed in pigs of this study. Although sow milk has even a higher carnitine concentration than human milk (120-180 vs. 30-95 μmol/L; Ramanau et al. 2005; Birkenfeld et al. 2006a, Rebouche and Paulson, 1986), concentrations of carnitine in plasma, liver and kidney in pigs were even decreasing during the suckling period. A possible explanation for this finding could be that carnitine absorbed from milk was deposited in the muscle as carnitine concentrations in skeletal muscle indeed increased during the suckling period. Normally, the movement of carnitine from blood into muscle is a slow process (Evans and Fornasini, 2003). Nevertheless, an uptake of carnitine from blood into muscle could also contribute to the decline of plasma carnitine concentration during the first weeks of life. After 4 weeks of life the pigs were switched from sows´ milk to a diet with relatively low carnitine concentrations. It is likely that the decline of carnitine concentrations in all tissues after 5 weeks of life could be due, at least in part, to the lower carnitine intake by the diet. Plasma and tissue carnitine concentrations of the 7 week old pigs of the present study were close to those of pigs in a body weight range between 20 and 30 kg used in two recent studies (Fischer et al., 2009a, b).

Determination of free carnitine and carnitine esters shows that the ratio of acetyl carnitine to free carnitine in plasma, kidney and heart is highest at birth and decreases thereafter. This ratio reflects the intramitochondrial relationship between acetyl-CoA and free CoA and is very sensitive to metabolic changes in the mitochondria (Böhles et al., 1994). The high ratio at birth, which is also noted in plasma of human neonates, is thought to reflect the increased production of acetyl-CoA produced by the enhanced fatty acid oxidation in the newborn period (Warshaw and Curry, 1980; Girard et al., 1992). The reduction of this ratio in liver and kidney during the first weeks of life suggests that there is a shift from fatty acid oxidation to utilization of glucose for energy production in these tissues during this time period. Interestingly, this ratio in plasma was unexpectedly increasing at an age of four weeks. We have no explanation for this observation, but it is possible that the increase of this ratio was due to the switch from sow´s milk to a vegetable diet with a relatively low native carnitine content.

Plasma and tissue carnitine concentrations are regulated by an interplay of absorption of carnitine from the diet, endogenous biosynthesis, uptake from blood into tissues, storage in muscle and excretion via the urine (Evans and Fornasini, 2003). Although we were not able to consider all these aspects of carnitine metabolism in detail, consideration of plasma and tissue carnitine concentrations shows that neonatal pigs in our study did not have an insufficient carnitine status. Indeed, concentrations of carnitine in plasma, liver and kidney at birth were higher than in the subsequent weeks of life which is in contrast to rats or humans (Davis et al. 1989; Flores et al., 1996; Rebouche, 1992). Plasma carnitine concentrations in neonatal and suckling pigs (being in the range between 14 and 21 μmol/L) were clearly below those of infants receiving carnitine-containing formulas (being in the range between 30 and 60 μmol/L, Olson et al. 1989) but they were in excess of those of growing-finishing pigs (Owen et al., 2001a) or pregnant or lactating sows (Doberenz et al., 2006; Birkenfeld et al. 2006a, b). Moreover, carnitine concentration in the liver was two-fold higher in neonatal pig compared to growing-finishing pigs with a body weight in excess of 100 kg (Owen et al., 2001a,b). The comparison of carnitine concentrations between young and older pigs clearly indicates that young pigs do not have an insufficient carnitine status.

In conclusion, the present study shows for the first time that liver and kidney are the only tissues in pigs with a considerable activity of BBD. In pigs, like in humans, these tissues might be therefore regarded as the major sites of carnitine synthesis. It is moreover shown that the activity of BBD in liver and kidney of pigs is lowest at birth. Nevertheless, carnitine concentrations in plasma, liver and kidney at birth are even higher than at the subsequent weeks. This study therefore does not confirm the view that neonatal pigs have a low carnitine status compared to older pigs.

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References

Angelini, C., Vergani, L., Martinuzzi, A. 1992. Clinical and biochemical aspects of carnitine deficiency and insufficiency: transport defects and inborn errors of beta-oxidation. Crit. Rev. Clin. Lab. Sci. 29, 217-242.

Auldist, D.E., Morrish, L., Eason, P., King, R.H. 1998. The influence of litter size on milk production of sows. Anim. Sci. 67, 333-337.

Baltzell J.K., Bazer F.W., Miguel S.G., Borum P.R. 1978. The neonatal piglet as a model for human neonatal carnitine metabolism. J. Nutr. 117, 754-757

Bassler R, Buchholz H., 1993. Methodenbuch Band III. Die chemische Untersuchung von Futtermitteln, 3. Ergänzungslieferung. VDLUFA-Verlag, Darmstadt, Germany.

Bertoli, M., Battistella, P.A., Vergani, L., Naso, A., Gasparotto, M.L., Romagnoli, G.F, Angelini, C. 1981. Carnitine deficiency induced during hemodialysis and hyperlipidemia: effect of replacement therapy. Am. J. Clin. Nutr. 34, 1496-1500.

Birkenfeld, C., Doberenz, J., Kluge, H., Eder, K., 2006a. Effect of L-carnitine supplementation of sows on L-carnitine status, body composition and concentrations of lipids in liver and plasma of their piglets at birth and during suckling period. Anim. Feed Sci. Technol. 129, 23-38.

Birkenfeld, C., Kluge, H., Eder, K., 2006b. L-carnitine supplementation of sows during pregnancy improves the suckling behaviour of their offspring. Br. J. Nutr. 96, 334-342.

Böhles, H., Evangeliou, A., Bervoets, K., Eckert, I., Sewell, A, 1994. Carnitine esters in metabolic disease. Eur. J. Pediatr. 153, S57-61.

Borum, P.R., 1981. Possible carnitine requirement of the newborn and the effect of genetic disease on the carnitine requirement. Nutr. Rev. 39, 385-390.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248-254.

Carter, A.L., Abney, T.O., Braver, H., Chuang, A.H., 1987. Localization of γ-butyrobetaine hydroxylase in rat testis. Biol. Reprod. 37, 68-72.

Davis, A.T. 1989. Fractional contributions to total carnitine in the neonatal rat. J. Nutr. 119, 262-267.

Doberenz, J., Birkenfeld, C., Kluge, H., Eder, K., 2006. Effects of L-carnitine supplementation in pregnant sows on plasma concentrations of insulin-like growth factors, various hormones and metabolites and chorion characteristics. J. Anim. Physiol. A. Anim. Nutr. 90, 487-499.

Englard, S., 1979. Hydroxylation of g-butyrobetaine to carnitine in human and monkey tissues. FEBS Lett. 102, 297–300.

Englard, S., Carnicero, H. H., 1978. γ-Butyrobetaine hydroxylation to carnitine in mammalian kidney. Arch. Biochem. Biophys. 190, 361–364.

Erfle, J.D., 1975. Hydroxylation of γ-butyrobetaine by rat and ovine tissue. Biochem. Biophys. Res. Commun. 64, 553-557.

Evans, A.M., Fornasini, G., 2003. Pharmacokinetics of L-carnitine. Clin. Pharmacokinet. 42, 941-967.

Fischer, M., Hirche, F., Kluge, H., Eder, K., 2009a. A moderate excess of dietary lysine lowers plasma and tissue carnitine concentrations in pigs. Br. J. Nutr. 101, 190-196.

Fischer, M., Varady, J., Hirche, F., Kluge, H., Eder, K., 2009b. Supplementation of L-carnitine in pigs: Absorption of carnitine and effect on plasma and tissue carnitine concentrations. Arch. Anim. Nutr. 63, 1-15.

Flores, C.A,, H,u C., Edmond, J., Koldovsky, O. 1996. Milk carnitine affects organ carnitine concentration in newborn rats. J. Nutr. 126, 1673-1682.

Galland, S., Le Borgne, F., Bouchard, F., Georges, B., Clouet, P., Grand-Jean, F., Demarquoy, J., 1999. Molecular cloning and characterization of the cDNA encoding the rat liver gamma-butyrobetaine hydroxylase. Biochim. Biophys. Acta 1441, 85-92.

Gesellschaft für Ernährungsphysiologie. 2006. Empfehlungen zur Energie- und Nährstoffversorgung von Schweinen, DLG-Verlag, Frankfurt/Main, Germany.

Girard, J., Ferré, P., Pégorier, J.P., Duée, P.H. 1992. Adaptations of glucose and fatty acid metabolism during perinatal period and suckling-weaning transition. Physiol. Rev. 72, 507-562.

Grube, M., Schwabedissen, H.M., Draber, K., Präger, D., Möritz, K.U., Linnemann, K., Fusch, C., Jedlitschky, G., Kroemer, H.K., 2007. Expression, localization, and function of the carnitine transporter octn2 (slc22a5) in human placenta. Drug Metab. Dispos. 33, 31-37.

Heo, K., Odle, J., Han, I.K., Cho, W., Seo, S., van Heugten, E., Pilkington, D.H., 2000. Dietary L-carnitine improves nitrogen utilization in growing pigs fed low energy, fat-containing diets. J. Nutr. 130, 1809-1814.

Hoppel, C.L., Davis, A.T., 1986. Inter-tissue relationships in the synthesis and distribution of carnitine. Biochem. Soc. Trans. 14, 673-674.

Koch, A., König, B., Stangl, G.I., Eder, K., 2008. PPARα mediates transcriptional upregulation of novel organic cation transporters-2 and -3 and enzymes involved in hepatic carnitine synthesis. Exp. Biol. Med. 233, 356-365.

Konishi, M., Hashimoto, H., 1992. Determination of pivaloylcarnitine in human plasma and urine by high-performance liquid chromatography with fluorescence detection. J Pharm Sci. 81(10), 1038-41.

Lahjouji, K., Elimrani, I., Lafond, J., Leduc, L., Qureshi, I.A., Mitchell, G.A., 2004. L-Carnitine transport in human placental brush-border membranes is mediated by the sodium-dependent organic cation transporter OCTN2. Am. J. Physiol. Cell. Physiol. 287, C263-269.

Lahjouji, K., Mitchell, G.A., Qureshi, I.A., 2001. Carnitine transport by organic cation transporters and systemic carnitine deficiency. Mol. Genet. Metab. 73, 287-297.

Lindstedt, G., 1967. Hydroxylation of γ-butyrobetaine to carnitine in rat liver. Biochem. 6, 1271–1282.

Lindstedt, G., Lindstedt, S., 1970. Cofactor requirements of γ-butyrobetaine hydroxylase from rat liver. J. Biol. Chem. 245, 4178–4186.

Lindstedt, G., Lindstedt, S., Nordin, I., 1982. γ-Butyrobetaine hydroxylase in human kidney. Scand. J. Clin. Lab. Invest. 42, 477–485.

Lindstedt, G., Lindstedt, S., Olander, B., Tofft, M., 1968. α-ketoglutarate and hydroxylation of γ-butyrobetaine. Biochim. Biophys. Acta 158, 503–505.

Luci, S., Hirche, F., Eder, K., 2008. Fasting and caloric restriction increases mRNA concentrations of novel organic cation transporter-2 and carnitine concentrations in rat tissues. Ann. Nutr. Metab. 52, 58-67.

Moorthy, A.V., Rosenblum, M., Rajaram, R., Shug, A.L. 1983. A comparison of plasma and muscle carnitine levels in patients on peritoneal or hemodialysis for chronic renal failure. Am. J. Nephrol. 3, 205-208.

Noël, H., Parvin, R., Pande, S.V., 1984. gamma-butyrobetaine in tissues and serum of fed and starved rats determined by an enzymic radioisotopic procedure. Biochem. J. 220, 701-706.

Novak, M., Monkus, E.F., Buch, M., Lesmes, H., Silverio, J. 1983. The effect of a L-carnitine supplemented soybean formula on the plasma lipids of infants. Acta Chir. Scand. Suppl. 517, 149-155.

Olson, A.L,, Nelson, S.E., Rebouche, C.J. 1989. Low carnitine intake and altered lipid metabolism in infants. Am. J. Clin. Nutr. 49, 624-628.

Olson, A.L., Rebouche, C.J., 1987. γ-Butyrobetaine hydroxylase activity is not rate limiting for carnitine biosynthesis in the human infant. J. Nutr. 117, 1024-1031.

Owen, K.Q., Jit, H., Maxwell, C.V., Nelssen, J.L., Goodband, R.D., Tokach, M.D., Tremblay, G.C., Koo, S.I., 2001a. Dietary L-carnitine suppresses mitochondrial branched-chain keto acid dehydrogenase activity and enhances protein accretion and carcass characteristics of swine. J. Anim. Sci. 79, 3104-3112.

Owen, K.Q., Nelssen, J.L., Goodband, R.D., Tokach, M.D., Friesen, K.G., 2001b. Effect of dietary L-carnitine on growth performance and body composition in nursery and growing-finishing pigs. J. Anim. Sci. 79, 1509-1515.

Penn, D., Bobrowski, P.J., Zhang, L., Schmidt-Sommerfeld, E., 1997. Neonatal nutritional carnitine deficiency: a piglet model. Pediatr. Res. 42, 114-121.

Penn, D., Schmidt-Sommerfeld, E., Pascu, F., 1981. Decreased tissue carnitine concentrations in newborn infants receiving total parenteral nutrition. J. Pediatr. 98, 976-978.

Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 29, e45.

Ramanau, A., Kluge, H., Eder, K. 2005. Effects of L-carnitine supplementation on milk production, litter gains and back-fat thickness in sows with a low energy and protein intake during lactation. Br. J. Nutr. 93, 717-721.

Rebouche, C.J. 1992.Carnitine function and requirements during the life cycle. FASEB J. 6, 3379-3386

Rebouche, C.J., Bosch, E.P., Chenard, C.A., Schabold, K.J., Nelson, S.E., 1989. Utilization of dietary precursors for carnitine synthesis in human adults. J. Nutr. 119, 1907-1913.

Rebouche, C.J., Engel, A.G., 1980. Tissue distribution of carnitine biosynthetic enzymes in man. Biochim. Biophys. Acta 630, 22-29.

Rebouche, C.J., Paulson, D.J. 1986. Carnitine metabolism and function in humans Annu. Rev. Nutr. 6, 41-66.

Rebouche. C.J., Seim, H., 1998. Carnitine metabolism and its regulation in microorganisms and mammals. Annu. Rev. Nutr. 18, 39-61.

Ringseis, R., Luci, S., Spielmann, J., Kluge, H., Fischer, M., Geissler, S., Wen, G., Hirche, F., Eder, K., 2008. Clofibrate treatment up-regulates novel organic cation transporter (OCTN)-2 in tissues of pigs as a model of non-proliferating species. Eur. J. Pharmacol. 31, 11-17.

Ringseis, R., Pösel, S., Hirche, F., Eder, K., 2007. Treatment with pharmacological peroxisome proliferator-activated receptor alpha agonist clofibrate causes upregulation of organic cation transporter 2 in liver and small intestine of rats. Pharmacol. Res. 56, 175-183.

Ruan, J., Guo, Y., Li, H., Hu, Y., Song, F., Huang, X., Kristiensen, K., Bolund, L., Wang, J., 2007. PigGIS: Pig Genomic Informatics System. Nucleic Acids Res. 35 (Database issue), D654-657.

Sandor, A., Minkler, P.E., Ingalls, S.T., Hoppel, C.L., 1988. An enzymatic method for the determination of butyrobetaine via conversion to carnitine after isolation by high performance liquid chromatography Clin. Chim. Acta. 176, 17-27.

Steiber, A., Kerner, J., Hoppel, C.L., 2004. Carnitine: a nutritional, biosynthetic, and functional perspective. Mol. Asp. Med. 25, 455-473.

Tamai, I., Ohashi, R., Nezu, J.I., Sai, Y., Kobayashi, D., Oku, A., Shimane, M., Tsuji, A., 2000. Molecular and functional characterization of organic cation/carnitine transporter family in mice. J. Biol. Chem. 275, 40064-40072.

van Kempen, T.A., Odle, J., 1995. Carnitine affects octanoate oxidation to carbon dioxide and dicarboxylic acids in colostrum-deprived piglets: in vivo analysis of mechanisms involved based on CoA- and carnitine-ester profiles. J. Nutr. 125, 238-250.

van Vlies, N., Ferdinandusse, S., Turkenburg, M., Wanders, R.J., Vaz, F.M., 2007. PPARα-activation results in enhanced carnitine biosynthesis and OCTN2-mediated hepatic carnitine accumulation. Biochim. Biophys. Acta 1767, 1134-1142.

van Vlies, N., Wanders, R.J., Vaz, F.M. 2006. Measurement of carnitine biosynthesis enzyme activities by tandem mass spectrometry: differences between the mouse and the rat. Anal. Biochem. 354, 132-139.

Vaz, F.M., Melegh, B., Bene, J., Cuebas, D., Gage, D.A., Bootsma, A., Vreken, P., van Gennip, A.H., Bieber, L.L., Wanders, R.J.A., 2002. Analysis of carnitine biosynthesis metabolites in urine by HPLC-electrospray tandem mass spectrometry. Clin. Chem. 48 (6 Pt 1) 826-34.

Vaz, F.M., Wanders, R.J., 2002. Carnitine biosynthesis in mammals. Biochem. J. 361, 417-429.

Warshaw, J.B., Curry, E. 1980. Comparison of serum carnitine and ketone body concentrations in breast- and in formula-fed newborn infants. J. Pediatr. 97, 122-125

4. Diskussion

4.1 Wirkung von Lysin auf den Carnitin-Status

In der Fütterungspraxis des Schweines ist Lysin die erst-limitierende Aminosäure. In der Carnitinsynthese hat Lysin als Ausgangspunkt der Synthese eine bedeutende Funktion (Tanphaichitr und Broquist, 1973). Allerdings ist fragwürdig, inwieweit freies Lysin im Rahmen einer Supplementierung die Carnitinsynthese steigert, da Säuger keine S-Adenosyl-L-Methionin-6-N-L-Lysin Methyltransferase besitzen, welche freies Lysin methyliert (Owen *et al*., 1996). Im Rahmen dieser Untersuchung von Owen *et al.* (1996) wurde jedoch festgestellt, dass die deutlichsten Effekte einer Carnitin-Zufuhr auf das Wachstum des Schweines bei geringer Lysin-Konzentration in der Diät zu verzeichnen sind.

Im Menschen erhöhen große Mengen von Lysin und Methionin möglicherweise die Rate der Carnitinsynthese (Rebouche *et al*., 1989). Weitere Humanstudien zeigten, dass bei geringer Lysinaufnahme durch bestimmte Nahrungspräferenzen, wie vegane Ernährung oder durch exzessive Reisaufnahme als Hauptproteinquelle, der Carnitin-Status des Körpers massiv beeinträchtigt wird (Tanphaichitr *et al*., 1980; Krajčovičová-Kudláčková *et al.*, 2000). In trächtigen und laktierenden Ratten wurde die Trimethyllysin-Biosynthese durch eine Diät mit geringer Lysin-Konzentration nicht beeinflusst (Davis, 1990), allerdings steigt bei Verabreichung einer Diät mit hoher Lysin-Konzentration die Trimethyllysin-Konzentration im Plasma und Skelettmuskel, während die Gesamt-Carnitinkonzentration im Plasma sinkt (Davis *et al.*, 1993). Die Dosis/Wirkungs-Beziehung von Lysin auf die Carnitin-Synthese ist somit umstritten und scheint speziesabhängig zu sein.

In der Studie 1 erhielten Schweine der Kontrollgruppe eine Lysinzufuhr, die sich an den Richtlinien des National Research Council (1998) orientierte (9,7g Lysin/kg), der Behandlungsgruppe wurde dagegen eine Diät mit einem moderaten Überschuss an Lysin (16,8g Lysin/kg) verabreicht. Hier konnte erstmals gezeigt werden, dass durch moderaten Lysinüberschuß die Konzentrationen von Carnitin in Plasma, Leber, Niere und Skelettmuskel beim Schwein sinken. Es ist wahrscheinlich, dass dieser niedrigere Carnitinstatus aus einer verminderten Verfügbarkeit des Carnitin-Precursors Butyrobetain resultiert. Im Muskel, der als Hauptsyntheseort des Butyrobetains gilt, ist die Synthese dieses Precursors vermutlich durch geringere mRNA-Expression des Enzyms TMLD, welches für die Hydroxylierung von Trimethyllysin verantwortlich ist, vermindert. Dies bestätigen auch die bereits bei Ratten beschriebenen erhöhten Trimethyllysin-Konzentrationen im Skelettmuskel nach hoher Lysin-Aufnahme (Davis *et al*., 1993). Das Plasma kann die Carnitin synthetisierenden Organe Leber

und Niere durch die vermutlich eingeschränkte Butyrobetain-Synthese im Skelettmuskel nur unzureichend mit Butyrobetain versorgen. Dadurch ist die Synthese des Carnitins in diesen Geweben mutmaßlich verringert. Weitere mögliche Ursache der verminderten Carnitin-Konzentration in Leber und Niere ist die geringere Produktion der Carnitin-Vorstufe Butyrobetain in diesen Geweben. Diese ist limitierender Faktor in der Carnitin-Synthese des Menschen (Rebouche *et al.*, 1989). Die vorliegende Studie liefert erste indirekte Hinweise, dass verminderte Butyrobetain-Konzentrationen ebenso die Carnitin-Synthese des Schweines beeinträchtigen.

Die γ-BBD ist das bedeutende Enzym zur Umwandlung von Butyrobetain in Carnitin. Im Menschen wurde die Aktivität dieses Enzyms in Leber, Niere und geringfügig im Gehirn nachgewiesen (Vaz und Wanders, 2002; Rebouche und Engel 1980). Durch Aktivitätsmessung des Enzyms konnte auch beim Schwein in Leber und Niere eine Carnitin-Synthese nachgewiesen werden. Trotz konstanter γ-BBD-Aktivität in der Niere und einer signifikanten Erhöhung der Aktivität der γ-BBD in der Leber sank die Carnitin-Konzentration in Plasma und Gewebe bei Schweinen mit moderatem Lysinüberschuss. Dies deutet darauf hin, dass beim Schwein, wie auch in Humanstudien nachgewiesen, die γ-BBD nicht limitierender Faktor der Carnitin-Synthese ist (Olsen und Rebouche, 1987; Rebouche *et al*., 1989). Die vierte Studie liefert dazu weitere Hinweise. Hierbei zeigte sich, dass bei Erreichen eines Aktivitäts-Plateaus der γ-BBD bei Ferkeln in den ersten Lebenswochen die Konzentration an Carnitin nicht zwangsläufig ansteigt. Die Größenordnung der γ-BBD-Aktivität in Studie 1 konnte in den Studien 2 und 4 bestätigt werden. Dabei ist in der Niere der Schweine dieser Studien eine höhere γ-BBD-Aktivität als in der Leber nachgewiesen worden. Diese Aktivitätsunterschiede sind ebenfalls in anderen Säugetieren wie Mensch, Katze und Rind zu finden (Vaz und Wanders, 2002).

In Studie 1 konnte erstmals gezeigt werden, dass ein moderater Lysinüberschuss in der Diät die Konzentration an Carnitin in Plasma und Gewebe von Schweinen vermindert. Mögliche Ursachen hierfür sind die geringeren Butyrobetain-Konzentrationen in Leber und Niere, da die Synthese dieser Vorstufe im Hauptsyntheseort Muskel mutmaßlich beeinträchtigt ist. Diese Forschungsergebnisse sind bedeutsam für die Bewertung der Lysin- und Carnitin-Zufuhr in der Fütterungspraxis des Schweines und verdeutlichen, inwieweit die Nährstoffzufuhr die Carnitin-Homöostase im Schwein beeinflussen kann.

4.2 Absorption und Gewebe-Konzentration von Carnitin

In der Literatur gibt es eine Vielzahl positiver Wirkungen eines Carnitin-Einsatzes bei wachsenden Schweinen (Birkenfeld *et al*., 2005; Owen *et al*., 1996, 2001a,b; Penn *et al.*, 1997; Rincker *et al*., 2003). Bei trächtigen Sauen haben Carnitin-Zusätze die Reproduktionsleistungen verbessert (Eder *et al*., 2001; Musser *et al*., 1999; Ramanau *et al.*, 2002, 2004). Bislang ist nicht bekannt, in welchem Ausmaß Carnitin-Supplemente beim Schwein absorbiert werden und inwieweit der Carnitin-Status dadurch beeinflusst werden kann. Zur Beantwortung dieser Fragestellungen wurde die Studie 2 durchgeführt. Neben einer Kontrollgruppe wurden die Schweine in dieser Studie in sechs weitere Gruppen gegliedert, denen jeweils Carnitin-Supplementationen in Höhe von 25, 50, 100, 200, 500 und 1000 mg Carnitin/kg Futter verabreicht wurden. Die Absorptionsrate des Carnitins wurde durch Konzentrationsänderungen des unverdaulichen Markers Titandioxid zwischen Diät und Chymus des Dünndarms ermittelt. Des Weiteren wurden die Konzentrationen von Carnitin, seiner Synthese-Metabolite und Derivate in Plasma und verschiedenen Geweben gemessen sowie die Aktivität der γ-BBD in Leber und Niere bestimmt. In der Untersuchung der Absorption von Carnitin beim Schwein wurde deutlich, dass der größte Anteil des Carnitins bereits im proximalen Segment des Dünndarms, dem Duodenum, absorbiert wurde. Die Absorptionsrate von Carnitin im Schwein betrug bei der Gabe von geringen Carnitin-Dosen (25-100 mg Carnitin/kg Futter) mehr als 95%. Selbst bei hohen Dosen von 200-1000 mg Carnitin/kg Futter war die Absorptionsrate von Carnitin beim Schwein höher als 90%. Diese Absorptionsraten von Carnitin beim Schwein sind bedeutend höher als beim Menschen, der bei Gabe von 1-6g Carnitin als Supplement nur 5-18% dieses Stoffes absorbiert (Evans und Fornasini, 2003). Die Raten zeigen, dass Schweine, im Gegensatz zum Menschen, eine sehr hohe Kapazität zur Absorption von Carnitin-Zusätzen besitzen. Selbst bei hohen Carnitin-Dosen tritt keine bzw. nur eine sehr geringe Sättigung der Carnitin-Absorption ein. Somit sind Carnitin-Supplementationen beim Schwein bis 1000 mg Carnitin/kg Futter ernährungsphysiologisch sinnvoll, da das Carnitin in hohen Mengen gespeichert und nicht ungenutzt ausgeschieden wird.

Carnitin wird im Dünndarm absorbiert und gelangt durch den aktiven und sättigbaren Transporter OCTN2 oder durch passive Diffusion zu den Zielgeweben. Die mRNA-Expression von OCTN2 wurde bei Schweinen im Dünndarm nachgewiesen (Ringseis *et al*., 2008b). Da pharmakologische Supplementationen von Carnitin beim Menschen passiv mit geringer Absorptionsrate aufgenommen werden (Evans und Fornasini, 2003; Li *et al*., 1992), absorbiert das Schwein Carnitin-Zusätze aufgrund der hohen Absorptionsrate vermutlich

aktiv durch den Transporter OCTN2. Dabei besitzt der OCTN2 im Dünndarm des Schweines möglicherweise keine sättigenden Eigenschaften und kann ungehindert Carnitin absorbieren. Weiterhin gibt es in der Literatur Hinweise darauf, dass neben OCTN2 der Transporter ATB^{0+} eine Rolle in der Carnitin-Absorption spielt (Taylor, 2001). ATB^{0+} besitzt zwar eine geringere Affinität zum Carnitin als OCTN2, allerdings ist seine Kapazität zum Transport dieses Stoffes gegenüber OCTN2 deutlich höher (Nakanishi *et al*., 2001). Bei Mäusen mit genetischen Defekten des OCTN2 ist die Bioverfügbarkeit von Carnitin nur 50% verringert, was somit ein weiteres Indiz für eine Beteiligung mehrerer Transporter bei der Absorption von Carnitin im Darm darstellt (Yokogawa *et al*., 1999). Die Wirkung mehrerer Transporter ist somit weitere mögliche Ursache für die hohe Absorptionskapazität von Carnitin im Dünndarm des Schweines.

Carnitin zirkuliert im Organismus in Abhängigkeit der Gewebe auf unterschiedliche Weise. Zum einen ist der Carnitin-Turnover im Muskel sehr langsam, dafür allerdings sehr umfangreich, während der Carnitin-Turnover in Leber, Niere und anderen Geweben schnell mit geringer Umsatzrate an Carnitin verläuft (Rebouche, 2004). Bislang gibt es nur wenige Untersuchungen zu Carnitin-Konzentrationen in Plasma und Geweben beim Schwein. Ziel der Studie 4 war es daher, die Konzentrationen von Carnitin in relevanten Geweben zu bestimmen. Es zeigte sich, dass der Skelettmuskel die höchste Konzentration an Carnitin beinhaltet, gefolgt von Herz, Dünndarm, Niere und Leber. Diese hohen Carnitin-Konzentrationen im Skelettmuskel verdeutlichen seine Funktion als Carnitinspeicher (Steiber *et al*., 2004). Die Gewebe-Konzentrationen von Carnitin sind in Leber, Niere, Skelettmuskel und Gehirn des Schweines generell geringer als in den entsprechenden Geweben des Menschen (Angelini *et al*., 1992; Bertoli *et al*., 1981; Moorthy *et al*., 1983) und lassen vermuten, dass Schweine einen niedrigeren Carnitin-Status besitzen. Im Vergleich zu Konzentrationen von Carnitin in der Ratte wird deutlich, dass Schweine ebenso weitaus geringere Konzentrationen in Plasma, Leber, Niere und Herz aufweisen (Luci *et al*., 2008; Ringseis *et al*., 2007). Mögliche Ursache hierfür ist die weitaus geringere Expression des PPARα in Schweinen im Vergleich zu Nagern (Holden und Tugwood, 1999). Durch Beeinflussung des Carnitin-Transporters OCTN2 ist der PPARα in der Lage, den Carnitin-Status in den Geweben regulieren zu können (Luci *et al*., 2006; Koch *et al*., 2008). Dies wurde auch in der Studie 3 der vorliegenden Arbeit nachgewiesen. Da die Expression des Transkriptionsfaktors PPARα mit solchen Regulationsmöglichkeiten im Schwein geringer ist als bei Ratten, sind vermutlich auch die Gewebekonzentrationen des Carnitins im Schwein niedriger.

Die Konzentrationen von Carnitin waren in Leber, Niere, Herz und Skelettmuskel in allen 4 Studien trotz unterschiedlichen Lebensalters von 7 (Studie 2 und 4) bzw. 12 (Studien 1 und 3) Lebenswochen vergleichbar. In den Studien 1-4 ist die Carnitin-Konzentration im Plasma des Schweines im Vergleich zu den Gewebe-Konzentrationen am geringsten. Im Menschen ist die höchste Konzentration von Carnitin im Skelettmuskel zu verzeichnen, wobei die hier vorliegenden Konzentrationen um den Faktor 100 höher sind als im Plasma (Angelini *et al*., 1992; Bertoli *et al*., 1981; Moorthy *et al*., 1983). Diese Relation findet sich in ähnlicher Weise in der vorliegenden Arbeit.

In der Studie 2 korreliert die Carnitinaufnahme über das Futter positiv mit der Konzentration von Carnitin und Acetyl-Carnitin im Plasma. Dieser Effekt zeigte sich bereits in einer Humanstudie von Lennon *et al*. (1986). In Leber, Herz, Niere und Skelettmuskel sind die Carnitin- und Acetyl-Carnitin-Konzentrationen in Abhängigkeit der Carnitin-Supplementation ebenfalls deutlich erhöht (Studie 2). Dies stimmt mit Ergebnissen vorheriger Studien überein (Ramanau *et al*., 2004; Doberenz *et al*., 2006). Bei Fütterung von 1000 mg Carnitin/kg Futter waren die Konzentrationen von Carnitin in den Geweben der Studie 2 im Vergleich zur Kontrollgruppe 3-6-fach höher. Bei Ratten zeigten sich dagegen nach Zufuhr von 5000 mg Carnitin/kg Futter geringere Konzentrationsanstiege in den Geweben (Ringseis *et al*., 2008a). Die niedrige Absorption des Carnitins von 40% begründet vermutlich den geringeren Anstieg der Konzentrationen in dieser Studie. Im Vergleich zur Absorption des Carnitins bei Ratten wird deutlich, dass Schweine eine weitaus höhere Kapazität zur Absorption dieses Stoffes besitzen.

Neben den Konzentrationen des Carnitins wurden die Konzentrationen der Synthese-Metabolite Trimethyllysin (Studien 1 und 2) und Butyrobetain (Studien 1-4) im Gewebe erfasst. Die höchsten Trimethyllysin-Konzentrationen wurden in Niere, Herz und Muskel der Studien 1 und 2 gemessen. Durch ihren hohen Anteil an Trimethyllysin liegt nahe, dass diese Gewebe hauptsächlich für die Umwandlung von Trimethyllysin in Butyrobetain verantwortlich sind. Die Niere besitzt als Syntheseort des Carnitins obligate Mengen an Trimethyllysin, speichert aber keine großen Mengen des umgewandelten Butyrobetains. Muskel und Herz beinhalten die höchsten Konzentrationen an Butyrobetain und sind somit Hauptsyntheseort und größter Speicher des für die Synthese limitierenden Carnitin-Vorläufers. Dabei ist die in den Studien 1-4 ermittelte Konzentration des Butyrobetains im Muskel 10-20-fach höher als in Ratte oder Maus. Die Konzentration des Butyrobetains im Herz der Schweine der Studien 2 und 4 ist 7-fach höher als im Herz von Maus oder Ratte (Davis und Monroe, 2005; Luci *et al*., 2008; Koch *et al*., 2008; Van Vlies *et al*., 2006). Diese Ergebnisse

verdeutlichen die hohe Kapazität des Schweines zur Produktion und Speicherung von Butyrobetain in Muskel und Herz und zeigen eine hohe Verfügbarkeit des limitierenden Vorläufers zur Carnitin-Synthese. Im Rahmen der Untersuchungen der Gewebe-Konzentration von Carnitin und seinen Derivaten im Organismus war in den Studien 1-4 die höchste Konzentration des Acetyl-Carnitins im Muskel zu verzeichnen. Der Muskel reguliert somit maßgeblich die Acetyl-CoA-Homöostase im Körper durch die Möglichkeit der reversiblen Veresterung von Carnitin mit Acetat.

Fazit der Untersuchungen zur Absorption und Gewebe-Konzentration von Carnitin im Schwein ist, dass wachsende Schweine hohe Kapazitäten zur Absorption von Carnitin besitzen. Das Plasma und die Gewebe des Schweines reagieren in Abhängigkeit der Carnitin-Zufuhr mit einem deutlichen Anstieg der Carnitin-Konzentration. Dies ist bedeutsam zur Beurteilung von physiologischen Effekten auf die Carnitin-Supplementation beim Schwein. Durch diese Erkenntnisse wird deutlich, dass eine Anreicherung des Futters mit Carnitin sinnvoll ist und durch die hohe Absorptionskapazität des Schweines entsprechend im Körper gepeichert wird. Generell besitzen Schweine im Vergleich zum Menschen geringere Gewebe-Konzentrationen von Carnitin. Dadurch ist zu vermuten, dass der Carnitin-Status des Schweines niedriger ist, als der des Menschen.

4.3 Regulation des Carnitin-Status durch den Transkriptionsfaktor PPARα

Ziel der Studie 3 war es, eine mögliche Beeinflussung des Carnitin-Transporters OCTN2 und des Enzyms γ-BBD durch Aktivierung des Transkriptionsfaktors PPARα beim Schwein zu untersuchen. Der Carnitin-Transporter OCTN2 wird durch Aktivierung des PPARα vermehrt exprimiert (Koch *et al*., 2007; Van Vlies *et al*., 2007). In Leber und Dünndarm der proliferierenden Spezie Ratte erhöhte sich die mRNA-Konzentration des mutmaßlichen Zielgenes OCTN2 nach PPARα-Aktivierung (Luci *et al*., 2006; Ringseis *et al*., 2007). Da die mRNA-Expression von PPARα in der Leber von Schweinen im Vergleich zur Ratte 10-fach geringer ist (Luci *et al*., 2007) und PPARα-Zielgene des Schweines als nicht-proliferierende Spezie geringer auf PPARα-Aktivierung ansprechen (Holden und Tugwood, 1999), war es fraglich, ob auch beim Schwein durch PPARα-Aktivierung das vermutliche Zielgen OCTN2 beeinflusst wird. Dabei ist zu berücksichtigen, dass der PPARα verstärkt in Geweben exprimiert wird, die in hohem Maße Fettsäureoxidation durchführen, wie Leber, Niere und Herz (Desvergne und Wahli, 1999). In der dritten Studie stellte sich heraus, dass durch Aktivierung des Transkriptionsfaktors PPARα die mRNA-Expression von OCTN2 in Leber, Skelettmuskel und Dünndarmenterozyten des Schweines erhöht ist. Somit wurde gezeigt, dass

auch bei der nicht-proliferierenden Spezie Schwein der Carnitin-Transport durch den Transkriptionsfaktor PPARα reguliert wird. Durch die erhöhte mRNA-Expression des Transporters OCTN2 steigerte sich die Carnitin-Aufnahme in Leber und Muskel, wodurch auch die Carnitin-Konzentration in diesen Geweben, im Vergleich zur Kontrollgruppe, höher war. Die Ergebnisse dieser Studie sind vergleichbar mit den Effekten, die bei Ratten nach PPARα-Aktivierung beobachtet wurden (Luci *et al*., 2006; Ringseis *et al*., 2007). Da der Mensch, wie auch das Schwein, zur nicht proliferierenden Spezie gehört, kann man durch die Ergebnisse der Studie 3 ähnliche Effekte einer PPARα-Aktivierung beim Menschen erwarten.
Neben der möglichen Regulierung des OCTN2 wurde im Rahmen der dritten Studie untersucht, ob durch PPARα-Aktivierung die Aktivität der γ-BBD beeinflusst wird. Es stellte sich heraus, dass die Aktivität der γ-BBD in der Leber der Schweine, denen Clofibrat als PPARα-Agonist verabreicht wurde, im Vergleich zur Kontrolle geringer war. Somit ist die Kapazität zur Carnitin-Synthese in der Leber durch PPARα-Aktivierung nicht angestiegen. Es ist wahrscheinlich, dass die Enzymaktivität nicht auf Transkriptionsebene durch Aktivierung des PPARα reguliert wird. Möglicherweise reagiert das Enzym selbst auf die erhöhten Carnitin-Konzentrationen, wie es schon in ähnlicher Weise bei Davis und Monroe (2005) beobachtet wurde.
Der Zustand des Fastens wirkt ebenso aktivierend auf den Transkriptionsfaktor PPARα. Ringseis *et al*. (2008b) zeigten, dass die γ-BBD-Aktivität bei Schweinen im Fastenzustand in Leber und Niere erhöht ist. Dies ist widersprüchlich zur Verringerung der γ-BBD-Aktivität nach PPARα-Aktivierung in der Leber der Schweine der Studie 3. Mögliche Ursachen hierfür sind bislang nicht geklärt. Es ist jedoch nicht davon auszugehen, dass bei einer Aktivierung des Transkriptionsfaktors PPARα die Aktivität der γ-BBD generell erhöht ist. Im Rahmen des Fastenversuchs konnten Ringseis *et al*. (2008b) nachweisen, dass dieser besondere metabolische Zustand die mRNA-Expression von OCTN2 in Leber, Niere, Skelettmuskel und Dünndarm erhöht. Dieser Anstieg der mRNA-Expression von OCTN2 zeigte sich ebenso nach PPARα-Aktivierung durch Clofibrat in der Studie 3 in Leber, Skelettmuskel und Dünndarm. Während dabei die Konzentrationen an Carnitin in Leber und Skelettmuskel erhöht waren, konnte im Fastenzustand trotz erhöhter mRNA-Expression von OCTN2 kein Konzentrations-Anstieg von Carnitin im Skelettmuskel nachgewiesen werden. Es bedarf noch weiterer intensiver Untersuchungen um zu klären, warum die Carnitin-Konzentration unabhängig von der Ursache der PPARα-Aktivierung im Skelettmuskel nicht konsequent ansteigt.

Im Rahmen der vorliegenden Arbeit konnte erstmals gezeigt werden, dass beim Schwein als nichtproliferierende Spezie durch PPARα-Aktivierung die mRNA-Expression des Carnitin-Transporters OCTN2 in Leber, Skelettmuskel und Enterozyten des Dünndarms erhöht ist. Durch die stimulierte Carnitin-Aufnahme in die Zellen sind die Carnitin-Konzentrationen in Leber und Skelettmuskel angestiegen. Dieser Effekt ist auch im Humanbereich zu vermuten, da der Mensch ebenfalls zur nichtproliferierenden Spezie gehört. Bei Gabe des lipidsenkenden Mittels Clofibrat ist in der Humanmedizin beispielsweise ein Anstieg der Carnitin-Konzentration in Leber und Muskel zu erwarten.

4.4 Aktivität der γ-Butyrobetaindioxygenase (γ-BBD)

Die γ-BBD vollzieht die Umsetzung von Butyrobetain in Carnitin als letzten Schritt der Carnitin-Synthese. In früheren Untersuchen wurde vermutet, dass die γ-BBD limitierend auf diese wirkt. Es zeigte sich allerdings, dass die γ-BBD beim Menschen im Überschuss vorhanden ist und dass vorrangig Butyrobetain bzw. Trimethyllysin limitierende Faktoren in der Carnitin-Synthese des Menschen sind (Rebouche *et al*., 1986; Davis und Hoppel, 1986; Olson und Rebouche, 1987; Rebouche *et al*., 1989). Leber, Niere und Gehirn gelten beim Menschen als relevante Organe in der Synthese des Carnitins, da nur in diesen Geweben deutliche Aktivitäten der γ-BBD nachgewiesen wurden (Rebouche und Engel, 1980). In der Ratte ist allein die Leber zur Umwandlung von Butyrobetain in Carnitin fähig (Vaz und Wanders, 2002; Van Vlies *et al*., 2006). In den vorliegenden Studien konnten beim Schwein in Leber (Studien 1-4) und Niere (Studien 1,2 und 4) Aktivitäten der γ-BBD nachgewiesen werden. Die Studien 1, 2 und 4 zeigen, dass die Niere eine weitaus höhere γ-BBD-Aktivität besitzt als die Leber. So haben beispielsweise neugeborene Ferkel der Studie 4 im Vergleich zur Leber eine 2,3-fach höhere γ-BBD-Aktivität in der Niere. Diese Aktivitätsunterschiede wurden auch in Humanstudien nachgewiesen (Rebouche und Engel, 1980; Vaz und Wanders, 2002). Des Weiteren wurde gezeigt, dass die Aktivität der γ-BBD in der Leber altersabhängig und in der Niere unabhängig vom Alter des Menschen reguliert wird (Olson und Rebouche, 1987; Rebouche und Engel, 1980). Dabei betrug die Enzymaktivität in der Leber von Säuglingen nur 12% im Vergleich zur vollständigen Aktivität im 15. Lebensjahr. In Studie 4 wurde die Aktivität der γ-BBD in Leber und Niere von neugeborenen Ferkeln erfasst. Es ist ersichtlich, dass, im Gegensatz zu den Ergebnissen der Humanstudien, die γ-BBD in der Niere der Schweine altersabhängig aktiv ist. In der Zusammenfassung aller untersuchter Enzymaktivitäten der Studien 1, 2 und 4 wird deutlich, dass die Enzymaktivität in der Niere vom Tag der Geburt bis zur 7. Lebenswoche um das 2,3-fache ansteigt und in der 12.

Lebenswoche um den Faktor 1,4 sinkt. Ebenso erhöht sich, in Abhängigkeit des Alters der Schweine, die Aktivität des Enzyms in der Leber und ist somit vergleichbar mit der Enzymregulation in der menschlichen Leber.

Die Studie 4 wurde durchgeführt, um grundlegende Kenntnisse zur Lokalisation und Aktivität der γ-BBD im Schwein zu erlangen. Dazu wurden die Protein-Konzentration, die mRNA-Expression sowie die Aktivität dieses Enzyms in Herz, Leber, Niere, Dickdarm, Dünndarm, Lunge, Skelettmuskel, Milz, Hoden und Nebenhoden bestimmt. In fast allen untersuchten Geweben, außer dem Muskel, konnte eine mRNA-Expression der γ-BBD nachgewiesen werden. In allen Geweben wurde γ-BBD-Protein nachgewiesen, wobei die Konzentrationen nicht überall mit der mRNA-Expression von γ-BBD und der letztendlichen Enzymaktivität übereinstimmten. Besonders auffällig war die höchste Proteinkonzentration und mRNA-Expression der γ-BBD im Nebenhoden. Dieses Gewebe zeigte jedoch eine sehr geringe Aktivität des Enzyms. Mögliche Gründe hierfür sind eine posttranslationale Inaktivierung des Enzyms oder gewebespezifische Verfügbarkeiten von molekularem Sauerstoff, zweiwertigem Eisen, 2-Oxoglutarat und stimulierender Katalase als Co-Faktoren der γ-BBD. Hinweise auf posttranslationale Inaktivierung des Enzyms geben Davis und Monroe (2005) bei Ratten, denen Pivalat und Carnitin verabreicht wurde. Durch die Supplementation wurde die mRNA-Expression der γ-BBD in der Leber nicht beeinflusst, dennoch war die Aktivität der γ-BBD in der Leber der Ratten, die Carnitin und Pivalat erhielten, signifikant niedriger. Die Enzymaktivität der γ-BBD wurde somit nicht auf Transkriptionsebene geregelt, sondern durch direkte Effekte des Enzyms selbst. Die posttranslationale Inaktivierung erfolgt möglicherweise durch Phosphorylierung oder Dephosphorylierung der Serin- oder Threoninreste des Proteins der γ-BBD. Weiterer Grund für die trotz ausgeprägter Protein-Konzentration und mRNA-Expression deutlich unterschiedlichen Aktivitäten der γ-BBD in den einzelnen Geweben sind möglicherweise verschiedene gewebsabhängige Isoformen des Enzyms. Lindstedt und Nordin (1984) zeigten diese Formen in der menschlichen Niere. Ebenso ist es möglich, dass gewebespezifische Inhibitoren das Enzym auf kompetitivem, nicht-kompetitivem oder allosterischem Wege hemmen können. In der Literatur ist des Weiteren eine gewebsabhängige Form des *splicing* der mRNA der γ-BBD beschrieben worden (Vaz und Wanders, 2002). Diese führt in folgender Translation zu γ-BBD-Protein mit ähnlicher Größe, allerdings ohne signifikante Aktivität des Enzyms.

Geringe Enzymaktivitäten sind in Studie 4 beim Schwein in Duodenum, Nebenhoden, Hoden und Gehirn nachgewiesen worden. In Skelettmuskel, Herz, Kolon, Lunge und Milz lagen die Aktivitäten unter der Nachweisgrenze. Skelettmuskel und Herz sind somit abhängig vom

OCTN2-vermittelten Carnitin-Transport aus den Carnitin synthetisierenden Organen Leber und Niere. Letztendlich wurden gut messbare Aktivitäten der γ-BBD nur in Leber (0,4 ± 0,1 nmol*mg protein^{-1}*min^{-1}) und Niere (2,37 ± 0,9 nmol*mg protein^{-1}*min^{-1}) nachgewiesen. In den Studien 1-3 konnte eine Aktivität dieses Enzyms in beiden Geweben bestätigt werden. Somit sind Leber und Niere vermutlich die Hauptorte der Carnitinsynthese des Schweines, wie es u.a. auch bei Mensch, Katze, Rind und Hamster nachgewiesen wurde (Englard, 1979; Rebouche und Engel, 1980; Vaz und Wanders, 2002).

Im Rahmen der vorliegenden Arbeit stellte sich die Frage, mit welcher Reaktionsfähigkeit und Sensitivität die γ-BBD auf Änderungen des Carnitin-Stoffwechsels beim Schwein reagiert. In der zweiten Studie wurde Schweinen Carnitin in Höhe von 25-1000 mg Carnitin/kg Futter verabreicht. Während in der Niere die Aktivität der γ-BBD schon bei einer Supplementierung von 25 mg Carnitin/kg Futter um 40% sank, wurde die γ-BBD-Aktivität in der Leber in Abhängigkeit der Supplementation des Carnitins nicht beeinflusst. Durch die verminderte Enzymaktivität in der Niere konnte allerdings keine Beeinträchtigung der Carnitin-Synthese beobachtet werden, da die Carnitin-Konzentrationen in Plasma und Gewebe der Behandlung geringfügig höher waren, als in der Kontrollgruppe. Dementsprechend war die Supplementierung von 25 mg Carnitin/kg Futter ausreichend, um die reduzierte Enzymaktivität zu kompensieren. Erstaunlich ist auch, dass die Enzymaktivität in der Niere durch die weiteren Supplementationsstufen von 50-1000 mg Carnitin/kg Futter nicht beeinflusst wurde. Diese Ergebnisse deuten darauf hin, dass die Aktivität der γ-BBD beim Schwein nicht limitierend für die Biosynthese des Carnitins ist und dass das Enzym γ-BBD relativ unempfindlich auf Änderungen im Carnitin-Metabolismus reagiert.

Fazit der Untersuchungen zur γ-BBD ist, dass Leber und Niere vermutlich die Hauptorte der Carnitin-Synthese beim Schwein darstellen. Die mRNA-Konzentration sowie die Protein-Konzentration der γ-BBD konnte zwar in mehreren Geweben nachgewiesen werden, allerdings ist nur in Leber und Niere eine deutliche Aktivität der γ-BBD erkennbar. Es bedarf weiterer Untersuchungen, um zu klären, warum in einigen Geweben trotz Nachweis von Protein und mRNA der γ-BBD keine Aktivität dieses Enzyms zu verzeichnen ist.

4.5 Carnitin-Status im neugeborenen Ferkel

Weiteres Ziel der Studie 4 war, zu untersuchen, ob neugeborene Ferkel gegenüber jungen Schweinen einen unzureichenden Carnitin-Status besitzen. Dazu wurden Plasma- und Gewebe-Konzentrationen von Carnitin sowie die Aktivität der γ-BBD in den ersten Lebenswochen des Ferkels untersucht. Vom Tag der Geburt bis zur 7. Lebenswoche wurde

von 5 Sauen pro Woche je ein Ferkel getötet und die Aktivität der γ-BBD in Leber und Niere bestimmt. Leber und Niere zeigten am Tag der Geburt die geringste Enzymaktivität. Dies stimmt mit Ergebnissen von Rebouche und Engel (1980) in der Leber des menschlichen Neugeborenen überein. In den ersten zwei Lebenswochen erhöhte sich die Aktivität der γ-BBD und erreichte ab der 2. Lebenswoche in Leber und Niere ein Plateau. Die Carnitin-Konzentrationen waren in Herz und Skelettmuskel am Tag der Geburt am geringsten. Im Gegensatz dazu zeigten Plasma, Leber und Niere am Tag der Geburt die höchsten Konzentrationen an Carnitin. Während die Konzentrationen in Herz und Skelettmuskel in den ersten Lebenswochen anstiegen, verringerten sich in diesem Zeitraum die Carnitin-Konzentrationen in Plasma, Leber und Niere. Es ist zu vermuten, dass eine Umverteilung des Carnitins aus dem Plasma zum Skelettmuskel erfolgt, so dass dieser seiner Funktion als Carnitin-Speicher gerecht wird. Ebenso ist es möglich, dass das Carnitin aus der Sauenmilch während der Laktation hauptsächlich im Muskel der Ferkel gespeichert wird. Dies würde zu einer ansteigenden Konzentration von Carnitin in dem Gewebe führen. Ursache für die hohen Konzentrationen von Carnitin in Plasma, Leber und Niere der Ferkel am Tag der Geburt ist wahrscheinlich die Versorgung des Fötus mit Carnitin während der Trächtigkeit durch maternalen Bluttransport. Beim Menschen werden verstärkt in der letzten Schwangerschaftsperiode hohe Mengen von Carnitin in Leber und Muskel des Fötus gespeichert (Nakano *et al*., 1989), da der Fötus selbst zur Oxidation langkettiger Fettsäuren fähig ist (Oey *et al*., 2005). Dabei wird Carnitin durch den Transporter OCTN2 von der Mutter auf den Fötus übertragen (Arenas *et al*., 1998; Girard *et al*., 1985; Grube *et al*., 2005; Lahjouji *et al*., 2004). Andererseits ist der Fötus selbst in der Lage, Carnitin zu synthetisieren, da in Niere, Leber und Rückenmark des Fötus TMLD und γ-BBD nachgewiesen wurden (Oey *et al*., 2006). Diese Mechanismen und die Fähigkeit zur Carnitin-Synthese sind vermutlich auch auf trächtige Sauen und deren Nachkommen übertragbar und begründen die hohen Carnitin-Konzentrationen in Plasma und Leber der Ferkel am Tag der Geburt.

Möglicherweise ist Butyrobetain im Schwein wie im Menschen (Olsen und Rebouche, 1987; Rebouche *et al*., 1989) limitierender Faktor der Carnitin-Synthese. Erste Hinweise lieferte dazu die Studie 1. Trifft dies zu, so sind die hohen Butyrobetain-Konzentrationen in Leber und Niere ebenso wahrscheinliche Ursache für den hohen Carnitin-Status am Tag der Geburt. Die höchsten Butyrobetain-Konzentrationen sind am Tag der Geburt im Muskel zu verzeichnen. Dies lässt vermuten, dass schon der Fötus hohe Kapazitäten zur Umwandlung des Trimethyllysins in Butyrobetain besitzt. Die Plasma-Konzentrationenen von Carnitin sind beim neugeborenen Ferkel 2 bis 3-fach höher als bei den Schweinen der Studien 1 u. 2 mit

einem Lebensalter von 7 bzw. 12 Wochen. Selbst im Vergleich zu ausgewachsenen Schweinen (Owen *et al*., 2001a) besitzen die neugeborenen Ferkel deutlich höhere Konzentrationen an Carnitin im Plasma. Die Konzentrationen des Carnitins sind in der Leber beim neugeborenen Ferkel 4 bis 5-fach höher im Vergleich zur Konzentration in der Leber von Schweinen der 7. bzw. 12. Lebenswoche (Studien 1 und 2). Ausgewachsene Schweine zeigten um den Faktor 2 geringere Carnitin-Konzentrationen in der Leber (Owen *et al.*, 2001a,b) als die neugeborenen Ferkel der Studie 4. Die Konzentration an Carnitin sind in der Niere bei neugeborenen Ferkeln und Schweinen der Studie 1 und 2 annähernd gleich.

Die verhältnismäßig hohen Carnitin-Konzentrationen im Ferkel lassen vermuten, dass der Carnitin-Status im neugeborenen Ferkel keine Defizite aufweist. Des Weiteren zeigt sich, dass das Verhältnis von Acetyl-Carnitin zu freiem Carnitin in Plasma, Niere und Herz am Tag der Geburt am höchsten ist und in den folgenden Wochen sinkt. Dies ist wahrscheinlich ein Indiz dafür, dass nach erhöhter Fettsäureoxidation in den ersten Lebenstagen die Verwertung von Glukose im Energiestoffwechsel an Bedeutung gewinnt. Das hohe Verhältnis von Acetyl-Carnitin zu freiem Carnitin wurde schon bei Neugeborenen beobachtet (Warshaw und Curry, 1980; Girard *et al*., 1992). Es resultiert mutmaßlich aus der erhöhten Konzentration von Acetyl-CoA, welches durch die gestiegene Fettsäure-Oxidation vermehrt produziert wird.

Mit dieser Studie konnte gezeigt werden, dass neugeborene Ferkel vermutlich ausreichende Carnitin-Konzentrationen in Plasma, Leber und Niere besitzen. Trotz altersabhängiger und somit geringer γ-BBD-Aktivität in Leber und Niere besitzen neugeborene Ferkel am Tag der Geburt offensichtlich keinen insuffizienten Carnitin-Status. Carnitin-Defizite waren am Tag der Geburt im Vergleich zu Konzentrationen in ausgewachsenen Schweinen nicht nachweisbar. Diese Ergebnisse sind relevant hinsichtlich möglicher Carnitin-Supplementationen beim neugeborenen Ferkel.

5. Zusammenfassung

Seit der Entdeckung des Carnitins vor über 100 Jahren ist das Interesse an dieser Substanz beträchtlich gestiegen. Die Erforschung der essentiellen Bedeutung des Carnitins im Energiestoffwechsel war die Grundlage für umfangreiche Untersuchungen zu Vorkommen, Synthese und Metabolismus dieses Stoffes im Körper. Durch die maßgebliche Beteiligung des Carnitins an der ß-Oxidation von Fettsäuren wurde vermutet, dass eine Verabreichung dieses Stoffes die Energiegewinnung bei Nutztieren fördert. In einer Vielzahl von Studien wurde nachgewiesen, dass sich eine Carnitin-Zufuhr beim landwirtschaftlichen Nutztier Schwein positiv auf dessen Leistungsparameter auswirkt. Jedoch fehlten bislang grundlegende biochemische Untersuchungen zur Carnitin-Synthese und zum Carnitin-Status des Schweines. Diese sind bedeutsam zur Beurteilung von physiologischen Zuständen und zur Bewertung von Carnitin-Supplementationen in der Fütterungspraxis des Nutztieres. Ziel der vorliegenden Arbeit war es daher, Kenntnisse zur Synthese und Homöostase des Carnitins im Schwein zu erlangen. In diesem Rahmen wurde erforscht, inwieweit ein moderater Überschuss an Lysin den Carnitin-Status im Schwein beeinflusst. In Studie 1 erhielten dazu jeweils 10 Schweine Futter mit 9,7 g Lysin/kg (Kontrolle) bzw. 16,8 g Lysin/kg (Behandlung), wobei sich die Lysin-Konzentration im Futter der Kontrollgruppe an den Richtlinien des National Research Council (1998) orientierte. In dieser Untersuchung wurde deutlich, dass die Zufuhr der essentiellen Aminosäure Lysin die Carnitin-Homöostase im Schwein beeinflussen kann. Es konnte erstmals gezeigt werden, dass ein moderater Überschuss an Lysin in der Diät die Plasma- und Gewebekonzentration von Carnitin und Butyrobetain im Schwein verringert. Durch verminderte mRNA-Expression der TMLD war die Umwandlung des Trimethyllysins in Butyrobetain im Skelettmuskel der Schweine, die eine Diät mit moderatem Lysinüberschuss erhielten, mutmaßlich beeinträchtigt. Dies hatte zur Folge, dass die Konzentration an Trimethyllysin im Muskel der Behandlung signifikant erhöht war. Die geringeren Carnitin-Konzentrationen in Plasma, Leber, Niere und Skelettmuskel resultierten vermutlich aus einer beeinträchtigten Synthese von Butyrobetain in Leber und Niere oder aus der verminderten Verfügbarkeit dieser Carnitin-Vorstufe, die hauptsächlich im Skelettmuskel synthetisiert wird. Die Ergebnisse dieser Studie sind bedeutsam zur Identifizierung von Nährstoffen, die die Carnitin-Homöostase beeinflussen können.

Eine weitere Zielstellung der Arbeit war, Kenntnisse zu Absorption und Regulation des Transports von Carnitin beim Schwein zu gewinnen. Dazu wurden die Studien 2 und 3 durchgeführt. In der Studie 2 wurde jungen Schweinen Carnitin in Supplementationen von 25, 50, 100, 200, 500 bzw. 1000 mg Carnitin/kg Futter verabreicht. Mittels eines unverdaulichen

Markers wurde die Absorption des Carnitins bestimmt. Ebenso wurden die Konzentrationen von Carnitin und seiner Derivate sowie der Vorstufen Butyrobetain und Trimethyllysin in Plasma, Leber, Niere, Herz und Skelettmuskel ermittelt. Es stellte sich heraus, dass Carnitin im Schwein hauptsächlich im proximalen Abschnitt des Dünndarms absorbiert wird. Für Carnitin-Gaben bis 100 mg Carnitin/kg Futter betrug die Absorptionsrate mehr als 95%. Selbst bei einer Dosis von 200 - 1000 mg Carnitin/kg Futter wurde mehr als 90% des Carnitins absorbiert. Dabei konnte eine dosisabhängige Erhöhung der Carnitin-Konzentration in Plasma und Gewebe nachgewiesen werden. Bei Schweinen, die die Höchstdosis an Carnitin erhielten, war die Konzentration an Carnitin in Plasma und Gewebe im Vergleich zur unbehandelten Kontrolle 3-6-fach höher. Bei der Supplementierung von 25 mg Carnitin/kg Futter verminderte sich die Aktivität der γ-BBD in der Niere um 40%. Dennoch konnte keine Beeinträchtigung der Carnitin-Synthese beobachtet werden, da die Carnitin-Konzentrationen in Plasma und Gewebe der Behandlung etwas höher waren, als in der Kontrollgruppe. Eine Carnitin-Zufuhr von 25 mg/kg Futter war dabei ausreichend zur Kompensation der reduzierten Enzymaktivität. Diese Ergebnisse sind ein Indiz dafür, dass Änderungen in der Aktivität der γ-BBD den Carnitin-Status im Schwein nur geringfügig beeinflussen.

Fazit der Studie 2 ist, dass Schweine eine hohe Kapazität zur Absorption von Carnitin besitzen. Da das Carnitin in hohen Mengen gespeichert und nicht ungenutzt ausgeschieden wird, sind Carnitin-Zusätze im Futter sinnvoll.

Bei der proliferierenden Spezie Ratte wird der Carnitin-Transporter OCTN2 durch Aktivierung des PPARα vermehrt exprimiert. Es stellte sich die Frage, inwieweit sich dieser Mechanismus auch bei Schweinen als nicht-proliferierende Spezie zeigt. Dazu wurde in Studie 3 Schweinen der PPARα-Agonist Clofibrat verabreicht. Es konnte nachgewiesen werden, dass sich durch PPARα-Aktivierung beim Schwein die mRNA-Konzentration von OCTN2 in Leber, Skelettmuskel und in den Enterozyten erhöht. Dies führte vermutlich zu dem signifikanten Anstieg von Carnitin in Leber und Skelettmuskel.

Ziel der Studie 4 war es, Kenntnisse zum Status und zur Synthese des Carnitins im Schwein zu erlangen. Dazu wurden die Konzentrationen von Carnitin sowie die mRNA-Expression, Protein-Konzentration und die Aktivität der γ-BBD in Herz, Leber, Niere, Dickdarm, Dünndarm, Lunge, Skelettmuskel, Milz, Hoden und Nebenhoden des Schweines bestimmt. Es zeigte sich, dass der Muskel als Carnitin-Speicher im Schwein die höchsten Carnitin-Konzentrationen besitzt, gefolgt von Herz, Dünndarm, Niere und Leber. Weiterhin stellte sich heraus, dass die Konzentrationen von Carnitin in Muskel, Leber, Niere oder Gehirn von Schweinen 3-6 fach geringer ist, als die Konzentrationen in den entsprechenden Geweben des

Menschen. Dadurch ist zu vermuten, dass Schweine generell einen geringeren Carnitin-Status im Vergleich zum Menschen besitzen.

Bei der Ermittlung der mRNA-Expression, der Protein-Konzentration sowie der Aktivität der γ-BBD wurde deutlich, dass Leber und Niere vermutlich die Hauptorte der Carnitin-Synthese beim Schwein sind. Nur in diesen beiden Geweben wurden neben der mRNA-Expression und Protein-Konzentration gut messbare Enzymaktivitäten von 0,4 ± 0,1 nmol*mg protein^{-1}*min^{-1} (Leber) und 2,37 ± 0,9 nmol*mg protein^{-1}*min^{-1} (Niere) nachgewiesen.

Um Aussagen zum Carnitin-Status im neugeborenen Ferkel treffen zu können, wurden in Studie 4 die Carnitin-Konzentrationen in Geweben von Ferkeln vom Tag der Geburt bis zur 7. Lebenswoche bestimmt. Weiterhin erfolgte die Messung der γ-BBD-Aktivität in Leber und Niere der Ferkel. Die Aktivität des Enzyms war am Tag der Geburt in Leber und Niere am geringsten. In den ersten zwei Lebenswochen erhöhte sich die Enzymaktivität und erreichte ab der zweiten Lebenswoche in beiden Geweben ein Plateau. Somit wurde erstmals nachgewiesen, dass die Aktivität der γ-BBD in Leber und Niere des Schweines altersabhängig ist.

Des Weiteren zeigte sich in Studie 4, dass in Plasma und Leber der neugeborenen Ferkel deutlich höhere Carnitin-Konzentrationen vorhanden sind, als in Plasma und Leber von ausgewachsenen Schweinen. Die ermittelten Carnitin-Konzentrationen in der Niere der Ferkel am Tag der Geburt sind vergleichbar mit den Konzentrationen von Carnitin in der Niere der älteren Schweine der Studien 1 und 2. Diese Ergebnisse verdeutlichen, dass neugeborene Ferkel keinen unzureichenden Carnitin-Status besitzen. Da die Carnitin-Konzentrationen trotz geringer Aktivität der γ-BBD am Tag der Geburt in Plasma, Leber und Niere am höchsten sind, ist zu vermuten, dass die γ-BBD-Aktivität nicht limitierend auf die Carnitin-Synthese des Schweines wirkt.

Die vorliegende Arbeit zeigt bedeutende Erkenntnisse zur Carnitin-Synthese und zum Carnitin-Status im Schwein. Die Ergebnisse sind hilfreich zur Beurteilung von Carnitin- und Lysin-Supplementen in der Fütterungspraxis dieses landwirtschaftlichen Nutztieres.

Weiterhin konnten durch die vorliegende Arbeit erste Aussagen zu Ort und Mechanismus der Carnitin-Synthese im Schwein getroffen werden. Diese neuen Aspekte bilden die Grundlage für zukünftige Untersuchungen zum Carnitin-Metabolismus des Schweines.

6. Summary

Since the discovery of carnitine more than 100 years ago the interest in this substance rose considerably. The study of the essential role of carnitine in the energy metabolism was the basis for extensive investigations to occurrences, synthesis and metabolism of this material in the body. Caused by the relevant participation of carnitine in the ß-oxidation of fatty acids it was assumed that an administration of this material promotes the power production with economically useful animals. A multiplicity of studies proved that a carnitine supply positively affects the achievement parameters of pigs as agricultural animals. However, so far fundamental biochemical investigations of the carnitine synthesis and the carnitine status of pigs were missing. These are important for the evaluation of physiological conditions and for the assessment of carnitine supplementations in the feeding practice of the economically useful animal. Thus, the aim of this work was to attain knowledge about the synthesis and homeostasis of carnitine in pigs. Within this framework it was investigated to what extent a moderate surplus of lysine affects the carnitine status in the pig. In the first study, the control group received diets with 9.7 g lysine/kg as suggested by the guidelines of the National Research Council (1998), and the treatment group received 16.8 g lysine/kg. The two groups comprised each ten pigs. In this investigation it became clear that the supply of the essential amino acid lysine can affect the carnitine homeostasis in the pig. It was shown for the first time that a moderate surplus of lysine in the diet reduces the plasma and tissue concentration of carnitine and butyrobetaine in the pig. Based on a decreased mRNA expression of the TMLD the transformation of trimethyllysine in butyrobetaine in the skeleton muscle of the pigs that received a diet with moderate lysine surplus was presumed impaired. In consequence, the concentration of trimethyllysine was significantly increased in the muscle of the treatment group. The smaller carnitine concentrations in plasma, liver, kidney and skeleton muscles of the treatment group probably resulted from an impaired synthesis of butyrobetaine in liver and kidney or from the reduced availability of this carnitine preliminary stage, which is synthesized mainly in the skeleton muscle. The results of this study are important for the identification of nutrients, which can affect carnitine homeostasis.

A further aim of the work was to attain knowledge about the absorption and regularization of the transport of carnitine within the pig. In addition the studies 2 and 3 were accomplished. In the study 2 carnitine was given to young pigs in supplementations of 25, 50, 100, 200, 500 and 1000 mg carnitine /kg diet. The absorption of carnitine was determined by means of an indigestible marker. Likewise, the concentrations of carnitine and its derivatives as well as the preliminary stages butyrobetaine and trimethyllysine in plasma, liver, kidney, heart and

skeleton muscle were determined. It turned out that carnitine in the pig is absorbed mainly in the proximal section of the small intestine. For carnitine additions up to 100 mg Carnitin/kg diet the absorption rate was more than 95%. Even with a dose of 200 - 1000 mg carnitine /kg diet more than 90% of carnitine were absorbed. A dose-dependent increase of carnitine concentrations in plasma and tissue was proven, whereby the concentration of carnitine in the plasma of the pigs which received the maximum dose of carnitine was three to six times higher than in the plasma and tissue of the untreated control group. Activity of γ-BBD was reduced in kidney about 40% by carnitine supplementation of 25 mg/kg diet. However, carnitine synthesis was not diminished. Carnitine concentrations in plasma and tissues were slightly higher in treatment than in control group. Carnitine supplementations of 25 mg/kg diet were sufficient to compensate the lower enzyme activity. The results are an indication that changes in the activity of γ-BBD have less impact on the carnitine status of the pigs.

In conclusion study 2 shows that pigs possess a high capacity for the absorption of carnitine. Since the carnitine is stored high quantities and is not discarded unused, carnitine additives in diets are suggestive.

In the proliferating specie rat an activation of PPARα increases mRNA concentrations of the carnitine transporter OCTN2. The question rose, to what extent this mechanism occurs also in pigs as a non-proliferating species. To test this, the PPARα agonist clofibrate was given to pigs in study 3. It was proven that the PPARα activation in the pig increases the mRNA concentration of OCTN2 in liver, skeleton muscle and enterocytes. This probably led to the observed significant rise of carnitine in liver and skeleton muscle.

The aim of the study 4 was it to attain knowledge to the status and the synthesis of the carnitine in pigs. In addition the concentrations of carnitine, the mRNA expression, the protein concentration as well as the activity of γ-BBD were determined in heart, liver, kidney, large intestine, small intestine, lung, skeleton muscle, spleen, testis and epididymis of pigs. This showed that the muscle possesses the highest carnitine concentrations as carnitine reservoir in the pig, followed of heart, small intestine, kidney and liver. Further it turned out that the concentrations of carnitine in muscles, liver, kidney or brain of pigs is three to six times smaller than the concentrations in the corresponding tissues of humans. Thus it is to be assumed that pigs generally possess a smaller carnitine status in comparison to humans.

By determination of the mRNA expression, the protein concentration as well as the activity of γ-BBD it became clear that liver and kidney are probably the principal places of the carnitine synthesis of pigs. Only in these two tissues well measurable enzyme activities of 0.4 ± 0.1

nmol*mg protein^{-1}*min^{-1} (liver) and 2.37 ± 0.9 nmol*mg protein^{-1}*min^{-1} (kidney) were proven besides the mRNA Expression and protein concentration.

In order to be able to estimate the carnitine status in the newborn piglet, in study 4 the carnitine concentrations in tissues were determined by piglets from the day of birth up to the 7th life week. Further the measurement of the γ-BBD-activity took place in liver and kidney of the piglets. The activity of the enzyme was smallest in liver and kidney on the day of birth. In the first two life weeks the enzyme activity increased and reached a plateau starting from the second life week in both tissues. Thus it was proven for the first time that the activity of the γ-BBD is age-dependent in liver and kidney of pigs.

The study 4 showed that carnitine concentrations in plasma and liver of the newborn piglets were even clearly higher than in plasma and liver of growing-finishing pigs. The determined carnitine concentrations are in the kidney of newborn piglets comparable with the concentrations of carnitine in the kidney of older pigs of the studies 1 and 2. These results show that newborn piglets do not possess an insufficient carnitine status. As the carnitine concentrations are highest on the day of the birth in plasma, liver and kidney despite a lower γ-BBD-activity, it is to be assumed that the γ-BBD-activity is not limiting the carnitine synthesis of the pig.

This work points out important findings on the carnitine synthesis and the carnitine status in pigs. The results are helpful for the evaluation of carnitine and lysine supplements in the feeding practice of this agricultural animal.

This work provides first assessments of place and mechanism of the carnitine synthesis in pigs. These new aspects are an important basis for future investigations to the carnitine metabolism in pigs.

7. Literaturverzeichnis

Angelini C, Vergani L, Martinuzzi A (1992) Clinical and biochemical aspects of carnitine deficiency and insufficiency: transport defects and inborn errors of beta-oxidation. Crit Rev Clin Lab Sci 29: 217-242.

Arenas J, Rubio JC, Martín MA, Campos Y (1998) Biological roles of L-carnitine in perinatal metabolism. Early Hum Dev 53: S43-50.

Bertoli M, Battistella PA, Vergani L, Naso A, Gasparotto ML, Romagnoli GF, Angelini C (1981) Carnitine deficiency induced during hemodialysis and hyperlipidemia: effect of replacement therapy. Am J Clin Nutr 34:1496-1500.

Birkenfeld C, Ramanau A, Kluge H, Spilke J, Eder K (2005) Effect of dietary L-carnitine supplementation on growth performance of piglets from control sows or sows treated with L-carnitine during pregnancy and lactation. J Anim Physiol Anim (Berl) 89(7-8): 277-283.

Birkenfeld C, Kluge H, Eder K (2006) L-carnitine supplementation of sows during pregnancy improves the suckling behaviour of their offspring. Br J Nutr 96(2): 334-342.

Bohles H, Segerer H, Fekl W (1984) Improved N-retention during L-carnitine-supplementated total parenteral nutrition. J Parent Enteral Nutr 8: 9-13.

Brass EP, Hoppel CL (1978) Carnitine metabolism in the fasting rat. J Biol Chem 253: 2688-2693.

Bremer J (1963) Carnitine in intermediary metabolism. The biosynthesis of palmitylcarnitine by cell subfractions. J Biol Chem 238: 2774-2779.

Bremer J (1983) Carnitin- metabolism and functions. Physiol Rev 63: 1420-1480.

Carter AL, Abney TO, Braver H, Chuang AH (1987) Localization of γ-butyrobetaine hydroxylase in rat testis. Biol Reprod 37: 68-72.

Carter, AL, Abney, TO and Lapp, DF (1995) Biosynthesis and metabolism of carnitine. J Child Neurol 10(Suppl 2): S3-S7.

Cederblad G, Fåhraeus L, Lindgren K (1986) Plasma carnitine and renal-carnitine clearance during pregnancy. Am J Clin Nutr 44: 379-383.

Cho WT, Kim JH, Bae SH, Han IK, Han YK, Heo KN, Odle J (1999) Effects of L-carnitine with different lysine levels on growth and nutrient digestibility in pigs weaned at 21 days of age. Asian-Australian J Anim Sci 12: 799-805.

Coffey MT, Shireman, RB, Herman DL, Jones EE (1991) Carnitine status and lipid utilization in neonatal piglets fed diets low in carnitine. J Nutr 121: 1047-1053.

Davis AT, Hoppel CL (1986) Effect of starvation on the disposition of free and peptide-linked trimethyllysine in the rat. J Nutr 116(5): 760-767.

Davis AT (1990) Tissue trimethyllysine biosynthesis and carnitine content in pregnant and lactating rats fed a lysine-limiting diet. J Nutr 120(8): 846-856.

Davis AT, Kruggel EM, Randall S (1993) Excess dietary lysine increases skeletal muscle and plasma trimethyllysine in rats. J Nutr 123(6): 1109-1116.

Davis AT, Monroe TJ (2005) Carnitine deficiency and supplementation do not affect the gene expression of carnitine biosynthetic enzymes in rats. J Nutr 135**:** 761-764.

Desvergne B, Wahli W (1999) Peroxisome proliferator-activated receptors: Nuclear control of metabolism. Endocr Rev 20: 649-688.

Doberenz J, Birkenfeld C, Kluge H, Eder K (2006) Effects of L-carnitine supplementation in pregnant sows on plasma concentrations of insuline-like growth factors, various hormones and metabolites and chorion characteristics. J Anim Physiol Anim Nutr.90: 487-499.

Duran M, Loof NE, Ketting D and Dorland L (1990) Secondary carnitine deficiency. J Clin Chem Clin Biochem 28: 359-363.

Eder K, Ramanau A, Kluge H (2001) Effect of L-carnitine supplementation on performance parameters in gilts and sows. J Anim Physiol Anim Nutr (Berl) 85(3-4): 73-80

Erfle JD (1975) Hydroxylation of γ-butyrobetaine by rat and ovine tissue. Biochem Biophys Res Commun 64: 553-557.

Englard S (1979) Hydroxylation of gamma-butyrobetaine to carnitine in human and monkey tissues. FEBS Lett 102(2): 297-300.

Enomoto A, Wempe MF, Tsuchida H, Shin HJ, Cha SH, Anzai N, Goto A, Sakamoto A, Niwa T, Kanai Y, Anders MW, Endou H (2002) Molecular identification of a novel carnitine transporter specific to human testis. Insights into the mechanism of carnitine recognition. J Biol Chem 277(39): 36262-36271.

Evans AM, Fornasini G (2003) Pharmacokinetics of L-carnitine. Clin Pharmacokinet 42(11): 941- 967.

Fraenkel G (1951) Effect and distribution of vitamin B_T. Arch Biochem Biophys 34(2): 457-467.

Fritz IB, Yue KTN (1963) Long chain carnitine acyltransferase and the role of acylcarnitine derivatives in the catalytic increase of fatty acid oxidation induced by carnitine. J Lipid Res 4: 279-288.

Galland S, Le Borgne F, Bouchard F, Georges B, Clouet P, Grand-Jean F, Demarquoy J (1999) Molecular cloning and characterization of the cDNA encoding the rat liver gamma-butyrobetaine hydroxylase. Biochim Biophys Acta 1441: 85-92.

Girard J, Duée PH, Ferré P, Pégorier JP, Escriva F, Decaux JF (1985) Fatty acid oxidation and ketogenesis during development. Reprod Nutr Dev 25(1B): 303-319.

Girard J, Ferré P, Pégorier JP, Duée PH (1992) Adaptations of glucose and fatty acid metabolism during perinatal period and suckling-weaning transition. Physiol Rev 72: 507-562.

Gross CJ, Henderson LM (1984) Absorption of D- and L-carnitine by the intestine and kidney tubule in the rat. Biochim Biophys Acta 772(2): 209-219.

Grube M, Schwabedissen HM, Draber K, Präger D, Möritz KU, Linnemann K, Fusch C, Jedlitschky G, Kroemer HK (2005) Expression, localization, and function of the carnitine transporter octn2 (slc22a5) in human placenta. Drug Metab Dispos 33(1): 31-37.

Gudjonsson H, Li BU, Shug AL, Olsen WA (1985) In vivo studies of intestinal carnitine absorption in rats. Gastroenterology 88(6): 1880-1887.

Gulewitsch W, Krimberg R (1905) Zur Kenntnis der Extractivstoffe der Muskeln. Z Physiol Chem 45: 326-328.

Hamilton JW, Li BU, Shug AL, Olsen WA (1986) Carnitine transport in human intestinal biopsy specimens: demonstration of an active transport system. Gastroenterology 91(1): 10-16.

Heo K, Odle J, Han IK, Cho W, Seo S, van Heugten E, Pilkington DH (2000) Dietary L-carnitine improves nitrogen utilization in growing pigs fed low energy, fat-containing diets. J Nutr 130(7): 1809-1814.

Hoffman LA, Ivers DJ, Ellersieck MR, Veum TL (1993) The effect of L-carnitine and soybean oil on performance and nitrogen and energy utilization by neonatal and young pigs. J Anim Sci 71: 132-138.

Holden PR, Tugwood JD (1999) Peroxisome proliferator-activated receptor alpha: role in rodent liver cancer and species differences. J Mol Endocrinol 22(1): 1-8.

Hoppel CL, Davis AT (1986) Inter-tissue relationships in the synthesis and distribution of carnitine. Biochem Soc Trans 14: 673-674.

Jakobs BS and Wanders RJ (1995) Fatty acid β-oxidation in peroxisomes and mitochondria: the first, unequivocal evidence for the involvement of carnitine in shuttling

propionyl-CoA from peroxisomes to mitochondria. Biochem Biophys Res Commun 213: 1035-1041.

Kerner J, Froseth JA, Miller ER, Bieber LL (1984) A study of the acylcarnitine content of sow`s colostrum, milk and newborn piglet tissues: Demonstration of high amounts of isovaleryl-carnitine in colostrum and milk. J Nutr 114: 854-861.

Khan-Siddiqui L & Bamji MS (1983) Lysine–carnitine conversion in normal and undernourished adult men – suggestion of a nonpeptidyl pathway. Am J Clin Nutr 37: 93–98.

Koch A, König B, Luci S, Stangl GI, Eder K (2007) Dietary oxidised fat up regulates the expression of organic cation transporters in liver and small intestine and alters carnitine concentrations in liver, muscle and plasma of rats. Br J Nutr 98(5): 882-889.

Koch A, König B, Stangl GI, Eder K (2008) PPAR alpha mediates transcriptional upregulation of novel organic cation transporters-2 and -3 and enzymes involved in hepatic carnitine synthesis. Exp Biol Med (Maywood) 233(3): 356-365.

Krajcovicová-Kudlácková M, Simoncic R, Béderová A, Babinská K, Béder I (2000) Correlation of carnitine levels to methionine and lysine intake. Physiol Res 49(3): 399-402.

Kutscher F (1905) Zur Kenntnis des Novains. Z Physiol Chem. 49: 47-49.

Lahjouji K, Elimrani I, Lafond J, Leduc L, Qureshi IA, Mitchell GA (2004) L-Carnitine transport in human placental brush-border membranes is mediated by the sodium-dependent organic cation transporter OCTN2. Am J Physiol Cell Physiol 287(2): C263-269.

Lamhonwah AM, Ackerley CA, Tilups A, Edwards VD, Wanders RJ, Tein I (2005) OCTN3 is a mammalian peroxisomal membrane carnitine transporter. Biochem Biophys Res Commun 338(4): 1966-1972.

Lennon DL, Shrago ER, Madden M, Nagle FJ, Hanson P (1986) Dietary carnitine intake related to skeletal muscle and plasma carnitine concentrations in adult men and women. Am J Clin Nutr 43(2): 234-238.

Li BUK, Lloyd ML, Gudjonsson H, Shug AL, Olsen WA (1992) The effect of enteral carnitine administration in humans. Am J Clin Nutr 55: 838-845.

Lindstedt S, Nordin I (1984) Multiple forms of gamma-butyrobetaine hydroxylase (EC 1.14.11.1) Biochem J 223(1):119-127.

Lindstedt G, Lindstedt S (1970) Cofactor requirements of gamma-butyrobetaine hydroxylase from rat liver. J Biol Chem 245(16): 4178-4186.

Luci S, Geissler S, König B, Koch A, Stangl GI, Hirche F, Eder K (2006) PPARalpha agonists up-regulate organic cation transporters in rat liver cells. Biochem Biophys Res Commun 350(3): 704-708.

Luci S, Giemsa B, Kluge H, Eder K (2007) Clofibrate causes an upregulation of PPAR-{alpha} target genes but does not alter expression of SREBP target genes in liver and adipose tissues of pigs. Am J Physiol Regul Integr Comp Physiol 293(1): R70-77.

Luci S, Hirche F, Eder K (2008) Fasting and caloric restriction increases mRNA concentrations of novel organic cation transporter-2 and carnitine concentrations in rat tissues. Ann Nutr Metab 52(1): 58-67.

Maeda T, Wakasawa T, Funabashi M, Fukushi A, Fujita M, Motojima K, Tamai I (2008) Regulation of Octn2 transporter (SLC22A5) by peroxisome proliferator activated receptor alpha. Biol Pharm Bull 31(6): 1230-1236.

Makowski L, Noland RC, Koves TR, Xing W, Ilkayeva OR, Muehlbauer MJ, Stevens RD, Muoio DM (2009) Metabolic profiling of PPAR{alpha}-/- mice reveals defects in carnitine and amino acid homeostasis that are partially reversed by oral carnitine supplementation. FASEB J 23: 586-604.

Mc Garry JD, Robles-Valdes C, Foster DW (1975) Role of carnitine in hepatic ketogenesis. Proc Natl Acad Sci USA 72: 4385-4388.

McGarry JD, Brown NF (1997) The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. Eur J Biochem 1997; 244:1-14.

Moorthy AV, Rosenblum M, Rajaram R, Shug AL (1983) A comparison of plasma and muscle carnitine levels in patients on peritoneal or hemodialysis for chronic renal failure. Am J Nephrol 3: 205-208.

Musser RE, Goodband RD, Tokach MD, Owen KQ, Nelssen JL, Blum SA, Dritz SS, Civis CA (1999) Effect of L-carnitine fed during gestation and lactation on sow and litter performance. J Anim Sci 77: 3289-3295.

Newton GL, Haydon KD (1988) Carnitine in nursery pig diets. J Anim Sci 66: 40.

Newton GL, Haydon KD (1989) Carnitine supplementation for finishing pigs. J Anim Sci 67: 267.

Nakanishi T, Hatanaka T, Huang W, Prasad PD, Leibach FH, Ganapathy ME, Ganapathy V (2001) Na^+ -and Cl^--coupled active transport of carnitine by the amino acid transporter $ATB^{(0,+)}$ from mouse colon expressed in HRPE cells and Xenopus oocytes. J Physiol 532(2): 283.

Nakano C, Takashima S, Takeshita K (1989) Carnitine concentration during the development of human tissues. Early Hum Dev 19(1): 21-27.

National Research Council (1998) Nutrient Requirement of Swine. Tenth Revised Edition. National Academie of Sciences, Washington DC.

Oey NA, den Boer ME, Wijburg FA, Vekemans M, Augé J, Steiner C, Wanders RJ, Waterham HR, Ruiter JP, Attié-Bitach T (2005) Long-chain fatty acid oxidation during early human development. Pediatr Res 57(6): 753-754.

Oey NA, van Vlies N, Wijburg FA, Wanders RJ, Attie-Bitach T, Vaz FM (2006) L-carnitine is synthesized in the human fetal-placental unit: potential roles in placental and fetal metabolism. Placenta 27(8): 841-846.

Olson AL, Rebouche CJ (1987) γ-Butyrobetaine hydroxylase activity is not rate limiting for camitine biosynthesis in the human infant. J Nutr 117: 1024-1031.

Owen KQ, Weeden TL, Nelssen JL, Blum SA, Goodband RD (1993) The effect of L-carnitine additions on performance and carcass characteristics of growing-finishing swine. J Anim Sci 71: 62.

Owen KQ, Nelssen JL, Goodband RD, Weeden TL, Blum SA (1996) Effect of L-carnitine and soybean oil on growth performance and body composition of early-weaned pigs. J Anim Sci 74(7): 1612-1619.

Owen KQ, Jit H, Maxwell CV, Nelssen JL, Goodband RD, Tokach MD, Tremblay GC, Koo SI (2001a) Dietary L-carnitine suppresses mitochondrial branched-chain keto acid dehydrogenase activity and enhances protein accretion and carcass characteristics of swine. J Anim Sci 79: 3104-3112.

Owen KQ, Nelssen JL, Goodband RD, Tokach MD, Friesen KG (2001b) Effect of dietary L-carnitine on growth performance and body composition in nursery and growing-finishing pigs. J Anim Sci 79: 1509-1515.

Penn D, Bobrowski PJ, Zhang L, Schmidt-Sommerfeld E (1997) Neonatal nutritional carnitine deficiency: a piglet model. Pediatr Res 42(1), 114-121.

Ramsay, RR, Gandour, RD, van der Leij, FR (2001) Molecular enzymology of carnitine transfer and transport. Biochim Biophys Acta; 1546: 21-43.

Ramanau A, Kluge H, Spilke J, Eder K (2002) Reproductive performance of sows supplemented with dietary L-carnitine over three reproductive cycles. Arch Anim Nutr 56(4): 287-296.

Ramanau A, Kluge H, Spilke J, Eder K (2004) Supplementation of sows with L-carnitine during pregnancy and lactation improves growth of the piglets during the suckling period through increased milk production. J Nutr 134 (1): 86-92.

Rebouche CJ and Engel AG (1980) Tissue distribution of carnitine biosynthetic enzymes in man. Biochim Biophys Acta 630: 22-29.

Rebouche CJ, Lehman LJ, Olson L (1986) ε-N-trimethyllysine availability regulates the rate of carnitine biosynthesis in the growing rat. J Nutr 116(5): 751-759.

Rebouche CJ, Bosch EP, Chenard CA, Schabold KJ, Nelson SE (1989) Utilization of dietary precursors for carnitine synthesis in human adults. J Nutr 119(12): 1907-1913.

Rebouche CJ, Chenard CA (1991) Metabolic fate of dietary carnitine in human adults: identification and quantification of urinary and fecal metabolites. J Nutr 121(4): 539-546.

Rebouche CJ (1996) Role of carnitine biosynthesis and renal conversation of carnitine in genetic and acquired disorders of carnitine metabolism. In carnitine: Pathobiochemical Basics and Clinical Applications (Seim, H. and Löster, H., eds.), pp.111-121, Ponte Press, Bochum.

Rebouche CJ, Seim H (1998) Carnitine metabolism and its regulation in microorganism and mammals. Annu Rev Nutr 18: 39-61.

Rebouche CJ (2004) Kinetics, pharmacokinetics, and regulation of L-carnitine and acetyl-L-carnitine metabolism. Ann N Y Acad Sci 1033: 30-41.

Ringseis R, Pösel S, Hirche F, Eder K (2007) Treatment with pharmacological peroxisome proliferator-activated receptor alpha agonist clofibrate causes upregulation of organic cation transporter 2 in liver and small intestine of rats. Pharmacol Res 56(2): 175-183.

Ringseis R, Lüdi S, Hirche F, Eder K (2008a) Treatment with pharmacological peroxisome proliferator-activated receptor alpha agonist clofibrate increases intestinal carnitine absorption in rats. Pharmacol Res 58(1): 58-64.

Ringseis R, Wege N, Wen G, Rauer C, Hirche F, Kluge H, Eder K (2008b) Carnitine synthesis and uptake into cells are stimulated by fasting in pigs as a model of nonproliferating species. J Nutr Biochem [Epub ahead of print].

Rincker MJ, Carter SD, Real DE, Nelssen JL, Tockach MD, Goodband RD, Dritz SS, Senne BW, Fent RW, Pettey LA, Owen KQ (2003) Effects of increasing dietary L-carnitine on growth performance of weanling pigs. J Anim Sci 81(9): 2259-2269.

Rodriguez CM, Labus JC, Hinton BT (2002) Organic cation/carnitine transporter, OCTN2, is diferentially expressed in the adult rat epididymis. Biol Reprod 67(1): 314-319.

Sewell AC, Böhles HJ (1995) Acylcarnitines in intermediary metabolism. Eur J Pediatr 154(11): 871-877.

Shaw RD, Li BU, Hamilton JW, Shug AL, Olsen WA (1983) Carnitine transport in rat small intestine. Am J Physiol 245(3): G376-381.

Slitt AL, Cherrington NJ, Hartley DP, Leazer TM, Klaassen CD (2002) Tissue distribution and renal developmental changes in rat organic cation transporter mRNA levels. Drug Metab Dispos 30(2): 212-219.

Steiber A, Kerner J, Hoppel CL (2004) Carnitine: a nutritional, biosynthetic, and functional perspective. Mol Aspects Med 25(5-6): 455-473.

Tamai I, Yabuuchi H, Nezu J, Sai Y, Oku A, Shimane M, Tsuji A (1997) Cloning and characterization of a novel human ph-dependent organic cation transporter, OCTN1. FEBS Lett 419: 107-111.

Tamai I, Ohashi R, Nezu J, Yabuuchi H, Oku A, Shimane M, Sai Y, Tsuij A (1998) Molecular and functional identification of sodium ion-dependent, high affinity human carnitine transporter OCTN2. J Biol Chem 273(32): 20378-20382.

Tanphaichitr V, Broquist HP (1973) Role of lysine and –N-trimethyllysine in carnitine biosynthesis. II. Studies in the rat. J Biol Chem 248(6): 2176-2181.

Tanphaichitr V, Lerdvuthisopon N, Dhanamitta S, Broquist HP (1980) Carnitine status in Thai adults. Am J Clin Nutr 33(4): 876-880.

Taylor PM (2001) Absorbing competition for carnitine. J Physiol 532: 283.

Tomita M, Sendju Y (1927) Über die Oxyaminverbindungen welche die Biuret Reaktionen zeigen. III. Spaltung der γ-amino-β-oxybuttersäure in die optisch aktive Komponente. Z Physiol Chem 169: 263-277.

Van Kempen TATG, Odle J (1993) Medium-chain fatty acid oxidation in colostrum-deprived neworn piglets: stimulatory effect of L-carnitine supplementation. J Nutr 123: 1531-1537.

Van Kempen TATG, Odle J (1995) Carnitine affects octanoate oxidation to carbon dioxide and dicarboxylic acids in colostrum-deprived piglets: in vivo analysis of mechanisms involved based on CoA- and carnitine-ester profiles. J Nutr 125(2): 238-250.

Van Vlies N, Wanders RJ, Vaz FM (2006) Measurement of carnitine biosynthesis enzyme activities by tandem mass spectrometry: Differences between the mouse and the rat. Anal Biochem 354(1): 132-139.

Van Vlies N, Ferdinandusse S, Turkenburg M, Wanders RJ, Vaz FM (2007) PPAR alpha-activation results in enhanced carnitine biosynthesis and OCTN2-mediated hepatic carnitine accumulation. Biochim Biophys Acta 1767(9): 1134-1142.

Vaz FM and Wanders RJ (2002) Carnitine biosynthesis in mammals. Biochem J 361: 417-429.

Vijayasarathy C, Khan-Siddiqui L, Murthy SN & Bamji MS (1987) Rise in plasma trimethyllysine levels in humans after oral lysine load. Am J Clin Nutr 46: 772–777.

Wagner CA, Lükewille U, Kaltenbach S, Moschen I, Bröer A, Risler T, Bröer S, Lang F (2000) Functional and pharmacological characterization of human $Na^{(+)}$-carnitine cotransporter hOCTN2. Am J Physiol Renal Physiol 279(3): F584-591.

Warshaw JB, Curry E (1980) Comparison of serum carnitine and ketone body concentrations in breast- and in formula-fed newborn infants. J Pediatr 97: 122-125.

Wolfe RG, Maxwell CV, Nelson EC (1978) Effect of age and dietary fat level on fatty acid oxidation in the neonatal pig. J Nutr 108: 1621-1634.

Wu X, Huang W, Prassad PD, Seth P, Rajan DP, Leibach FH, Chen J, Conway SJ and Ganapathy V (1999) Functional characteristics and tissue distribution pattern of organic cation transporter 2 (OCTN2), an organic cation/carnitine transporter. J Pharmacol Exp Ther 290: 1482-1492.

Yokogawa K, Higashi Y, Tamai I, Nomura M, Hashimoto N, Nikaido H, Hayakawa J, Miyamoto K, Tsuji A (1999) Decreased tissue distribution of L-carnitine in juvenile visceral steatosis mice. J Pharmacol Exp Ther 289(1): 224-230.

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Erklärung

Hiermit erkläre ich, dass ich die vorliegende Dissertation „Experimentelle Untersuchungen zum Carnitin-Metabolismus beim Schwein“ selbständig angefertigt habe und diese nicht bereits für eine Promotion oder ähnliche Zwecke an einer anderen Universität eingereicht habe. Alle hierzu benutzten wissenschaftlichen Arbeiten und Hilfsmittel sind genau und vollständig angegeben worden.
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Des Weiteren erkläre ich, dass keine Strafverfahren gegen mich anhängig sind.

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Maren Fischer

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