African Journal of Agricultural Research Vol. 7(10), pp. 1450-1455, 12 March, 2012
Available online at http://www.academicjournals.org/AJAR
DOI: 10.5897/AJAR11.1014
ISSN 1991-637X © 2012 Academic Journals

Full Length Research Paper

Effects of activation of peroxisome proliferator-activated receptor-α by clofibrate on carnitine homeostasis in laying hens

Mohamed Shibani[1]*, Janine Keller[1], Holger Kluge[2], Bettina König[2], Gabriele I. Stangl[2], Robert Ringseis[1] and Klaus Eder[1]

[1]Institute of Animal Nutrition and Nutrition Physiology, Justus-Liebig-Universität Gießen, 35392 Gießen, Germany.
[2]Institute of Agricultural and Nutritional Sciences, Martin-Luther-Universität Halle-Wittenberg, 06108 Halle (Saale), Germany.

Accepted 28 September, 2011

In this study, the hypothesis was investigated that activation of peroxisome proliferator-activated receptor (PPAR)-α regulates homeostasis of carnitine in laying hens. Therefore, laying hens received either a control diet or a diet supplemented with 0.15% clofibrate as a synthetic PPARα agonist for 4 weeks. Feed intake was not different between both groups of hens while egg production rate was slightly reduced in the group of hens treated with clofibrate ($P < 0.05$). Hens treated with clofibrate had an increased expression of the classical PPARα target genes carnitine palmitoyltransferase-1 and acyl CoA oxidase in the liver compared to control hens ($P < 0.05$), indicative of an activation of PPARα. In hens treated with clofibrate, mRNA concentration of novel organic cation transporter (OCTN)-2, the most important carnitine transporter, in the liver as well as carnitine concentrations in plasma, liver, egg yolk and albumen were increased compared to control hens ($P < 0.05$). mRNA concentrations of enzymes of hepatic carnitine synthesis as well as concentrations of the carnitine precursors trimethyllysine and γ-butyrobetaine in plasma, liver and muscle were unchanged in hens treated with clofibrate, suggesting that activation of PPARα did not influence carnitine biosynthesis. In conclusion, this study shows that activation of PPARα up-regulates expression of OCTN2 in the liver of laying hens, such as in mammalian species and causes an increase of carnitine concentrations in liver, plasma and egg.

Key words: Peroxisome proliferator-activated receptor α, clofibrate, laying hen, carnitine, novel organic cation transporter 2.

INTRODUCTION

Carnitine (L-3-hydroxy-4-N-N-N-trimethyl-aminobutyrate) is an essential metabolite, which has a number of indispensable functions in intermediary metabolism (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 (Vaz and Wanders, 2002). 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. The released TML is further oxidized to γ-butyrobetaine (BB) by the action of trimethyllysine dioxygenase (TMLD), 3-hydroxy-N-trimethyllysine aldolase and 4-N-trimethylaminobut-yraldehyde dehydrogenase (TMABA-DH). BB is hydroxylated by γ-butyrobetaine dioxygenase (BBD) to form carnitine. In most animal species, this last reaction occurs primarily in the liver and kidney. In rats and humans, it has been found that the availability of BB is rate limiting for carnitine synthesis whereas the activity of BBD normally exceeds the amounts of carnitine synthesized (Olson and Rebouche, 1987; Rebouche et al., 1989). Therefore, concentrations of carnitine-precursors are of great relevance in the consideration of

*Corresponding author. E-mail: mohamedzik9999@yahoo.de. Tel: +49-641-9939266.

carnitine synthesis.

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 (SLC) 22A family, localised to the apical membrane of cells. In mammalian species, three OCTN have been identified so far, OCTN1, OCTN2 and OCTN3 (Tamai et al., 1997; Tamai et al., 1998; Tamai et al., 2000). OCTN1 and OCTN2 are expressed in several tissues such as kidney, intestine, skeletal muscle, heart, liver and brain (Tamai et al., 2000). OCTN3 is expressed exclusively in kidney, small intestine and testes (Tamai et al., 2000; Duran et al., 2005). Due to its high binding affinity for carnitine and its wide expression, OCTN2 is the most physiologically important carnitine transporter. OCTN1 contributes less to carnitine transport than OCTN2 due to its low carnitine transport activity.

We and others have recently found in mice and pigs, that hepatic enzymes of carnitine synthesis as well as OCTN2 are up-regulated by activation of peroxisome proliferator activated receptor (PPAR)-α, which in turn led to increased concentrations of carnitine in tissues (van Vlies et al., 2007; Koch et al., 2008; Ringseis et al., 2007; Ringseis et al., 2008a; Maeda et al., 2008). PPARα is a transcription factor belonging to the nuclear receptor superfamily which is highly expressed in tissues with high fatty acid oxidation such as liver or muscle. PPARα target genes are mainly involved in cellular fatty acid uptake and intracellular fatty acid transport, mitochondrial and peroxisomal fatty acid oxidation, ketogenesis and gluconeogenesis. PPARα is activated by both native compounds such as n-3 polyunsaturated fatty acids (PUFA), conjugated linoleic acids (CLA) and oxidized fatty acids or by synthetic agonists such as fibrates which are in humans used for the therapy of hypertriglyceridemia (Desvergne and Wahli, 1999).

It has been shown that PPARα is expressed also in chicken liver and that it has a high homology with mouse, rat and human PPARα (Diot and Douaire, 1999; Meng et al., 2005). It has been suggested that PPARα plays a similar role in chicks for the homeostasis of energy and lipid metabolism during fasting as in mammals (Cogburn et al., 2007; Desert et al., 2008). However, a potential role of PPARα on carnitine homeostasis in chicks has not yet been investigated. To address this issue is of importance because it cannot be deduced from findings in mice and pigs that carnitine homeostasis is also regulated by PPARα in birds because regulation of the avian genes coding for enzymes and transporters involved in carnitine homeostasis has not been investigated yet. Even if the regulatory region, e.g., the promoter, of the avian genes is similar to that of other species, the avian genes might be regulated differentially because the response of PPARα target genes also strongly depends on the expression level of PPARα, its heterodimerization partner as well as its co-activators which can vary in a highly species-specific manner.

The aim of the present study was to investigate the hypothesis that treatment of laying hens with a synthetic PPARα agonist causes also an up-regulation of enzymes involved in carnitine synthesis and transport which in turn leads to increased concentrations of carnitine in tissues and eggs. Therefore, we fed laying hens diets supplemented with clofibrate as a synthetic PPARα agonist and determined mRNA concentrations of hepatic enzymes involved in carnitine synthesis (TMLD, TMABA-DH, BBD) and transport (OCTN1, OCTN2) in various tissues as well as carnitine concentrations in plasma, tissues and egg.

MATERIALS AND METHODS

Animals and treatment

An experiment was conducted with 20 Lohmann White layers with an age of 20 weeks and an average body weight of 1998 g (± 159, SD). The hens were allotted to two groups of 10 each, a control group and a group treated with clofibrate as synthetic PPARα agonist. Both groups received a nutritionally adequate diet consisting of (in g/kg diet) wheat (469), extracted soy bean meal (130), corn (120), peas (80), calcium carbonate (75), extracted sunflower meal (70), sunflower oil (30), dicalcium phosphate (12.5), vitamin and mineral premix (10), fiber (10), sodium chloride (2) and DL-methionine (0.5). This diet contained 11.4 MJ metabolizable energy and 169 g crude protein per kg [as determined by the official German VDLUFA methods (Naumann and Bassler, 1976)]. Sunflower oil was used as a source of dietary oil, as it supplied sufficient linoleic acid but had a low concentration of longchain highly unsaturated fatty acids which could act as native activate PPARα agonists. The carnitine concentration of this diet was < 5 mg/kg. Group 1 (control group) received this diet without any further supplement; group 2 (clofibrate group) received the diet supplemented with 1.5 g clofibrate [ethyl 2-(4-chlorophenoxy)-2-methylpropionate; Fluka Chemie GmbH, Buchs, Switzerland] per kg.

This dose was chosen based on the observation from a recent study in laying hens (König et al., 2007), in which a clofibrate dose of 5 g/kg caused a marked decline in feed intake and a complete stop of egg production. To avoid these confounding effects, which would have made it impossible to study the effect of PPARα activation on carnitine homeostasis, we chose a clofibrate dose of 1.5 g/kg diet which is sufficient to cause PPARα activation but does not induce confounding effects. The hens were kept one bird per cage in an environmentally controlled room at 18°C. The room was lit for 14 h daily at an intensity of 20 to 30 lx. Feed and water (via nipple drinkers) were available *ad libitum*. The experiment was conducted over a 4 week period. All procedures followed established guidelines for the care and handling of animals and were approved by the veterinary council of Saxony-Anhalt.

Sample collection

To determine egg weight, weight of yolk and albumen and carnitine concentrations, two eggs from each hen were sampled at the end of week 4. One hen of the clofibrate group died in week 2 of the experiment for unknown reasons. To determine weight of yolk and albumen, eggs were cooked in water for 10 min. After the end of week 4, hens were anaesthesized and then decapitated. As fasting leads to activation of PPARα, the hens were not food deprived before killing. Blood was collected in heparinized tubes; plasma

Table 1. Characteristics of the specific primers used for RT-PCR analysis.

Gene[1]	Forward primer (from 5' to 3')	Reverse primer (from 5' to 3')	bp	Annealing temperature (℃)	NCBI GenBank
ACO	ACGCCCAAATTACTCAGGTG	GGATTTCTTTGCCCACTCAA	173	60	NM_001006205
β-actin	ATGAAGCCCAGAGCAAAAGA	GGGGTGTTGAAGGTCTCAAA	223	62	L08165
BBD	ACCCTGTCCTGCAGCACCCA	TGGCCGCACTTCTTCTGCCG	314	62	XM_4245432
CPT-1	GATTTGGACCTGTGGCTGAT	CTGCTTTCATTCGCTGTTCA	262	60	NM_001012898
OCTN1	CCTGGTGATGCTTGGAAAAT	TCGTAGGCACCCAGGTAAAC	171	58	NM_001146131
OCTN2	TCCATTCGTCTGCTGTTCTG	TCGCTGGGGTCAAAGATTAC	298	58	NM_001045828
PPARα	AGGCCAAGTTGAAAGCAGAA	GTCTTCTCTGCCATGCACAA	217	60	NM_001001464
TMABA-DH	CCCTGCAGCAGCCCCTCAAC	AGATCCCGCCAGTCCTGCGT	359	60	XM_422248
TMLD	CAAGCCCAAGGCTGTCCGGG	ACACTTGGATGCCGCAGGGC	458	62	NM_001012575

[1]ACO = Acyl-CoA oxidase; BBD = butyrobetaine dioxygenase; CPT = carnitine palmitoyltransferase; OCTN = organic cation transporter; PPAR = peroxisome proliferator-activated receptor; TMABA-DH = trimethylaminobutyraldehyde dehydrogenase; TMLD = trimethyllysine dioxygenase.

Table 2. Food intake, body weights, body weight changes and egg production of control hens and hens fed a control diet or a diet supplemented with clofibrate for 4 weeks.

Group	Control(n=10)	Clofibrate(n=9)
Initial weight (g)	1950 ± 177	2046 ± 140
Final weight, week 4 (g)	2032 ± 193	2005 ± 160
Weight gain (g)	82 ± 56[a]	-40 ± 115[b]
Feed intake (g/d)	114 ± 7	110 ± 12
Egg production (%)		
Week 1	83 ± 16	86 ± 19
Week 2	86 ± 12	81 ± 12
Week 3	86 ± 10[a]	77 ± 13[b]
Week 4	76 ± 15[a]	59 ± 18[b]
Weeks 1-4	83 ± 8[a]	75 ± 6[b]
Egg weight (g	67.8 ± 4.6	66.7 ± 3.0
Yolk weight (g)	17.3 ± 1.5	16.3 ± 0.9
Albumen weight (g)	38.9 ± 4.3	37.0 ± 3.4

Values are means ± SD. Means without the same superscript letters (a, b) are significantly different (P<0.05).

was separated by centrifugation at 1,500 × g for 10 min at 4℃. Samples of liver and *musculus pectoralis* were excised and immediately snap frozen in liquid nitrogen. Aliquots of liver and muscle for RNA isolation were stored at –80℃; other samples were stored at –20℃.

RT-PCR Analysis

Total RNA was isolated from liver and skeletal muscle by TRIZOL reagent (Invitrogen, Karlsruhe, Germany) according to the manufacturer`s protocol. cDNA synthesis was carried out as described (König and Eder, 2006). The mRNA concentration of genes was measured by real-time detection PCR using SYBR® Green I and the Rotor Gene 2000 system (Corbett Research, Mortlake, Australia) as described previously in detail (Ringseis et al., 2007). Relative quantification was performed using the $2^{-\Delta\Delta CT}$-method (Livak and Schmittgen, 2001). Ct-values of target genes and the reference gene (ß-actin) for normalization were obtained using Rotorgene Software 5.0. Relative expression ratios are expressed as fold changes of mRNA abundance in the control group compared to the clofibrate group. Gene-specific primer pairs obtained from Eurofins MWG Operon (Ebersberg, Germany) were designed using Primer3 and BLAST. Characteristics of gene-specific primers (Operon Biotechnologies, Cologne, Germany) are shown in Table 1.

Analysis of carnitine and metabolic precursors of carnitine

Concentrations of free carnitine, acetyl carnitine, propionyl carnitine, TML and BB in plasma, liver, muscle, egg yolk and albumen were determined by tandem mass spectrometry using deuterated analoga as internal standard (Hirche et al., 2009). Carnitine-d_3 (N-methyl- d_3) was supplied by Cambridge Isotope Laboratories (Andover, MA), acetyl carnitine-d_3 and propionyl carnitnie-d_3 were products of Larodan Fine Chemicals (Malmö, Sweden).

Statistical analyses

Treatment effects were evaluated by Student´s t-test. Means were considered significantly different for P < 0.05. Values in the text are given as means ± SD. In the clofibrate group, one hen died during the experiment. Therefore, the number of replications in this group was only 9. In the clofibrate group, eggs from only 4 hens were available for analysis of carnitine.

RESULTS

Body weights, food intake and laying performance

Initial body weights of the hens and daily feed intake during the trial was not different between the two groups of hens (Table 2). Hens of the clofibrate group slightly lost weight during the experimental period whereas those of the control group gained 82 g in average (Table 2). Final body weights of the hens at the end of the experimental period were however not different between

Table 3. Relative mRNA concentrations of PPARα, PPARα target genes involved In β-oxidation (ACO, CPT-1) and novel organic cation transports (OCTN1, OCTN2) in liver and muscle and hepatic enzymes of carnitine synthesis (TMLD, TMABA-DH, BBD) in hens fed a control diet or a diet supplemented with clofibrate for 4 weeks (control = 1.00).

Group	Control (n=10)	Clofibrate (n=9)
Liver		
PPARα	1.00 ± 0.25	0.96 ± 0.32
ACO	1.00 ± 0.23[b]	1.86 ± 0.56[a]
CPT-1	1.00 ± 0.49[b]	1.52 ± 0.51[a]
OCTN1	1.00 ± 0.16	0.86 ± 0.15
OCTN2	1.00 ± 0.44[b]	1.78 ± 0.82[a]
TMLD	1.00 ± 0.39	0.96 ± 0.30
TMABA DH	1.00 ± 0.37	0.91 ± 0.23
BBD	1.00 ± 0.16	1.23 ± 0.39
Muscle		
PPARα	1.00 ± 0.16	1.38 ± 0.32
ACO	1.00 ± 0.14	1.02 ± 0.33
CPT-1	1.00 ± 0.63[b]	2.86 ± 1.30[a]
OCTN1	1.00 ± 0.27	1.18 ± 0.53
OCTN2	1.00 ± 0.13	1.13 ± 0.13

Values are means ± SD. Means without the same superscript letters (a, b) are significantly different (P<0.05). Abbreviations: ACO = Acyl-CoA oxidase; BBD = butyrobetaine dioxygenase; CPT = carnitine palmitoyltransferase; OCTN = organic cation transporter; PPAR = peroxisome proliferator-activated receptor; TMABA DH = trimethylaminobutyraldehyde dehydrogenase; TMLD = trimethyllysinedioxygenase.

Table 4. Concentrations of carnitine in liver, muscle and plasma and amounts of carnitine in egg yolk, albumen and whole egg sampled from hens fed a control diet or a diet supplemended with clofibrate for 4 weeks.

Group	Control (n=10)	Clofibrate (n=9)
Liver (nmol/g)	365 ± 121[b]	550 ± 180[a]
Muscle (nmol/g)	151 ± 36	181 ± 47
Plasma (µmol/l)	21.0 ± 7.4[b]	29.5 ± 7.8[a]
Egg yolk (nmoles/egg)	237 ± 50[b]	326 ± 81[a]
Albumen (nmoles/egg)	47 ± 17[b]	161 ± 131[a]
Whole egg (nmoles/egg)	275 ± 60[b]	487 ± 129[a]

Values are means ± SD. Means without the same superscript letters (a, b) are significantly different (P < 0.05). Liver, muscle, plasma: n=10 for control group, n=9 for clofibrate group. Egg yolk, albumen, whole egg: n=10 for control group, n=4 for clofibrate group.

the two groups (Table 2). In the first 2 weeks of the experiment, egg production rate did not differ between the two groups of hens (Table 2). Thereafter, egg production rate was declining in the group of hens treated with clofibrate. In weeks 3 and 4 and in average of the 4 weeks, egg production rate was significantly lower in hens treated with clofibrate than in control hens (P < 0.05, Table 2). Weights of eggs, yolk and albumen did not differ between the two groups of hens (Table 2).

mRNA concentrations of PPARα and PPARα target genes involved in ß-oxidation in liver and muscle

In liver, mRNA concentration of PPARα did not differ between the two groups of hens (Table 3). In liver, an up-regulation of the classical PPARα target genes carnitine palmitoyltransferase (CPT)-1 and acyl CoA oxidase (ACO) involved in mitochondrial and peroxisomal ß-oxidation, respectively, was observed in the group treated with clofibrate (P < 0.05, Table 3). Hens treated with clofibrate had also an increased expression of OCTN2 in the liver (P < 0.05) while mRNA concentrations of all the enzymes involved in carnitine synthesis (TMLD, TMABA DH, BBD) and of OCTN1 were not altered compared to control hens (Table 3).

In muscle, expression of PPARα mRNA was also not different between the two groups of hens (Table 3). Hens of the clofibrate group had an increased expression of CPT-1 in muscle (P < 0.05) while mRNA concentration of ACO was unchanged compared to control hens. mRNA concentrations of OCTN1 and OCTN2 in muscle were not different between the two groups of hens (Table 3).

Concentration of carnitine and its metabolic precursors in plasma, tissues and egg

Hens treated with clofibrate had higher concentrations of carnitine in liver and plasma and higher amounts of carnitine in egg yolk, albumen and whole egg than hens of the control groups (P < 0.05, Table 4). In muscle, concentration of carnitine was also 20 to 30% higher in hens treated with clofibrate than in control hens; this difference, however, was not significant (Table 4). Concentrations of carnitine precursors TML and BB in liver, muscle and plasma did not differ between the two groups of hens.

DISCUSSION

The aim of the present study was to test the hypothesis that PPARα also regulates carnitine homeostasis in laying hens. For this end, we fed hens diets supplemented with 0.15% of clofibrate as a synthetic PPARα agonist. Clofibrate was chosen in this study because administration of fibrates is a widely accepted approach to elucidate in any species, whether or not a specific metabolic pathway is regulated by PPARα. The increased mRNA concentrations of the classical PPARα target genes CPT-1 and ACO in the liver show that this dose was sufficiently high to induce PPARα activation. It was shown that treatment with clofibrate leads to an

up-regulation of OCTN2 in the liver and to increased carnitine concentrations in liver, plasma, and egg. It was moreover observed that mRNA concentrations of enzymes of carnitine synthesis in the liver, as well as concentrations of TML and BB, the precursors of carnitine, in the liver are not influenced by clofibrate. These observations suggest that treatment with clofibrate did not influence endogenous carnitine synthesis in laying hens. The fact that OCTN1 was also not influenced by clofibrate agrees with a study in mice that shows that OCTN1, in opposite to OCTN2, is not activated by clofibrate (Koch et al., 2008).

Therefore, increased carnitine concentrations in the liver of hens treated with clofibrate might be due to an increased uptake of carnitine from plasma into liver cells by OCTN2. Similar findings have been made in rats in which clofibrate treatment also up-regulated OCTN2 in liver without having an effect on mRNA concentrations of enzymes of carnitine synthesis (Luci et al., 2006; Ringseis et al., 2007). We cannot completely exclude the possibility that effects of clofibrate were independent of its ability to activate PPARα. Nevertheless, with respect to studies in rats, mice and pigs, it is highly probable that hepatic OCTN2 in laying hens was up-regulated by activation of PPARα. Indeed, it has been recently shown that murine OCTN2 possesses a functional PPAR response element in its promoter region, demonstrating that it is a direct PPARα target gene (Wen et al., 2010). In mice and rats, treatment with PPARα agonists causes also an up-regulation of OCTN2 in kidney and small intestine which in turn increases the rate of reabsorption of carnitine in the kidney and absorption of carnitine from the diet (Ringseis et al., 2007; Koch et al., 2008; Ringseis et al., 2008b). In the present study, expression of OCTN2 in these tissues was not determined. However, it is possible that an increased absorption of carnitine from the diet and an increased reabsorotion of carnitine from the urine could provide an explanation for increased plasma carnitine concentrations. In contrast to liver, treatment with clofibrate did not increase expression of OCTN2 in muscle although there was an up-regulation of CPT-1 indicative of PPARα activation. One reason for the lack of effect of PPARα on OCTN2 might be that PPARα is generally less expressed in muscle compared to liver and that the extent of up-regulation of CPT-1 in muscle by clofibrate was smaller than in liver.

Moreover, we must be aware of the fact that pectorial muscle which was used as a sample in this study is a fast twitch, mainly glycolytic tissue. It is possibly that PPARα agonists have stronger effects on target genes, including OCTN2, in slow twitch red muscles such as thigh muscle. We found moreover that clofibrate treatment leads to increased carnitine concentrations in egg yolk and albumen. To the best of our knowledge, transfer of carnitine from blood into egg has not yet been determined. Thus, it is not clear whether OCTN2 is expressed in the oocyte membrane or not. If OCTN2 is indeed expressed in this membrane, it is possible that it is up-regulated by PPARα activation, which could be an explanation for an increased transfer of carnitine from plasma into the egg. Even if carnitine is transferred into the egg by diffusion, increased egg carnitine concentrations could simply reflect increased plasma carnitine concentrations.

Overall, the findings of this study suggest that PPARα has an essential role in the regulation of carnitine homeostasis in hens as in other species. During PPARα activation, induced either by treatment with agonists or by fasting, β-oxidation of fatty acids is strongly increased which in turn increases the demand of carnitine. An up-regulation of OCTN2 might be a means to increase carnitine concentrations in tissues and thus to meet the increased demand of carnitine for β-oxidation. Since PPARα is also activated by natural ligands such as n-3 PUFA and CLA, it is possible that similar alterations in carnitine homeostasis can be observed in hens fed diets containing fish oil or CLA.

ACKNOWLEDGMENTS

Mohamed Shibani was supported by a grant from Alfatah-University Libya.

REFERENCES

Cogburn LA, Porter TE, Duclos MJ, Simon J, Burgess SC, Zhu JJ, Cheng HH, Dodgson JB, Burnside J (2007). Functional genomics of the chicken - A model organism. Poult. Sci., 86: 2059-2094

Désert C, Duclos MJ, Blavy P., Lecerf F, Moreews F, Klopp C, Aubry M, Herault F, Le Roy P, Berri C, Douaire M, Diot, C, Lagarrigue S (2008). Transcriptome profiling of the feeding-to-fasting transition in chicken liver. BMC Genomics, 9: 611.

Desvergne B, Wahli W (1999). Peroxisome proliferator-activated receptors: Nuclear control of metabolism. Endocrine Rev., 20: 648-688.

Diot C, Douaire M (1999). Characterization of a cDNA sequence encoding the peroxisome proliferator activated receptor alpha in the chicken. Poult. Sci., 78: 1198-1202.

Durán JM, Peral MJ, Calonge ML, Ilundáin AA (2005). OCTN3: A Na^+-independent L-carnitine transporter in enterocytes basolateral membrane. J. Cell Physiol., 202: 929-935.

Hirche F, Fischer M, Keller J, Eder K (2009). Determination of carnitine, its short chain acyl esters and metabolic precursors trimethyllysine and gamma-butyrobetaine by quasi-solid phase extraction and MS/MS detection. J. Chromatogr., 877: 2158-62.

Koch A, König B, Stangl GI, 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. (Maywood), 233: 356-365.

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.

König B, Kluge H, Haase K, Brandsch C, Stangl GI, Eder K (2007). Effects of clofibrate treatment in laying hens. Poult. Sci., 86: 1187-95.

Livak KJ, Schmittgen TD (2001). Analysis of relative gene expression data using realtime quantitative PCR and the $2^{-\Delta\Delta}$Ct method. Methods, 25: 402-408.

Luci S, Geissler S, König B, Koch A, Stangl GI, Hirche F, Eder K (2006). PPARα agonists up-regulate organic cation transporters in rat liver cells. Biochem. Biophys. Res. Commun., 305: 704-708.

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 α. Biol. Pharm. Bull., 31: 1230-1236.

Meng H, Li H, Zhao JG, Gu ZL (2005). Differential expression of peroxisome proliferator-activated receptors alpha and gamma gene in various chicken tissues. Domest. Anim. Endocrinol., 28: 105-110.

Naumann C, Bassler R (1976). Methodenbuch Band III. Die chemische Untersuchung von Futtermitteln, mit 1. Ergänzungslieferung (1983), 2. Ergänzungslieferung (1988), 3. Ergänzungslieferung (1993) und 4. Ergänzungslieferung (1997). VDLUFA-Verlag, Darmstadt, Germany.

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

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

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.

Ringseis R, Wege N, Wen G, Rauer C, Hirche F, Kluge H, Eder K (2008a). Carnitine synthesis and uptake into cells are stimulated by fasting in pigs as a model of nonproliferating species. J. Nutr. Biochem., 20: 840-847.

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

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.

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

Tamai I, Yabuuchi H, Nezu H, 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, 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 JI, Sai JI, Kobayashi J, 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 Vlies N, Ferdinandusse S, Turkenburg M, Wanders RJ, Vaz. FM (2007). PPARα-activation results in enhanced carnitine biosynthesis and OCTN2-mediated hepatic carnitine accumulation. Biochim. Biophys. Acta, 1767: 1134-1142.

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

Wen G, Ringseis R, Eder K (2010). Mouse OCTN2 is directly regulated by peroxisome proliferator-activated receptor α (PPARα) via a PPRE located in the first intron. Biochem. Pharmacol., 79: 768-776.