

6. Summary

The *peroxisome proliferator-activated receptor- α* (PPAR α) is a member of the family of nuclear transcription factors and plays a crucial role in the regulation of lipid metabolism, ketogenesis, carbohydrate metabolism and inflammatory processes. Eicosanoids, free and polyunsaturated fatty acids are natural ligands of PPAR α . Furthermore, dietary oxidized fat has been shown to activate PPAR α in studies with rats. The lipid lowering properties of PPAR α activation are used since several decades by application of fibrates, which are known to act as synthetic ligands of the receptor. It is well established, that fibrates and oxidized fatty acids induce peroxisome proliferation and oxidative stress in liver of rats and mice. In these proliferating species, long-term administration of fibrates cause hepatocarcinogenesis. In contrast, non-proliferating species, like human, primates and pig, are refractory for these effects. Several quantitative and structural differences have been suggested as reasons for these species differences. This fact is limiting in the assignment of the findings from studies with rodents for humans. The aim of the first part of the present work was to investigate the effects of synthetic and natural ligands of PPAR α in the pig, representing a non-proliferating species.

Therefore, we performed an experiment in which 18 pigs received a control diet or control diet supplemented with 5 g clofibrate/kg diet for 28 days. The aim of *study 1* was to investigate the effects on lipid metabolism. The administration of synthetic PPAR α -agonist clofibrate increased plasma concentration of ketone bodies, decreased triglyceride and cholesterol concentrations in plasma and lipoproteins and moderately increased the relative mRNA concentration of known target genes of PPAR α in liver and adipose tissue, compared to control pigs ($P < 0.05$). In both tissues, the expression of *sterol regulatory element binding protein-1* (SREBP-1), SREBP-2, *insulin-induced gene-1* (Insig-1), Insig-2, *acetyl-CoA carboxylase* (ACC), LDL receptor and *3-hydroxy-3-methylglutaryl* (HMG)-CoA reductase was not affected by clofibrate. Furthermore, relative mRNA concentrations of apolipoproteins A-I, A-II and C-III, cholesterol-7 α -hydroxylase and microsomal triglyceride transfer protein in liver and of lipoprotein lipase in skeletal muscle of pigs were unchanged by clofibrate treatment. The mRNA concentrations of PPAR α were similar in pig and human liver, but 10-fold lower than concentrations in rat liver. In pigs, the mRNA concentrations of PPAR α were similar in liver and adipose tissue. In conclusion, the effects of clofibrate on plasma lipids in pigs are consistent to a great extent with other animal models and humans. However, the

biochemical mechanisms which are responsible for the hypolipidemic effects, might be in part different from those in other species.

In **study 2** it was shown for the first time, that clofibrate treatment increased the hepatic glucuronidation of thyroid hormones in pigs as non-proliferating species, resulting in decreased plasma concentrations of T₃ and T₄ compared to control pigs ($P < 0.05$). Thyroid weight and histological parameters of thyroid gland did not differ between control pigs and pigs treated with clofibrate, but increased relative mRNA concentrations of TSH-responsive genes (sodium iodide symporter, cathepsin B, TSH receptor, thyroid peroxidase) were observed in thyroid of pigs treated with clofibrate ($P < 0.05$). In liver, relative mRNA concentrations of proteins involved in plasma thyroid hormone transport (transthyretin, albumin) and of thyroid hormone receptor α_1 were decreased after clofibrate treatment ($P < 0.05$). In conclusion, it can be suggested that clofibrate acts as a disruptor of thyroid hormone axis in pigs. These disturbances of thyroid function should be seen critical with respect to humans.

The aim of **study 3** was to investigate several effects in pigs which are suggested to be responsible for the fibrate-induced development of hepatocarcinogenesis in rodents. Liver weights and number of peroxisomes in liver were increased in pigs treated with clofibrate ($P < 0.05$). The relative mRNA concentration of *acyl-CoA oxidase* (ACO) and catalase activity were increased and concentration of hydrogen peroxide was decreased in liver of pigs after clofibrate treatment ($P < 0.05$). Hepatic concentrations of total and reduced glutathione and lipid peroxidation products did not differ between control pigs and pigs treated with clofibrate, but relative mRNA concentrations of bax, c-jun and c-myc were increased and relative mRNA concentration of bcl-xl was decreased in liver after clofibrate treatment ($P < 0.05$). However, the increased hepatic expression of proto-oncogenes should be seen critical. Because fibrates are used as hypolipidaemic drugs in humans, this aspect should be examined more detailed in further studies.

Whether the dietary intake of a moderat oxidised fat causes an activation of PPAR α in non-proliferating species, should be investigated in **study 4**. Therefore, an experiment was performed with 18 pigs which were fed either a diet containing 90 g/kg diet of a fresh fat or the same diet with 90 g/kg diet of a moderat oxidised fat (24 h; 180°C) for 28 days. In pigs fed the moderat oxidised fat, the hepatic relative mRNA concentration of mitochondrial HMG-CoA synthase and plasma concentrations of ketone bodies were increased compared to pigs fed the fresh fat ($P < 0.05$). In liver, relative mRNA concentrations of ACO and *carnitine-palmitoyl transferase-1* (CPT-I) tended to be higher in pigs received the moderat

oxidised fat ($P < 0.10$), but failed to decrease lipid concentrations in plasma. In pigs fed the moderat oxidised fat, the activity of catalase, the concentration of conjugated dienes and the number of peroxisomes were increased and concentration of α -tocopherol was decreased in liver compared to pigs fed the fresh fat ($P < 0.05$). The relative mRNA concentrations of SREBP-1 and its target genes in liver and of SREBP-2 and its target genes in small intestine were increased in pigs recieved the moderat oxidised fat ($P < 0.05$). Taken together, it could be shown for the first time that the dietary intake of a moderat oxidised fat activates PPAR α in non-proliferating species and might induce lipogenesis in liver and small intestine of pigs. Furthermore, no influence on thyroid function or plasma thyroid hormone concentrations could be observed after dietary intake of a moderat oxidised fat, as it was shown in previous studies for rats and pigs. To investigate a possible direct influence of oxidised fatty acids on thyroid function, experiments with primary thyroid cells from pigs were performed in **study 5**, using 13-hydroperoxy-9,11-octadecadienic acid (13-HPODE) as primary oxidation product of linoleic acid (18:2n-6). The activities of superoxide dismutase and glutathione peroxidase were increased after incubation of 13-HPODE compared to control ($P < 0.05$). The relative mRNA concentrations of sodium iodide symporter, TSH receptor and thyroid peroxidase, as well as cell viability and iodide upake were unchanged, but the relative mRNA concentration of dual oxidase-2 and hydrogen peroxide production were decreased in thyrocytes treated with 13-HPODE compared to control cells ($P < 0.05$). Because the formation of hydrogen peroxide is rate-limiting in thyroid hormone synthesis, an inhibitory effect of 13-HPODE on thyroid function might be suggested.

The aim of the second part of the present work was to investigate the basic mechanisms, which are responsible for the increased hepatic concentrations of carnitin, as it was observed for animals after fibrate administration or fasting in previous studies. In **study 6**, an experiment was performed with 16 rats which recieved 250 mg clofibrate/kg in sunflower oil or only sunflower oil in control group additionally to commercial standard diet for 4 days. Treatment of synthetic PPAR α agonist clofibrate increased the relative mRNA concentrations of *novel organic cation transporter-1* (OCTN1), OCTN2, CPT-I and CPT-II and the carnitine concentrations in liver of rats ($P < 0.05$). Furthermore, incubation of FAO hepatoma cells with synthetic PPAR α agonist WY-14,643 resulted in increased relative mRNA concentration of OCTN2 and increased cellular carnitine concentrations ($P < 0.05$). In liver of rats and in FAO hepatoma cells, relative mRNA concentrations of proteins involved in carnitne synthesis were unchanged by treatment of synthetic PPAR α agonists. In conclusion, we could

demonstrate for the first time, that activation of PPAR α was accompanied by strong increased hepatic expression of OCTNs, which are responsible for the cellular carnitine uptake. The findings, that concentrations of carnitine were increased in the liver and decreased in plasma of rats are indicative for elevated activities of carnitine transporters. Primarily the increase of OCTN2 (*SLC22A5*), which possesses high affinity for carnitine, might be responsible for increased tissue concentrations.

In **study 7**, an experiment was performed with 18 rats either fed a commercial standard diet with oxidised sunflower oil or fresh sunflower oil in control group for 6 days. Relative mRNA concentrations of the known PPAR α target genes ACO, CPT-I and CPT-II in liver and small intestine and relative mRNA concentrations of OCTN1 and OCTN2 in liver and of OCTN2 in small intestine were increased in rats administered the oxidised fat compared to control group ($P < 0.05$). Furthermore, increased carnitine concentrations in liver and decreased carnitine concentrations in plasma, skeletal muscle and heart were observed in rats fed oxidised fat ($P < 0.05$). In conclusion, these results demonstrate for the first time, that dietary oxidised fat increases hepatic expression of OCTNs and carnitine concentrations in the liver of rats. The increased expression of OCTN2 in small intestine might suggest an increased absorption of carnitine.

The aim of **study 8** was to investigate the effects of fasting or caloric restriction, both physiological conditions of PPAR α activation, on carnitine concentrations and the expression of OCTNs. Therefore, an experiment was performed for 10 days with 36 rats either received *ad libitum* (control group), 10.5 g/d (70% of energy requirement for maintenance) or 6 g/d (40% of energy requirement for maintenance) of a commercial standard diet. A fourth group received the diet *ad libitum* for nine days and was then fasted for 24 h. Fasting and caloric restriction resulted in increased relative mRNA concentrations of PPAR α target gene ACO in liver, kidney and heart of rats compared to control group ($P < 0.05$). In rats receiving 70% of energy requirement for maintenance, relative mRNA concentrations of OCTN2 and carnitine concentrations were higher in liver and kidney than in control rats ($P < 0.05$). In animals receiving 40% of energy requirement for maintenance, relative mRNA concentrations of OCTN2 and carnitine concentrations were increased in liver, kidney, heart and skeletal muscle compared to control group ($P < 0.05$). Fasted rats had higher relative mRNA concentrations of OCTN2 and carnitine concentrations in liver, kidney and heart than control rats ($P < 0.05$). The increase of OCTN2 in kidney might be an evidence for elevated resorption as a mechanism for the maintenance of carnitine in these conditions.

6. Summary

As a result of the *studies 6-8*, it can be concluded that maintenance of carnitine is ensured by transcriptional regulation of transporters in situations of increased cellular requirement. Whether there was only a marginal effect on the expression in these studies, however, an increased activity of enzymes involved in carnitine synthesis can not be excluded completely. Furthermore, these studies support strong evidence particularly for OCTN2 to represent a direct target gene of PPAR α . This assumption could be verified by the identification of a functional PPRE in the promoter region of OCTN2 and should be investigated further studies.