6. Summary

During heating and deep-frying of food the used fat is exposed to high temperatures over a long period and can undergo oxidation. The uptake of oxidised fat results in an accumulation of primary and secondary lipid peroxidation products (LPOP) in human and animal tissues. LPOP impair the vitamin E status in rats due to the increased consumption of that antioxidant in the course of the lipid peroxidation and the hepatic triglyceride and cholesterol metabolism. The effects of oxidised fats on vitamin E and lipid metabolism of pregnant and lactating animals and their offspring are not well understood and are the focus of the work presented here. In these investigations rats were used as a model as their metabolism and reproduction processes are very similar to that of humans. Oxidation of used fats was prepared at low temperature over a long period of time as these kinds of fat have not shown growth retardation in rats neither in previous studies nor in these studies. This indicates that the used fat was moderately oxidised. Feeding female rats diets with fresh fat (control) or oxidised fat before and during pairing, pregnancy and/ or lactation with a controlled feeding system ensured an equal diet intake. The oxidation level of the used fat was determined by measuring the primary (peroxide value, acid value, conjugated dienes) and secondary (thiobarbituric acid-reactive substances, carbonyls, polar compounds) LPOP. The oxidised fats used in the experiments were found to have predominantly primary LPOP.

Prenatal and postnatal nutrition can have long-term effects on growth and metabolism of offspring (foetal programming). In experiment 1 it has been investigated whether feeding oxidised fat to female rats during reproduction impairs the growth and vitamin E status of the offspring at different developing stages (newborns, suckling pups [20 days], adults [10 weeks]), and whether high vitamin E supplementation improves the vitamin E status of dams and offspring. The diets were enriched with either 25 mg or 250 mg α-tocopherol equivalents/kg diet. The α-tocopherol concentrations were measured in liver, plasma, milk and mammary gland in dams and in liver and plasma in the offspring. In dams, supplementation of 250 mg α-tocopherol equivalents to oxidised fats improved the vitamin E status in tissues. However, the α-tocopherol concentrations were strongly reduced in tissues of dams fed the oxidised fat compared to those ones fed with fresh fat. A high vitamin E supplementation can not totally counteract the increased vitamin E catabolism when feeding oxidised fat during reproduction. The high vitamin E supplementation of the maternal diet in both groups fed with fresh and oxidised fat was still seen in the liver of newborns, suckling
pups and male adults by high α-tocopherol concentrations. However, decreased α-tocopherol concentrations after feeding oxidised fat to dams were not found in the offspring and a transport of LPOP from dams to the offspring neither along the placenta nor via the milk can be probably excluded. Also, there was no long-term impairment of the growth of the offspring from birth until an age of 10 weeks after feeding oxidised fats to dams.

At the end of pregnancy and during lactation the hepatic fatty acid oxidation decreases in humans and rats. In mice the decreased fatty acid oxidation is correlated with a decreased mRNA concentration of the transcription factor peroxisome proliferator-activated receptor α (PPARα), an important regulator of lipid metabolism. Because such investigations have not been carried out in rats, in experiment 2 female rats were fed ad libitum a commercial diet until the end of pregnancy or until day 10 or 20 of lactation, and the mRNA concentrations of PPARα and its target genes (carnitine-palmitoyltransferase1a [CPT1a], acyl-CoA oxidase [ACO], cytochrome P450 4A1 [CYP4A1]) were measured in the livers. Female virgin rats were used as control. In lactating rats, but not in pregnant rats, decreased mRNA concentrations of PPARα and its target genes CPT1a and CYP4A1 were found. Thus, the decreased fatty acid oxidation during lactation could be a consequence of a decreased expression of PPARα. This down regulation could be due to changes in hormone metabolism during lactation.

Oxidised fat reduces triglyceride concentrations in the liver of rats. Reasons for this are a PPARα-activation with an increased expression of genes of fatty acid oxidation and a decreased expression of the transcription factor sterol regulatory element-binding protein-1c [SREBP-1c] and the gene fatty acid synthase [FAS]. Synthetic PPARα-ligands (clofibrate) can pass through the placenta and cause PPARα-activation in the liver of pregnant rats and their foetuses. In experiment 3 it has been investigated whether oxidised fat causes PPARα-activation, and/ or a decrease in fatty acid synthesis in pregnant rats and their foetuses. Clofibrate was fed as a control for PPARα-activation. In the liver of pregnant rats and their foetuses the mRNA concentrations of PPARα and its target genes (ACO, CPT1a, CYP4A1, medium-chain acyl-CoA dehydrogenase [MCAD], long-chain acyl-CoA dehydrogenase [LCAD]) and SREBP-1c and FAS have been measured. In addition, the triglyceride concentrations in liver and plasma have also been determined.
Summary

It was shown for the first time, that oxidised fat caused PPARα-activation with up-regulation of the PPARα-target genes in the liver of pregnant rats and their foetuses. Because fatty acid synthesis was not influenced by oxidised fat in the liver of pregnant rats and their foetuses, the reduced triglyceride concentrations in liver and plasma of pregnant rats and in liver of fetuses seem to be only a result of PPARα-activation and increase of fatty acid catabolism. In conclusion, oxidised fat is able to pass through the placenta and can act as a PPARα-agonist, with a similar intensity as clofibrate.

Oxidised fat reduces significant triglyceride concentrations in milk of lactating rats. The reason for this effect has not been known yet and thus in experiment 4 the triglyceride metabolism was investigated in liver and mammary gland of lactating rats after feeding oxidised fat. In order to proof PPARα-activation, in the liver and mammary gland the mRNA concentrations of PPARα and its target genes have been measured in both organs. Furthermore, the concentrations of triglycerides in liver, plasma and milk were determined. Hepatic fatty acid synthesis was determined by measuring mRNA concentrations of SREBP-1c and FAS.

It was shown for the first time that oxidised fat cause PPARα-activation in the liver, but not in the mammary gland of lactating rats. Hepatic fatty acid synthesis only tended to decrease, and leads to the conclusion that the decreased triglyceride concentrations in the liver and plasma are predominantly a result of the hepatic PPARα-activation. The decreased triglyceride concentrations in the milk after feeding oxidised fat are not a result of an increased fatty acid catabolism in mammary gland.

Milk triglycerides come from three different sources: 1. biosynthesis of medium-chain fatty acids (C8-C14) and their esterification in mammary gland, 2. uptake of long-chain fatty acids (C18-C22) from triglycerides from lipoproteins through lipoprotein lipase [LPL], 3. uptake of non-esterified-fatty-acids [NEFA] from triglycerides from adipose tissue through fatty acid transporters from plasma into the mammary gland. It is already known that fatty acid synthesis in the mammary gland from lactating rats after feeding oxidised fat is not influenced. In the present study, the unchanged mRNA concentrations of SREBP-1c and FAS and the unchanged concentrations of medium-chain fatty acids in the milk confirm the previous study. Decreased mRNA concentrations of LPL and fatty acid transporters in mammary gland and decreased concentrations of long-chain fatty acids in milk and of NEFA in plasma have been measured. These results indicate that decreased triglyceride concentrations in the milk after feeding oxidised fat are due to reduced uptake of fatty acids.
Summary

from lipoproteins and of NEFA from plasma by mammary gland. Parallel to these investigations, the mRNA concentrations of LPL and fatty acid transporters in the liver have been determined; here oxidised fat caused an increase of mRNA concentrations of LPL and fatty acid transporters. This adjustment of the transport of fatty acids to the liver instead to the mammary gland during lactation after feeding oxidised fat might be due to the enhanced demand of fatty acids in the liver caused by the PPARα-activation.

The reduced concentrations of milk triglycerides caused decreased body weights of suckling pups. The different effects of maternal oxidised fat on the body weight of the offspring in experiments 1 and 4 could be the result of a different content of PUFA in the prepared fats. The content of PUFA was much lower in experiment 1, compare to experiment 4.

Oxidised fat reduces significant cholesterol concentrations in liver and plasma of lactating rats. The reason for this effect was unknown and thus it was the object of experiments 3 and 4. In the livers of pregnant and lactating rats, the unchanged mRNA concentrations of SREBP-2 and its target genes hydroxy-ethyl-glutaryl-CoA-reductase (experiment 3,4) and low-density-lipoprotein-receptor (experiment 4) do not indicate reduced cholesterol synthesis or reduced uptake of cholesterol into cells. Fetal metabolism was not influenced by oxidised fat.

In conclusion, the results show that maternal uptake of oxidised fat can impair the physiologic processes in pregnancy and lactation and can have long-term effects on growth and metabolism in offspring.