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# THE EFFECT OF DIETARY PARTIALLY HYDROGENATED MARINE OIL ON THE *in vivo* AND *in vitro* FATTY ACID METABOLISM IN THE LIVER

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## ABSTRACT

Two groups of rats were fed for 30 days on a diet containing 20% by weight of either partially hydrogenated marine oil (PHMO) supplemented with linoleic acid, or palm oil (PO).

In the PHMO-group the liver lipids showed decreased levels of 20:1 and 22:1 compared to the dietary fat, and a corresponding increase in 18:1 and polyenoic fatty acids. The high dietary levels of 16:0 and 18:1 in the PO-group were reduced in the liver lipids, and counterbalanced by increased levels of 18:0 and polyenoic fatty acids. The PL of both groups had highest levels of 20:4 compared to the other lipid classes. A decreased conversion of 18:2 to 20:4 was seen in the PHMO-group, reflected by higher 18:2/20:4 ratios, especially in FFA- and PL-fractions.

Hepatocytes incubated with 18:1 cis or trans resulted in higher levels of 16:0 in the TG in both dietary groups compared to the liver values. In the FFA and PL the increased levels of 18:0 were most marked. 18:1 trans was incorporated in all lipid classes, most pronounced in the PHMO-group. Increased ratios of 18:2/20:4 were found in all lipids of the hepatocytes compared to liver tissue values.

Secreted lipids TG and PL, but also FFA were found. The FFA fraction was dominated by the incubation fatty acids, 18:1 cis and 18:1 trans, but 16:0, 18:0, 18:2 and 20:4 were also found. Higher amounts of 20:4 were found in the secreted PL and TG in both dietary groups, and the TG had a generally different fatty acid pattern than the liver hepatocyte TG, whereas the secreted PL resembled the tissue PL.

## INTRODUCTION

Partially hydrogenated marine oils (PHMO) are used in substantial amounts in Northern Europe for margarine production. This fat contains 25–

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Table 1. Body and liver weights of rats fed 20% (by wt.) of either partially hydrogenated marine oil (PHMO) or palm oil (PO) for 30 days. The results are given as mean±SD of the number in the parenthesis.

| Dietary group | PHMO | PO |
|---|---|---|
| Body weight, g (5) | 274.7±16.1 | 273.7±10.6 |
| Weight gain, g/day (5) | 5.6± 0.4 | 5.6± 0.2 |
| Liver weight (2) | 9.0± 0.5 | 8.5± 0.5 |

30% of very long chain monoenoic fatty acids (20:1 and 22:1) and up to 50% trans isomers (1, 2). Several studies have aimed to elucidate the influence of this fat on the lipid metabolism in rat hearts and livers (1–6) and in other experimental animals (7, 8). Poor growth has been ascribed to the deficiency of essential fatty acids (9) and temporary heart lipidosis to the content of docosenoic acids (22:1) (10, 11). Low levels of 22:1 have been found in the liver after feeding PHMO, indicating that these fatty acids are either exported, chain shortened or oxidized due to an adaptive process of increased oxidative capacity in the liver (3, 6, 11, 12). An inhibition of the convertion of 18:2 ω 6 to 20:4 ω 6 has also been associated with feeding high amounts of PHMO fat containing monoenoic fatty acids and trans isomers to rats (15, 16).

As the adaptive changes take place mainly in the liver (11) the object of this study was the elucidation of the fatty acid distribution in liver lipid classes from rats adapted to high fat diets with PHMO or PO, before and after hepatocyte incubation. The hepatocytes were incubated with *cis* 18:1 ω 9 or *trans* 18:1 ω 9. The two dietary fats were equal with regard to the amounts of the essential linoleic acid (18:2 ω 6) (10% by wt.).

In previous studies (6, 12–14) the fatty acid liver metabolism in rats adapted to PHMO-diet was studied realtive to an input of 22:1 whereas the adaptation to trans isomers was focused on in the present study.

## MATERIALS AND METHODS

### *Chemicals*

Collagenase type I (spec. act. 150 U/mg), bovine serum albumin, essential fatty acid free (A-6003), oleic acid (18:1 ω 9 cis) and elaidic acid (18:1 ω 9 trans) were from Sigma Chemicals Co. All other chemicals were of analytical grade, mainly from Merck AG.

### *Animals and Diets*

Male weanling Wistar rats (120 g) (Møllegaard, Denmark) were fed a semisynthetic diet for 30 days containing by weight: 20% fat (partially

hydrogenated marine oil (PHMO) or palm oil (PO), both adjusted to 10 % linoleic acid, DeNoFa-Lilleborg, Norway) (Table 2), 15% protein (hen egg white), 56% dextrinized potato starch, 1% vitamin mixture, 4% salt mixture and 4% wheat bran. The rats were fed *ad libitum*. Prior to the experiment the rats were fasted overnight, anaesthezised with Mebumal and weighted. Two rats from each group were used for liver lipid analyses and three were taken for hepatocyte preparation.

*Preparation and incubation of hepatocytes*

Rat liver cells were prepared by a two step perfusion technique essentially according to Seglen (15) using 0.05% collagenase and purified and incubated as described previously (12). From the final cell suspension 5–6 x $10^6$ cells/ml were incubated for 3 hours in 50 ml stoppered Erlenmeyer flasks containing 5 ml of a medium with 1% glucose, 1% bovine serum albumin and 1 mM of albumin bound oleic or elaidic acid. After the incubation the hepatocytes and the incubation medium were separated by centrifugation at 2000 rpm for 10 minutes.

Table 2. The fatty acid composition of the dietary fats and the liver lipids (TG, FFA, PL) from rats fed 20% by wt. of either partially hydrogenated marine oil (PHMO) or palm oil (PO).

| | PHMO | | | | PO | | | |
|---|---|---|---|---|---|---|---|---|
| | | Liver lipids | | | | Liver Lipids | | |
| | Diet.fat | TG | FFA | PL | Diet.fat | TG | FFA | PL |
| 14:0 | 5.0 | – | 1.9 | – | 1.3 | – | 2.0 | – |
| 16:0 | 12.1 | 17.6 | 13.4 | 9.2 | 35.4 | 16.2 | 18.5 | 11.4 |
| 16:1 | 9.0 | 2.6 | 3.5 | 1.9 | – | 1.7 | 1.0 | 1.2 |
| 18:0 | 3.3 | 2.6 | 9.8 | 12.8 | 4.8 | 2.2 | 22.1 | 26.1 |
| 18:1*t* | 7.6 | 8.5 | 7.0 | 5.2 | – | – | – | – |
| 18:1 *c* | 9.6 | 38.1 | 13.9 | 10.7 | 45.9 | 50.8 | 24.0 | 6.4 |
| 18:2 | 10.1 | 13.9 | 16.4 | 14.2 | 10.7 | 13.2 | 7.8 | 6.0 |
| 20:1 *c*+*t* | 15.5[1] | 4.5 | 3.6 | 1.7 | – | 1.2 | 1.0 | 1.0 |
| 20:2+3 | 5.1 | *tr* | 2.2 | 1.9 | – | – | – | *tr* |
| 20:4 | – | 5.9 | 17.4 | 31.8 | – | 5.8 | 17.9 | 35.3 |
| 22:1 *c*+*t* | 15.3[1] | 2.6 | 3.5 | – | – | – | – | – |
| 22:2–6 | 4.2 | 1.0 | 3.6 | 7.6 | – | 4.6 | 4.3 | 9.6 |
| non-calc. | 4.2 | 2.7 | 3.8 | 3.0 | 1.9 | 4.3 | 1.4 | 3.0 |
| 18:2/20:4 | – | 2.4 | 0.9 | 0.4 | – | 2.3 | 0.4 | 0.2 |

The data are expressed as % of total fatty acids, means of 2 determinations in each group.
Abbreviations, TG: triglycerides, FFA: free fatty acids, PL: phospholipids.
*c* = cis, *t* = trans, *tr* = trace (<1%).
[1] incl. 20:0, resp. 22:0 (each about 1.5%).

The initial viability was better than 90 %, and maintained throughout the experimental period by the addition of albumin, glucose and amino acid mixture to the media (16).

*Analytical procedures*

The incubation media were treated with charcoal according to Chen (17), and lipids from the carbon complex as well as from the dietary fats, homogenized livers and hepatocytes were extracted with chloroform-methanol (2:1) according to Folch (18). Lipid classes were separated by TLC on Silica Gel G-60 plates (Merck No. 5115), solvent system; hexan-diisopropyl ether-acetic acid (70:20:1.5). Phospholipids (PL), triglycerides (TG) and free fatty acids (FFA) were scraped off and methylated with $BF_3$/MeOH (19). Gas-Liquid Chromatography (GLC) of the methyl esters were performed in a Perkin-Elmer GLC Model 900, with a 10 feet glas column packed with SP 2330 on Supelcoport 100/120 (170–210°C, 4 min. initial and thereafter 2°C/min). The results are expressed as mean of 3 determinations per group.

## RESULTS AND DISCUSSION

In Table 2 is given the fatty acid composition of the dietary fats. Adequate amounts of linoleic acid were present in the palm oil, and in the PHMO-diet assured by the addition of sun flower seed oil. This corresponds to about 4% of dietary energy as 18:2 ω 6, sufficient to avoid essential fatty acid deficiency. The partially hydrogenated marine oil is characterized by high amounts of monoenoic fatty acids, including 20:1 (14%) and 22:1 (14%). According to previous reports (1, 20) up to 50% of the monoenes are trans isomers due to the hydrogenation process. Further 10% of very long chain polyenoic fatty acids isomers (mainly dienes and trienes) are present. In contrast, the palm oil contains high amounts of 16:0 (35%) and 18:1 (46%), and no trans fatty acids. The experiment was based on rats adapted to a high fat diet with 20% (by wt.) of the above mentioned fats. During the 30 days feeding period growth and liver weights were equal in the two dietary groups (Table 1).

The effect of trans fatty acids on growth is related to the composition of the diet, in particular, its content of essential fatty acids (21–23). With rats fed adequate amounts of essential fatty acids, trans monoenoic isomers have little effect on the growth. Rats do not discriminate between the cis and trans isomers of fatty acids during digestion and absorption (23). But the dietary intake, the amount and composition of trans isomers effect lipid metabolism and the incorporation of fatty acids into different tissue lipids. Recent research has provided data on some aspects of fatty acid isomer metabolism (20–24), though not all issues are resolved.

*In vivo* and *in vitro* studies with hydrogenated marine oil diets deficient or adequate in linoleic acid, have been performed, but have mainly been focused on the effects of the high content of very long chain fatty acids (e.g. 20:1 and 22:1), and their possible role in inducing increased peroxisomal oxidation.

The fatty acid composition of the liver lipids (TG, PL and FFA) from the PHMO-group all had decreased levels of the very long chains 20:1 and 22:1, and correspondingly higher amounts of oleic acid (18:1) and an accumulation of the essential fatty acids 18:2 ω 6 and 20:4 ω 6 (Table 2). A chain shortening of the very long monoenes mainly to 18:1 is assumed to occur when rats are fed a diet containing partially hydrogenated marine oil (3–6, 12–14) and this pathway together with an effective esterification and secretion of lipids (3, 12) results in a fast clearing of 20:1 and 22:1 from the liver lipids.

A further *de novo* synthesis of 16:0 and 18:0 is seen, and a chain elongation combined with desaturation gives appreciable amounts of arachidonic and other long chain polyenoic acids. Trans monoenes were present in all lipid fractions, mainly as elaidic acid, trans 18:1 ω 9 (5–9% of the total fatty acids). In the liver lipids from the palm oil fed rats (Table 2), decreased levels of 16:0 and 18:1, together with increased stearic acid (18:0) and polyenoic fatty acids were most marked compared to the dietary fat. Although equal amounts giving sufficient daily intake of the essential linoleic acid was ensured in both groups, an accumulation of 18:2 ω 6 in the PHMO-group relative to the PO-group was noticable. The relative ratios between 18:2 and 20:4 given in Table 2 were generally about 50% higher in the PHMO-group, and give clear evidence for an inhibited chain elongation and desaturation of 18:2 in this group. Similar results were found by Svensson (20) and Kirstein et al. (25) who studied the effect of dietary fats on the conversion of 18:2 ω 6 to 20:4 ω 6 in rat liver microsomes. These authors claimed that both the $\Delta 6$ and the $\Delta 5$ desaturase activities were reduced in the rats fed PHMO. Svensson further suggested that the desaturation activity is better related to the intake of 20:1 and 22:1 isomers than to the total intake of trans isomers.

Trans fatty acids resemble saturated fatty acids in their mode of incorporation into lipids *in vivo* (26) and are readily catabolized as shown by Coots (27). The trans isomers of 18:1 other than elaidic acid can be desaturated by $\Delta 9$ desaturates to yield trans, cis dienes, and may thus compete with stearic acid (18:0) which is the normal substrate for this desaturase (28). Hence, trans 18:1 (except elaidic acid) may inhibite $\Delta 9$ and $\Delta 6$ desaturates and thereby exert negative effects on the synthesis of 20:4 ω 6.

It is apparent that substantial amounts of dietary trans fatty acids have effects on the relative levels between fatty acids in the liver lipids. These effects increase with the dietary level of trans fatty acids and/or when the essential fatty acids requirements are not met. To study the dietary effects on the metabolism of isomers of 18:1, hepatocytes were incubated 3 hours with

Table 3. The fatty acid composition (%) of the lipids in hepatocytes incubated 3 hours with cis 18:1 *w* 9 or trans 18:1 *w* 9.

| Dietary fat | PHMO | | | | | | PO | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lipid class | TG | | FFA | | PL | | TG | | FFA | | PL | |
| 18:1*w*9 | *c* | *t* | *c* | *t* | *c* | *t* | *c* | *t* | *c* | *t* | *c* | *t* |
| 16:0 | 23.9 | 21.0 | 13.5 | 11.8 | 17.4 | 15.1 | 28.2 | 25.6 | 17.6 | 14.9 | 18.9 | 19.6 |
| 16:1 | 7.3 | 7.2 | 1.7 | 2.2 | 3.2 | 2.5 | 1.0 | 1.0 | 1.3 | 1.2 | *tr* | *tr* |
| 18:0 | 2.9 | 2.3 | 28.0 | 21.8 | 21.4 | 22.0 | 2.1 | 2.5 | 16.8 | 17.1 | 30.6 | 32.3 |
| 18:1*t* | 8.0 | *15.8* | 8.6 | *19.4* | 5.7 | *10.1* | – | *5.6* | – | *22.5* | – | *2.0* |
| 18:1*c* | *30.3* | 24.8 | *15.4* | 12.6 | *13.9* | 10.7 | *47.7* | 45.5 | *40.8* | 22.0 | *9.8* | 9.1 |
| 18:2 | 18.1 | 18.4 | 9.9 | 13.0 | 10.8 | 12.6 | 14.0 | 13.5 | 9.6 | 8.7 | 6.1 | 6.4 |
| 20:1*c*+*t* | 5.1 | 4.5 | 1.7 | 2.6 | 1.6 | 1.5 | *tr* | *tr* | 1.3 | *tr* | – | – |
| 20:2+3 | *tr* | *tr* | – | – | 1.3 | 1.6 | – | – | – | – | *tr* | *tr* |
| 20:4 | 2.1 | 2.2 | 13.7 | 10.6 | 18.9 | 16.3 | 3.2 | 3.3 | 5.6 | 5.9 | 24.4 | 20.6 |
| 22:1*c*+*t* | 1.9 | 2.1 | 2.1 | 1.5 | *tr* | *tr* | – | – | – | – | – | – |
| 22:2–6 | *tr* | *tr* | 2.1 | 1.2 | 2.4 | 2.7 | 1.1 | 1.1 | 3.6 | 3.2 | 7.2 | 6.3 |
| non-calc. | 0.7 | 1.7 | 3.3 | 3.3 | 3.4 | 4.9 | 2.7 | 1.9 | 3.4 | 4.5 | 3.0 | 3.7 |
| 18:2/20:4 | 8.6 | 8.4 | 0.7 | 1.2 | 0.6 | 0.8 | 4.4 | 4.1 | 1.7 | 1.5 | 0.3 | 0.3 |

For abbreviations see Table 2.
The results are mean of 2–3 determinations in each group.

Table 4. The fatty acid composition (%) of the lipids in the incubation medium after 3 hours incubation with cis 18:1 *w* 9 or trans 18:1 *w* 9.

| Dietary fat | PHMO | | | | | | PO | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lipid class | TG | | FFA | | PL | | TG | | FFA | | PL | |
| 18:1*w*9 | *c* | *t* | *c* | *t* | *c* | *t* | *c* | *t* | *c* | *t* | *c* | *t* |
| 14:0 | 3.5 | *tr* | – | – | – | – | 2.0 | 1.4 | – | – | – | – |
| 16:0 | 9.1 | 8.4 | 9.6 | 6.7 | 21.9 | 18.2 | 7.5 | 10.0 | 10.2 | 8.2 | 24.5 | 23.3 |
| 16:1 | 2.1 | 2.3 | 2.0 | 1.7 | 3.7 | 2.4 | 1.5 | *tr* | – | – | 1.6 | 1.4 |
| 18:0 | 8.4 | 7.4 | 9.7 | 7.2 | 20.9 | 21.0 | 10.5 | 9.3 | 10.9 | 10.5 | 30.1 | 28.1 |
| 18:1*t* | 6.0 | *40.4* | – | *63.4* | 5.5 | *10.5* | – | *30.3* | – | *51.3* | – | *3.4* |
| 18:1*c* | *43.1* | 19.1 | *56.4* | 3.9 | *12.9* | 12.3 | *59.8* | 36.0 | *61.2* | 14.2 | *11.4* | 12.1 |
| 18.2 | 10.6 | 9.2 | 11.1 | 8.2 | 11.8 | 13.1 | 7.1 | 4.4 | 4.9 | 3.5 | 6.2 | 6.5 |
| 20:1*c*+*t* | 1.3 | 1.1 | *tr* | *tr* | 2.1 | 1.3 | – | – | – | – | – | – |
| 20:2+3 | 2.6 | 1.7 | – | – | 1.2 | 1.2 | – | – | – | – | – | – |
| 20:4 | 8.5 | 7.0 | 9.3 | 6.8 | 14.8 | 12.9 | 6.8 | 5.5 | 9.8 | 7.5 | 18.4 | 17.4 |
| 22:1*c*+*t* | *1.0* | *tr* | – | – | *tr* | *tr* | – | – | – | – | – | – |
| 22:2–6 | – | – | – | – | 3.2 | 2.8 | 2.7 | 2.2 | 1.3 | 1.2 | 5.5 | 6.6 |
| non-calc. | 3.8 | 3.4 | 1.9 | 2.1 | 2.0 | 4.3 | 2.3 | 0.9 | 1.7 | 3.6 | 2.3 | 1.2 |
| 18:2/20:4 | 1.2 | 1.3 | 1.2 | 1.2 | 0.8 | 1.0 | 1.0 | 0.8 | 0.5 | 0.5 | 0.3 | 0.4 |

For abbrevations see Table 2.
The results are mean of 2–3 determinations of each group.

albumin bound cis 18:1 and trans 18:1, respectively, and the fatty acid pattern in the cell lipids as well as in the secreted lipids were determined. Trans 18:1 was incorporated into all lipid classes of the hepatocytes in both dietary groups (Table 3). 10 to 16% trans 18:1 (of the total fatty acids) were found in the phospholipids and triglycerides, respectively, in the PHMO-group, whereas only 2 to 6% were found in the PO-group. The free fatty acid fraction contained appreciable higher amounts of trans 18:1, 18 and 23% in the PHMO and PO-group, respectively. Cis 18:1 as well as trans 18:1 inhibit the 18:2 to 20:4 conversion. A more pronounced negative effect of trans 18:1 as proposed by other investigators was not confirmed in this experiment. The phospholipids and triglycerides in the PHMO-group gave only half the values for the ratio of 18:2 and 20:4, relative to the PO-group. Possibly, high amounts of trans 18:1 isomers given to hepatocytes from PHMO-fed rats is likely to induce even an enhanced inhibition on the chain elongation and desaturation process. The reason for this may be the oxidation of 20:1 and 22:1 acids in the PHMO livers yielding high amounts of 18:1 and 16:1, which in turn together with the remaining long-chain monoenes may complete with 18:2 ω 6 in the $\Delta$ 6 desaturation process, the rat limiting step in the formation of arachidonic acid (29).

Compared to the original liver lipids increased levels of the saturated acids, 16:0 and 18:0 were found, particularly in the phospholipids in both dietary groups and after both incubations. As the incubations were carried out with glucose and amino acids in the medium, these nutrients may have contributed to the formation of acetyl CoA, and therefore to *de novo* synthesis of 16:0 and 18:0. In the triglycerides, the increased amounts of 16:0 and polyenoic acids were most marked. The free fatty acid fraction resembled the liver lipids and the dietary fats, except for the trans 18:1 incubations where this acid was incorporated as an addition to the others. Generally, the FFA had a composition «in between» of TG and PL, as would be expected as FFA's are used for TG as well as PL synthesis. It must be mentioned that a hydrolysis of the lipids might have occurred during the storage and preparation of the samples.

The lipids secreted from the hepatocytes were also analysed with regard to the fatty acid composition (Table 4). As expected the free fatty acid fraction was dominated by the incubation fatty acids; 50–60% of either cis 18:1 or trans 18:1. However, other fatty acids e.g. 16:0, 18:0, 18:2 and 20:4 were also found in this fraction, indicating that hepatocytes do secrete non-esterified fatty acids. This is in accordance with the results from our study on perfused rat livers (30).

Secreted triglycerides are supposed to constitute the major part of the very low density lipoproteins (VLDL) (31), and this fraction showed a different fatty acid pattern compared to the original liver TG and to the hepatocyte TG. For both groups were found increased levels of the incubation acids 18:1

cis or 18:1 trans, particularly the latter in the group fed PHMO. Compared to the tissue values the ratio 18:2/20:4 was lower, because of a preferred incorporation of 20:4 ω 6 relative to 18:2 ω 6 in the secreted TG. Lastly, both groups had much lower contents of 16:0 (7–10% vs. 20–30%) and higher levels of 18:2 (7–10% vs. 2–3%). Thomassen et al. (3) found evidence for the existence of two TG pools in the liver; one for storage and one for secretion. Our findings of different fatty acid pattern in liver tissue/hepatocytes TG relative to secreted TG, is in good agreement with this report. They found, however, higher amounts of 20:1 and 22:1 secreted in the VLDL-TG from the perfused PHMO-liver. The level for 20:4 ω 6 was negligible, compared to our results (7–9% 20:4), probably reflecting a dietary EFA deficiency with only 2.6 % linoleic acid in the PHMO-fat.

Little is known with respect to the fatty acid composition in the phospholipids secreted as VLDL-constituents from PHMO-fed rats. Phospholipids are not stored in the liver, as the TG in lipid droplets, but are incorporated into the membrane lipids or subsequently secreted as newly synthesized PL, accounting for a minor part of VLDL (33). Hence, the fatty acid composition of the PL resembles that in the hepatocyte PL (Tables 3 and 4). The incubation fatty acids, cis 18:1 and trans 18:1, had little influence on the phospholipid composition and the main difference between the medium and hepatocyte PL was higher level of 16:0 and lower level of 20:4. The ratio 18:2/20:4 increased in both groups relative to the original liver values, indicating a preferred secretion of 20:4 compared to 18:2.

As we previously suggested (12) the PL's in the liver/hepatocytes have a more complex composition than the secreted PL, probably because of the presence of several polar components of fatty acids in the tissue polar fraction.

## CONCLUSIONS

Two groups of rats were fed for 30 days a diet containing 20% by weight of either partially hydrogenated marine oil (PHMO) or palm oil (PO), both with 4 energy % as linoleic acid. Hepatocytes were prepared from both groups and incubated for 3 hours with oleic acid (cis 18:1 ω 9) or elaidic acid (trans 18:1 ω 9).

Compared to the dietary PHMO only minor amounts of 20:1 and 22:1 were present in the liver lipids, the reason being an effective chain shortening of these acids yielding increased levels of 18:1. For both groups medium chain fatty acids were chain elongated and desaturated shown by appreciable amounts of polyenoic acids, particular 18:2 and 20:4, present in all lipids.

When incubating with cis 18:1 or trans 18:1 decreased conversion of 18:2 ω 6 to 20:4 ω 6 was seen, reflecting an inhibitive effect of large amounts of 18:1

competing with 18:2 ω 6 in the △ 6 desaturation process, the rate limiting step in the formation of arachidonic acid.

Increased levels of 18:2 ω 6 in the PHMO-group relative to the PO-group may be a result of an inhibitive effect of the different dietary cis and trans mono, di- and trienes present in the PHMO.

A *de novo* synthesis of 16:0 and 18:0 via acetyl CoA from glucose and amino acids present in the incubation medium gave higher levels of these fatty acids in the hepatocytes than normally present in the liver.

The incorporation of trans 18:1 was evident in all lipid fractions, but most pronounced in the group fed PHMO.

Lipids secreted from the hepatocytes (PL and TG) contained higher levels of 20:4 ω 6 than the livers/hepatocytes did. Free fatty acids were also secreted for the cells.

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