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ORIGINAL CONTRIBUTION

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Effect of randomization of mixtures of butter oil and vegetable oil on absorption and lipid metabolism in rats

Abstract *Background* The nutritional effect of the regiospecific distribution of fatty acids in edible fats is currently discussed due to an increased use of interesterification of fats for human consumption. However, disagreeing results have been reported which may be due to the varying composition of the dietary fats compared. Data on the fate of such lipids beyond the bloodstream is rather scarce and animal model studies are needed. *Aim of the study* To compare the metabolism of butter oil and mixtures of butter and rapeseed oil, native or randomized, in a rat model. The regiospecific fatty acid distribution present in dietary fats was followed through absorption, chylomicron formation, and deposition in adipose tissue and in different liver lipids (triacylglycerols, phosholipids, and cholesterol esters). *Methods* Rats were fed for 6 weeks from weaning either butter oil (BO), a butteroil-rapeseed oil mixture 65:35 w/w (BR) or a randomized mixture of BR (tBR). Half of the animals were used for organ analysis, the rest for a postprandial study with the same fats and isolation of chylomicrons. The regiospecific distribution of the fatty acids present in the dietary fats was followed during metabolism by analyses of chylomicrons, depot fat and liver lipids, using regiospecific cleavage followed by TLC separation and quantification by GC. *Results* Randomization of edible fat mixtures leading to equal distribution of fatty acids between TG positions is directly reflected in the composition of chylomicrons. During clearing by lipoprotein lipase this positional distribution is abolished and the regiospecific composition of triacylglycerols in adipose tissue is completely identical for BR and tBR. Chylomicron remnants, which are taken up by the liver, are correspondingly fully degraded to free fatty acids by hepatic lipase, and distribution of fatty acids in liver triacylglycerols, phospholipids and cholesterol esters are identical for the groups fed either BR or tBR. The group fed BO with a low content of linoleic acid is on the borderline of essential fatty acid-deficiency. *Conclusion* Randomization (interesterification) of butter oil with rapeseed oil (65:35 w/w) for use as edible fat did not have any impact on the fatty acid composition beyond the chylomicron step when compared to the native mixture.

Key words Randomized (interesterified) fat – fatty acids – chylomicrons – adipose tissue – liver lipids

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Introduction

Mixtures of butter and vegetable oils are popular fats due to the combination of taste from butter and the improved nutritional value from the plant oil, furnishing mono- and polyunsaturated fatty acids counteracting the adverse effect of the saturated fatty acids on blood lipid parameters (1–3).

The direct mixing of such oils, differing in melting points, may pose some technical problems, especially with highly unsaturated oils used in high proportions. To ensure a better "mouth feel" and stabilization (4), a chemical randomization can be carried out, giving rise

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to equal positional distribution of the fatty acids in the triacylglycerols and a leveling of the melting points of the fats.

Previously, the structure of triacylglycerols (TG) was found to influence plasma cholesterol concentrations (5–7) and the absorption efficiency and even the site of absorption in the intestine (8–10). However, different opinions have been reported (11–13); the nutritional effect of the interesterification process therefore seems to need further verification.

We recently compared the influence of feeding butter oil, butter oil with 35% rapeseed oil and the corresponding transesterified mixture on blood lipid patterns of normal young volunteers (to be published). Both of the two mixtures consistently resulted in lower plasma concentrations of total cholesterol, LDL cholesterol and apo-B and higher values for HDL cholesterol and apo-A1 in comparison with butter oil alone, but no differences between the two mixtures.

Due to the conflicting reports on the effect of transesterification we wanted to ensure that such fat did not have an adverse effect on the lipid profile in metabolizing organs. The purpose of the present experiments was therefore to use the same experimental fats as mentioned above for the human trial to verify the influence of changing the regiospecific fatty acid distribution of otherwise identical fats on lipid composition and thereby membrane function in tissues not available from humans. Changes, as increased saturation of plasma membrane fatty acids, have previously been reported to affect membrane function (14, 15) which could have long term health consequences.

Naturally occurring triacylglycerols, with milk fat as an exception, contain most of the saturates in the *sn*-1 and *sn*-3 positions of the glycerol backbone. When TGs are degraded by pancreatic lipase in the intestine, the fatty acids in the *sn*-1 and *sn*-3 positions are hydrolysed and absorbed as such while most of the fatty acids at the *sn*-2 position are absorbed as 2-monoacylglycerols (16–18). As the monoacylglycerols are re-acylated in the enterocyte via the 2-monoacylglycerol pathway, the resulting chylomicron TGs retain most of the original fatty acid in the *sn*-2 position (19, 20). It is therefore important to examine whether transesterification, a process which is becoming more common in the food industry in a strive to make spreads and other edible fats according to the health recommendations, have an impact on the metabolism beyond the chylomicrons.

In the present experiment we examined the effect of a randomly interesterified mixture of triacylglycerol fatty acids from butter oil and rapeseed oil compared to a simple mixture of the same ingredients and to pure butter oil. Lipid metabolism was followed after digestion and distribution of fatty acids in chylomicrons, adipose tissue and liver determined.

Materials and methods

Animals and diets

Sixty weanling male albino SPF rats of the Wistar strain, obtained from Møllegård Breeding Laboratories (Lille Skensved, Denmark), were divided into three groups of twenty animals and maintained on one of three experimental diets for six weeks. The animals were caged two by two at 25°C and a relative humidity of 45%, with 12/12 hours dark-light cycle. Diets and water supplied *ad libitum*.

All rats were given a semisynthetic diet comprising by weight: 20% of the experimental fat, 40% corn starch (Maizena Co, DK), 10% sucrose (Danisco A/S, DK), 4% cellulose fibers, 20% casein (Milko, DK), 5% salts and trace elements, 0.5% vitamins (21) and 0.5% choline chloride. The feed was prepared in batches of 4 kg no more than two weeks before use, and was stored at –80°C until the evening before use, when it was taken out and allowed to thaw slowly overnight. The animals were given fresh feed every day.

All procedures for the animal experiments were approved by the official authorities.

Dietary fats

The dietary fats were either butter oil (BO), a mixture of butter oil and low erucic rape seed oil (65:35) (BR) or the same chemically transesterified (tBR). The butter was kindly supplied by MD Foods (Rødkærsbro, DK). The blending and transesterification of fats were done by Aarhus Olie A/S (Aarhus DK). A single lot of each fat was purchased and prepared to cover the entire study.

Regiospecific analyses of test fats were carried out according to Becker et al. (22). Due to the presence of short chain fatty acids the methyl esters were prepared with a KOH catalyzed transmethylation according to Christopherson and Glass (23).

The separation of fatty acid methyl esters was performed on a Hewlett-Packard 5880A instrument with FID and split injection on a 30 m × 0.32 mm fused silica capillary column coated with a 0.2 μm film of SP2380 (Supelco Inc., Bellefonte, PA, USA). Initial oven temperature was maintained at 35 °C for 3 min before being raised to 155 °C at a rate of 20 °C/min, then after 2 min the temperature was increased at a rate of 10 °C/min to 225 °C and maintained for 7 min.

The concentration of fatty acids was estimated by calculation with a butter oil standard (BCR, Brussels, Belgium). Fatty acids methyl esters were identified by comparing retention times with those of known standards (Nu-Check Prep, Elysian, MN).

Isolation of blood and tissues

After six weeks of feeding, half of the animals, randomly selected, were killed after an overnight fasting. They were anesthesized by abdominal injection of 80 µl Mebumal (50 g/l, Nycomed DAK, Copenhagen, DK) per 100 g body mass. After opening of the chest, blood was collected from the heart using the EDTA Venoject system (Terumo Corp., Leuven, Belgium). The liver was isolated, rinsed with cold 0.9 % (w/w) saline, blotted dry and weighed before frozen in liquid nitrogen. Perirenal fat was excised, rinsed and frozen similarly. Organs were kept at –80 °C until immediately before analysis.

Each of the remaining 30 rats (10 per group), also in the fasted state, had access to 6 g of the fats (pure) they previously had ingested during the feeding experiment, to mimic a postprandial state, and to evaluate the absorption of the test fats. The fat was eaten within one hour. Approximately 1.5 h after the ingestion, a blood sample was drawn from the heart as described above for chylomicron preparation.

Lipid analysis

Chylomicrons

Plasma was isolated by centrifugation at 1500 g for 20 min. For the isolation of chylomicrons 2.5 ml plasma was overlayed by a NaCl solution of density 1.004 g/ml and centrifuged at 27 000 g for 1 h at 15 °C in a Sorvall RC5C centrifuge. The top layer with the chylomicrons (approx. 1 ml) was aspirated and extracted by vigorous mixing with 19 volumes of chloroform/methanol, 1:1 (v/v). The phases were dried with anhydrous sodium sulphate and evaporated to dryness. The lipid was redissolved in chloroform/methanol 95:5 (v/v) with 0.005 % BHT and stored at –80 °C until analysis. Chylomicron triacylglycerols (TG) from rats belonging to the same group were pooled and three determinations of the fatty acid composition in the *sn*-2 position were carried out (24). Total TG fatty acids were determined as described for dietary fats.

Adipose tissue (perirenal fat)

Lipids were extracted with chloroform:methanol 2:1 (v/v) and fatty acid methyl esters prepared and GC carried out as described for liver (below).

Liver

The liver was extracted according to Folch et al. (25). The fatty acids in triacylglycerols (TG), phospholipids (PL) and cholesterol esters (CE) were determined after lipid class separation by thin layer chromatography on silica 60 plates (Merck, Darmstadt, Germany). The solvent system was hexane/diethyl ether/acetic acid 80:20:1 (by volume). The TG, PL and CE bands were scraped off and directly transmethylated using 12 % BF_3 in methanol (26). Fatty acid methyl esters were analyzed by GC using a Hewlett-Packard 5830 chromatograph with split injection, a 30 m × 0.32 mm fused silica column with 0.2 µm film of SP2380 (Supelco Inc. Bellefonte, PA) and FID. The oven temperature was programmed to rise at a rate of 2°C/min from 140°C to 160°C where it was maintained for 2 min before rising again at 3°C/min to 200°C.

Fatty acids methyl esters were identified by comparing their retention times with those of known standards (Nu-Check Prep, Elysian, MN).

Statistical analysis

Statistical evaluation of the data obtained from different experimental groups, which had received diets containing different fats, was carried out using a software package (Statgraphics, STSC Inc., Rockville, MD, USA). The assumption of normality was checked by the chi square test. A one way analysis of variance (ANOVA) was carried out after the assumption of variance homogenity had been verified using Bartlett's test. Where there was a significant ($p < 0.01$) difference in means in the data, the three groups were compared pair-wise by a two tailed t-test. The significance level for the test was chosen so that the probability for making a type one error for one or more entries in the table as a whole was less than 5 %. The results are presented as means ± standard deviations.

Results and discussion

Growth

During the feeding the rats gained weight at approximately the same rate. When sacrificed the rats weighed (mean ± SD): BO: 241 g ± 28, BR: 256 g ± 20, tBR: 249 g ± 28. These differences are not significant on a 5 % level (ANOVA and t-test). The decreasing tendency for BO might be a reflection of the dietary regimen which is on the borderline of EFA deficiency, as shown by the presence of 20:3(n-9), Mead acid (27) in liver TG and PL (see later).

Dietary TG

Fatty acid compositions of the three dietary fats are given in Table 1. The butter oil (BO) had about 60 % saturated fatty acids with a slightly lower content of palmitic acid than normally found in Danish butter. There was a higher than average content of oleic acid,

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Tab. 1 Composition and regiospecific distribution of fatty acids in the dietary test fats (wt%)[1]

	BO			BR			tBR		
Fatty acids	TG	2-MG	1(3)-MG	TG	2-MG	1(3)-MG	TG	2-MG	1(3)-MG
4:0	4.2	0.0	6.6	2.7	0.0	4.1	2.5	2.4	2.5
6:0	2.3	0.0	3.6	1.5	0.0	2.2	1.4	1.3	1.5
8:0	1.3	0.0	2.0	0.9	0.0	1.3	0.8	0.7	0.9
10:0	2.7	1.4	3.5	1.8	0.9	2.3	1.8	1.4	1.9
12:0	3.1	3.2	3.0	2.0	2.6	1.7	2.0	1.7	2.1
14:0	10.1	17.8	5.6	6.3	11.7	3.5	6.3	5.4	6.7
16:0	23.2	30.8	18.8	16.3	19.6	14.6	16.5	17.0	16.3
16:1 (n–7)	1.2	2.2	0.6	0.8	1.4	0.5	0.8	0.9	0.8
18:0	11.8	6.0	15.1	8.6	3.8	11.0	8.4	8.4	8.4
18:1 (n–9)	22.7	15.7	26.8	34.2	24.4	39.2	34.3	34.7	34.1
18:2 (n–6)	1.4	2.1	1.0	8.6	13.6	6.0	8.6	8.6	8.5
18:2 conj.[2]	1.6	2.6	1.0	1.0	1.3	0.9	1.0	0.8	1.0
18:3 (n–3)	0.8	1.9	0.2	4.3	7.7	2.6	4.0	4.2	3.9

BO Butter oil; *BR* Butter oil/rapeseed oil (65:35) blend; *tBR* transesterified BR.
TG Triacylglycerol, *2-MG sn*-2 monoacylglycerol, *1(3)-MG sn*-1 or *sn*-3 monoacylglycerol
[1] Means of duplicate analysis. Fatty acids present in minor amounts are not given.
[2] Conjugated linoleic acid

whereas the amount of linoleic acid was slightly lower than normal. The butter-rapeseed test fat (BR) was prepared by blending butter oil with rapeseed oil, giving a test fat with increased amounts of 18:1(n-9), 18:2(n-6) and 18:3(n-3). The fatty composition of the blend agrees well with values calculated from butter and rapeseed oil analysis. A part of this blend was transesterified (randomized) to make tBR. There was no difference in the total fatty acid composition of BR and tBR, indicating that no losses had occurred during interesterification.

The regiospecific analysis of butter oil TGs showed that palmitic and myristic acids were concentrated in the *sn*-2 position of the TG, whereas oleic acid, stearic acid and the short chain fatty acids were located in the outer positions. The analysis of the *sn*-2 position from BR test fat showed that most of the polyunsaturated fatty acid (PUFA) from rapeseed oil was found there. After transesterification of the 65:35 butter and rapeseed oil blend (tBR), the differences between the fatty acid composition of total TG, 2-monoacylglycerol and 1(3) MG were very small, indicating that an effective randomization had taken place.

Chylomicron triacylglycerols (CM-TG)

The structure of the dietary triacylglycerols may be important for the general metabolism but especially for the postprandial clearing. It has been reported the atherogenic effect of interesterified butter and peanut oil was significantly reduced when compared to the native oils (28), but others did not observe any effects on lipoprotein concentrations (29).

Except for arachidonic acid, rats having ingested BR and tBR had fatty acid profiles of the CM TG (Table 2) that could not be distinguished, but were significantly different from the BO profiles with lower contents of C14-, C16- and C18-saturates and higher amounts of C18-unsaturates. This was a direct reflection of the composition of the dietary fat. Arachidonic acid concentration may be higher in the tBR group because more of the precursor linoleic acid is made available. The fatty acid in the *sn*-2 position is conserved within the monoacylglycerol during fat absorption, whereas the *sn*-1(3) positions are hydrolysed and absorbed as free fatty acids which can be elongated. Long chain (n-3)-fatty acid deposition was not significantly different, but their presence in the chylomicrons, in spite of the fact that the parent oils contained negligible quantities of these fatty acids, requires explanation. Presumably they were made by elongation and desaturation of 18:3(n-3) in the enterocytes but they could also be obtained by exchange with cellular membranes or lipoproteins. The highest level of PUFA was found in the CM from animals fed tBR, relative to BR, which is consistent with higher concentration of 18:3(n-3) and 18:2(n-6) in the *sn*-1 and *sn*-3 positions of tBR and their greater release in the intestinal lumen and higher concentration in the enterocytes.

The differences between rapeseed oil containing fats and BO were reflected in the *sn*-2 position of the triacylglycerols in the chylomicrons. The rats having eaten BO had a higher content of saturates and a lower concentration of unsaturates. Fatty acids with four to ten carbon atoms made up approximately 4 % of the experimental diets but have as reported (30) very little effect on plasma and organ lipids. In accordance, short chain fatty acids were not present in CM but appeared to go directly to the liver via the portal vein.

The fatty acid distribution in the *sn*-2 position was

Tab. 2 Fatty acid composition of chylomicrons (wt%)[1]

	BO		BR		tBR	
Fatty acids	TG	2-MG	TG	2-MG	TG	2-MG
C12:0	1.8 ± 0.1$^{£c}$	1.1 ± 0.2	1.1 ± 0.1*	0.8 ± 0.3	1.1 ± 0.1*	0.5 ± 0.5
C14:0	7.5 ± 0.3$^{£c}$	11.7 ± 0.3$^{£c}$	5.0 ± 0.2*	8.0 ± 0.7*	4.6 ± 0.2*	4.9 ± 0.9*
C14:1 (n-5)	0.5 ± 0.1	1.5 ± 0.0^{c}	0.3 ± 0.1	0.5 ± 0.4	0.4 ± 0.1	0.3 ± 0.1*
C15:0	0.6 ± 0.1	1.6 ± 0.0$^{£c}$	0.8 ± 0.1	1.1 ± 0.1*	0.8 ± 0.1	0.9 ± 0.0*
C16:0	24.8 ± 1.0$^{£c}$	30.4 ± 0.4$^{£c}$	18.6 ± 0.7*	21.1 ± 0.1*^{c}	19.0 ± 0.8*	19.4 ± 0.1*$^{£}$
C16:1 (n-7)	1.6 ± 0.1$^{£c}$	1.9 ± 0.0$^{£c}$	1.0 ± 0.1*	1.2 ± 0.0*^{c}	1.0 ± 0.1*	1.0 ± 0.0*$^{£}$
C17:0	1.1 ± 0.1$^{£c}$	1.0 ± 0.0	0.8 ± 0.1*	0.6 ± 0.0	0.7 ± 0.1*	0.6 ± 0.3
C18:0	11.5 ± 0.5$^{£c}$	6.6 ± 0.1$^{£}$	8.7 ± 0.4*	4.6 ± 0.1*^{c}	8.8 ± 0.4*	7.2 ± 0.2$^{£}$
C18:1 (n-9)	32.2 ± 1.3	30.6 ± 0.2$^{£c}$	37.7 ± 1.5	34.8 ± 0.7*^{c}	36.9 ± 1.5	40.2 ± 1.1*$^{£}$
C18:1 isomer[2]	1.4 ± 0.1$^{£c}$	0.9 ± 0.0^{c}	2.0 ± 0.1*	0.8 ± 0.1^{c}	2.1 ± 0.1*	1.6 ± 0.1*$^{£}$
C18:2 (n-6)	3.2 ± 0.1$^{£c}$	3.3 ± 0.1$^{£c}$	10.0 ± 0.4*	14.2 ± 0.3*^{c}	10.2 ± 0.4*	12.7 ± 0.4*$^{£}$
C18:2 conj[3]	1.6 ± 0.1^{c}	1.0 ± 0.0$^{£}$	1.4 ± 0.1	0.7 ± 0.0*^{c}	1.2 ± 0.1*	1.0 ± 0.0$^{£}$
C18:3 (n-3)	0.7 ± 0.1$^{£c}$	0.7 ± 0.0$^{£c}$	3.8 ± 0.2*	5.0 ± 0.1*^{c}	3.3 ± 0.1*	3.5 ± 0.1*$^{£}$
C20:1 (n-9)	0.5 ± 0.1	0.0 ± 0.0	0.4 ± 0.1	0.0 ± 0.0	0.6 ± 0.1	0.1 ± 0.0
C20:4 (n-6)	0.9 ± 0.1$^{£c}$	0.5 ± 0.0^{c}	1.4 ± 0.1*^{c}	0.7 ± 0.0^{c}	1.7 ± 0.1*$^{£}$	0.9 ± 0.1*$^{£}$
C20:5 (n-3)	0.5 ± 0.1	0.2 ± 0.0$^{£}$	0.5 ± 0.1	0.1 ± 0.0*	0.5 ± 0.1	0.2 ± 0.0
C22:5 (n-3)	0.3 ± 0.1	0.2 ± 0.0	0.4 ± 0.1	0.2 ± 0.0	0.4 ± 0.1	0.3 ± 0.0
C22:6 (n-3)	0.9 ± 0.1	0.2 ± 0.0	0.8 ± 0.1	0.2 ± 0.0	1.0 ± 0.1	0.2 ± 0.0

BO Butter oil; *BR* Butter oil/rapeseed oil (65:35) blend; *tBR* transesterified BR.
TG Triacylglycerol, *2-MG sn*-2 monoacylglycerol
[1] Chylomicrons from ten rats were pooled. Mean of three determinations ± SD.
[2] Mainly 18:1 (n-7).
[3] Conjugated linoleic acid
* Significantly different from BO ($p < 0.003$), as determined by a two sided t-test.
$^{£}$ Significantly different from BR ($p < 0.003$).
^{c} Significantly different from tBR ($p < 0.003$).

significantly different after BR and tBR. The composition of the *sn*-2 position in the dietary fat (Table 1) was distinctly reflected in the 2-MGs (Table 2).

Our results are in accordance with other studies (13, 31, 32) reporting that TGs of fats with the same fatty acid composition but distinct regiospecific distribution give chylomicrons with similar total fatty acid composition but retain the original fatty acids in the *sn*-2 position.

In our experiments 16:0 and 18:0 were present in the CM TG in the same concentration as in the test fats. In other studies on rats (33, 34) and humans (35), it was found that stearic acid was not well absorbed (< 90 %), possibly because of formation of calcium stearate. Others, however, have found that 18:0 is well absorbed in mixed triacylglycerols (36, 37). The high absorption we found for 16:0 could be attributed to its presence in the *sn*-2 position of BO and BR. Since this ester bond is not hydrolysed, calcium soaps of 16:0 cannot be formed. Stearic acid, on the other hand, being concentrated in the *sn*-1(3) positions of the dietary TG may give rise to soap formation. However, in our experiments the composition of the CM TG does not indicate a great loss. When one considers that the majority of the fatty acids with less than 12 C-atoms are lost through the portal vein (38, 39) and do not show up in the CM, the concentration of stearic acid has probably decreased less than 10 % relative to the experimental fats. Whether this is due to poor absorption, conversion to oleic acid, which is actually increased in the CM, or fatty acid exchange in the intestinal cell is not possible to determine.

Perirenal adipose tissue

The total fatty acid composition of the triacylglycerols of the perirenal fat (Table 3) was greatly influenced by the fatty acid composition of the experimental fats.

The composition after BO feeding is for all fatty acids significantly different from BR and tBR, whereas fatty acids from triacylglycerols in these two groups are almost identical in composition. The presence of C14:1 and 16:1, and especially the amount of C18:1 is much more pronounced in depot fat than in CM. The PUFAs are on the contrary less abundant; they are apparently preferentially used for membrane synthesis.

In the 2-MG fraction, oleic acid comprises $^{2}/_{3}$ of all fatty acids in all groups and, besides the polyunsaturated fatty acids, are concentrated in this position. This is fundamentally different from the CM composition. There are absolutely no differences between perirenal adipose tissue TGs of rats fed BR or the randomized fat (tBR) which means that also the mobilization during fasting will be equal for these two dietary groups. This confirms the findings of Hodge et al. (13), who reported that the

Tab. 3 Fatty acid composition of perirenal fat from rats fed different experimental diets (wt%)[1]

	BO		BR		tBR	
Fatty acid	TG	2-MG	TG	2-MG	TG	2-MG
C12:0	1.9 ± 0.1[£ç]	0.8 ± 0.3	1.4 ± 0.1*	0.8 ± 0.4	1.3 ± 0.1*	0.7 ± 0.2
C14:0	8.5 ± 0.3[£ç]	3.5 ± 0.5[ç£]	5.9 ± 0.2*	2.4 ± 0.3*	5.8 ± 0.2*	2.1 ± 0.3*
C14:1	1.2 ± 0.1[£ç]	1.0 ± 0.1[ç£]	0.8 ± 0.1*	0.5 ± 0.2*	0.8 ± 0.1*	0.6 ± 0.1*
C15:0	1.2 ± 0.0[£ç]	0.5 ± 0.1[ç]	0.9 ± 0.0*	0.3 ± 0.1	0.9 ± 0.0*	0.3 ± 0.1*
C16:0	29.0 ± 0.7[£ç]	12.5 ± 1.1[ç£]	22.3 ± 0.8*	8.9 ± 1.0*	22.3 ± 0.9*	8.1 ± 0.7*
C16:1(n-7)	5.2 ± 0.5[£ç]	5.8 ± 1.0[£ç]	3.1 ± 0.4*	2.7 ± 0.4*	3.1 ± 0.6*	2.8 ± 0.6*
C17:0	0.6 ± 0.0[£ç]	0.2 ± 0.2	0.4 ± 0.0*	0.1 ± 0.1	0.4 ± 0.0*	0.1 ± 0.1
C17:1	0.7 ± 0.1[£ç]	0.9 ± 0.2[£ç]	0.4 ± 0.1*	0.5 ± 0.2*	0.4 ± 0.0*	0.5 ± 0.1*
C18:0	5.9 ± 0.4[£ç]	2.2 ± 0.3[£ç]	4.8 ± 0.3*	1.6 ± 0.3*	4.8 ± 0.4*	1.4 ± 0.1*
C18:1(n-9)	41.6 ± 1.0£[ç]	66.8 ± 2.8[£ç]	47.6 ± 1.1*	62.9 ± 1.0*	48.1 ± 1.1*	63.8 ± 1.1*
C18:2(n-6)	1.8 ± 0.2[£ç]	3.4 ± 0.2[£ç]	8.5 ± 0.4*	15.4 ± 1.1*	8.4 ± 0.3*	15.7 ± 0.6*
C18:3(n-3)	0.5 ± 0.0[£ç]	0.7 ± 0.1[£ç]	2.7 ± 0.1*	3.3 ± 0.4*	2.5 ± 0.1*	3.2 ± 0.2*
C18:2conj.[2]	1.9 ± 0.2[£ç]	1.6 ± 0.5[£ç]	1.1 ± 0.1*	0.9 ± 0.3*	1.0 ± 0.1*	0.8 ± 0.3*
C20:1	0.0 ± 0.0[£ç]	0.0 ± 0.0	0.2 ± 0.0*	0.0 ± 0.0	0.2 ± 0.0*	0.0 ± 0.0

BO Butter oil; *BR* Butter oil/rapeseed oil (65:35) blend; *tBR* transesterified BR.
TG Triacylglycerol, *2-MG sn*-2 monoacylglycerol
[1] Mean ± SD. N = 10.
[2] Conjugated linoleic acid.
* Significantly different from BO ($p < 0.004$).
[£] Significantly different from BR ($p < 0.004$).
[ç] Significantly different from tBR ($p < 0.004$).

metabolism of native and randomized butterfat chylomicrons was similar. The saturated fatty acids specifically found in the *sn*-2-position of the test fats and also reflected in the CM have totally disappeared in the depot fat triacylglycerols due to the total hydrolysis of CM-TG by the lipoprotein lipase and *de novo* synthesis of triacylglycerols in the adipose tissue.

Rat liver triacylglycerols

The fatty acid composition of rat liver triacylglycerols is shown in Table 4. In the final clearing after a meal, the chylomicron remnants which still contain relatively high amounts of triacylglycerols are taken up by the liver via the apoE receptor. They are fully cleaved by either hepatic lipase or other lipases present in the liver. The fatty acids are now ready for incorporation into various liver lipids, i. e. phospholipids for membrane formation, but also for re-formation of TG, which returns to the circulation in the VLDL lipoproteins.

The fatty acid composition of liver triacylglycerols is for most components comparable to that of depot fat, but a significant higher content of linoleic acid is present in liver TG and small amounts of long chain PUFAs. The BO group shows persistently significant deviations from the two other groups, which on the contrary are nearly identical in composition. The randomization of the dietary fat has not influenced the TG pool in the liver.

Tab. 4 Fatty acid composition in rat liver triacylglycerols (wt%)[1]

Fatty acid	BO	BR	tBR
14:0	2.6 ± 0.6[£ç]	1.9 ± 0.3*	1.9 ± 0.2*
14:1	0.5 ± 0.1[£ç]	0.3 ± 0.1*	0.4 ± 0.2*
15:0	0.7 ± 0.1[£ç]	0.4 ± 0.1*	0.5 ± 0.1*
16:0	26.8 ± 1.4[£ç]	21.4 ± 1.0*[£]	22.7 ± 1.0*[ç]
16:1 (n-7)	4.8 ± 1.4[£ç]	1.9 ± 0.5*	2.3 ± 0.5*
17:0	0.5 ± 0.1[£ç]	0.4 ± 0.1*	0.4 ± 0.1*
18:0	3.4 ± 0.8	3.1 ± 0.7	2.7 ± 0.6
18:1 (n-9)	49.6 ± 3.2[£ç]	43.8 ± 2.0*	44.0 ± 1.4*
18:1 isom[2]	0.8 ± 0.8[£ç]	2.3 ± 0.3*	2.5 ± 0.3*
18:2 (n-6)	2.2 ± 0.5[£ç]	12.2 ± 1.6*	11.1 ± 1.2*
18:3 (n-3)	0.4 ± 0.5[£ç]	2.6 ± 0.3*	2.4 ± 0.4*
20:3 (n-9)	0.3 ± 0.2[£ç]	0.1 ± 0.1*	0.0 ± 0.0*
20:4 (n-6)	0.4 ± 0.2[£ç]	0.8 ± 0.2*	0.7 ± 0.2*
20:5 (n-3)	0.3 ± 0.3[£ç]	1.0 ± 0.3	0.9 ± 0.2*
22:5 (n-3)	0.1 ± 0.2[£ç]	0.7 ± 0.2*	0.7 ± 0.2
22:6 (n-3)	0.9 ± 0.6[£ç]	1.8 ± 0.6*	1.5 ± 0.5*

BO Butter oil; *BR* Butter oil/rapeseed oil (65:35) blend; *tBR* transesterified BR.
[1] Mean ± SD. N = 10.
[2] Mainly 18:1 (n-7).
* Significantly different from BO ($p < 0.003$).
[£] Significantly different from BR ($p < 0.003$).
[ç] Significantly different from tBR ($p < 0.003$).

Rat liver phospholipids (PL)

In the rat liver phospholipids (Table 5) BR and tBR gave fatty acid patterns very similar to each other and different from that obtained after BO. This is consistent with a previous study (40) in which it was reported that randomization of either fish oil or peanut oil had no sig-

Tab. 5 Fatty acid composition in rat liver phospholipids (wt%)[1]

Fatty acid	BO	BR	tBR
14:0	0.5 ± 0.1[£¢]	0.3 ± 0.0*	0.4 ± 0.1*
14:1	0.1 ± 0.1[£¢]	0.0 ± 0.0*	0.0 ± 0.0*
15:0	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.0
16:0	15.9 ± 0.8[£¢]	14.9 ± 0.6*	14.6 ± 0.8*
16:1 (n-7)	2.0 ± 0.6[£¢]	0.7 ± 0.1*	0.6 ± 0.1*
17:0	0.5 ± 0.1	0.5 ± 0.0	0.5 ± 0.0
18:0	18.4 ± 1.5[£¢]	20.0 ± 0.8	21.0 ± 1.6*
18:1 (n-9)	13.3 ± 1.3[£¢]	8.4 ± 0.7*	8.2 ± 1.3*
18:1 isom[2]	2.7 ± 0.7[£¢]	2.2 ± 0.2*	2.1 ± 0.3*
18:2 (n-6)	7.7 ± 0.4[£¢]	13.6 ± 1.5*	13.1 ± 0.8*
18:3 (n-3)	0.1 ± 0.2	0.2 ± 0.0	0.2 ± 0.0
20:3 (n-9)	2.8 ± 0.3[£¢]	0.5 ± 0.1*	0.5 ± 0.1*
20:3 (n-6)	1.8 ± 0.2[¢]	1.9 ± 0.2[¢]	1.6 ± 0.2*[£]
20:4 (n-6)	12.3 ± 1.1[£¢]	19.9 ± 1.3*	20.9 ± 1.2*
20:5 (n-3)	3.9 ± 0.5[£¢]	3.2 ± 0.4*[¢]	2.6 ± 0.3*[£]
22:4 (n-3)	0.3 ± 0.1[£¢]	0.2 ± 0.0*	0.2 ± 0.0*
22:5 (n-3)	1.0 ± 0.1[£¢]	1.4 ± 0.1*	1.3 ± 0.2*
22:6 (n-3)	11.2 ± 1.0[£¢]	8.3 ± 0.8*	8.5 ± 0.7*

BO Butter oil; *BR* Butter oil/rapeseed oil (65:35) blend; *tBR* transesterified BR.
[1] Mean ± SD. N =10.
[2] Mainly 18:1 (n-7).
* Significantly different from BO ($p < 0.003$).
[£] Significantly different from BR ($p < 0.003$).
[¢] Significantly different from tBR ($p < 0.003$).

Tab. 6 Fatty acid composition in rat liver cholesterol esters (wt%)[1]

Fatty acid	BO	BR	tBR
14:0	2.3 ± 0.6[£]	3.0 ± 0.4*	2.5 ± 0.8
14:1	0.4 ± 0.3[£]	0.1 ± 0.2*	0.2 ± 0.2
15:0	3.9 ± 1.7[£]	5.5 ± 1.2*	4.7 ± 2.9
16:0	20.0 ± 4.0	18.1 ± 1.3	17.4 ± 2.9
16:1 (n-7)	7.9 ± 1.7[£¢]	3.1 ± 0.6*[¢]	4.0 ± 0.7*[£]
17:0	0.8 ± 0.3	0.5 ± 0.3	0.6 ± 0.2
18:0	5.9 ± 1.5[£]	7.1 ± 0.6*	6.2 ± 1.6
18:1 (n-9)	43.0 ± 4.6[£]	38.4 ± 3.8*	40.4 ± 4.2
18:1 isom[2]	3.0 ± 0.6[£¢]	1.7 ± 0.3*	1.8 ± 0.3*
18:2 (n-6)	2.4 ± 0.4[£¢]	8.0 ± 1.5*	6.9 ± 0.8*
18:3 (n-3)	0.7 ± 0.9[£¢]	2.2 ± 0.4*	2.2 ± 0.4*
20:4 (n-6)	1.4 ± 0.6[£¢]	4.4 ± 1.3*	3.5 ± 0.8*
20:5 (n-3)	0.5 ± 0.3[£¢]	1.2 ± 0.3*	1.0 ± 0.3*

BO Butter oil; *BR* Butter oil/rapeseed oil (65:35) blend; *tBR* transesterified BR.
[1] Mean ± SD. N=10.
[2] Mainly 18:1 (n-7).
* Significantly different from BO ($p < 0.004$).
[£] Significantly different from BR ($p < 0.004$).
[¢] Significantly different from tBR ($p < 0.004$).

nificant effect on liver phospholipids. After feeding BO a lower level of the (n-6) fatty acids 18:2 and 20:4, higher levels of 16:1(n-7), 18:1(n-9), 20:3(n-9) and 22:6(n-3) were found. In the case of butterfat only a rather limited supply of (n-6) and (n-3) fatty acids was available and the rats compensated by synthesising (n-7) and (n-9) acids *de novo* (27). This indicates that the rats were at the borderline of EFA deficiency when fed butter fat alone. The amount of 22:6(n-3) in the PL after BO feeding is greater than found for BR and tBR, despite the approximate 2:1 ratio between (n-6) and (n-3) in all three fats and the greater concentration of 18:3(n-3) in BR and tBR. This may be explained by the great importance of the competitive metabolic interactions between polyunsaturated fatty acid families during elongations, desaturations and esterifications in the final PL synthesis (27).

The interesterification (BR versus tBR) did not introduce differences in the fatty acid pattern of liver phosholipids and thereby the membrane structure. This certifies the security of using randomized fats for human consumption.

Rat liver cholesterol esters

The data (Table 6) showed that oleic and palmitic acids were the dominating fatty acid species in all groups. This indicates that liver CE is a depot for cellular cholesterol rather than an important fatty acid pool. The rapeseed oil-containing fats gave cholesterol esters with significantly ($p < 0.004$) more polyunsaturated fatty acids than BO reflecting the low supply of EFA from the butter. The concentration of arachidonic acid was three times higher for BR and tBR compared to BO but rather low. There were no significant differences between BR and tBR except for a slightly higher content of palmitoleic acid after tBR feeding, which underlines the equality of the two rapeseed oil-containing fats after the uptake in the liver.

Concluding remarks

The present results show that randomization of edible fat mixtures leading to a change in the regiospecific distribution of the fatty acids significantly affects the positional distribution of fatty acids in chylomicrons. However, during the clearing phase where lipoprotein lipase releases fatty acids for use in peripheral organs or for deposition in adipose tissues, this regiospecific difference is abolished as shown by the finding of equal composition, including positional distribution, of the triacylglycerols in the perirenal fat, after the ingestion of BR or the randomized counterpart tBR. No preferential degradation according to position was observed.

The remaining remnants engulfed by the liver are also fully digested to free fatty acids, which results in an equal supply of fatty acids to the liver pool, to be used for membrane synthesis, for lipid species of VLDL particles or for energy purposes. This finding is consistent with reports on other fats (40–43). We therefore conclude that interesterification of butter with a vegetable oil, for use as a nutritionally improved edible fat for human con-

sumption, does not have any impact on lipid metabolism beyond the chylomicron step, at least in rats.

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