Butter Blend Containing Fish Oil Improves the Level of n-3 Fatty Acids in Biological Tissues of Hamster

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Many studies have shown beneficial effects of long chain n-3 polyunsaturated fatty acids (PUFA) on human health. Regardless of the positive effects of n-3 PUFA, the intake of these fatty acids remains low. An approach to increase the intake of n-3 PUFA in the population is to incorporate fish oil into food. In the present study, fish oil was incorporated into butter blends by enzymatic interesterification. The aim of the study was to investigate the effects of this butter product in comparison with a commercial butter blend and a product produced by interesterification but without fish oil. Golden Syrian hamsters received hamster feed blended with one of the three butter products. After 6 weeks of feeding, the fatty acid compositions of plasma, erythrocytes, liver, brain, and visceral fat were determined. The intake of butter product with fish oil resulted in a higher level of n-3 PUFA in plasma, erythrocytes, and liver. The incorporation of n-3 PUFA was significantly higher in phospholipids than in triacylglycerols. The results suggest that enriching butter blends with small amounts of fish oil can be used as an alternative method for improving the level of n-3 PUFA in biological tissues.

INTRODUCTION

Fatty fish contain high amounts of the long-chain n-3 polyunsaturated fatty acids (PUFA), especially eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3). A high habitual intake of these fatty acids has been correlated with beneficial effects on the plasma lipid profile leading to a reduced risk of coronary heart disease (*1*). Furthermore, n-3 PUFA were shown to influence the brain and visual development during infancy (*2*), have immunomodulating effects (*3*), and decrease the inflammation in rheumatoid arthritis (*4*), and recent research suggests that n-3 PUFA are important for mental health as well (*5*).

Despite the increasing weight of evidence suggesting beneficial effects of n-3 PUFA, fish intake remains low in many parts of Western society. Dietary guidelines from the American Heart Association recommend an intake of two fish meals (preferably fatty fish) per week; similar suggestions also exist in Denmark. Recommendations from Western countries and organizations for daily intake of 20:5n-3 and 22:6n-3 are 0.3–0.5 g (*1*). The consumption of fish is, however, far from reaching the recommended level in many countries. In the United States, the total intake of n-3 PUFA is approximately 1.6 g/day and only 0.1–0.2 g/day come from 20:5n-3 and 22:6n-3 (*1*). The same tendency of low intake of 20:5n-3 and 22:6n-3 is seen in the Danish population (*1*).

Incorporation of fish oil into food products provides a means of increasing n-3 fatty acid intake, particularly in populations where fish consumption remains low. There have been a number of studies that have examined the effects of n-3 fatty acid-enriched food products (*6*, *7*). In Denmark, the consumption of blended butter products, that is, Kærgården consisting of rapeseed oil and butter fat (25:75, wt %), has become increasingly popular. A strategy for increasing the intake of n-3 fatty acids in the population is to add these fatty acids to blended butter products.

Interesterification of fats can change triacylglycerol (TAG) profiles (*8*) and thereby the physical properties of fat. However, it is not well-studied how the interesterification affects the nutritional value of the fats, especially after adding fish oil. The aim of the present study was to investigate the long-term effects of interesterified butter products in comparison with a simple blending as well as to investigate the effects of adding a low amount of fish oil to a blended butter product, with focusing on the incorporation of n-3 PUFA in different tissues.

MATERIALS AND METHODS

Butter Products. A commercial butter blend, Kærgården (Arla Foods amba, Viby, Denmark), consisting of 75% butter fat and 25% rapeseed oil, was used in this study. Two butter blends were produced by enzymatic interesterification, which was carried out on a Packed Bed Reactor employing previously optimized conditions (*9*) and Lipozyme TL IM (Novozymes A/S, Bagsvared, Denmark) as a biocatalyst. Two types of interesterification products [a blend of butter

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Table 1. Fatty Acid Composition of Butter Products and Feed (g/100 g)[a]

fatty acid	butter products			feed		
	commercial butter blend	interesterified butter	interesterified butter + fish oil	commercial butter blend	interesterified butter	interesterified butter + fish oil
8:0	0.4	0.4	0.4	0.5	0.5	0.4
10:0	1.5	1.5	1.4	1.2	1.3	1.1
12:0	2.2	2.3	2.3	1.6	1.7	1.7
14:0	7.9	7.7	7.9	5.6	5.6	5.7
14:1n-5	0.6	0.7	0.7	0.5	0.5	0.5
15:0	0.8	0.8	0.9	0.6	0.6	0.6
16:0	25.3	24.5	25.1	22.6	22.2	22.6
16:1n-7	1.5	1.6	1.9	1.3	1.3	1.5
18:0	9.1	8.4	8.5	6.8	6.3	6.5
18:1trans	1.6	1.9	2.0	1.2	1.3	1.3
18:1n-9	34.8	35.8	33.5	30.8	30.7	29.3
18:1n-7	1.6	1.5	1.6	1.5	1.4	1.5
18:2n-6	7.7	7.8	7.2	20.1	20.8	20.3
18:3n-3	3.7	3.7	3.5	3.8	4.0	3.9
20:1n-9	0.5	0.5	1.0	0.6	0.6	0.9
20:5n-3	–	–	0.5	0.3	0.3	0.6
22:6n-3	–	–	0.6	0.1	–	0.7
others	0.8	0.9	1.0	0.9	0.9	0.9
ΣSFA	47.2	45.6	46.5	38.9	38.2	38.6
ΣMUFA	40.6	42.0	40.7	35.9	35.8	35.0
Σn-6	7.7	7.8	7.2	20.1	20.8	20.3
Σn-3	3.7	3.7	4.6	4.2	4.3	5.2

[a] Values are means, $n = 3$. Others represent fatty acids constituting less than 0.5. A dash (–) means not detected. ΣSFA, ΣMUFA, Σn-6, and Σn-3 are the sum of saturated, monounsaturated, n-6, and n-3 polyunsaturated fatty acids, respectively.

fat (70%) and rapeseed oil (30%) and a blend of butter fat (70%), rapeseed oil (25.5%), and fish oil (4.5%) by weight] were prepared. The resulting products were subjected to deodorization at 120 °C and vacuum of 5 mbar for 2 h to remove the free fatty acids (*10*). The butter production using the resultant interesterified products was implemented in a pilot plant at Holstebro Mejeri (Arla Food A/S, Denmark), following a typical procedure for butter production (*11*). The feedstock consisted of 80% oil phase (interesterified products) and 20% water phase (17.75% water, 1.5% salt, 0.5% skim milk powder, and 0.25% Dimodan OT). The resulting products were stored at −40 °C.

Animals, Diets, and Experimental Design. The experiment was approved by the Danish National Committee for Animal Experiments and was conducted with 8−9 week old male Golden Syrian hamsters purchased from Harlan Scandinavia (Allerød, Denmark). The hamsters were housed individually in polyethylene cages in a temperature- (21 ± 3 °C) and humidity- (50 ± 15%) controlled room with a 12 h day−night rhythm. The hamsters were fed a commercial pelleted hamster maintenance diet (Altromin 7020, Brogaarden, Gentofte, Denmark) during a 5 day acclimatization period.

The hamsters were randomly divided into three groups of 10 animals each. For the following 6 weeks, they were given free access to diets composed of a powdered hamster maintenance diet (Altromin 7021, Brogaarden) supplemented with 10 g butter product per 100 g (either the commercial butter blend, the interesterified butter product, or the interesterified butter product with added fish oil). Diets were stored at −20 °C and replaced every day. The hamsters were weighed weekly.

The day before initiation of the feeding experiment, the hamsters were fasted overnight. The following morning, the hamsters weighed 93.8 ± 0.8 g, and they were anesthetized with 0.45 mL/100 g of a mixture composed of ketamin (50 mg/mL; Intervet International B.V., Boxmeer, Netherlands) and xylazin (20 mg/mL; Intervet International B.V.) in a 10:1.25 ratio (vol/vol). Blood was drawn from the retro-orbital venous plexus using ethylenediaminetetraacetic acid (EDTA)-containing hematocrit tubes (Modulohm A/S, Herlev, Denmark), and plasma was isolated using a 1−15 Sigma hematocrit tube centrifuge. Following blood collection, the hamsters were given 0.02 mL/100 g of a 5 mg/mL atipamezol solution (Antisedan, Orion, Espoo, Finland).

At the end of the experimental period, hamsters were fasted overnight and anesthetized with the ketamin−xylazin mixture, and blood was collected in EDTA-containing glasses by cardiac puncture. Plasma was obtained after centrifugation. Erythrocytes were washed three times

with 150 mM NaCl and 1 M EDTA. Livers, brains, and visceral adipose tissues were dissected and immediately frozen in liquid nitrogen and then at −80 °C until analysis.

Fatty Acid Composition Determinations. The fatty acid composition of butter products (gently melted before lipid extraction), feed, total plasma, erythrocyte, adipose tissue, and brain was determined by gas chromatography (*12*). Samples were extracted with chloroform and methanol (*13*) and methylated with a BF_3-catalyzed method (*12*). Organs were homogenized before lipid extraction. Plasma and liver lipid extracts were separated on thin-layer chromatography (TLC) plates before methylation for determination of the fatty acid composition of TAG and phospholipids (PL) (*12*). Erythrocyte membranes were burst with redistilled water before lipid extraction.

Statistical Analysis. Results are presented as means ± standard errors of the mean (SEM), $n = 10$. Statistical analysis was performed using GraphPad PRISM version 3.02 (GraphPad software, San Diego, CA). Data were analyzed by one-way analysis of variance followed by Tukey's multiple comparison post-test. Differences were considered significant at $P < 0.05$.

RESULTS

Composition of Dietary Butter Products and Feed. The interesterified butter product with added fish oil contained small amounts of 20:5n-3 and 22:6n-3 at the expense of 18:1n-9 in comparison with the two other products (**Table 1**). Otherwise, the butter products, and thereby also the feed, were comparable in fatty acid compositions.

Body and Organ Weights. Final body weights and weights of livers and brains were not influenced by the different dietary treatments (**Table 2**).

Fatty Acid Compositions of Plasma and Organs. The fatty acid composition of plasma and organs was influenced by the addition of fish oil to the interesterified butter product, while the effect of interesterification vs simple physical blending of butter fat and rapeseed oil was very limited (**Tables 3** and **4**). No long chain PUFA were detected in the adipose tissue; the only detected n-3 PUFA was 18:3n-3. In all of the other analyzed tissues, except for the brain, the levels of 20:5n-3 and 22:6n-3 and the summarized n-3 fatty acid contents were

Alternative Source of n-3 Fatty Acids

J. Agric. Food Chem., Vol. 55, No. 18, 2007 **7617**

Table 2. Body and Organ Weights (g) of Hamsters Fed Diets Containing Different Butter Products[a]

	commercial butter blend	interesterified butter	interesterified butter + fish oil
final body weight	109.6 ± 3.3	111.8 ± 2.7	118.7 ± 3.9
liver weight	3.17 ± 0.07	3.10 ± 0.09	3.21 ± 0.10
brain weight	1.01 ± 0.02	1.05 ± 0.01	1.05 ± 0.01

[a] Values are means ± SEM, $n = 10$.

Table 3. Selected Fatty Acids (g/100 g) in Total Plasma, Plasma PL and TAG, and Erythrocytes of Hamsters Fed Diets Containing Different Butter Products[a]

fatty acid	commercial butter blend	interesterified butter	interesterified butter + fish oil
	total plasma		
18:1n-9	13.3 ± 0.4	13.9 ± 0.4	13.7 ± 0.7
18:2n-6	35.5 ± 0.6	34.5 ± 0.7	34.7 ± 0.7
18:3n-3	1.8 ± 0.1 b	1.8 ± 0.1 b	2.4 ± 0.2 a
20:4n-6	9.7 ± 0.3 a	9.3 ± 0.4 a	7.5 ± 0.4 b
20:5n-3	0.8 ± 0.1 b	0.8 ± 0.1 b	1.5 ± 0.1 a
22:5n-3	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.1
22:6n-3	4.9 ± 0.3 b	5.0 ± 0.3 b	6.5 ± 0.2 a
Σn-3	8.1 ± 0.4 b	8.2 ± 0.4 b	11.1 ± 0.3 a
Σn-6	45.8 ± 0.8	44.4 ± 0.7	42.7 ± 1.0
	plasma PL		
18:1n-9	7.3 ± 0.3 a	6.7 ± 0.2 a,b	5.9 ± 0.3 b
18:2n-6	26.5 ± 1.0	25.9 ± 0.8	25.8 ± 0.5
18:3n-3	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
20:4n-6	13.9 ± 0.6 a,b	14.7 ± 0.4 a	13.1 ± 0.2 b
20:5n-3	0.7 ± 0.1 b	0.7 ± 0.1 b	1.4 ± 0.2 a
22:5n-3	0.9 ± 0.0	1.0 ± 0.0	0.9 ± 0.0
22:6n-3	7.1 ± 0.5 b	8.1 ± 0.5 b	10.9 ± 0.5 a
Σn-3	9.0 ± 0.5 b	10.1 ± 0.5 b	13.4 ± 0.4 a
Σn-6	41.7 ± 1.3	42.0 ± 0.6	39.8 ± 0.5
	plasma TAG		
18:1n-9	27.9 ± 0.4 a	27.7 ± 0.6 a	24.1 ± 0.8 b
18:2n-6	31.3 ± 0.9	31.0 ± 0.8	33.3 ± 1.4
18:3n-3	3.5 ± 0.3	3.4 ± 0.2	3.7 ± 0.3
20:4n-6	1.4 ± 0.1	1.5 ± 0.1	1.2 ± 0.1
20:5n-3	0.6 ± 0.1 b	0.6 ± 0.0 b	1.1 ± 0.2 a
22:5n-3	0.8 ± 0.0	0.7 ± 0.0	0.8 ± 0.2
22:6n-3	2.1 ± 0.1 b	1.9 ± 0.1 b	3.1 ± 0.2 a
Σn-3	7.0 ± 0.3 a,b	6.6 ± 0.3 b	8.7 ± 0.7 a
Σn-6	32.9 ± 0.9	32.8 ± 0.9	34.7 ± 1.5
	erythrocytes		
18:1n-9	15.1 ± 0.4 a	14.5 ± 0.1 a,b	13.7 ± 0.3 b
18:2n-6	19.8 ± 0.6	19.7 ± 0.2	19.0 ± 0.6
18:3n-3	0.4 ± 0.0	0.5 ± 0.0	0.5 ± 0.1
20:4n-6	17.5 ± 1.1	19.0 ± 0.4	17.8 ± 0.3
20:5n-3	0.8 ± 0.1 b	1.0 ± 0.1 b	1.6 ± 0.1 a
22:5n-6	0.3 ± 0.01 a	0.3 ± 0.06 a	0.03 ± 0.03 b
22:5n-3	1.8 ± 0.1 b	1.9 ± 0.0 b	2.1 ± 0.1 a
22:6n-3	4.2 ± 0.3 b	4.6 ± 0.2 b	6.5 ± 0.2 a
Σn-3	7.2 ± 0.5 b	8.0 ± 0.2 b	10.7 ± 0.4 a
Σn-6	41.6 ± 1.2	42.9 ± 1.1	40.6 ± 0.7

[a] Values are means ± SEM, $n = 10$. Values in a row without a common letter differ significantly ($P < 0.01$). Σn-3 and Σn-6 are the sum of n-3 and n-6 PUFAs, respectively.

significantly higher in the group fed the butter product with added fish oil in comparison with the other two groups ($P < 0.01$; with the exception of the summarized n-3 fatty acids content in plasma and liver TAG). In addition, in erythrocytes and liver TAG, the concentration of 22:5n-3 was elevated in the group fed the butter product with added fish oil in comparison with the other groups ($P < 0.01$). The increase in n-3 PUFA was at the expense of 18:1(n-9) (except for total plasma) and in the analyzed PL fractions of plasma and liver also with decreases in 20:4n-6. The summarized content of 20:

Table 4. Selected Fatty Acids (g/100 g) in Adipose Tissue, Liver PL and TAG, and Brains of Hamsters Fed Diets Containing Different Butter Products[a]
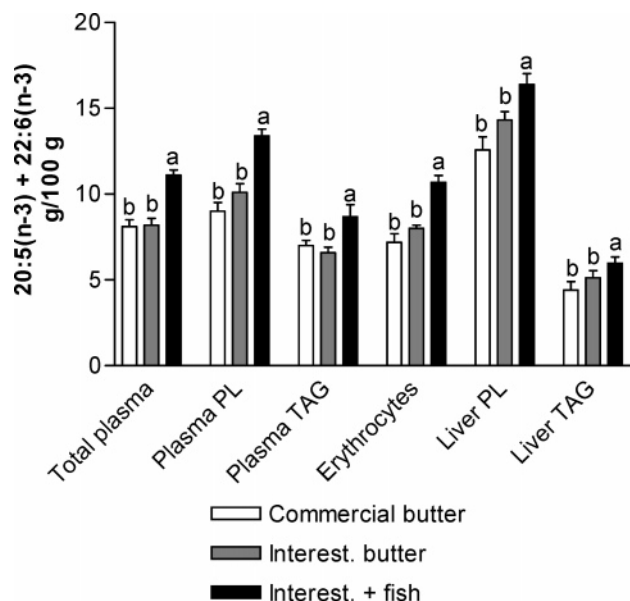
fatty acid	commercial butter blend	interesterified butter	interesterified butter + fish oil
	adipose tissue		
16:0	22.0 ± 0.1	22.2 ± 0.2	22.6 ± 0.2
18:1n-9	40.6 ± 0.2 a	40.1 ± 0.2 a	39.1 ± 0.2 b
18:2n-6	19.9 ± 0.4	20.2 ± 0.3	20.8 ± 0.3
18:3n-3	1.95 ± 0.05 b	2.08 ± 0.02 a	2.07 ± 0.04 a
20:1n-9	0.5 ± 0.0 b	0.5 ± 0.0 b	0.6 ± 0.0 a
SFA	30.2 ± 0.4	30.3 ± 0.3	30.5 ± 0.2
MUFA	47.9 ± 0.2 a	47.4 ± 0.2 a,b	46.6 ± 0.2 b
Σn-3	1.95 ± 0.05 b	2.08 ± 0.02 a	2.07 ± 0.04 a
Σn-6	19.9 ± 0.4	20.2 ± 0.3	20.8 ± 0.3
	liver PL		
18:1n-9	7.6 ± 0.2 a	7.2 ± 0.3 a,b	6.7 ± 0.2 b
18:2n-6	26.5 ± 0.5	25.5 ± 0.5	24.8 ± 0.6
18:3n-3	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
20:4n-6	14.4 ± 0.4 a	14.6 ± 0.3 a	12.9 ± 0.4 b
20:5n-3	0.8 ± 0.0 b	1.0 ± 0.1 b	1.4 ± 0.1 a
22:5n-3	1.0 ± 0.0	1.1 ± 0.0	1.0 ± 0.0
22:6n-3	10.9 ± 0.6 b	12.3 ± 0.4 b	14.2 ± 0.6 a
Σn-3	13.0 ± 0.6 b	14.6 ± 0.4 b	17.0 ± 0.6 a
Σn-6	42.1 ± 0.7 a	41.4 ± 0.7 a,b	38.8 ± 1.0 b
	liver TAG		
18:1n-9	31.8 ± 0.6 a	31.2 ± 0.7 a	27.8 ± 0.5 b
18:2n-6	29.0 ± 0.7	29.5 ± 0.6	30.2 ± 0.5
18:3n-3	2.5 ± 0.3	3.0 ± 0.2	2.8 ± 0.1
20:4n-6	1.1 ± 0.1	1.0 ± 0.1	1.1 ± 0.1
20:5n-3	0.2 ± 0.0 b	0.2 ± 0.1 b	0.5 ± 0.1 a
22:5n-3	0.6 ± 0.1 b	0.6 ± 0.0 b	0.8 ± 0.1 a
22:6n-3	1.4 ± 0.1 b	1.4 ± 0.1 b	2.2 ± 0.2 a
Σn-3	4.8 ± 0.5 b	5.2 ± 0.4 a,b	6.4 ± 0.3 a
Σn-6	30.5 ± 0.9	31.1 ± 0.6	31.8 ± 0.5
	brain		
18:1n-9	15.1 ± 0.2	15.4 ± 0.3	15.5 ± 0.2
18:2n-6	1.3 ± 0.1	1.2 ± 0.0	1.1 ± 0.1
18:3n-3	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.0
20:4n-6	11.9 ± 0.2	11.7 ± 0.2	11.5 ± 0.1
20:5n-3	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1
22:5n-3	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
22:5n-6	0.8 ± 0.0 a	0.7 ± 0.0 a	0.6 ± 0.0 b
22:6n-3	14.5 ± 0.3	14.0 ± 0.3	14.2 ± 0.2
Σn-3	14.7 ± 0.3	14.3 ± 0.2	14.6 ± 0.2
Σn-6	16.8 ± 0.1 a	16.2 ± 0.2 b	15.9 ± 0.1 b

[a] Values are means ± SEM, $n = 10$. Values in a row without a common letter differ, $P < 0.01$. Σn-3 and Σn-6 are the sum of n-3 and n-6 PUFAs, respectively.

5n-3 and 22:6n-3 was significantly higher in all analyzed tissues with the exception of brain tissue, in the group fed the butter product with added fish oil in comparison with the other two groups ($P < 0.001$; **Figure 1**). The hamsters receiving the commercial butter product had a significantly higher summarized n-6 content of the brain, as compared to the other dietary groups (**Table 4**).

DISCUSSION

The aim of the present hamster study was to evaluate the nutritional effects of enriching an interesterified butter blend with fish oil by focusing on the incorporation of very long chain n-3 PUFA into various tissues and tissue fractions (PL vs TAG). The fatty acid composition of plasma and erythrocytes can be used as biomarkers for fish intake (*14*, *15*). In the present study, the plasma and erythrocyte compositions of fatty acids revealed a higher concentration of n-3 PUFA in the hamsters receiving the butter blend with added fish oil, even though only 4.5 g/100 g of fish oil was incorporated into the butter blend resulting in

Figure 1. 1. Summarized concentration of 20:5n-3 and 22:6n-3 (g/100 g) in plasma and organs of hamsters after 6 weeks of feeding of ordinary hamster chow supplemented with 10 g/100 g of either a commercial butter blend, an interesterified butter product, or an interesterified butter product added fish oil. Values (means ± SEM, $n = 10$) not sharing a common superscript letter were significantly different ($P < 0.001$).

5.2 g/100 g of n-3 PUFA. The other two products contained 4.2 g/100 g n-3 PUFA. Examination of the fatty acid composition of the PL and TAG of plasma revealed an increase in 20:5n-3, 22:6n-3, and summarized concentration of n-3 PUFA, in both lipid classes, although to a higher degree in the PL. This is in accordance with results obtained by Leaf et al. (*16*) and can be explained by the PL high need for polyunsaturation, because of their role in the cell membranes. The extent of incorporation of n-3 PUFA into liver PL and TAG was also clearly seen in the group fed the fish oil.

The fatty acid compositions of the brains of the hamsters were not highly affected by the different butter products. Several mechanisms may be involved. First, it could be due to slow turnover of 22:6n-3 in the brain. Studies with rats have revealed that recovery of 22:6n-3 levels in brain after deprivation is slow. Wister rats were kept on a diet containing either sunflower oil or soya oil for three generations, and the examination of the offspring of the third generation after 60 days revealed that rats kept on the sunflower oil had a three-fold reduction in their n-3/n-6 ratio, as compared to soya oil-fed rats. The recovery in content of n-6 and n-3 progressed slowly to reach normal values after 2 months in the deprived rats after replacement of their diet with the soya oil diet (*17*). It suggests that the alteration of brain lipids is slow in rats and likely also in hamsters. Second, it could be due to a sufficient amount of 22:6n-3 in mature hamster brain. Edmond et al. (*18*) demonstrated that the rat brain appeared to be completely autonomous with respect to synthesis of nonessential fatty acids during development of the brain, but the activity of Δ6-desaturase, the rate-limiting step in the synthesis pathway of 22:6n-3, seemed to decrease during development (*19*). Once 22:6n-3 is synthesized in the brain, it is very efficiently retained, because of the fundamentality to the concentrating process of 22:6n-3 in the brain (*20*). If the mature hamster brain already has a sufficient amount of 22:6n-3, it is not easily affected by dietary fatty acids.

The production of the butter blend enriched with fish oil was done by enzymatic interesterification. To exclude any effects

obtained because of the enzymatic interesterification itself, a third group of hamsters was included in the study, which was fed a diet containing a butter blend consisting of almost the same composition as the commercial butter but produced by enzymatic interesterification instead of simple blending. Physical properties of the butter product were modified by the enzymatic interesterification (*21*). However, enzymatic interesterification resulted in limited nutritional effects when compared with the commercial product. This is in accordance with previous studies performed by Cristophe et al. (*22*) and Becker et al. (*23*). No effects on plasma lipid levels and fatty acid composition of TAG and PL in plasma were observed when comparing enzymatically interesterified butter or randomized product with native butter.

In conclusion, the intake of an interesterified butter product with added fish oil resulted in incorporation of fatty acids characteristic for fish oil in plasma, erythrocyte, and liver. The incorporation of 20:5n-3 and 22:6n-3 was higher in PL than in TAG.

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