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Research Article

Postprandial lipid responses of butter blend containing fish oil in a single-meal study in humans

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The postprandial effects of a butter product containing fish oil were investigated in a single-meal, randomized crossover study with a commercial butter product as the control. Twelve healthy males consumed two test meals with ^{13}C-labelled cholesterol (45 mg) and either an interesterified butter blend with fish oil (352 mg n-3 long-chain PUFA (LCPUFA)) or the commercial butter blend. Blood samples were collected after the meals and in the fasting condition on the test day and the following morning, and were analysed for cholesterol absorption, plasma lipid profile and fatty acid composition. No significant difference in the postprandial plasma fatty acid composition was observed between the groups, neither difference in cholesterol absorption, plasma cholesterol or the cholesterol contents of plasma lipoproteins. The incorporation of fish oil in the butter resulted in a significant lower concentration of triacylglycerols in the plasma 2 h after the meal in comparison with the commercial butter blend ($p = 0.02$); there was, however, no significant difference 24 h after the meal. In conclusion, fish oil-enriched butter blend provides a source to increase the intake of n-3 LCPUFA in the population, but has no acute effect on cholesterol absorption and plasma cholesterol concentration in human.

Keywords: Blended butter products / Cholesterol absorption / Fish oil / n-3 LCPUFA / Plasma lipid profile

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1 Introduction

Butter fat ranks third in the worldwide production of edible fats and oils and is a major product of the Danish dairy industry. Human metabolic studies have shown that butter fat is hypercholesterolemic when compared to other fat sources [1, 2]. An acute study also showed that ingestion of different dietary lipids modulated postprandial lipid responses differently, saturated fatty acids induced higher postprandial cholesterolemia, whereas PUFAs prevented postprandial cholesterol increase in New Zealand white rabbits [3]. Substituting butter fat with vegetable oils in the diet reduced not only total cholesterol levels, but also the levels of low-density lipoprotein (LDL) cholesterol and triacylglycerols (TAGs) [4]. Butter blends containing rapeseed oil have become increasingly popular in the Danish population [5, 6], not only because of their spreadability, but also because of the nutritional beneficial effects of rapeseed oil. The intake of butter blends in Denmark is 21 000 tons/year, which equals an approximate consumption of 3.9 kg/person/year [7].

Intervention study in healthy men showed that fish oils decreased postprandial lipaemia [8]. Similar results were also reported in moderately hypertriglyceridemic adults [9]. Harris [10] gave a critical review on fish oils and plasma lipid and lipoprotein metabolism in human. n-3 long-chain PUFAs (LCPUFAs) have many beneficial effects such as reducing inflammation responses [11, 12], reducing the risk of coronary heart disease [13], and recent research suggest that n-3 LCPUFA are important for mental health as well [14]. A high intake of these fatty acids is therefore encouraged. The Danish health authorities recommend a weekly intake of 200 – 300 g of fatty fish [15], which is in good agreement with the recommendations from US about 0.65 g/day of 20:5n-3 and 22:6n-3 [16].

Regardless of the accumulating evidence suggesting positive effects of n-3 LCPUFA, the intake of n-3 LCPUFA in the population is low [6]. Enrichment of butter blends with

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Abbreviations: IAUC, incremental area under the curve; **LCPUFA,** long-chain PUFA; **LDL,** low-density lipoprotein; **TAG,** triacylglycerol

fish oil may provide the means to increase the intake. Furthermore, addition of fish oil may enhance the nutritional value of blended butter products or reduce some of the negative effects from the butterfat in the blended butter product.

The aim of the present study was to study the postprandial effects of butter blends containing 4.5% fish oil or no fish oil in a single-meal study in healthy men. The effect of the two butter products was assessed on the absorption of ^{13}C-labelled cholesterol from the test meals, the postprandial responses in plasma cholesterol and TAG, along with the lipoprotein content of cholesterol and the plasma fatty acid profile.

2 Materials and methods

2.1 Ethics

The study protocol followed the tenets of the Declaration of Helsinki II and was approved by The Ethics Committee of the Copenhagen district, Denmark (KA 05014g). All volunteers were healthy young males who gave written consent before participating in the study.

2.2 Subjects

Twelve healthy men aged 25 – 34 years, with BMI of 23.3 ± 2.5 participated in the study. Inclusion criteria for the participants: aged between 20 and 40 years, normal weight (BMI < 25), nonsmokers. None of the participants had recently donated blood, was suffering from a chronic disease, or taking medicine during the study. Furthermore, the participants did not use fish oil supplements and their habitual consumption of fish was limited to a maximum of two servings *per* week.

2.3 Study design

The study was performed as a randomized, crossover design where all the participants consumed two test meals; one containing the commercial product Kærgården and the other with the interesterified butter product containing fish oil. The two test meals were given with a wash out period of at least 1 wk.

At the days of the experiments, the participants arrived at the clinic in the morning after an overnight fast. After having their height and weight measured, a cannula was inserted into a vein in the arm, from which blood samples were collected during the day. All blood samples were taken into EDTA vacutainer and plasma was separated by centrifugation (1500 × *g*) for 15 min at 4°C. After drawing of a fasting blood sample, the test meals were served. Blood samples were collected at an hourly interval until 7 h after ingestion of the test meal. A light lunch was served after drawing of the fourth hour blood sample. At the end of the test day, the participants were instructed to avoid high cholesterol containing food and fish products for the rest of the day, and to fast from 9:00 p.m. to the next morning, when the participants returned for collection of a 24 h blood sample.

2.4 Test meals

A commercial butter blend, Kærgården (Arla Foods amba, Viby, Denmark), was used as the control fat. It contains 17% water and 80% fat (75% butter fat and 25% rapeseed oil). A butter blend was produced from butter fat (70%), rapeseed oil (25.5%) and fish oil (4.5%) by enzymatic interesterification, which was carried out on a Packed Bed Reactor employing previous optimized conditions [17] and Lipozyme TL IM as a biocatalyst. The resulting product was subjected to deodorization at 120°C and vacuum of 5 mbar for 2 h to remove the free fatty acids [18]. Butter was produced with the resultant interesterified blend in a pilot plant at Holstebro Mejeri (Arla Food A/S, Denmark), following a typical procedure for butter production [19]. The feedstock consists 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). Both butter products were stored at – 40°C until their use in test meals.

The participants ingested 40 g of either of the two butter products (32 g fat), *ca.* 352 mg n-3 LCPUFA from the product with the fish oil. The butters were served on three pieces of white bread (144 g) with one table spoon of strawberry jam. Forty-five milligrams of ^{13}C-isotope-labelled cholesterol (3,4-^{13}C2, 99%, powder, Cambridge Isotope Laboratories) was sprinkled out on the top of the food. The energy content of the test meal was 2804 kJ (670 kcal) of which 8% came from proteins, 44% from carbohydrates and 48% from fat. The participants were allowed to drink either one cup of coffee or tea along with the meal. Water was permitted *ad libitum* throughout the day. An almost fat-free lunch was served after the fourth hour blood sample, which consisted of cucumber (~100 g), half an apple (~64 g) and two pieces of flat bread (~18 g). The energy content of the lunch was 425 kJ (102 kcal) of which 11% came from proteins, 84% from carbohydrates and 5% from fat (< 1 g fat).

2.5 Cholesterol content of butter products

The cholesterol content of the two butter products was determined by GC as described by Fletouris *et al*. [20].

2.6 Fatty acid composition

Fatty acid composition of butter products (gently melted before lipid extraction) and total plasma was determined after extraction with chloroform and methanol [21], methylation with BF_3-catalysed method, and analysis by GC [22].

2.7 Plasma lipids

The contents of total cholesterol and TAG in plasma were determined using a Cobas Mira (Roche, USA) and enzymatic kits (total cholesterol and TAG from Horiba ABX Diagnostics). The cholesterol distribution among plasma lipoproteins was determined by online fast phase LC (FPLC) as described previously [23]. The cholesterol content in LDL and HDL was determined by external standards (LDL and HDL cal from Horiba ABX Diagnostics). The intra- and interassay coefficients of variation were less than 3% using a reference human plasma sample.

2.8 Cholesterol absorption

The lipids in plasma were extracted as mentioned above after adding the internal standard stigmastanol (Sigma), followed by saponification catalysed with KOH. The cholesterols were analysed by GC/combustion/isotope ratio MS (GC/C/IRMS) (Delta^{PLUS}; Thermo-Finnigan, Bremen, Germany) for quantification of cholesterol and determination of the $^{13}C/^{12}C$ ratio for cholesterols. Samples were analysed on a fused-silica capillary column (Zebron ZB-5, 60 m × 0.25 mm, 0.2 μm film thickness, Phenomenex, Torrance, CA) with the following temperature program: the initial temperature 280°C was maintained for 1 min and then raised to 310°C at 2°C/min, where it was maintained for 20 min. The injector was used at 320°C and in the split mode with a ratio of 1:15. The $^{13}C/^{12}C$ ratio of cholesterol carbon atoms was quantified. The stability of the mass spectrometer was checked everyday by standard on–off test (18 injections of CO_2) and B-scan (control of all mass values, especially water, nitrogen and argon) and with CO_2 injections in the beginning and end of each run. Correct determinations of sample $^{13}C/^{12}C$ ratios were checked by standard samples with known δ values. The calculations were done as previously described [24].

2.9 Statistical analysis

The postprandial responses of plasma total TAG, cholesterol and ^{13}C-labelled cholesterol were analysed as incremental areas under the curves (IAUC) [25]. The responses were calculated as IAUC by the trapezoid model after subtraction of the baseline value, to get changes after the meals. To compare the two butter products, a paired *t*-test of the IAUC obtained was performed. The fatty acid composition of plasma and the sum of 20:5n-3 and 22:6n-3 in plasma were evaluated by a paired *t*-test where the value at each time point was compared. Statistical analysis was performed using GraphPad Prism version 3.02 (GraphPad Software, San Diego, CA, USA). Differences were considered significant at $p < 0.05$.

Table 1. Major fatty acid composition of butter products (wt%)

Fatty acid	Commercial butter blend	Interesterified blend add. fish oil
4:0	1.6	1.5
6:0	1.3	1.2
10:0	2.0	1.8
12:0	2.6	2.6
14:0	7.9	7.7
16:0	25.1	23.7
16:1(n-7)	1.4	1.8
18:0	8.6	7.8
18:1(n-12)	1.6	1.0
18:1(n-9)	31.9	31.9
18:1(n-7)	1.6	1.6
18:2(n-6)	6.9	6.8
18:3(n-3)	3.4	3.4
20:5(n-3)	–	0.5
22:6(n-3)	–	0.6
SFA	51.1	48.5
MUFA	38.1	39.7
(n-6)	7.4	7.2
(n-3)	3.4	4.6
(n-3)/(n-6)	0.5	0.6

Values are the means of three determinations. A dash (-) means: not detected. SFA, MUFA, n-6 PUFA and n-3 PUFA are the sum of saturated, monounsaturated, (n-6) polyunsaturated and (n-3) PUFAs, respectively. (n-3)/(n-6) PUFA is the ratio of the total sum of (n-3) to (n-6) fatty acids.

3 Results

3.1 Cholesterol contents and fatty acid composition of butter products

The concentration of cholesterol in the commercial product Kærgården was 1.2 mg/g, and 1.3 mg/g in the interesterified product added fish oil. The major fatty acid compositions of the two butter blends are shown in the Table 1. The interesterified butter product contained a low concentration of fish oil, supplying LCPUFA 20:5n-3 and 22:6n-3. The butter blends were otherwise comparable in their fatty acid composition.

3.2 Fatty acid composition of plasma

All plasma samples of the subjects after ingestion of the two butter products were analysed for their fatty acid compositions. The fatty acid composition in the fasting state of the subjects was similar for the two experimental days (data not shown). The concentration of n-3 LCPUFA (20:5n-3 and 22:6n-3) tended to be higher from 2 to 6 h after ingestion of the butter product containing fish oil compared with that after taking the commercial butter blend and it reached significance ($p = 0.049$) 3 h after meal ingestion (Fig. 1).

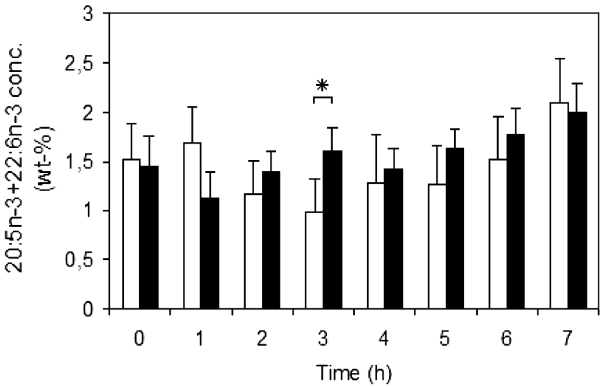

Figure 1. Summarized content of C20:5n-3 and C22:6n-3 (wt%) in plasma of subjects having either consumed the commercial butter product (white bars) or the interesterified butter product added fish oil (black bars). Columns represent means (±SEM, $n = 12$). A star (*) indicates that means differ significantly, $p < 0.05$.

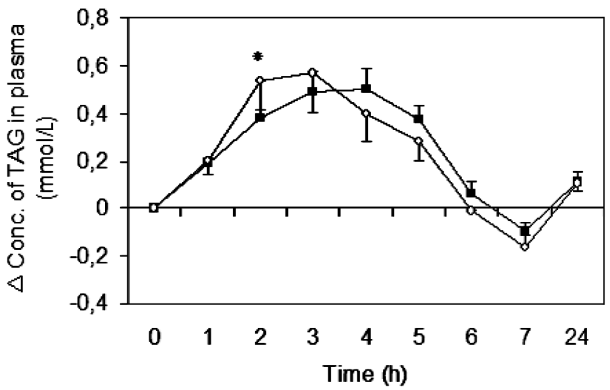

Figure 2. Incremental plasma TAG after consumption of test meals containing either the commercial butter product (○) or the interesterified butter product containing fish oil (■). Values are means at a given time with the baseline values subtracted, the vertical bars represent the SEM, $n = 12$. A star (*) indicates that means differ significantly, $p < 0.05$.

Table 2. Cholesterol contents of lipoproteins

	Commercial butter blend	Butter blend added fish oil	*p*-values
Baseline:			
HDL (mmol/L)	1.17 ± 0.12	1.11 ± 0.09	0.68
LDL (mmol/L)	3.44 ± 0.27	3.07 ± 0.24	0.32
LDL/HDL	3.19 ± 0.32	2.86 ± 0.23	0.33
24 h:			
HDL (mmol/L)	1.21 ± 0.08	1.14 ± 0.07	0.44
LDL (mmol/L)	3.75 ± 0.35	3.38 ± 0.30	0.24
LDL/HDL	3.14 ± 0.22	3.00 ± 0.25	0.40

Cholesterol contents of lipoproteins and ratio of LDL- to HDL-cholesterol at baseline and 24 h after having consumed commercial butter product or an interesterified butter product added fish oil. Values are means ± SEM, $n = 12$.

3.3 Cholesterol absorption, plasma concentration and lipoprotein profile

The absorption of exogenous cholesterol was monitored in subjects by GC/C/IRMS after administering ^{13}C-labelled cholesterol. The incremental area under the absorption curve was not significantly different between the two dietary treatments (data not shown). There was no significant increase in the total concentration of plasma cholesterol during the day after ingestion of the butter blends ($p = 0.20$). The IAUC response after the commercial and the interesterified product with fish oil was -0.54 ± 1.77 and 4.63 ± 1.88 mmol × h/L, respectively ($p = 0.08$). No significant differences were observed in the lipoprotein profile of the plasma cholesterol at baseline or 24 h after the test meals between the days they participated in the test (Table 2).

3.4 TAG in plasma

The concentration of TAG in plasma in the fasting state was 0.81 ± 0.28 and 0.73 ± 0.18 mmol/L for the days the subjects had the commercial butter product and the interesterified butter product added fish oil, respectively ($p = 0.19$). The maximum levels of plasma TAG were observed at 3 h after consumption of the commercial butter blend, and at 4 h after consumption of the interesterified butter blend added fish oil. The means differed significantly in the third hour ($p = 0.02$) (Fig. 2). However, the incremental areas under the postprandial plasma total TAG curves did not differ after consumption of the commercial butter blend and the interesterified butter blend added fish oil ($p = 0.71$).

4 Discussion

The aim of the present study was to investigate the postprandial effects of ingestion of a butter blend in which fish oil was incorporated by enzymatic interesterification. The plasma cholesterol concentration has been reported to be positively related with the intake of saturated fatty acids [26], and more cholesterol was detected in lipid-rich lipoproteins after a meal containing saturated fatty acids than after a meal containing PUFA [27]. The hypothesis was that the saturated fatty acids in the butter may have negative effects, but that these could be counter balanced by adding fish oil to the butter blend. Addition of fish oil to the butter blend increased the content of 20:5n-3 and 22:6n-3 and concomitantly decreased the relative content of other fatty acids, primarily 16:0 and 18:0. However, we did not observe any difference in cholesterol absorption and plasma cholesterol in the subjects after the two test meals. Instead, a small change in the postprandial plasma TAG response was observed.

Previous studies have shown that dietary lipids affect cholesterol absorption and cholesterol concentration in plasma. Long-term intervention study showed that substitution of MUFAs for saturated fatty acids reduced plasma cholesterol concentrations [28], diet rich in PUFAs also reduced LDL-cholesterol concentration [29]. A short-term study showed that rapeseed oil tends to decrease cholesterol absorption and serum levels of cholesterol compared to olive oil [30]. Acute ingestion of different dietary lipids showed that PUFA prevented postprandial cholesterol increase in New Zealand white rabbits [3]. Controversial results have been reported regarding the effects of fish oil on cholesterols. Vognild *et al*. [31] and Hansen *et al*. [32] found that neither total cholesterol, nor the HDL-cholesterol concentrations were altered after 12 or 7 wk of supplementation with fish oil. Supplement of fish oil for patients with coronary artery disease increased plasma LDL-cholesterol [33]. A study in African Green monkeys has demonstrated that dietary fat could influence cholesterol absorption. The monkeys were fed diets enriched in lard, oleinate (oleic acid-rich safflower oil) or fish oil at two levels of dietary cholesterol. The study revealed a reduction in cholesterol absorption from the high cholesterol containing diet (0.77 mg/kcal), when the monkeys were fed oleinate or fish oil, compared to lard [34]. At lower level of dietary cholesterol (0.05 mg/kcal), however, cholesterol absorption was not affected by the type of dietary fat. In the present study we investigated the possible effect of a single dose of only 0.35 g of n-3 LCPUFA on cholesterol absorption when this was supplied as 0.14 – 0.15 mg cholesterol/kcal in the meals. We did not observe the effect on cholesterol absorption either. The above-mentioned results suggest that both the level of n-3 LCPUFA and cholesterol should be considered for discussions.

To our knowledge this is the first study regarding the effect of butter blends containing fish oil on cholesterol absorption. The plasma dual stable-isotope ratio method has been used previously, this method however involves intravenous injection of isotope-labelled cholesterol with simultaneous oral administration of a differently labelled cholesterol, which results in high study cost. In the present study a simpler single-isotope method was used to trace the absorption of cholesterols after injection of the two different butter blends. ^{13}C-labelled cholesterol was absorbed similarly from the two different diets. The results suggest that 4.5% fish oil in the butter blend does not play an important role in acute cholesterol absorption. It has however been shown that n-3 PUFA *via* effects on transcription factors can upregulate 7a-hydroxylase mRNA in mouse liver leading to an increase in bile acid and cholesterol excretion [35]. Therefore it cannot be rule out that a long-term consumption of the butter blend containing fish oil may affect the cholesterol levels in plasma.

The only immediate effect of the fish oil in the present study was a change in the postprandial TAG-response observed in the blood sample collected after 3 h. However, the IAUC after ingestion of the two different butter products did not differ significantly. Ingestion of fish oil for longer periods has shown consistently reducing TAG levels of plasma [10], and it has been suggested that the major effect of n-3 fatty acids is to suppress the secretion of apoB [9]. Short-term study has also shown that n-3 PUFA significantly reduced plasma TAG levels when a high dose of fish oil concentrate was used [8], otherwise differences in single-meal fatty acid composition exert little or no effect on postprandial changes in plasma lipids [36 – 38]. Recently Zampelas *et al*. [39] reported that Northern and Southern Europeans had different postprandial lipaemic responses. Saturated fatty acids increased the concentration of TAG in plasma [27]. Butterfat with short- and medium-chain fatty acids, however, resulted in lower postprandial lipaemia in a single-meal study in young men than oils containing unsaturated fatty acids [40]. This may contribute to the fact that we did not see any effect of n-3 LCPUFA. Many factors may contribute to the lack of influence of fish oil in the present study, which include the low dose of fish oil in comparison with the high background saturated fat from the butter blend. Therefore, there was no obvious advantage of adding fish oil to the butter blend for short-term applications.

In conclusion, there was no significant difference in the postprandial response in plasma TAG, total cholesterol and cholesterols in different lipoproteins between the normal butter blend and the butter blend containing 4.5% fish oil. ^{13}C-labelled cholesterols were absorbed similarly in the two different diets. However, the absence of acute postprandial effect of adding fish oil in butter blend cannot rule out potential benefits for long-term consumption of this butter blend in comparison with normal butter blend. This, however, need to be further studied.

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