

Metabolic effects of interesterified dietary fat in humans:
a literature review

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Summary

Partial hydrogenation of vegetable oils is used to convert liquid oils into semi-solid fats. This process results in the formation of *trans* fatty acids, which have unfavorable effects on cardiovascular disease. Alternatively, interesterification can be used to modify the characteristics of edible oils and fats for food manufacturing purposes. During interesterification the arrangement of the fatty acids on the glycerol backbone of a triacylglycerol (TAG) molecule is changed, with no change in the overall fatty acid composition of the fat, so that no *trans* fatty acids are introduced. It is important to know the effects of interesterified fats on metabolic health in humans. This review summarizes studies in which men or women were fed interesterified fats and oils with palmitic acid (16:0) or stearic acid (18:0) as the major fatty acids of interest.

The majority of longer-term studies showed no effect of interesterification on the ability of fats to influence the fasting serum lipoprotein profile. In one study, however, an interesterified 18:0-rich fat adversely affected the fasting serum total to HDL cholesterol ratio, a well-known risk marker for cardiovascular disease, when compared to palm olein. This effect could not be explained by differences in fatty acid profiles.

Results from short-term studies on postprandial lipemia are less consistent. The majority of studies that did see a change, found a decrease in postprandial lipemia with interesterified fat. It was suggested that this decrease was due to a reduction in high melting point solids, rather than to an effect of interesterification per se. A smaller lipid response after a meal may lower cardiovascular risk. Postprandial responses of plasma levels of glucose, insulin, and FVII (a marker for coagulation tendency) response were also not systematically affected by interesterification. However, one study showed adverse effects on fasting and postprandial glucose concentrations of an interesterified fat rich in 18:0 as compared to palm olein.

In conclusion, results of one study need to be confirmed or rejected. Also, it is advised to include in future studies effects on inflammatory markers. Overall, however, the studies discussed do not suggest that at current intakes interesterified fats have adverse effects on the metabolic parameters examined.

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1. Introduction

Partial hydrogenation of vegetable oils has been widely applied by the food industry. During this process, *cis*-unsaturated fatty acids are partially converted into saturated fatty acids, which result in the conversion of liquid vegetable oils into semi-solid fats. Until recently, such fats were used for the production of foods such as margarines, frying fats and oils and bakery foods. Partially hydrogenated vegetable oils have more desirable physical properties, such as a longer shelf life and increased stability during deep-frying. In addition, they are semi-solid, which may enhance palatability. Unfortunately, partial hydrogenation also converts the *cis*-unsaturated double bonds into *trans*-unsaturated double bonds. These so-called *trans* fatty acids have an unfavorable effect on the serum lipoprotein profile (1). Further, consumption of *trans* fatty acids is strongly related to an increased risk of coronary heart disease (2). This has led food authorities to legislate on the labelling of *trans* fatty acids and to issue advice on limiting intakes (3). Also, the food industry and consumers have reacted so that intakes of *trans* fatty acids have substantially decreased during the recent years (4).

As an alternative to partial hydrogenation, the food industry can use chemical interesterification (or randomization) to modify the physical and functional characteristics of edible fats and oils. During chemical interesterification the arrangement of the fatty acids on the glycerol backbone of a triacylglycerol (TAG) molecule is changed, with no change in the overall fatty acid composition of the fat, so that no *trans* fatty acids are introduced. The product of this process is a TAG where the fatty acids are randomly distributed over the glycerol backbone (5, 6). Typically, in most vegetable oils unsaturated fatty acids occupy the *sn*-2 position and saturated fatty acids the *sn*-1 or *sn*-3 (*sn*-1,3) positions (**Table 1**). Thus, interesterification of vegetable fats and oils - or of mixtures of these oils with other fats (e.g. fully hydrogenated oils) - will lead to products with a higher proportion of saturated fatty acids at *sn*-2 (and consequently a lower proportion of unsaturated fatty acids at *sn*-1,3) than the native vegetable oil (7). It is therefore important to have detailed knowledge about the metabolic and health effects of these interesterified TAGs. This review now thoroughly summarizes human studies that have been conducted so far on the effects of interesterified TAGs on a variety of blood parameters related to metabolic health.

Table 1. Positional distribution of fatty acids in dietary triacylglycerols.*

Source	Position	Fatty acid (% mol)					
		Myristic acid (14:0)	Palmitic acid (16:0)	Stearic acid (18:0)	Oleic acid (18:1)	Linoleic acid (18:2)	α -Linolenic acid (18:3)
Palm oil	1		60	3	27	9	-
	2		13	<1	68	18	-
	3		72	8	14	3	-
Olive oil	1		13	3	72	10	1
	2		1	-	83	14	1
	3		17	4	74	5	-
Soybean oil	1		14	6	23	48	9
	2		1	<1	22	70	7
	3		13	6	28	45	9
Shea butter	1 and 3		4	80	10	5	-
	2		1	3	79	17	-
Cocoa butter	1		34	50	12	1	1
	2		2	2	87	9	-
	3		37	53	9	<1	-
Pig (outer back)	1	1	10	30	51	6	-
	2	4	72	2	13	3	-
	3	-	-	7	73	8	-
Beef (depot)	1	4	41	17	20	4	1
	2	9	17	9	41	5	1
	3	1	22	24	37	5	1
Cow's milk	1	11	36	15	21	1	-
	2	20	33	6	14	3	-
	1	7	10	4	15	<1	-

*References (7-9)

2. Metabolism

The positional distribution of fatty acids in dietary TAGs can affect the process of intestinal absorption. This process begins with the hydrolysis of TAG in the stomach by lingual and gastric lipases, which preferentially hydrolyze the *sn*-3 ester bond resulting in formation of *sn*-1,2 DAGs. These DAGs and the remaining TAGs are hydrolyzed in the duodenal lumen by pancreatic lipase, which acts mainly on the *sn*-1 and *sn*-3 position of TAG molecules, releasing 2-monoglycerides (MAG) and free fatty acids (FFA) (10).

Uptake of MAG and FFA by intestinal epithelial cells occurs by passive diffusion through the plasma membrane or via an active transport system present in the epithelium. Within the epithelial cells, the fate of fatty acids depends on the chain length. Fatty acids of short and medium chain length (≤ 10 carbon atoms) enter the portal vein. Long-chain fatty acids (≥ 12 carbon atoms), in particular those with ≥ 14 carbon atoms, or their MAG, are converted back into TAG.

These TAGS are packed within the enterocyte into lipoproteins, which are stable for transport in the aqueous blood environment. The intestine secretes mainly chylomicrons (CM) and the liver very-low density lipoproteins (VLDL). During fasting, VLDLs are the major TAG-rich lipoproteins in the circulation and following a fat-containing meal, CM become the major ones.

Enzymes on cells lining the blood vessels (lipases) catalyze the hydrolysis of TAG into glycerol and FFA. These can subsequently be absorbed in peripheral tissues, such as adipose tissue and skeletal muscle tissues for energy and storage. Lipases also help in the production of low-density lipoprotein (LDL) from VLDL. LDL is the major cholesterol transporting lipoprotein in the plasma and is responsible for the delivery of sterols to the cells for growth, and for hormone or bile acid production. HDLs are synthesized by the liver and are capable of picking up cholesterol from cells. HDL is considered the 'good' cholesterol, as it can remove cholesterol from lesions and returning it to the liver for excretion or re-utilization. This is in contrast to LDL, which is considered to be the 'bad' cholesterol, as it is responsible for bringing the cholesterol to the arteries where it can accumulate and cause plaque forming (atherosclerosis).

Hydrolyzed long-chain saturated fatty acids present in the intestine have melting points above body temperature and may therefore less readily absorbed. In addition, they can form insoluble soaps with Ca^{2+} and Mg^{2+} . Therefore, it has been suggested that long-chain saturated fatty are better absorbed if attached at the *sn*-2 than at the *sn*-1,3 positions (11, 12). In animals and in very young infants it has been documented that 16:0 and 18:0 are more efficiently absorbed when attached to the *sn*-2 as compared to *sn*-1,3 (13, 14).

CM-TAG retains some of the original fatty acids at the *sn*-2 position. Fatty acids attached to the *sn*-2 position might be preferentially transported to the liver instead of the extra-hepatic organs because LPL prefers to hydrolyze fatty acids at *sn*-1 and *sn*-3. The residual TAGs that remain in the CM-remnant after hydrolysis are therefore an important source of hepatic fatty acids. As the hepatocyte is the major site of action of fatty acids on VLDL metabolism, it has been postulated that saturated fatty acids in the *sn*-2 position might affect hepatic lipid metabolism differently than the same fatty acids in the *sn*-1 or *sn*-3 position (6, 12, 15).

3. Palmitic acid (16:0)

Several studies have been carried out to examine the effects of the positional distribution of palmitic acid (16:0) on fasting lipoproteins and postprandial lipemia (**table 2**).

Zock et al. (15) have compared the effects of palm oil and interesterified palm oil on serum lipid and lipoprotein concentrations in 60 healthy normolipidemic volunteers. For palm oil, 82% of 16:0 was esterified at the *sn*-1,3 position and 18% at the *sn*-2 position. For the modified palm oil diet, 35% of 16:0 was attached to the two outer carbon atoms and 65% at *sn*-2. Volunteers received the two diets in random order for three weeks. Dietary periods were not separated by a wash-out period. All foodstuffs were supplied individually. However, subjects were allowed to consume a limited number of food items, free from fat and cholesterol, which provided 9 to 10% of total daily energy. The experimental fats provided 70% of total fat intake or 28% of daily energy. For the whole study population, serum total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides concentrations did not

change. In men, however, serum total and LDL cholesterol concentrations increased marginally - though statistically significant - by respectively 0.10 mmol/L and of 0.08 mmol/L on the modified palm oil diet. The authors however concluded that despite a large difference in the position of 16:0 on the TAG molecule serum lipoprotein concentration were hardly affected. In another study, **Nestel et al.** (16) have compared the effects of two high-palm oil blends in 27 hypercholesterolemic men. These blends were identical in their fatty acid compositions, but one blend was interesterified. For the native blend, 22% of 16:0 was attached to *sn*-2 in contrast to the interesterified blend where 52% of 16:0 was esterified at *sn*-2. This enrichment of 16:0 at position *sn*-2 was at the expense of unsaturated fatty acids (18:1 and 18:2). The men received the test diets in random order for three weeks and these dietary periods were not separated by a wash-out period. The test diets provided 31-32% of total energy as fat and the experimental fats provided almost 60% of total fat intake. The process of interesterification did not influence the plasma lipoprotein profile. **Christophe et al.** (17) have compared the effects on the serum lipoprotein profile of butter with those of enzymatically interesterified butter. For this, 32 healthy men consumed for 18 days a diet in which all spreads, and cooking and baking fats were replaced by butter. Subjects were then equally divided over two groups. One group continued to consume the butter-rich diet and the other group consumed interesterified butter. Daily intake of the experimental fats for the two groups were respectively 16.5% and 18.5% of energy. No effects on the serum lipoprotein profile were observed.

Effects of the positional distribution of palmitic acid in dietary TAG on postprandial lipemia have also been studied. Postprandial lipemia represents the increase in serum TAG concentrations after ingestion of a meal and this increase is mostly expressed as area under the curve (AUC) during a certain time period. An extreme postprandial response is associated with an increased risk to develop atherosclerosis. CM-remnants can contribute to the formation of foam cells, while postprandial lipemia may also increase the risk for cardiovascular diseases by increasing factor VII coagulant (FVIIc) activity.

Zampelas et al. (18) monitored the postprandial response in 16 men over a 6 h time period after consumption of test meals containing dietary TAG in which 16:0 was predominantly at the *sn*-1 (control blend) or at the *sn*-2 position (Betapol). The liquid

meals provided 40 gram test fat, which accounted for 54% of total energy. In the control meal, only 6% of 16:0 was esterified at *sn*-2, in contrast to the interesterified fat in which 73% of 16:0 was attached to *sn*-2. The results showed that there were no statistically significant differences between the two test meals for the AUCs as calculated from postprandial TAG responses in total plasma, and in CM-rich and CM-poor plasma. Thus, these results suggested that enrichment of the *sn*-2 position of dietary fatty acids with 16:0 is not an important determinant of postprandial lipemia when dietary TAG were fed in amounts that can be consumed as part of a standard meal. **Yli-Jokipii et al.** (11) have also investigated whether interesterified palm oil influenced postprandial lipemia by measuring in 10 healthy women the lipemic response over 6 h. Except for a small percentage from skim milk, all the fat (55 gram per m². Assuming a body surface area of 1.6 m² for women, this would equal 90 gram) was derived of the test fats. In the native palm oil, 10% of the major isomers was 16:0 esterified at *sn*-2. For the interesterified fat, this value was 31%. The TAG with 16:0 predominantly at *sn*-1,3 (native palm oil) caused a larger incremental AUC of total plasma TAG concentrations compared with that of the interesterified palm oil. 16:0 randomly distributed. Thus, it can be concluded that moving 16:0 from *sn*-1,3 to *sn*-2 reduced the postprandial TAG response. **Berry et al.** (19) conducted two randomized cross-over trials in 20 men, in which postprandial lipid responses were compared between native palm oil and interesterified palm oil, and between interesterified palm oil and high-oleic sunflower oil. The meals contained 50 gram of the test fats, which accounted for 53% of total energy. In native palm oil, 7.2% of the *sn*-2 position was occupied with 16:0 in contrast to interesterified palm oil in which 37.2% of the *sn*-2 position contained 16:0. The results suggested that, compared with native palm oil, interesterified palm oil tended to decrease postprandial plasma TAG concentrations although the difference between the AUC did not reach statistical significance ($P = 0.075$). The incremental AUC for plasma TAG concentrations was significantly lower after the interesterified palm oil diet compared with the high-oleic sunflower oil diet. The authors concluded that interesterification of palm oil did not result in adverse changes in postprandial lipemia compared with native palm oil and high-oleic sunflower oil. Finally, **Yli-Jokipii et al.** (20) have compared the effects of lard and interesterified lard on postprandial lipemia in seven women and two men over 8 h. Except for a small percentage from low-fat cheese, all the fat (55 gram per m² or about 90 gram) was derived of the test fats. In the native

lard, 68.7% of the fat molecules had 16:0 at *sn*-2. For the interesterified lard, this value was 49.7%. The results indicated an increase in postprandial TAG concentrations as a result of interesterification, moving 16:0 from *sn*-2 to *sn*-1,3.

Both **Yli-Jokipii et al.** (11) and **Berry et al.** (19) offered the same explanation for the reduced postprandial lipid response following a meal rich in interesterified palm oil. It was discussed that interesterified palm oil contains a higher proportion of solid fat at 37 °C compared with native palm oil. Due to this higher melting point, interesterified palm oil may be emulsified less readily in the intestinal tract, resulting in a slower absorption and lowered postprandial plasma TAG concentrations. **Yli-Jokipii et al.** (11) reported that at 20 °C the solid fat content of the interesterified palm oil was 17.8% and of palm oil 0%. At body temperature both oils were liquid. **Berry et al.** (19) found that at 37 °C interesterified palm oil had a solid fat content of 15%, native palm oil of 4%, and high-oleic sunflower oil of 1%. In contrast, **Yli-Jokipii et al.** (20) found less striking differences: at 35 °C lard had a solid fat content of 12.5% and interesterified lard of 11%.

3.1 Conclusion

It can be concluded that interesterification of palm oil does not adversely affect fasting serum lipid and lipoprotein concentrations in humans. Zock et al. (15) did find an increase in serum total cholesterol levels and LDL cholesterol levels in men after consumption of interesterified palm oil. However, there was a large difference in positional distribution between the two test fats. In daily life, these differences in positional distribution of 16:0 will be much smaller, while intakes of interesterified fat blends will be much less. Therefore, the studies carried out study so far do not indicate that shifting 16:0 from *sn*-1,3 to *sn*-2 materially affect the ability of palm oil to influence the fasting profile of serum lipoproteins.

The effect of interesterification on postprandial lipid concentrations is less clear. While Zampelas et al. (18) did not find a difference in postprandial lipemia between native and interesterified palm oil, both Yli-Jokipii et al. (11) and Berry et al. (19) found a reduced postprandial lipid response after ingestion of meals enriched with interesterified palm oil. Both authors ascribed the differences in postprandial lipemia to differences in solid fat content of the test fats, and not to the process of

interesterification per se. For lard, although the solid fat contents of the experimental fats were very similar, postprandial lipemia was larger after modified lard (20). Thus, this study suggested that a shift of 16:0 from *sn*-2 to *sn*-1,3 increased the postprandial response. This effect is the mirror image of that seen in the experiments with palm oil where interesterification moves 16:0 from *sn*-2 to *sn*-1,3. In other words, the position of 16:0 in the TAG molecule may determine the effect on postprandial lipemia, not whether or not the fat has been interesterified.

Table 2. Human studies on the effects of the positional distribution of dietary palmitic acid (16:0) on fasting and postprandial lipid metabolism

Authors	No. of men/ women	Type of study and design	Days on diet / hours after meal	Major FA	Positional distribution / major fatty acid	Fat source	Test fat as % of daily energy intake	Conclusion
Zock <i>et al.</i> , 1995 (15)	23 / 37	Fasting/ cross-over	21 days	16:0	I: 82% of 16:0 at <i>sn</i> -1,3 + 18% at <i>sn</i> -2 II: 35% of 16:0 at <i>sn</i> -1,3 + 65% at <i>sn</i> -2	I: Palm oil II: Modified palm oil (Betapol)	28	Only in men, TCH and LDL-C increased marginally on diet II
Nestel <i>et al.</i> , 1995 (16)	27 / 0	Fasting/ cross-over	21 days	16:0	I: 22% of 16:0 at <i>sn</i> -2 II: 52% of 16:0 at <i>sn</i> -2	I: Palm oil II: Interesterified palm oil	20	No difference in lipoprotein profile between the diets
Cristophe <i>et al.</i> , 2000 (17)	32 / 0	Fasting / Parallel	28 days	16:0	Not specified	I: Butter II Interesterified butter	17	No difference in lipoprotein profile between the diets
Zampelas <i>et al.</i> , 1994 (18)	16 / 0	Post-prandial/ cross-over	6 hrs	16:0	I: 6% of 16:0 at <i>sn</i> -2 II: 73% of 16:0 at <i>sn</i> -2	I: Palm oil II: Modified palm oil (Betapol)	54	TAG responses in total plasma, CM-rich and CM-poor fractions were similar after both meals
Yli-Jokipii <i>et al.</i> , 2001 (11)	0 / 10	Post-prandial/ cross-over	6 hrs	16:0	I: 10% of the major isomers had 16:0 at <i>sn</i> -2 II: 31% of the major isomers had 16:0 at <i>sn</i> -2	I: Palm oil II: Interesterified palm oil	Not known	Diet I caused a greater increase on plasma total TAG compared to diet II
Berry <i>et al.</i> , 2007 (19)	20 / 0	Post-prandial/ cross-over	6 hrs	16:0	I: 7.2% of 16:0 at <i>sn</i> -2 II: 37.2% of 16:0 at <i>sn</i> -2	I: Palm oil II: Interesterified palm oil	50	A non-significant decrease on diet II compared to diet I ($P = 0.075$) in plasma

Authors	No. of men/ women	Type of study and design	Days on diet / hours after meal	Major FA	Positional distribution / major fatty acid	Fat source	Test fat as % of daily energy intake	Conclusion
								TAG concentration
Berry <i>et al.</i> , 2007 (19)	18 / 0	Post-prandial/ cross-over	6 hrs	16:0	I: 37.2% of <i>sn</i> -2 position was 16:0 and 45.3% 18:1 II: 91.2% of <i>sn</i> -2 position 18:1	I: Interesterified palm oil II: High-oleic sunflower oil	50	A lower plasma TAG concentration after diet I compared to diet II
Yli-Jokipii <i>et al.</i> , 2001 (20)	7 / 2	Post-prandial/ cross-over	8 hrs	16:0	I: 68.7% of the major isomers had 16:0 at <i>sn</i> -2 II: 49.7% of the major isomers had 16:0 at <i>sn</i> -2.	I: Lard II: Interesterified lard	Not known	A lower AUC of VLDL TAG after diet I compared to diet II.

4. Stearic acid (18:0)

Research has also been conducted to examine the effects of the positional distribution of another saturated fatty acid, namely stearic acid (18:0), on fasting lipoproteins and postprandial lipemia (**table 3**).

Grande et al. (21) have examined in 30 men the effects of natural cocoa butter with those of imitation cocoa butter. This latter fat was made by interesterifying complete hydrogenated soybean oil, olive oil and safflower oil. Diets were consumed for 18 days in randomized order. Daily intake of the experimental fats was 83 g or 27% of energy. The results showed comparable effects of the two diets on serum total cholesterol and TAG concentrations.

Summers et al. (22) have compared the postprandial lipid response of meals enriched with two different synthetic TAGs in 14 healthy female volunteers. A meal contained 984 kcal of which the test fat provided 58% of energy. The fat consisted of a synthetic TAG containing stearic acid either predominantly at the *sn*-1,3 position (StOO) or at the *sn*-2 position (OStO). Both meals were given to the volunteers at two occasions in random order. Blood samples were taken at different time points until 6 h after consumption of the meals. Plasma TAG and CM-TAG concentrations rose after the meals to the same extent, reaching peak values at 240 to 300 minutes.

Sanders et al. (5) therefore compared the postprandial lipid response of native and interesterified cocoa butter in 17 male subjects. In this study, they used two meals: one meal enriched with the cocoa butter (18:0 at *sn*-1,3) and one with the interesterified cocoa butter. The meals contained 50 gram of test fat, which accounted for 63% of total energy. In the native cocoa butter, 18:1 was mainly present at *sn*-2 (80%), while the interesterified cocoa butter contained 18:0 (38%), 18:1 (30%) and 16:0 (26%) at *sn*-2. The consequence of interesterification was a diminished postprandial response of plasma TAG concentrations. This suggests that TAGs containing stearic acid at the *sn*-2 position are more slowly absorbed than TAGs containing stearic acid predominantly at the *sn*-1,3 position, analogous to what is reported for 16:0. In 2005, **Berry and Sanders** (12) published a review where they ascribed the lowered postprandial lipemia of the interesterified TAGs used to the higher solid fat content of these TAGs. They hypothesized that the physical properties of the fats determined the rate of absorption and that the proportion of

solid fat at 37 °C is negatively related to the extent of postprandial lipemia. Randomisation of shea butter or cocoa butter increases the proportion of tri-saturated TAGs, which have a higher melting point. Fats that contain crystalline solids at body temperature may affect micelle formation and retard the process of absorption and consequently result in reduced postprandial lipemia. After this review, **Berry et al.** (23) published a study in which diets rich in native and interesterified shea butter, and an oleic-acid rich sunflower oil blends were compared in 16 men. Native shea butter contains 18:0 predominantly at positions *sn*-1 and *sn*-3, while in the interesterified blend 18:0 is randomly distributed. The meals provided 50 gram of test fat, which accounted for 53% of total energy. The native shea blend contained 76.2% 18:1 at *sn*-2, while this percentage was decreased to 46.3% in the interesterified shea blend. Almost no 18:0 was present at *sn*-2 in the native blend, while the interesterified blend contained 18:0 randomly distributed (31% at *sn*-2). The postprandial lipid response was lower following the native shea blend, compared with the high-oleic acid sunflower oil. However, responses after consumption of the native and interesterified blends were similar. In comparison with the high-oleic acid sunflower oil, postprandial lipemia did decrease after both shea blends. The results of this study differed from those of previous studies performed by this group, as postprandial lipemia was not reduced after consumption of an interesterified dietary TAG. However, in previous studies the solid fat content of the native fats was substantially lower. Both shea blends had a high proportion of solid fat at 37 °C (22.2% for the native shea blend and 41.2% for the interesterified shea blend), whereas the solid fat content of the high-oleic acid sunflower oil at 37 °C was 0%. An explanation could be that the effect of TAG on postprandial lipemia levels off at higher solid fat contents. **Berry et al.** (23) also measured fasting lipoprotein levels. There were no differences between the diets in plasma total cholesterol concentrations, LDL cholesterol, HDL cholesterol and plasma TAG concentrations, suggesting that interesterification has no influence on fasting lipoprotein levels in humans.

4.1 Conclusion

Two studies were described in which the effects of interesterification on fasting serum lipoprotein levels were studied (21, 23). The results of these studies indicated that interesterification of 18:0-rich fat (so that 18:0 moved from *sn*-1,3 to *sn*-2) did not influence fasting lipoprotein levels. Results regarding the effect of interesterification

of 18:0-rich fats on postprandial lipemia are more difficult to interpret. Summers et al. (22) did not find an effect of interesterification on postprandial lipemia. Sanders and Berry (5, 23) have conducted several studies on the effects of interesterification. Interesterification of cocoa butter and a synthetic TAG resulted in decreased lipemia, while interesterification of a shea blend did not have an effect. Both native and interesterified shea have a relatively high solid fat content at 37 °C. It was therefore postulated that the solid fat content of fats and oils is negatively related to the rate of fat absorption, which may reduce postprandial lipemia.

Table 3. Human studies on the effects of the positional distribution of dietary stearic acid (18:0) on fasting and postprandial lipid metabolism

Authors	No. of men/ women	Type of study and design	Days on diet / hours after meal	Major FA	Positional distribution/ major fatty acid	Fat source	Test fat in % of daily energy intake	Conclusion
Grande <i>et al.</i> , 1970 (21)	30 / 0	Fasting/ cross-over	18 days	18:0	I: - II: Interesterified III: - IV: Interesterified	I: Cocoa butter and safflower oil II: Interesterified imitation cocoa butter III: Natural palm oil IV: Interesterified imitation palm oil	31.25	No differences in lipid parameters between diet I and diet II. Comparison between diet III and IV shows that 16:0 has a cholesterol-raising effect relative to 18:0.
Summers <i>et al.</i> , 1999 (22)	0 / 14	Post-prandial/ cross-over	6 hrs	18:0	I: 18:0 predominantly at <i>sn</i> -1,3 II: 18:0 predominantly at <i>sn</i> -2	I: Synthetic TAG II: Synthetic TAG	58	No difference between meals in concentration plasma TAG and CM-TAG
Sanders <i>et al.</i> , 2003 (5)	17 / 0	Post-prandial/ cross-over	6 hrs	18:0	I: 18:1 mainly present at <i>sn</i> -2 (80%) II: 18:0 (38%), 18:1 (30%) and 16:0 (26%) at <i>sn</i> -2	I: Cocoa butter II: Interesterified cocoa butter	63	A lower plasma TAG concentration and no increase in FVIIa after diet II compared to diet I
Berry <i>et al.</i> , 2007 (23)	16 / 0	Fasting/ cross-over	21 days	18:0	I: 76.2% at <i>sn</i> -2 was 18:1 and almost no 18:0 was present at	I: Shea blend II: Interesterified shea blend	285 kcal/day	No differences in TCH, LDL-C, HDL-C and plasma TAG concentrations

Authors	No. of men/ women	Type of study and design	Days on diet / hours after meal	Major FA	Positional distribution/ major fatty acid	Fat source	Test fat in % of daily energy intake	Conclusion
					<i>sn-2</i> II: 46.3% at <i>sn-2</i> was 18:1 and 31% was 18:0			between the two diets
Berry <i>et al.</i> , 2007 (23)	16 / 0	Post-prandial/ cross-over	8 hrs	18:0	I: 76.2% at <i>sn-2</i> was 18:1 and almost no 18:0 was present at <i>sn-2</i> II: 46.3% at <i>sn-2</i> was 18:1 and 31% was 18:0 III: 83.9 mol% was 18:1, 8.0% was 18:2 and 1.6 was 18:0	I: Shea blend II: Interesterified shea blend III: Oleic acid-rich sunflower oil	52	TAG response lower after diet I compared to diet III, while the response was similar compared to the diet II

5. Interesterified fats

Two studies were identified which compared interesterified fats and both showed very different results (**table 4**).

Meijer and Weststrate (6) compared in 60 young normocholesterolemic adults the effects on fasting serum lipids and lipoproteins of a blend of commonly used edible vegetable fats with those of the same blend after random interesterification. In contrast to other studies, intake of the blends was closer to habitual intakes and were supplied at two levels of energy (4 and 8% of energy). The fat blend was composed of 36% (w/w) coconut fat, 33% palm oil, 22% dry-fractionated palm oil-stearin fraction and 9% low-trans partially hydrogenated rapeseed oil. Part of this blend was used as such and the remainder was interesterified. Interesterification resulted in relatively more myristic acid (14:0) and palmitic acid (16:0) at the *sn*-2 position at the expense of lauric acid (12:0) and oleic (18:1) acid. Neither the type of fat blend, nor the level of energy resulted in significant changes in serum lipid values. Recently, **Sundram et al.** (24) have compared the effects on serum lipids and lipoproteins of a fat rich in *trans* fatty acids, an interesterified fat, and unmodified palm olein. The interesterified fat was prepared by the full hydrogenation of refined soybean oil to convert all unsaturated C18 fatty acids into 18:0, which was then blended with refined soybean oil and palm olein. In the interesterified fat, a saturated fatty acid was present at the *sn*-2 position in 21% of all TAG molecules (15% 18:0, 6% 16:0). The *sn*-2 position of TAG molecules in the palm olein fat was predominantly occupied with *cis*18:1 and palmitic acid was present at *sn*-1,3. 18:0 was not present at *sn*-2 and 9% of all TAG molecules had 16:0 at *sn*-2. The total saturated fatty acid content at the *sn*-2 position was more than twice as high in the interesterified fat in comparison to the palm olein fat. As the positional distribution of *trans* fatty acids in the partially hydrogenated fat could not be measured, the authors assumed that partial hydrogenation resulted in substantial numbers of *trans* fatty acids at *sn*-2. This assumption was based on the fact that most of the monounsaturated fatty acids, including *trans*-18:1, were located at *sn*-2. The results revealed that the diets did not affect plasma total cholesterol and TAG concentrations. Compared with the palm olein diet, plasma HDL cholesterol was lowered after consumption of the interesterified diet and partially hydrogenated fat

diet, while LDL cholesterol was significantly increased after consumption of the partially hydrogenated test diet. Both test diets increased the total to HDL cholesterol ratio, and the LDL to HDL cholesterol ratio. These results indicated that the distribution of cholesterol among lipoproteins is altered by the test diets, as the total serum cholesterol concentration was not changed. The partially hydrogenated test diet was characterized by an unnatural insertion of *trans*18:1 at *sn*-2. The increase in the LDL to HDL cholesterol ratio is a typical pattern seen during *trans*-fat consumption and this study supports the hypothesis that *trans* fatty acids have a negative impact on plasma lipoproteins. The authors also concluded that 18:0 should not be seen as a neutral saturated fatty acid, at least when randomized or when it becomes the major dietary saturated fatty acid. Finally, the authors concluded that both modified fats adversely altered the metabolism of plasma lipoproteins and that future research is needed before interesterification can be designated as the process of choice for replacing partial hydrogenation to harden vegetable oils for use in foods.

5.1 Conclusion

Meijer and Weststrate (6) supplied fat blends at a low energy level and did not find an effect of randomization on fasting lipoprotein levels. Sundram et al. (24) found 18:0-rich randomized fat to decrease HDL cholesterol levels and increase - though not significantly - LDL cholesterol levels. This study suggested that both *trans*-fats as well as interesterified fats increase the total to HDL cholesterol ratio and the LDL to HDL cholesterol ratio compared to palm olein.

Table 4. Human studies on the effects of interesterified fats on fasting and postprandial lipid metabolism

Authors	No. of men/ Women	Type of study and design	Days on diet / hours after meal	Major FA	Positional distribution	Fat source	Test fat in % of daily energy intake	Conclusion
Meijer and Westrate, 1997 (6)	30 / 30	Fasting/ cross-over	21 days	-	More 18:0 and 16:0 at <i>sn</i> -2 at the expense of 12:0 and 18:1 after randomization	I: Fat blend composed of 36% (w/w) coconut fat, 33% palm oil, 22% dry-fractionated palm oil-stearin fraction and 9% low-trans partially hydrogenated rapeseed oil II: Interesterified fat blend	4% and 8%	No differences in TCH, LDL-C, HDL-C and plasma TAG concentration between fat blends and energy levels
Sundram <i>et al.</i> , 2007 (24)	11 / 20	Fasting/ cross-over	28 days	-	I: <i>sn</i> -2 was mainly occupied with <i>cis</i> 18:1 and 16:0 was present at <i>sn</i> -1,3 II: assumed that <i>trans</i> 18:1 at <i>sn</i> -2 III: SAFA present at <i>sn</i> -2 in 21% of all TAG molecules (15% 18:0, 6% 16:0)	I: Palm olein II: Partially hydrogenated soybean oil III: Interesterified fat	31% of energy from fat, of which more than 70% test fat	Diets II and III altered the metabolism of lipoproteins and glucose in an adverse way compared to diet I

6. Glucose metabolism

Increased postprandial glucose concentrations are seen as a risk marker to develop type 2 diabetes mellitus. After a meal, insulin concentrations increase to facilitate glucose uptake by tissues. Like for glucose, increased postprandial insulin concentrations are considered as a risk marker for type 2 diabetes mellitus.

Some of the studies already described in this review have also assessed the postprandial glucose response to the different meals. **Zampelas et al.** (18) measured the glucose response over 6 hours following a liquid meal containing dietary TAG in which palmitic acid was predominantly on the *sn*-1 or *sn*-2 position. Following ingestion of either meal, plasma glucose peaked at 40 minutes and returned to baseline within 140 minutes. Insulin responses were measured as well and were also very similar between the two meals. **Summers et al.** (22) also included postprandial glucose and insulin responses in their study. They used a synthetic TAG, containing stearic acid predominantly at the *sn*-1,3 or at the *sn*-2 positions. They measured the response for 6 hours after ingestion of both meals and plasma glucose concentrations rose after both meals, reaching a peak at 30 minutes. Neither glucose nor insulin responses differed between the two meals, suggesting again that positional distribution of fatty acids does not affect the postprandial glucose response. **Berry et al.** (23) conducted a study in which volunteers adhered to a diet for three weeks and underwent a postprandial test at the end of the dietary period. They compared native and interesterified shea butter at a low and a high intake. Fasting glucose and insulin levels as well as postprandial concentrations did not differ significantly between the high-stearic acid diet and low-stearic acid diet or between interesterified and native shea blends. **Sundram et al.** (24) measured fasting glucose levels after dietary periods of four weeks. During each dietary period, a subgroup of 19 subjects also underwent after 2 weeks a postprandial test made from the experimental fat. Fasting glucose levels increased significantly after the interesterified fat diet in comparison with the palm olein and partially hydrogenated soybean oil diet. Glucose also increased after the partially hydrogenated soybean oil diet compared to the palm olein diet. The rise was 3% for the palm olein, 9% for partially hydrogenated soybean oil and 22% for interesterified fat diet, corresponding with an increase after the interesterified fat diet from 5.47 to 6.67 mmol/L. During the

postprandial test, glucose concentrations increased after the interesterified fat meal compared to the other two meals and remained elevated till 8 hours after ingestion of the meals. The palm olein and partially hydrogenated soybean oil meals showed a similar glucose response and both returned to baseline values after 6 hours. The area under the curve was 40% greater for the interesterified fat meal compared to the other two meals. Insulin was also measured postprandially. A peak was seen for all meals after 2 hours. However, this peak was significantly lower after both the partially hydrogenated soybean oil and interesterified fat meal compared to palm olein meal.

6.1 Conclusion

Sundram et al. (24) found an unfavorable fasting and postprandial glucose response after consumption of a interesterified fat compared to an unmodified saturated fat. In the other studies (18, 22, 23), postprandial glucose and insulin responses were not influenced by the process of interesterification.

7. FVII response

Only a very limited number of the studies discussed so far have also investigated whether interesterification of fats influenced the response of factor VII coagulant activity (FVIIc). FVIIc is one of the central proteins in the coagulation cascade and its activated form is known as FVIIa. Elevated FVIIc activity is a risk marker for the development of cardiovascular diseases. Several reviews have concluded that no consensus exists on the effects of the various fatty acids on fasting FVIIc or FVIIa. Total fat intake, however, may be positively related to FVIIc and FVIIa during both the fasting and postprandial phase (25).

Meijer and Weststrate (6) have compared a blend of commonly used edible vegetable fats with the same blend after interesterification and they also included haemostatic parameters in their analysis. There were, however, no differences in fasting FVIIa concentration between the two fat blends at the end of the dietary periods. The effects of interesterification on FVIIa were studied by **Sanders et al.** (5) by comparing native cocoa butter with interesterified cocoa butter. FVIIa did not increase after consumption of interesterified cocoa butter, while there was an increase after the ingestion of a meal enriched with native cocoa butter. As interesterification also decreased the postprandial plasma TAG response, they hypothesized that 18:0-rich TAGs with 18:1 at *sn*-2 and 18:0 at *sn*-1,3 are absorbed more rapidly - leading to activation of FVII - than a fat with a long-chain saturated fatty acid esterified at *sn*-2. **Berry et al.** (23) compared the chronic and acute effects of interesterification of a 18:0-rich TAG. For this study they used meals enriched with native and randomized shea butter. There was no increase in FVIIa concentrations after both meals in contrast to an increase observed after consumption of a meal rich in high-oleic sunflower oil after 3 and 6 hours. **Berry et al.** (19) also investigated the effect of interesterification of 16:0-rich TAG on FVIIa responses. They compared meals enriched native palm oil, interesterified palm oil, and high-oleic sunflower oil. FVIIa concentrations increased after the native palm oil meal as well as after the interesterified palm oil meal, while the increase was not different between the meals. The increase in FVIIa was however lower after the meal enriched with interesterified palm oil compared to the high-oleic sunflower oil meal at 6 hours. They concluded that interesterification of palm oil does not result in adverse changes in FVII

activation.

7.1 Conclusion

Interesterification of fats does not influence the postprandial FVII response in an adverse way. The extent of increase in plasma TAG was not proportional to the increase in FVII response (5).

8. Overall conclusion

The main aim of this paper was to review thoroughly human studies that have examined the effects of interesterified dietary fats on a variety of blood parameters related to metabolic disturbances.

The majority of longer-term studies did not suggest that interesterification of dietary fats - either rich in 16:0 or 18:0 - changed the effects of these fats on the fasting serum lipoprotein profile. In one study, however, an interesterified fat rich in 18:0 had an adverse effect on the fasting serum total to HDL cholesterol ratio, a well-known risk marker for cardiovascular disease, as compared to palm olein. This effect could not be explained by differences in the fatty-acid profile of these two fats.

Results from studies on postprandial lipemia are less consistent. The majority of studies that did see a change, found a decrease in postprandial lipemia with interesterified fat. It was suggested that this decrease was due to a reduction in high melting point solids, rather than to an effect of interesterification per se. A smaller lipid response after a meal may lower cardiovascular risk. Postprandial responses of plasma levels of glucose, insulin, and FVII (a marker for coagulation tendency) response were also not systematically affected by interesterification. However, one study showed adverse effects on fasting and postprandial glucose concentrations of an interesterified fat rich in 18:0 as compared to palm olein.

In conclusion, results of one study (24) need to be confirmed or rejected. Also, it is advised to include in future studies effects on inflammatory markers. Overall, however, the studies discussed do not suggest that at current intakes interesterified fats have adverse effects on the metabolic parameters examined.

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Appendix: Details of the studies discussed

Article: Berry SEE, Woodward R, Yeoh C, Miller GJ, Sanders TAB. Effect of interesterification of palmitic acid-rich triacylglycerol on postprandial lipid and factor VII response. *Lipids* 2007; 42: 315-23.

Design: Cross-over postprandial test
Days on diet: -
Duration of postprandial test: 6 hours
Number of subjects: 20 men

	Major fatty acid	Positional distribution	Other fatty acids
Diet 1	16:0	7.2% of <i>sn</i> -2 position was occupied with 16:0	18:0, 18:1
Diet 2	16:0	37.2% of <i>sn</i> -2 position was occupied with 16:0	18:0, 18:1
Diet 3	18:1	91.2% of <i>sn</i> -2 position was occupied with 18:1	18:0, 18:2

Fat source:

Diet 1: Native palm oil
Diet 2: Interesterified palm oil
Diet 3: High-oleic sunflower oil
Intake (En%) of test fat: 50 g (450 kcal) of test fat, which provided 53 En%
Type of diet: Fats were incorporated into two muffins.

Characteristics subjects:

- Age: 31.9 years
- BMI: 24.0 kg/m²
- Total cholesterol: 4.5 mmol/L
- LDL cholesterol: 2.6 mmol/L
- HDL cholesterol: 1.4 mmol/L
- TAG: 1.2 mmol/L

Measured parameters:

Plasma total cholesterol, LDL cholesterol, TAG, glucose, insuline and FVIIa were determined.

Main results:

In one experiment, native and interesterified palm oil were compared side-by-side. In a second study, interesterified palm oil and high-oleic sunflower oil were compared side-by-side.

Compared with native palm oil, interesterified palm oil tended to decrease postprandial plasma TAG concentrations, as the incremental area under curve (iAUC) was smaller ($P = 0.075$). The iAUC for plasma TAG concentrations was significantly lower following the interesterified palm oil compared with the high-oleic sunflower oil.

FVIIa concentrations increased after the native palm oil meal as well as after the

interesterified palm oil meal, while the increases were not different between the meals. The increase in FVIIa was however lower after the meal enriched with interesterified palm oil compared to the high-oleic sunflower oil meal at 6 hours. No differences were observed for plasma total cholesterol, LDL cholesterol, and HDL cholesterol, and glucose. Also for insulin, the iAUC were comparable.

Major conclusion:

Interesterification of palm oil does not result in adverse changes in postprandial lipemia and FVIIa compared with native palm oil and high-oleic sunflower oil. Compared with high-oleic acid sunflower oil, the interesterified palm oil diet even had a more favourable response in postprandial TAG and FVIIa.

Remarks:

The solid fat content of interesterified palm oil at 37 °C was 15%, of native palm oil 4%, and of high-oleic sunflower oil 1%.
A direct side-by-side comparison between native palm oil and high-oleic sunflower oil was not be made.

Article: Berry SEE, Miller GJ, Sanders TAB. The solid fat content of stearic acid-rich fats determine their postprandial effects. *Am J Clin Nutr* 2007; 85:1486-94.

Design: Cross-over study (“diet”) with four week wash-out period and postprandial test
Days on diet: 21 days
Duration of postprandial test: 8 hours
Number of subjects: 16 men

	Major fatty acid	Positional distribution	Other fatty acids
Diet 1	18:0	76.2% at <i>sn-2</i> is 18:1 and almost no 18:0 was present at <i>sn-2</i>	18:1, 18:2
Diet 2	18:0	46.3% at <i>sn-2</i> is 18:1 and 31% is 18:0	18:1, 18:2
Diet 3	18:1	83.9% is 18:1, 8.0% is 18:2 and 1.6% is 18:0	18:0, 18:2

Fat source:

Diet 1: Shea blend
Diet 2: Interesterified shea blend
Diet 3: Oleic acid-rich sunflower oil
Daily intake (En%) of test fat: For “diet”, 30 g of test fat (270 kcal/day); for postprandial test, 50 g (450 kcal) of test fat was provided, equalling 53% of energy intake
Type of diet: For “diet” and postprandial test, fats were incorporated in 2 muffins.

Characteristics subjects:

- Age: 26.8 years
- BMI: 23.7 kg/m²
- Total cholesterol: 4.5 mmol/L
- LDL cholesterol: 2.6 mmol/L
- HDL cholesterol: 1.5 mmol/L
- TAG: 0.9 mmol/L

Measured parameters:

Concentrations of plasma total cholesterol, HDL cholesterol, TAG, glucose, and insulin were measured. Method of analysis for LDL cholesterol not specified. For the postprandial test, FVIIa was also determined.

Main results:

Diet: Randomized and unrandomized shea butter had no effects on the fasting parameters measured.

Postprandial test: Postprandial TAG responses were similar between the two shea

blends. Also, no differences were observed for plasma total cholesterol, HDL cholesterol, LDL cholesterol, glucose, insulin and FVIIa. The postprandial increases in plasma TAG and FVIIa were lower following the native shea blend, compared with the high-oleic acid sunflower oil.

Major conclusion:

The present study does not suggest adverse effects of the position distribution of stearic acid within dietary TAG on fasting or postprandial plasma parameters. Compared to high-oleic acid sunflower oil, unrandomized shea blends decreased postprandial TAG and FVIIa concentrations.

Remarks:

Both shea blends had a high proportion of solid fat content (randomized 41%, native 22%), whereas the solid fat content of the high-oleic acid oil was 0% at 37 °C. It was hypothesized that fats rich in crystalline solids at body temperature may affect micelle formation and retard the process of absorption, which consequently results in reduced postprandial lipemia.

Article: Christophe AB, De Greyt WF, Delanghe JR, Huyghebaert AD. Substituting enzymatically interesterified butter for native butter has no effect on lipemia or lipoproteinemia in man. *Ann Nutr Metab* 2000; 44:61-7.

Design: Parallel-design study
Days on diet: 28 days run-in period followed by an experimental period of 28 days
Duration of postprandial test: -
Number of subjects: 32 men, 16 in each group

	Major fatty acid	Positional distribution	Other fatty acids
Diet 1	Saturated fatty acids	Not reported	18:1
Diet 2	Saturated fatty acids	Not reported	18:1

Fat source:
Diet 1: Butter fat
Diet 2: Enzymatically interesterified fat
Daily En% intake of test fat: During the experimental period, butter provided 16.5 En% (22.1 g/day) and the interesterified butter 18.5 En% (23.2 g/day).
Type of diet: All spreading, cooking and baking fats were replaced by butter.

Characteristics subjects:

- Age: 32.2 years
- BMI: 23.1 kg/m²
- Total cholesterol: 5.36 mmol/L
- LDL cholesterol: 3.59 mmol/L
- HDL cholesterol: 1.19 mmol/L
- TAG: 1.27 mmol/L

Measured parameters:
 Serum total cholesterol, HDL cholesterol, TAG, free cholesterol, phospholipids, apoA-I and apoB were determined. LDL cholesterol was calculated as the difference between total and cholesterol in the supernatant after precipitation of LDL cholesterol.

Main results:
 Changes in the measured parameters were not different between the control and intervention groups.

Major conclusion:
 Interesterification of butter fat does not affect the fasting metabolic parameters measured.

Remarks:
 None.

Article: Grande F, Joseph TA, Keys A. Comparison of effects of palmitic and stearic acids in the diet on serum cholesterol in man. *Am J Clin Nutr* 1970; 23: 1184-93.

Design: Cross-over study with no wash-out period
Days on diet: 18 days
Duration of postprandial test: -
Number of subjects: 30 men

	Major fatty acid	Positional distribution	Other fatty acids
Diet 1	18:0	-	16:0, 18:1
Diet 2	18:0	Interesterified	16:0, 18:1

Fat source:

Diet 1: Cocoa butter and safflower oil
Diet 2: Interesterified imitation cocoa butter
Daily intake (En%) of test fat: 27%
Type of diet: Mixed solid diets

Characteristics subjects:

- Age: 63 years
- BMI (kg/m²): 25.5 kg/m²
- Total cholesterol: -
- LDL cholesterol: -
- HDL cholesterol: -
- TAG: -

Measured parameters:

Serum total cholesterol, phospholipids and TAG were measured.

Main results:

The serum cholesterol, TAG and phospholipids were practically identical when subjects consumed the diets enriched with either natural cocoa butter or interesterified cocoa butter.

Major conclusion:

Interesterification of cocoa butter does not change the effects of 18:0 on serum total and TAG concentrations.

Remarks:

This was a strictly controlled dietary study.

Article: Meijer GW, Weststrate JA. Interesterification of fats in margarine: effect on blood lipids, blood enzymes, and hemostasis parameters. *Eur J Clin Nutr* 1997; 51:527-34.

Design: Cross-over study with no wash-out period
Days on diet: 21
Duration of postprandial test: -
Number of subjects: 30 men and 30 women

	Major fatty acid	Positional distribution	Other fatty acids
Diet 1	-		18:2, <i>Cis</i> 18:1, 16:0
Diet 2	-	Interesterification resulted in more 14:0, 16:0 and 18:0 at <i>sn</i> -2, at the expense of 12:0 and 18:1	18:2, <i>Cis</i> 18:1, 16:0

Fat source:

Diet 1: Fat blend was composed of 36% (w/w) coconut fat, 33% palm oil, 22% dry-fractionated palm oil-stearin fraction, and 9% low-trans partially hydrogenated rapeseed oil

Diet 2: Interesterified fat blend

Daily intake (En%) of test fat: 4% and 8%

Type of diet: Fat blends were incorporated into margarine as an ingredient in cake, and as margarines for spreading on bread

Characteristics subjects (baseline):

- Age: 36 years
- BMI: 23.7 kg/m²
- Total cholesterol: 5.0 mmol/L
- LDL cholesterol: -
- HDL cholesterol: -
- TAG: -

Measured parameters:

Thirty parameters were determined including serum total cholesterol, HDL cholesterol, TAG and glucose. LDL cholesterol was calculated with the Friedewald equation. Blood clinical chemical characteristics were determined as well as haemostatic parameters (e.g. PAI-I and FVIIa).

Main results:

Interesterification did not affect in a adverse way the parameters of interest. Effects did not depend on energy intake.

Major conclusion:

Interesterification of a blend of commonly used vegetable oils did not affect fasting blood lipids, glucose, clinical chemical and haemostatic parameters in healthy humans, when compared with an non-esterified fat blend with the same fatty acid composition.

Remarks:

None.

Article: Nestel PJ, Noakes M, Belling GB, McArthur R, Clifton PM. Effect on plasma lipids of interesterifying a mix of edible oils. *Am J Clin Nutr* 1995; 62: 950-5.

Design: Cross-over study with no wash-out period
Days on diet: 21 days
Duration of postprandial test: -
Number of subjects: 27 men

	Major fatty acid	Positional distribution	Other fatty acids
Diet 1	16:0	22% of 16:0 at <i>sn</i> -2	<i>Cis</i> 18:1, 18:2, 12:0
Diet 2	16:0	52% of 16:0 at <i>sn</i> -2	<i>Cis</i> 18:1, 18:2, 12:0
Diet 3	18:2	-	18:2, 16:0

Fat source:

Diet 1: Native palm oil
Diet 2: Interesterified palm oil
Diet 3: High-linoleic acid
Daily intake (En%) of test fat: 20%
Type of diet: Test fats were provided as margarines and incorporated into cookies

Characteristics subjects (baseline):

- Age: 49 years
- BMI: 26.3 kg/m²
- Total cholesterol: 5.97 mmol/L
- LDL cholesterol: 4.30 mmol/L
- HDL cholesterol: 0.92 mmol/L
- TAG: 1.82 mmol/L

Measured parameters:

Plasma total cholesterol, HDL cholesterol and TAG were measured. LDL cholesterol was calculated with the Friedewald equation.

Main results:

Interesterification of palm oil did not affect plasma lipid and lipoprotein concentrations. Compared to the diet rich in linoleic acid, both “palm-oil” diets raised plasma total cholesterol, LDL and HDL cholesterol concentrations.

Major conclusion:

Interesterification of palm oil does not change the effects of 16:0 on the plasma lipid and lipoprotein profile.

Remarks:

None.

Article: Sanders TAB, Berry SEE, Miller GJ. Influence of triacylglycerol structure on the postprandial response of factor FVII to stearic acid-rich fats. *Am J Clin Nutr* 2003; 77:777-82.

Design: Cross-over postprandial test
Days on diet: -
Duration of postprandial test: 6 hours
Number of subjects: 17 men

	Major fatty acid	Positional distribution	Other fatty acids
Diet 1	18:0	18:1 mainly present at <i>sn</i> -2 (80%)	16:0, 18:1
Diet 2	18:0	18:0 (38%), 18:1 (30%) and 16:0 (26%) at <i>sn</i> -2	16:0, 18:1

Fat source:
Diet 1: Native cocoa butter
Diet 2: Interesterified cocoa butter
Intake (En%) of test fat: 50 g or 63%
Type of diet: Solid food (muffin) and milkshake

Characteristics subjects:

- Age: 38.2 years
- BMI: 24.5 kg/m²
- Total cholesterol: 4.51 mmol/L
- LDL cholesterol: 2.72 mmol/L
- HDL cholesterol: 1.29 mmol/L
- TAG: 1.04 mmol/L

Measured parameters:

Plasma total cholesterol, HDL cholesterol, TAG and FVIIa were measured.

Main results:

Plasma TAG concentrations increased to a lesser extent after consumption of the interesterified cocoa butter meal than after consumption of the native cocoa butter meal. At 3h and 6h, FVII:a was higher after the native cocoa butter meal than after the randomized cocoa butter meal. There were no differences in total cholesterol and HDL cholesterol concentrations at 3h and 6h between the two meals.

Major conclusion:

Results suggest that TAGs containing 18:0 in the *sn*-2 position are less well absorbed or that their release in the circulation is delayed. Compared with the native cocoa butter, interesterified cocoa butter did not increase FVIIa. It appears that stearic acid-rich TAG with oleic acid at *sn*-2 is absorbed and metabolized more rapidly leading to activation of FVII than TAGs with long-chain fatty acids at *sn*-2.

Remarks:

Difference in solid fat content at 37 °C, native cocoa butter has 1% of solid fat content

in contrast to interesterified, where this 37% is solid fat content. (Analyzed in study by Berry SEE, Miller GJ, Sanders TAB. The solid fat content of stearic acid-rich fats determine their postprandial effects. Am J Clin Nutr 2007; 85: 1486-1494).

Article: Summers LKM, Fielding BA, Herd SL, Ilic V, Clark ML, Quinlan PT, Frayn KN. Use of structured triacylglycerols containing predominantly stearic and oleic acids to probe early events in metabolic processing of dietary fat. *J Lipid Res* 1999; 40:1890-8.

Design: Cross-over postprandial test
Days on diet: -
Duration of postprandial test: 6 hours
Number of subjects: 14 women

	Major fatty acid	Positional distribution	Other fatty acids
Diet 1	18:0	18:0 mainly at <i>sn</i> -1,3	C18:1, C18:2
Diet 2	18:0	18:0 mainly at <i>sn</i> -2	C18:1, C18:2

Fat source:

Diet 1: Synthetic TAG
Diet 2: Synthetic TAG
Intake (En%) of test fat: 60 g or approximately 58%
Type of diet: Solid food and milkshake

Characteristics subjects:

- Age (y): 49 years
- BMI (kg/m²): 27.5 kg/m²
- Total cholesterol: 4.8 mmol/L
- LDL cholesterol: -
- HDL cholesterol: 1.2 mmol/L
- TAG: 1.1 mmol/L

Measured parameters:

Plasma TAG, CM-TAG, glucose, insulin, and non-esterified fatty acids were measured. Also, whole-blood lactate, glycerol, and 3-hydroxybutyrate were determined.

Main results:

The test fats did not affect any of the parameters measured.

Major conclusion:

Metabolic events after LPL hydrolysis of CM-TAG are largely unaffected by the nature or the position of fatty acids within dietary TAG.

Remarks:

None.

Article: Sundram K, Karupaiah T, Hayes KC. Stearic acid-rich interesterified fat and trans-rich fat raise the LDL/HDL ratio and plasma glucose relative to palm olein in humans. *Nutr Metab* 2007; 4: 3.

Design: Cross-over study with no wash-out period
Days on diet: 28 days
Duration of postprandial test: 8 hours
Number of subjects: 11 men and 20 women

	Major fatty acid	Positional distribution	Other fatty acids
Diet 1	18:1	<i>sn</i> -2 was predominantly occupied with <i>cis</i> 18:1 and 16:0 was present at <i>sn</i> -1,3	16:0
Diet 2	<i>trans</i> 18:0	assumed that <i>trans</i> 18:0 are present at <i>sn</i> -2	16:0
Diet 3	18:0	SAFA present at <i>sn</i> -2 in 21% of all TAG molecules (15% 18:0, 6% 16:0)	16:0, 18:2

Fat source:

Diet 1: Palm olein
Diet 2: Partially hydrogenated soybean oil
Diet 3: Interesterified fat, made from 40% fully hydrogenated soy bean oil, 30% refined soybean oil, and 30% palm olein.

Daily intake (En%) of test fat: 31% of energy from fat, of which >70% test fat
Type of diet: Mixed solid foods prepared by caterer

Characteristics subjects (baseline):

- Age: 30 years
- BMI: 22 kg/m²
- Total cholesterol: 5.05 mmol/L
- LDL cholesterol: 3.17 mmol/L
- HDL cholesterol: 1.48 mmol/L
- TAG: 0.89 mmol/L
- Glucose 5.43 mmol/L

Measured parameters:

Plasma total cholesterol, HDL cholesterol and TAG were measured. LDL cholesterol was calculated with the Friedewald equation. Plasma glucose and insulin concentrations were determined.

Main results:

The diets did not affect plasma total cholesterol and TAG concentrations, while HDL cholesterol was lower after consumption of the interesterified (IE) diet as well as after consumption of the partially hydrogenated soybean oil (PHSO) diet compared with the palm olein (POL) diet. LDL-C concentration increased significantly after consumption of the PHSO diet.

Fasting glucose levels increased significantly after the IE diet in comparison with the POL and PHSO diet. Glucose also increased after the PHSO diet compared to the POL diet. During the postprandial test, glucose concentrations increased after the IE meal compared to the other two meals, while the POL and PHSO meals showed a similar glucose response. Insulin responses were the same after the three meals. Fasting insulin was the lowest on the IE-diet as compared with the other two diets.

Major conclusion:

Both modified fats (IE and PHSO) adversely altered lipid and glucose metabolism. Future research is needed before interesterification can be designated as the process of choice for replacing partial hydrogenation to harden vegetable oils for use in foods.

Remarks:

Assumptions were made concerning the positional distribution of fatty acids for the partially hydrogenated soybean oil, as standards for the *trans* FA-containing TAG separated by the HPLC method used were not available.

Article: Yli-Jokipii K, Kallio H, Schwab U, Mykkanen H, Kurvinen J, Savolainen MJ, Tahvonen R. Effects of palm oil and transesterified palm oil on chylomicron and VLDL triacylglycerol structures and postprandial lipid response. *J Lipid Res* 2001; 42:1618-25.

Design: Cross-over postprandial test
Days on diet: -
Duration of postprandial test: 6 hours
Number of subjects: 10 women

	Major fatty acid	Positional distribution	Other fatty acids
Diet 1	16:0	10% of the major isomers had 16:0 at <i>sn</i> -2	18:1, 18:2
Diet 2	16:0	31% of the major isomers had 16:0 at <i>sn</i> -2	18:1, 18:2

Fat source:
Diet 1: Palm oil
Diet 2: Randomized palm oil
Intake of test fat: 55 g per m² body area
Type of diet: Liquid meal

Characteristics subjects:

- Age (y): 26.9 years
- BMI (kg/m²): 20.6 kg/m²
- Total cholesterol: 3.95 mmol/L
- LDL cholesterol: 2.24 mmol/L
- HDL cholesterol: 1.49 mmol/L
- TAG: 0.72 mmol/L

Measured parameters:

Postprandial plasma total cholesterol, and TAG in total plasma, CM and VLDL-rich fractions, glucose, insulin and free fatty acids were determined.

Main results:

The incremental area under the curve (iAUC) for plasma TAG was significantly greater after the native palm oil than after the transesterified palm oil meal ($P=0.047$). iAUC for other parameters were identical. However, the peak for insulin was at 90 min after the native palm oil and at 60 min after the meal rich in randomized palm oil.

Major conclusion:

Native palm oil increased the postprandial TAG response compared to randomized palm oil.

Remarks:

Both fats were liquid at body temperature, but at 20 °C the solid fat content of the transesterified palm oil was 17.8% and of palm oil 0%.

Article: Yli-Jokipii KM, Schwab US, Tahvonen RL, Kurvinen JP, Mykkänen HM, Kallio HP. Chylomicron and VLDL TAG structures and postprandial lipid response induced by lard and modified lard. *Lipids* 2003; 38:693-703.

Design: Cross-over postprandial test
Days on diet: -
Duration of postprandial test: 8 hours
Number of subjects: 7 women, 2 men

	Major fatty acid	Positional distribution	Other fatty acids
Diet 1	16:0	68.7% of the major isomers had 16:0 at <i>sn-2</i>	18:0, 18:1
Diet 2	16:0	49.7% of the major isomers had 16:0 at <i>sn-2</i>	18:0, 18:1

Fat source:
Diet 1: Lard
Diet 2: Randomized lard
Intake of test fat: 55 g per m² body area
Type of diet: Liquid meal

Characteristics subjects:

- Age (y): 24 years
- BMI (kg/m²): 21.5 kg/m²
- Total cholesterol: 4.4 mmol/L
- LDL cholesterol: 2.5 mmol/L
- HDL cholesterol: 1.5 mmol/L
- TAG: 0.9 mmol/L
- Glucose: 4.9 mmol/L

Measured parameters:

Postprandial plasma total and VLDL cholesterol, and TAG in total plasma and VLDL-rich fractions, glucose, insulin and free fatty acids were determined.

Main results:

The incremental area under the curve (iAUC) of plasma TAG tended to be larger after modified lard than after lard (P=0.086). The iAUC of VLDL TAG was larger after modified lard. Plasma FFA rose faster after lard than after modified lard. Otherwise, there were no statistically significant differences.

Major conclusion:

Modified lard may increase the postprandial TAG response compared to native lard.

Remarks:

Measured by pulse NMR, 27.6% of lard and 18.3% of modified lard were solid at 30 °C, 12.5 and 11.0% at 35 °C, and 8.3 and 6.5% at 40 °C.
Wash-out period of 4 weeks.

Article: Zampelas A, Williams CM, Morgan LM, Wright J. The effect of triacylglycerol fatty acid positional distribution on postprandial plasma metabolite and hormone responses in normal adult men. *Br J Nutr* 1994; 71:401-10.

Design: Cross-over postprandial test
Days on diet: -
Duration of postprandial test: 6 hours
Number of subjects: 16 men

	Major fatty acid	Positional distribution	Other fatty acids
Diet 1	16:0	6% of 16:0 at <i>sn</i> -2	18:1, 18:2
Diet 2	16:0	73% of 16:0 at <i>sn</i> -2	18:1, 18:2

Fat source:

Diet 1: Palm oil
Diet 2: Modified palm oil (Betapol)
Intake (En%) of test fat: 40 g or 54 En%
Type of diet: Liquid meal

Characteristics subjects:

- Age: 24.8 years
- BMI: 22.7 kg/m²
- Total cholesterol: 4.69 mmol/L
- LDL cholesterol: 2.90 mmol/L
- HDL cholesterol: 1.29 mmol/L
- TAG: 0.80 mmol/L
- Glucose: 5.00 mmol/L

Measured parameters:

Plasma total cholesterol, HDL cholesterol, TAG, non-esterified fatty acids, apoprotein, glucose, insulin and glucose-dependent insulinotropic polypeptide (GIP) concentrations were determined. LDL was calculated with the Friedewald equation.

Main results:

No differences in postprandial responses were found for any of the parameters measured (including TAG responses in total plasma, CM-rich, and CM-poor fractions).

Major conclusion:

Enrichment of the *sn*-2 position of dietary TAG with 16:0 is not an important determinant of postprandial responses. when dietary TAG is fed in amounts normally consumed as part of a standard meal.

Remarks:

This was a strictly controlled dietary study.

Article: Zock PL, de Vries JHM, de Fouw NJ, Katan MB. Positional distribution of fatty acids in dietary triglycerides: effects on fasting blood lipoprotein concentrations in humans. *Am J Clin Nutr* 1995; 61:48-55.

Design: Cross-over study with no wash-out period
Days on diet 21 days
Duration of postprandial test: -
Number of subjects: 23 men and 37 women

	Major fatty acid	Positional distribution	Other fatty acids
Diet 1	16:0	82% of 16:0 at <i>sn</i> -1,3 + 18% at <i>sn</i> -2	<i>Cis</i> 18:1, 18:2
Diet 2	16:0	35% of 16:0 at <i>sn</i> -1,3 + 65% at <i>sn</i> -2	<i>Cis</i> 18:1, 18:2

Fat source:

Diet 1: Native palm oil
Diet 2: Modified palm oil (Betapol)
Daily intake (En%) of test fat: 28%
Type of diet: Mixed solid diets

Baseline characteristics subjects:

- age: 31 years
- BMI: 22.7 kg/m²
- Total cholesterol: 4.63 mmol/L
- LDL cholesterol: -
- HDL cholesterol: 1.52 mmol/L
- TAG: 0.92 mmol/L

Measured parameters:

Serum total cholesterol, HDL cholesterol and TAG were measured. LDL cholesterol was calculated with the Friedewald equation.

Main results:

For the whole study population, serum total cholesterol, HDL cholesterol, LDL cholesterol and TAG concentrations, and the HDL to LDL cholesterol ratio did not change. In men only, serum total and LDL cholesterol increased marginally - though significantly - by respectively 0.10 mmol/L and of 0.08 mmol/L on the modified palm oil diet. Responses between men and women were however not statistically different.

Major conclusion:

The positional distribution of palmitic acid within dietary TAG had little effects on serum lipid and lipoprotein concentrations. Also in men, the slight increases in serum total and LDL cholesterol were not considered to be detrimental in view of the high intakes of the experimental fats (which will be difficult to achieve with everyday diets).

Remarks:

None.