Significance of the SN-2 Hypothesis

Voon Phooi Tee* and Kalanithi Nesaretnam*

INTRODUCTION

The body uses fats for long-term energy storage because they provide about six times as much energy as an equal weight of stored, hydrated glycogen (McMurray, 2000). Many different fats and oils exist as sources of triacylglycerols in the human diet. These oils originate from fruits (palm oil and olive oil) or from seeds (corn oil, rapeseed oil and soyabean oil). Animal and fish fats are other examples of fats. Animal fats like butter and lard are solid at room temperature while vegetable oils like corn, soyabean and peanut oils are liquid. However, their structures are closely related.

Fats and oils are made up of a mixture of triacylglycerols (TAG), which in turn consist of a glycerol backbone to which three fatty acids are esterified. The distribution of the fatty acids on the glycerol backbone of the TAG which is referred to as the stereospecific number, (sn) -1, -2 and -3, plays a significant role as a marker of its composition and properties (Goh, 2006). *Figure 1* shows the schematic structure of the TAG where three fatty acids are bonded to a glycerol backbone.

The fatty acids in fats and oils are classified as saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) or polyunsaturated fatty acids (PUFA). With this classification, palm kernel oil, which is made up of 88% SFA (lauric acid, C12:0 and myristic acid, C14:0) and has very little MUFA and PUFA, is considered a saturated fat. Olive oil on the other hand has 80% oleic acid (C18:1) and only 9% PUFA and 10% SFA, and is therefore classified as an oil that is predominantly monounsaturated. Sunflower oil has 70% linoleic acid (C18:2) and only 12% SFA, and hence is termed a polyunsaturated oil.

In vegetable oils, oleic acid (C18:1, a member of MUFA) is predominantly situated at the sn-2 position, while in animals fats it is predominantly palmitic acid or stearic acid (C16:0 or C18:0-saturated fat) at this position. *Figure 2* shows the fatty acids composition (FAC) for palm olein, lard, human milk and cow's milk. Even though palm olein and lard have similar proportion of SFA, MUFA and PUFA, they differ significantly in the positional distribution of the fatty acids on the TAG molecule (*Figure 3*). Palm olein triglycerides contain only 7%-11% palmitic acid at the sn-2 position while 60%-70% is oleic acid. On the other hand, in human milk, palmitic acid is predominantly present in the sn-2 position (53%-57%) while cow's milk fat contains less palmitic acid (38%) at that position, as reported by Straarup *et al.* workers (2006). They found that infant formulations in the Danish market did not match human milk although they were formulated to mimic its FAC. Most of the palmitic acid in the TAG molecules was located at the sn-1 and sn-3 positions (75%-97%). It has become apparent that the FAC alone does not tell you the whole story. The positional distribution of the fatty acids in the TAG is more important.

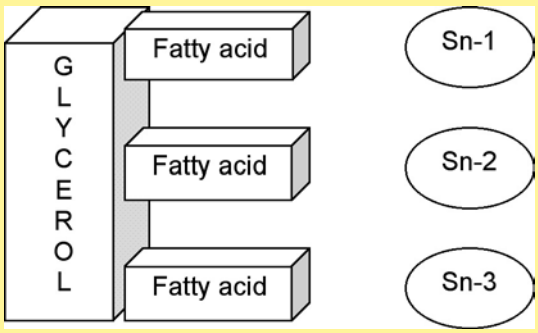
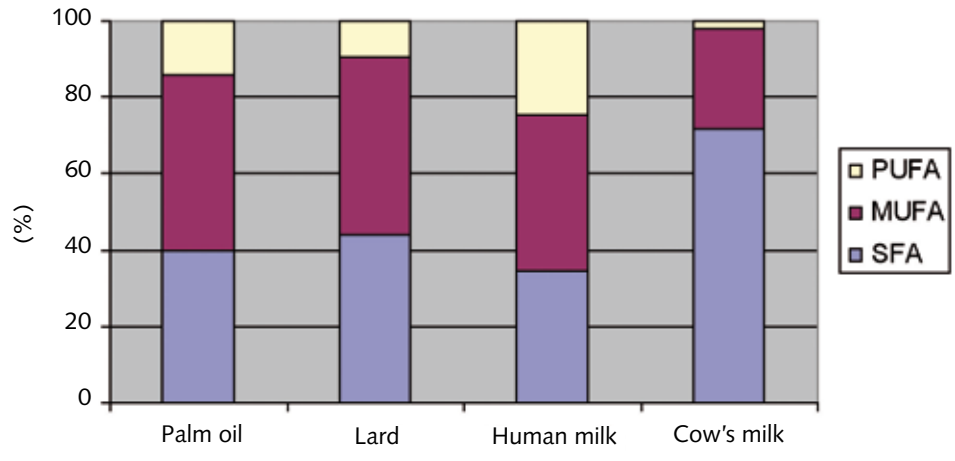


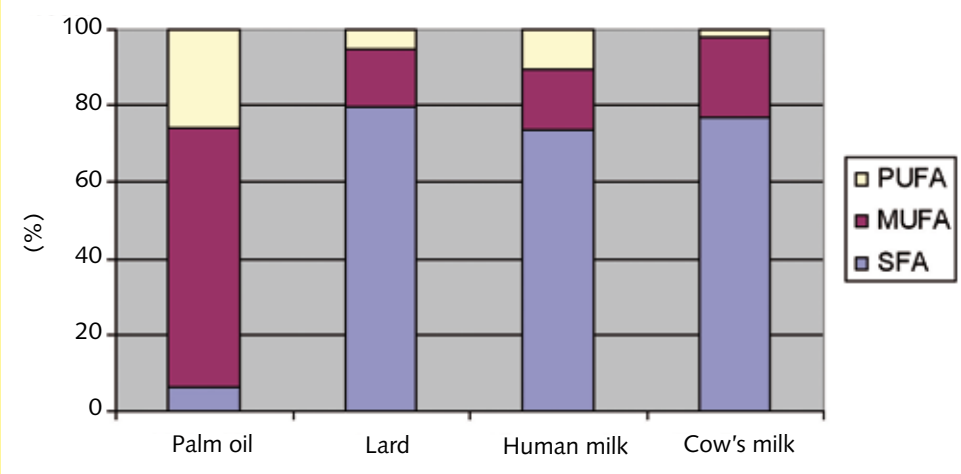
Figure 1. Triacylglycerols (TAG) structure showing the stereospecific numbering of sn-1, -2 and -3.

* Malaysian Palm Oil Board,
P. O. Box 10620,
50720 Kuala Lumpur,
Malaysia.
E-mail: sarnesar@mpob.gov.my



Source: Straarup *et al.* (2006).

Figure 2. Fatty acid compositions of palm olein, lard, human milk and cow's milk.



Source: Straarup *et al.* (2006).

Figure 3. Sn-2 fatty acid composition of palm olein, lard, human milk and cow's milk.

LIPID DIGESTION AND METABOLISM

The digestion of fat occurs when the enzyme lipase is present. The lipases involved in this process are lingual, gastric, pancreatic and co-pancreatic lipases that are found in the mouth, stomach and small intestine, respectively.

In the stomach, pre-digestion of 10%-30% of fat occurs in the presence of lingual and gastric lipases, and bile salts produced from the liver. Lingual and gastric lipases prefer to cleave the sn-3 fatty acids, resulting in the formation of 1,2-diacylglycerols (1,2-DAG) and sn-3 free fatty acids. The acidic medium in the stomach will facilitate the conversion of sn-1,2-DAG to sn-1,3-DAG. The sn-1,3-DAG and sn-3 free fatty acids (if <12 carbons) are readily absorbed in the intestine. The schematic diagram below shows the hydrolysis route of TAG at different locations.

In the stomach,

TAG —(Gastric or lingual lipase)→ sn-1,2-DAG + sn-3 free fatty acids
(Acyl migration occurs in the acidic medium in the stomach)
↓
sn-1,3-DAG

In the small intestine, particularly in the duodenum,

TAG —(1,3-specific pancreatic and co-lipase)→ 2-Monoacylglycerols (2-MAG) + sn-1 and sn-3 free fatty acids

In the small intestine, particularly in the duodenum, digestion of 70%-90% of the fats takes place in the presence of pancreatic and co-pancreatic lipases. Pancreatic lipase hydrolyse the sn-1 fatty acids, while co-pancreatic lipase prefers to hydrolyse sn-3 fatty acids in TAG. The products from the hydrolysis of TAG are 2-MAG, sn-1 and sn-3 free fatty acids.

The sn-2 fatty acid in the form of 2-MAG is transported by a type of lipoprotein called the chylomicrons. Chylomicrons contain 93% of new TAG (solely from the food source) in its core. These new TAG are result of the resynthesis of 2-MAG and free fatty acids (majority long-chain SFA) present in the intestine. Short and medium chain fatty acid absorption is not via chylomicrons. Chylomicrons are then secreted into the blood stream via the lymphatic system. The presence of lipoprotein lipase which lines on the blood vessel walls will hydrolyze the new TAG in chylomicron. Chylomicron remnants, 2-MAG and free fatty acids are then produced. Chylomicron remnants which carry the cholesterol ester and TAG will be transported back to the liver, while 2-MAG and free fatty acids will be used for the liver TAG synthesis or energy supply and storage. Eating long-chain SFA and elaidic acid (*trans* isomer of oleic acid) situated at the sn-2 position of TAG might slow down the hydrolysis of chylomicron TAG, liver uptake and the clearance of chylomicron remnants. The presence of a large amount of chylomicron remnants in the blood could lead to an increased plasma cholesterol level and atherogenesis which can cause detrimental effects to health.

In the intestines, sn-1 and sn-3 short and medium chain free fatty acids are absorbed directly after hydrolysis. Long chain SFA will either be absorbed or predominantly react with 2-MAG for the resynthesis of new TAG and chylomicron formation. Sn-1 and sn-3 long chain free SFA will not be absorbed or have a delayed absorption as their melting points are higher than body temperature. Furthermore, with the presence of calcium in the intestine, these long chain free SFA tend to precipitate as calcium soaps and are then excreted (*Table 1*).

Surprisingly, pancreatic lipase and its co-lipase have low activity on long chain PUFA that have more than 20 carbons, especially arachidonic acid (ARA, C20:4n-6), eicopentaenoci acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3), although they are located at the sn-3 position (Bottino *et al.*, 1967). These longchain PUFA are present in the form of 2,3-DAG instead of sn-3 fatty acid itself. These 2,3-DAG will only be hydrolyzed by hepatic lipase in the liver. They are retained in the chylomicron remnants that will be transported to the liver to produce 2-MAG and free long chain PUFA. The slow hydrolysis of the long chain PUFA at the sn-3 position in TAG reduces the supply of sn-2 MAG and delays the resynthesis of new TAG in the intestine. Hence, sn-3 positioned long chain PUFA are not directly absorbed by the body, while the sn-2 long chain PUFA will be directly absorbed in the form of 2-MAG. This is important especially when introducing DHA in infant formula for better and faster absorption.

The absorption and digestion of fat in infants are slightly different from those of adults. At birth, infants have to adapt to the high fat content of breast milk after relying mainly on glucose as an energy source during fetal development. The pancreatic secretion of lipase is low and the immature liver is unable to provide sufficient bile salts to solubilize the digested lipids. Hence, newborn babies digest fats less efficiently than adults. However, breast milk contains lipoprotein lipase and bile-salt-stimulated-lipase that might be able to assist the baby to digest milk TAG. In addition, the baby also secretes a TAG lipase from glands in the stomach and tongue. The presence of milk lipase (only in babies) in the intestinal lumen will hydrolyse 2-MAG to glycerol and free fatty acids for direct absorption before the resynthesis of new TAG happens. This milk lipase will shorten the route of digestion and absorption of sn-2 long chain SFA in infants. Besides, human milk with palmitic acid (long chain SFA) predominantly at the sn-2 position forms mixed micelles with bile salts in the milk itself. This again

TABLE 1. SUMMARY OF ABSORPTION FOR SOME COMMON FATTY ACIDS AT SN-1 AND SN-3 POSITIONS IN TRIACYLGLYCEROLS

Common name	Fate after hydrolysis
Short chain fatty acids (C4-C6)	Absorbed directly
Medium chain fatty acids (C8-C10)	Absorbed directly
Long chain saturated fatty acids	Delayed absorption by minor phosphatidic acid pathway or form calcium soaps and are excreted
Long chain polyunsaturated fatty acids	Delayed formation of TAG and reduced supply of 2-MAG

TABLE 2. STUDIES ON THE NUTRITIONAL IMPACTS OF COMMON DIETARY FATTY ACIDS IN HUMAN, INFANT AND ANIMAL MODELS

Model	Test fats or fatty acids involved	Results	References
Infant nutrition	Palmitic acid at sn-1 and sn-3 positions in infant formula *vs*. human milk.	Infant formula with palmitic acid in sn-1 and sn-3 positions causes the formation of calcium-fatty acid complexes that are poorly absorbed by infant compared to human milk.	Lewis *et al*. (1977), Chappel *et al*. (1986)
	Palmitic acid at the sn-2 position in a formula called Betapol.	Palmitic acid at the sn-2 position reduces the excretion of palmitate and associated calcium soaps in stools and stool hardness, increases calcium absorption, resulting in greater skeletal mineral deposition as compared to normal infant formula. However, higher levels of HDL-cholesterol were seen in infants fed with breast milk compared to infants fed with formula milk which have less palmitic acid at the sn-2 position.	Nelson *et al.* (1999), Kennedy *et al.* (1999)
Adult nutrition	Palm olein *vs*. lard (~7% and 70% palmitic acid at the sn-2 position, respectively).	Palm olein group results showed lower serum total cholesterol, LDL-cholesterol and total cholesterol/HDL ratio compared to the lard group in normocholesterolemic subjects. However, the intake of dietary cholesterol from lard was higher than palm olein group with 313 mg day^{-1} and 226 mg day^{-1} respectively could also explain this.	Jian *et al*. (1997)
	Palmitic acid at sn-2 *vs*. palmitic acid at sn-1 and sn-3 positions in the presence of n-6 PUFA.	Dietary fats containing palmitic acid at the sn-2 position may result in a slightly lower fasting total cholesterol than diets with palmitic acid at the sn-1 and sn-3 positions, while the level of n-6 PUFA influences endogenous cholesterol synthesis.	Forsythe *et al*. (2007)
	Palmitic *vs*. lauric *vs*. myristic *vs*. stearic acids at sn-2 position.	No significant changes were detected in the blood lipids and lipoprotein parameters in all the fats.	Meijer *et al*. (1997)
	Stearic acid at sn-2 position (postprandials' trial).	Consumption of stearic acid in the form of structured TAG leads to elevated plasma triglycerides and factor FVII:c than a meal enriched with cocoa butter or oleate. The stereospecific structure of the ingested TAG was largely preserved in chylomicron-TAG.	Lucinda *et al.* (1999); Sanders *et al*. (2001)
	EPA and DHA at sn-2 position.	It is recommended to add DHA at the sn-2 position for preferential and rapid supply into plasma TAG and phospholipids.	Sadou *et al*. (1995)
Animal nutrition	Different amounts of palmitic acid at sn-2 position.	Piglets fed with higher sn-2 palmitic acid have higher plasma total cholesterol and triglycerides concentration.	Innis *et al*. (1997)
	Different amounts of palmitic acid at sn-2 position.	Atherogenicity increased in rabbits fed with 8%-14% sn-2-palmitic acid compared to rabbits fed with 2% sn-2 palmitic acid.	Kritchevsky *et al*. (1998)
	Palm oil *vs*. interesterified palm oil (palmitic acid at sn-2 *vs*. palmitic acid at sn-1 and sn-3).	The sn-2 palmitic acid-fed piglets have higher total cholesterol and HDL-cholesterol compared to sn-2 oleic acid-fed piglets.	Innis *et al*. (1993)
	Palmitic acid *vs*. stearic acid at sn-2 position.	Sn-2 palmitic acid is more atherogenic than stearic acid at sn-2 position.	Kritchevsky *et al*. (1997)
	Stearic acid at sn-2, sn-1 and sn-3 positions.	Stearic acid at sn-1 and sn-3 positions resulted in increased loss of fats and calcium in the feces due to formation of insoluble calcium soaps compared to stearic acid at sn-2 position in rats.	Apgar *et al.* (1987), Brink *et al.* (1995)
	Seal oil *vs*. fish oil (DHA at sn-1 and sn-3 *vs*. DHA at sn-2 positions, respectively).	DHA content was similar in both seal and fish oil-fed rats.	Jensen *et al*. (1996)
	n-3 PUFA at sn-1 and sn-3 posiitons *vs*. n-3 PUFA at sn-2 position.	There was no difference in the absorption profiles and *in vitro* rate of lipase activity in rats.	Porsgaard *et al.* (2005)
	EPA and DHA at sn-1, sn-3 and sn-2 positions.	EPA and DHA predominantly at the sn-2 position were more readily absorbed than at the sn-1 and sn-3 positions in rats.	Christensen *et al.* (1995)

allows good and rapid absorption of TAG by infants (Filter *et al*., 1969; Bracco *et al.*, 1994; Innis *et al*., 1994). If the infant formulation contains long chain SFA at the sn-1 and sn-3 positions, absorption will be delayed as the milk lipase may only act on 2-MAG formed in the intestine before new TAG is resynthesized. Hence, the tendency to form long chain calcium soap causes hard stools or constipation, and less calcium absorption in infants. The TAG structure of human milk is unique as it leads to optimize absorption of palmitic acid. That is why sn-2 palmitic acid is preferred in infants over sn-1 and sn-3 palmitic acid. Thus, an improved infant formulation that mimics the TAG positional distribution of human milk is required. Betapol is one example of such an infant formulation.

EFFECTS OF STEREOSPECIFIC FATS ON LIPID PROFILE

Clinical trials on humans and animal testing have been carried out to determine the effects of stereospecific fats on lipid profile. *Table 2* summarizes the outcome of these trials. The position of SFA at TAG may exert two effects on plasma cholesterol. If the long chain SFA occur at the sn-1 and sn-3 positions, they are either neutral or tend to lower cholesterol levels. If the long chain SFA occur at the sn-2 position, they generally tend to increase the cholesterol level.

SN-2 HYPOTHESIS AND PALM OIL

In palm oil, the long chain SFA (palmitic acid) is predominantly situated at the sn-1 and sn-3 positions, and is mainly excreted through the formation of calcium soaps (evidence shown in human infant and animals, no evidence of this in human adults yet). The other main fatty acid in palm oil, oleic acid, is situated at the sn-2 position, and at this position, it will be absorbed into the body and does not alter the blood lipid profile as shown in studies with olive oil (~ 80% oleic acid at sn-2) (Ng *et al.*, 1992; Choudhury *et al.*, 1995). Hence, it is wrong to group palm oil with the traditional sources of saturated fats. In addition, plasma lipid response to a palm oil-rich diet was found to be mild in intensity, and appeared to be more dependent on age, gender, increased body mass index (BMI), daily cholesterol ingestion and the synthetic nature of the oils. More scientific evidence with adequate and well controlled study designs are required to clear the misconception of palm oil and its nutritional implications especially on human adults. This is currently the focus of research at MPOB.

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