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REVIEW

sn-2 Hypothesis: a Review of the Effects of Palm Oil on Blood Lipid Levels

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Abstract: Saturated fats are commonly claimed to raise human blood cholesterols and contribute to cardiovascular disease. Previous literature data were highlighted that although palm oil is 50% saturated, it does not behave like a saturated fat. Human trials were conducted to compare the effects on serum cholesterol levels given by palm olein and monounsaturated oils. It was postulated that saturation/ unsaturation of the fatty acids situated at *sn*-2 positions of triglycerides in the fat molecules determine the induced blood lipid levels but not the overall saturation of oils. The results showed that the lipid parameters (LDL and HDL) effects induced by these oils are similar with no significant differences. This study provides concrete evidence that the unsaturation levels of these oils at *sn*-2 position of TG are similar (90-100%) which are claimed to be responsible for the lipid parameters. In conclusion, the public negative perception on believing that the overall saturation of oils is detrimental to health should be corrected because in fact the unsaturation at *sn*-2 positions of the saturated vegetable fat such as palm olein and cocoa butter make them behave like mono-unsaturated oils, unlike saturated animal fats that possess a high content of saturated fatty acids at *sn*-2 position.

Key words: *sn*-2 position, overall saturation, palm oil, monounsaturated oils, perception

1 Introduction

Palm oil is a good ingredient for food manufacturers due to its nutritional profiles and versatility[1)]. It has a variety of food uses including cooking oils, margarines, shortenings and cocoa butter equivalents. It is an important source of downstream non-food products such as surfactants, candles, soaps, cosmetics, detergents, emulsifiers, lubricants, paints, pharmaceutical and biofuels[2)]. Palm oil derivatives are used extensively as food ingredients in ice-cream, salad dressings, biscuits, instant noodles and condensed milk[3)]. Palm oil has a high oxidative stability and health benefits, which are contributed by the abundance of natural source of carotenoids, tocopherols and tocotrienols. The existence of natural vitamin E in palm oil warrants a longer shelf-life for palm-based food products. Furthermore, tocotrienols have been reported to exert anti-oxidant[4, 5)] and natural inhibitors of cholesterol synthesis[6)]. Besides, carotenoids have been proven scientifically to possess anticancer[7, 8)] properties.

Saturated fatty acids (SFA) are often perceived to be correlated with increased risk of heart-related diseases. The American Heart Association's Nutrition Committee suggested a reduction of daily saturated fats intake for adults due to the assumption that saturated fats will elevate serum cholesterol levels and consequently increase the risk of coronary heart diseases (CHD)[9)]. Palm olein is often classified as a saturated fat as it contains approximately 47.2% of SFA and 52.8% of unsaturated fatty acid (UFA), at which its fatty acid composition is similar to lard[10)]. However, the structure of palm olein is unique with SFA bound principally at the *sn*-1 and *sn*-3 positions while UFA at the *sn*-2 position. Even though palm olein contains considerably a high level of SFA, yet, it behaves more like a mono-unsaturated oil such as olive oil in terms of serum cholesterol levels[11)]. Reiser (1973)[12)] performed a comprehensive examination on the literature data to assess the correlation of saturated fats with the serum cholesterol concentration and came across the fact that not all of the

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saturated fats possess detrimental effect on plasma lipids, with surprise. Reiser also noticed that a highly saturated fat such as cocoa butter, which comprises approximately 63.6% of SFA, exhibited neutral effects on serum cholesterols in human subjects. Therefore, the misconception of deleterious effect of saturated fats should be reviewed.

2 Classification of Fats and Oils

Fats and oils are made up of a mixture of triglycerides (TG), which consists of a glycerol backbone to which three fatty acids are esterified. The positions of fatty acid attached to the glycerol backbone are referred by stereospecific numbering (*sn*)-1, -2 and -3, as shown in Fig. 1[13]. The stereospecific position of fatty acids in TG has a great impact on the properties of oils and fats. The fatty acids in fats and oils are generally characterized as SFA, monounsaturated fatty acids (MUFA), or polyunsaturated fatty acids (PUFA). For example, palm kernel oil is classified as a saturated fat as it consists of 85.4% SFA, 13.5% MUFA and a minimum amount of PUFA. On the other hand, olive oil is classified as monounsaturated oil as it is made up of 76.8% MUFA, 8.3% PUFA and 14.9% SFA. In addition, sunflower oil is classified as a polyunsaturated oil as it contains predominantly PUFA (63.5%) with only 10.7% of SFA, as presented in Table 1. MUFA is predominantly attached at the *sn*-2 position of TG in vegetable oils while SFA at the *sn*-1,3 positions. Even though palm olein has a similar profile of overall fatty acid compositions with lard, the stereospecific positions of fatty acids on the TG molecule differ greatly[14]. Palm olein contains only 4.3–10.4% of SFA at the *sn*-2 position of TG and the major fatty acids attached at this position is UFA (89.6-95.7%) (see Table 1). Conversely, lard has the highest amount of SFA (88.8%) at the *sn*-2 position of TG. Beef fat, another type of animal fat, contains less SFA at the *sn*-2 position of TG with approximately 46.6% (see Table 1). As a summary, it is noteworthy that the positional distribution of fatty acids in the TG molecule does give an impact to the properties of fats and oils, and should not be classified based on the overall fatty acid composition[1, 13].

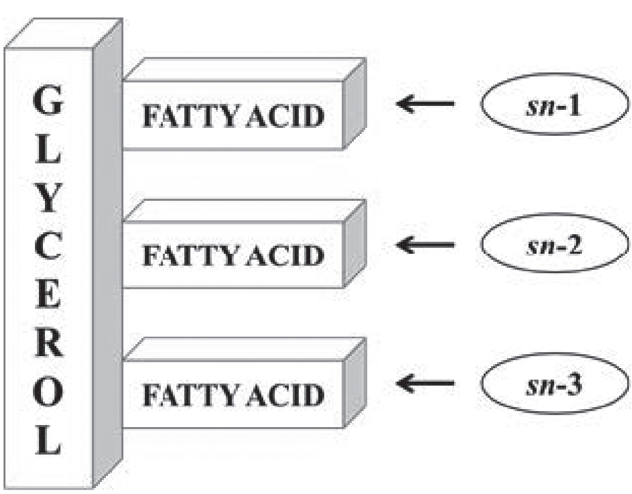

Fig. 1 Stereospecific numbering (*sn*) of fatty acids in triglyceride structure[13].

3 Concepts of *sn*-2 hypothesis

The exceptional neutral effects of human serum cholesterol resulted by high level of SFA may be due to the digestibility, which is influenced by the fatty acid distribution on TG[15]. According to Padley[16] and World Health Organization (WHO), the stereospecific position of fatty acids in the TG molecule determines the physical characteristic of fats, as well as affects the absorption of fatty acids, lipid metabolism and fat distribution in tissues. The different digestibility and absorption of fatty acids, which are attached to different positions at TG backbone, play major roles in serum cholesterol levels. This statement is able to justify that the cocoa butter with high level of SFA does not elevate the serum cholesterol concentration. To date, no scientific study reveals that all of the fats containing high SFA will lead to the cardiovascular diseases for the reason that the metabolic and serum cholesterol response distinctively to the positional distribution of SFA[17].

TG is easily hydrolyzed by the pancreatic lipase and gives rise to two units of free fatty acids, which are released from the *sn*-1 and *sn*-3 positions of TG, together with one unit of 2-monoglyceride[18]. These free units will be re-synthesized into TG in endoplasmic reticulum once they cross the enterocyte barriers by diffusion. The ingested TG and fat-soluble substances will be packed into lipoproteins called chylomicrons in the body system. The major role of chylomicrons is to carry the lipids in the circulatory system through lymphatic vessels and consequently delivered the absorbed TG to the cells of the body. In short, lipoprotein lipase, an enzyme that is present in the capillary endothelial cells, is responsible to hydrolyze TG in chylomicrons and other lipoproteins into 2-monoglyceride and fatty acids and subsequently diffuse the hydrolyzed substances into the body cells[19]. Intestinal cholesterol absorption is important because of the clinical relevance of cholesterol. When passing through the small intestine, an approximately 50% of total cholesterol will be typically absorbed while the leftover will then be excreted in the form of faeces[20].

Conversely, fatty acids that are situated at the *sn*-2 position of TG are prone to be transported to the liver instead of adipose tissue, attributed to the stereospecific activity of lipoprotein lipase for the *sn*-1 and *sn*-3 positions of TG[21, 22]. In other words, the glyceride that will be absorbed in the

Table 1 Positional fatty acid composition (mol/100 mol total fatty acids) of the dietary oils.

	Composition (mol%)		
	sn-1,3	*sn*-2	*sn*-1,2,3
Palm Kernel Oil			
Saturated	90.2	75.6	85.4
Monounsaturated	8.8	23.2	13.5
Polyunsaturated	1.0	1.2	1.1
Palm Olein IV56			
Saturated	67.1	10.4	47.2
Monounsaturated	26.8	65.8	40.5
Polyunsaturated	6.1	23.8	12.3
Palm Olein IV64			
Saturated	60.7	8.5	43.3
Monounsaturated	34.5	63.9	44.3
Polyunsaturated	4.8	27.6	12.4
Palm Olein IV72			
Saturated	51.3	4.3	35.8
Monounsaturated	41.9	67.1	50.2
Polyunsaturated	6.8	28.6	14.0
Olive Oil (OO)			
Saturated	22.1	n.d.	14.9
Monounsaturated	71.5	88.0	76.8
Polyunsaturated	6.4	12.0	8.3
Sunflower Seed Oil (SFO)			
Saturated	16.0	n.d.	10.7
Monounsaturated	27.0	23.3	25.8
Polyunsaturated	57.0	76.7	63.5
Canola Oil (CAN)			
Saturated	11.7	n.d.	7.7
Monounsaturated	66.7	51.7	61.6
Polyunsaturated	21.6	48.3	30.7
Corn Oil (CO)			
Saturated	22.0	n.d.	14.5
Monounsaturated	27.7	26.0	27.1
Polyunsaturated	50.3	74.0	58.4
Soybean Oil (SBO)			
Saturated	20.1	n.d.	12.9
Monounsaturated	26.6	21.4	24.8
Polyunsaturated	53.3	78.6	62.3
Peanut Oil (PE)			
Saturated	28.0	n.d.	18.9
Monounsaturated	53.4	59.5	55.3
Polyunsaturated	18.6	40.5	25.8
Coconut Oil (CNO)			
Saturated	93.7	93.5	93.5
Monounsaturated	6.3	6.5	6.5
Polyunsaturated	n.d.	n.d.	n.d.
Lard			
Saturated	32.5	88.8	49.0
Monounsaturated	55.9	11.2	42.8
Polyunsaturated	11.6	n.d.	8.2
Beef Fat			
Saturated	68.7	46.6	61.4
Monounsaturated	30.2	49.6	36.6
Polyunsaturated	1.1	3.8	2.0
Cocoa Butter			
Saturated	96.1	n.d.	63.6
Monounsaturated	3.9	87.2	32.1
Polyunsaturated	n.d.	12.8	4.3
Safflower Oil			
Saturated	14.7	n.d.	9.8
Monounsaturated	12.7	11.4	12.2
Polyunsaturated	72.6	88.6	78.0

Note: Regio-specific analyses of the dietary oils were performed using a NMR spectrometer JEOL ECZ-600MHz as described by Teh *et al.*[10]; n.d.= not detectable.

intestine is 2-monoglyceride. This is in opposition to the weak absorption of long chain SFA, which are derived from *sn*-1 and *sn*-3, due to the poor solubility of these SFA and likely to form insoluble magnesium and calcium soaps instead of being absorbed into the body. Furthermore, hepatocyte is the major site of actions of fatty acids for the metabolism of low density lipoprotein (LDL). Therefore, SFA at the *sn*-2 position of dietary TG are more susceptible to raise the LDL concentrations than the same SFA which are located at the *sn*-1 or *sn*-3 positions.

4 Comparison of serum cholesterol levels contributed by palm olein and various vegetable oils in human clinical trials

4.1 Palm olein vs Olive oil

Five randomized crossover human clinical trials were carried out by different groups of researchers from year 1992 to 2015 and the summary is presented in Table 2. A comparative study (2×6 weeks intervention) on serum cholesterol levels in healthy normocholesterolemic Malaysian adults aged from 21 to 41 was performed by Ng and his co-workers in 1992[23]. The results indicated that sub-

Table 2 Experimental design, subjects and test oils.

Reference	Year	No. of subjects		Targets		Study design	% energy from fats (% energy from test oils)	Types of oils	Serum lipid parameters				
		Men	Women	Age (Average age)	TC (mmol/L)				TC	TG	HDL	LDL	TC:HDL
Ng *et al*.	1992	20	13	21-41	4.1-5.7	x	34 (23)	PO IV56- OO	NS	NS	NS	NS	Nil
Choudhury *et al*.	1995	10	11	19-44 (27)	4.4-6.7	x	30 (17)	PO IV56- OO	NS	NS	* (>)	NS	Nil
Tholstrup *et al*.	2011	32	0	19-64 (30)	<5.2	x	35 (17)	PO IV64-OO	* (>)	* (<)	NS	* (>)	NS
								PO IV64- Lard	NS	NS	NS	NS	NS
								OO- Lard	* (<)	NS	NS	* (<)	NS
Voon *et al*.	2011	9	36	(30)	4.0- 5.4	x	31 (21)	PO IV64- OO	NS	NS	NS	NS	NS
								PO IV64-CNO	NS	NS	NS	NS	NS
								OO-CNO	* (<)	NS	* (<)	* (<)	NS
Sun *et al*.	2017	48	52	25-55 (40)	3.6-5.0	x	29 (17)	PO IV72-OO	NS	NS	NS	NS	Nil
Lucci *et al*.	2016	160	0	62-65	5.3-5.3	//	26 (Nil)	Hybrid Palm Oil-OO	NS	NS	NS	NS	Nil
Marzuki *et al*.	1991	110	0	16-17	3.8-4.0	x	35 (Nil)	PO IV56-SBO	NS	NS	NS	NS	NS
		7	0	16-17	6.0-6.1	x	35 (Nil)	PO IV56-SBO	NS	* (<)	NS	NS	NS
Ghafoorunissa *et al.*	1995	12	0	29-39 (35)	3.1-4.8	x (8-week)	27 (Nil)	PO IV56-PE	NS	NS	NS	NS	Nil
		12	12	30-52 (43)	3.1-6.7	x (16-week)	32 (Nil)	PO IV56-PE	NS	NS	NS	NS	Nil
Zhang *et al*.	1997	120	0	18-25	2.8-5.0	//	30 (23)	PO IV64-SBO	NS	NS	NS	NS	NS
								PO IV64-PE	NS	NS	* (>)	NS	* (<)
								PO IV64-Lard	* (<)	NS	NS	* (<)	* (<)
		31	20	32-68	5.5-7.0	x	30 (19)	PO IV64 (week-0)-(week-6)	* (<)	NS	NS	* (<)	* (<)
								PO IV64 (week-6)-(week-12)	NS	NS	NS	NS	* (<)
								PE (week-0)-PE (week-6)	NS	NS	NS	NS	NS
								PE (week-6)-PE (week-12)	NS	NS	NS	NS	NS
Wood *et al*.	1993	29	0	30-60 (41)	4.3-5.9	x	38 (19)	PO IV56-SFO	* (>)	* (>)	* (>)	NS	Nil
Sundram *et al*.	1995	23	0	19-24 (22)	3.8-5.1	x	32 (20)	PO IV56-CAN	NS	NS	NS	NS	Nil

Note: TC = Total cholesterol; TG = Triglycerides; HDL = High density lipoprotein; LDL = Low density lipoprotein; x = Cross-over, Latin-square or rotating diets; // = Parallel; * = Significant different ($p<0.05$) between two test oils; NS = no significant different; Nil = Not reported in the article; > = higher; < = lower.

jects fed with both palm olein IV56 and olive oils diets had comparative levels in total cholesterol (TC), TG, high density lipoprotein (HDL) and LDL.

Later, a similar trial with 30 day-30 day intervention was performed by Choudhury *et al.* (1995), a group of researchers from Australia but with lower fat intake[11]. The palm olein and olive oil consumption for 10 men and 11 women with an average age of 27 was 57% of total fat intake. The results were consistent with that of studies done by Ng *et al.* (1992), except for HDL. The volunteers, who consumed diet made of palm olein, demonstrated serum profiles with significantly higher HDL than those who consumed diet made of olive oil.

Two randomized cross-over interventions were reported in the same year, 2011 by the researchers from Denmark[24] and Malaysia[25]. A randomized 3×3 weeks intervention was conducted in 32 healthy Danish men[24]. The test fats/oils, namely palm olein IV64, olive oil and lard were compared at 57% of total fat intake. The TC and LDL of human subjects in the palm olein group were significantly higher than those in the olive oil group. On the contrary, TG of the subjects in the palm olein group was significantly lower than those in the olive oil group. The subjects who were fed with lard-rich diet had significantly higher TC and LDL in their serum profile if compared to those who were fed with olive oil-rich diet. Nevertheless, the serum cholesterol levels of TC, TG, LDL and HDL of human subjects consuming lard and palm olein diets were comparable.

On the other hand, Malaysian researchers conducted a double-blinded, randomized 3×5 weeks cross-over dietary intervention in 45 healthy individuals[25]. The experimental fats/oils, namely palm olein IV64, olive oil and coconut oil were compared at 21% of total dietary energy. Interestingly, subjects in palm olein and olive oil groups had comparable serum cholesterol levels ($p > 0.05$), whereas subjects who consumed coconut oil diet demonstrated significantly higher TC, HDL and LDL in their serum if compared to those who consumed olive oil diet ($p < 0.05$).

Recent literature data published by Lucci *et al.* (2016)[26] showed that hybrid *Elaeis oleifera* X *E. guineensis* palm oil and extra virgin olive oil had comparable effects on plasma lipids, TC, LDL, HDL and TG ($p > 0.05$). Recent human trial on the comparison of the effects of palm olein IV72 and olive oil on plasma lipids were reported by Sun *et al.* (2017)[27]. A controlled, randomized 2×5 weeks cross-over intervention with 2 weeks washout period was conducted in 100 normocholesterolemic volunteers, aged from 25 to 55. The test diets contained 30% dietary energy from fat where 60% of which came from test oils. The findings were in good agreement with previous trials where subjects who had the intake of palm olein-rich diet and olive oil-rich diet demonstrated comparable serum cholesterol levels ($p > 0.05$), except for the trial reported by Tholstrup *et al.*[24]. Even so, another marker, ratio of TC:HDL showed that there was no significant difference between volunteers consuming palm olein and olive oil diets, statistically.

4.2 Palm olein *vs* Soybean oil *vs* Peanut oil

A 5-week cross-over human clinical trial was conducted by Marzuki *et al.* (1991)[28] to evaluate the influence of palm olein IV56 and soybean oil diets on plasma lipid profiles in 110 male adolescent students in Malaysia. It was found that the serum cholesterol levels, TC, TG, LDL and HDL for both groups did not differ significantly. Seven subjects had serum cholesterol levels exceeding the baseline set for normocholesterolemic subjects. The data of serum cholesterol levels for these subjects were reanalysed and the authors found that the TG levels of the subjects in soybean oil group was exceptionally higher than that of palm olein group ($p < 0.05$).

A few years later, Ghafoorunissa *et al.* (1995)[29] performed two cross-over studies to assess the effects of palm olein and groundnut oil on plasma lipid profile in middle-aged healthy Indian subjects. The 8-week cross-over intervention was conducted in 12 men where the energy intake from fat was 27%, whereas the 16-week cross-over intervention was conducted in 12 men and 12 women where the energy intake from fat was 32%. No statistically difference was observed on the plasma lipid profiles of the subjects between palm olein and groundnut diets for both short- and long-term studies, even though both studies were varied in terms of number of participants, duration and energy intake from fats.

A 6-week parallel human clinical study was also performed by Zhang and co-workers[30] to evaluate the effects of palm olein IV64, soybean oil, peanut oil and lard-enriched diets in 120 normocholesterolemic young men with serum cholesterol concentration ranged from 2.8 to 5.0 mmol/L[30]. The test oils were incorporated at approximately 75-80% of 30% fat calories into Chinese diets. The subjects in palm olein and soybean oil groups showed comparable serum cholesterol levels ($p > 0.05$). The findings were in good agreement with the previous study performed by Marzuki *et al.* (1991)[28]. The subjects in the peanut oil group had statistically lower HDL and higher TC:HDL ratio if compared to that of palm olein group. In addition, the TC, LDL and TC:HDL ratio of subjects consuming lard-enriched diet were significantly higher than those consuming palm olein-enriched diet. The outcomes differed from the findings report by Tholstrup *et al.*[24].

Zhang *et al.* (1997)[30] also conducted another randomized 2×6 weeks crossover intervention with 3 weeks of washout period to investigate the effects of palm olein and peanut oil-enriched diets on serum lipids in 51 hypercholesterolemia subjects with serum cholesterol ranged from 5.5 to 7.0 mmol/L. There was no significant difference between the entry and end values of subjects consuming palm olein on TG and HDL. Meanwhile, significant reductions of TC, LDL and TC:HDL ratio of the subjects in the palm olein group were observed at the end of the sixth week. The ratio of TC:HDL was found to be reduced significantly in the subjects consuming palm olein diet after a 3-week washout period while peanut oil diets demonstrated no significant influence on serum cholesterol levels throughout the experiment. In short, subjects consuming palm olein-enriched diet possessed better serum cholesterol profile compared to those consuming soybean oil-, peanut oil- and lard-enriched diets, as reported in literature data.

4.3 Palm olein *vs* Sunflower seed oil *vs* Canola oil

A Latin square designed study was done in 29 middle-aged male subjects to compare the effects of dietary fats containing palm olein IV56 and sunflower seed oil on serum cholesterols in 1993[31]. Nineteen percent of the total energy was obtained from the test oils. Volunteers who consumed palm olein-rich diet possessed significantly higher TC, TG and HDL than those who consumed sunflower seed oil group. Nevertheless, subjects with the intake of palm olein diet have no significant influence on plasma lipid profile compared to the baseline values.

A double-blinded, randomized 3×4 weeks cross-over study was conducted on 23 healthy normolipemic men. The results revealed that the subjects fed with palm olein IV56 and canola oil demonstrated comparable effects on TC, LDL, TG and HDL[32]. It was noted that palm olein diet reduced serum TG relative to the entry values (baseline) by approximately 10%. Thus, we can summarize that palm olein behaves like both mono-unsaturated oils (e.g. olive, peanut and canola oils) and poly-unsaturated oils (e.g. soybean and sunflower seed oils).

5 Predictions of effects/responses of dietary fats on serum cholesterol levels

Several predictive studies were carried out from year 1957 to 2016 to evaluate the responses of dietary fats on serum cholesterol levels (see Table 3). Ahrens *et al.* (1957) reported that the total degree of unsaturation levels of dietary fats plays an important role in regulating plasma lipid levels. The researchers suggested that the serum cholesterol level is inversely correlated to the iodine number of dietary fat[33]. On the other hand, Gunning *et al.* (1964)

Table 3 Predictions of effects of dietary fats on serum cholesterol levels.

Authors	Year	Statements / Equations
Ahrens *et al.*	1957	Serum cholesterol α 1/IV
Keys *et al.*	1957	Δ serum cholesterol = 2.76 Δ SFA + 0.05 Δ MUFA - 1.35 Δ PUFA - 1.68 → Δ serum cholesterol = 2.74 Δ SFA - 1.35 Δ PUFA
Gunning *et al.*	1964	Serum cholesterol α $1/\sqrt{IV}$
Keys *et al.*	1965	Δ serum cholesterol = 1.2 (2 Δ S'FA - Δ PUFA) + 1.5 Δ Z
Hegsted *et al.*	1965	Δ serum cholesterol = 2.32 Δ SFA + 0.32 Δ MUFA - 1.46 Δ PUFA + 6.51 Δ C + 0.83
McGandy *et al.*	1970	Lipid metabolism affects by 1) Chain length, 2) Saturation level & 3) Positional distribution of fatty acids at the glyceride backbone
Mensink *et al.*	1992	Δ TC (mmol/L) = 0.039 Δ SFA - 0.003 Δ MUFA - 0.015 Δ PUFA Δ LDL (mmol/L) = 0.033 Δ SFA - 0.006 Δ MUFA - 0.014 Δ PUFA Δ HDL (mmol/L) = 0.012 Δ SFA + 0.009 Δ MUFA + 0.007 Δ PUFA Δ TG (mmol/L) = - 0.025 Δ SFA - 0.022 Δ MUFA - 0.028 Δ PUFA
Mensink *et al.*	2003	Δ TC (mmol/L) = 0.036 Δ SFA - 0.006 Δ MUFA - 0.021 Δ PUFA Δ LDL (mmol/L) = 0.032 Δ SFA - 0.009 Δ MUFA - 0.019 Δ PUFA Δ HDL (mmol/L) = 0.010 Δ SFA + 0.008 Δ MUFA + 0.006 Δ PUFA Δ TG (mmol/L) = - 0.021 Δ SFA - 0.019 Δ MUFA - 0.026 Δ PUFA Δ TC:HDL (mmol/L) = 0.003 Δ SFA - 0.026 Δ MUFA - 0.032 Δ PUFA
Teh *et al.*	2016	Fatty acids at sn-2 position gave significant effects on serum cholesterol levels rather than overall fatty acid compositions of the triglyceride

Note: IV= Iodine number; Δ= Changes; SFA= Saturated fatty acid; MUFA= Mono-unsaturated fatty acid; PUFA= Poly-unsaturated fatty acid;
S'FA= Percentage of calories of saturated fatty acids excluding stearic acid; Z= Square root of dietary cholesterol in mg/1000 cal; C= Dietary cholesterol.

compared the effects of avocado oil, corn oil, soybean oil, cottonseed oil, safflower oil and coconut oil on plasma lipid levels in human subjects[34]. They postulated that the serum cholesterol level is inversely proportional to the square root of iodine number instead of iodine number of dietary fat.

Keys *et al.* (1957)[35] reviewed the effects of dietary fats on serum cholesterols of man based on the literature data where the majority of included studies were conducted by Keys and his co-researchers. Different types of dietary fats studied and reviewed were mixed food fats of standard American diets, fish oil, safflower oil, sunflower seed oil, corn oil, cottonseed oil, olive oil, hydrogenated coconut oil and beef fat. The authors proposed that MUFA does not contribute significant effect on the plasma cholesterols level. The multiple regression equation developed by Keys *et al.* (1957) was presented as Δ serum cholesterol = 2.76 Δ SFA + 0.05 Δ MUFA − 1.35 Δ PUFA − 1.68, where Δ SFA, Δ MUFA and Δ PUFA indicated the changes of percentage of calories of the saturated, mono-unsaturated and poly-unsaturated fatty acids, respectively. The constant value of 1.68 and the coefficient of Δ MUFA were eliminated from the regression equation because both variables were not significantly different. The multiple regression equation became Δ serum cholesterol = 2.74 Δ SFA − 1.35 Δ PUFA.

A few years later, Keys *et al.* (1965)[36] analyzed 63 sets of data using least square multiple regression analysis. It was noteworthy that dietary cholesterol influenced serum cholesterol level. This explanation was depicted by the predictive equation, Δ cholesterol = 1.2 (2 Δ S'FA − Δ PUFA) + 1.5 Δ Z, where S'FA was the percentage of calories of SFA excluding stearic acid and Z was the square root of dietary cholesterol in mg/1000 cal.

Hegsted *et al.* (1965)[37] conducted a human clinical study to evaluate the quantitative effect of coconut oil, olive oil, safflower oil, cocoa butter, butter fat and different combinations of tested fats on serum cholesterols in men aged from 38 to 57 years old. The test diets which contained 22% to 38% fat calories were incorporated into habitual American diets. The multiple regression equation, Δ serum cholesterol = 2.32 Δ SFA + 0.32 Δ MUFA − 1.46 Δ PUFA + 6.51 Δ C + 0.83, where C was the dietary cholesterol in 100 mg units developed from the study to assess the effects of the SFA, MUFA, PUFA and cholesterol. The discrepancy on the effects of MUFA between Keys *et al.* (1965) and Hegsted *et al.* (1965) is noticeable. The significant coefficient value of MUFA implies that MUFA gives effect on blood cholesterol level[37].

McGandy *et al.* (1970)[38] performed a similar human feeding study with different test fats. The test fats used in the trial were olive oil, safflower oil, butter fat, medium-chain triglyceride (MCT) oil, different combinations of tested fats and randomized transesterification products of natural tested fats with lauric, palmitic and/or stearic acids. It is notable that lauric and stearic acids are less hypercholesterolemic than myristic and palmitic acids. However, this assumption does not apply to cocoa butter, which predominantly consists of stearic acid with a total SFA of 63.6%. Hence, the authors suggested that the positional distribution of fatty acids at the TG backbone would affect the lipid metabolism, in addition to the fatty acid chain length and saturation level.

Mensink and Katan (2003)[39] reported that the previous predictive equations were inadequate because of the effects of dietary fats on LDL and HDL cholesterols were not accounted. Therefore, they selected 27 clinical trials that were published in between year 1970 and 1991 and performed a meta-analysis to analyze the effects of dietary fatty acids on serum cholesterol levels. The multiple regression analysis indicated that SFA raised TC and LDL, whereas both MUFA and PUFA lowered TC and LDL at different extent when carbohydrates were isoenergetically replaced with SFA, MUFA or PUFA. All the three fatty acids, SFA, MUFA and PUFA increased HDL level distinctively.

Another meta-analysis of 60 controlled trials were carried out by Mensink *et al.* (2003)[40]. The test fats included in the analysis were safflower oil, high oleic safflower oil, corn oil, coconut oil, beef fat, soybean oil, synthetic fat, blends, hydrogenated safflower oil, hydrogenated soybean oil, hydrogenated canola oil, hydrogenated sunflower seed oil, butter, canola oil, *trans* fatty acids, rice bran oil, MCT, palm oil, rapeseed oil, sunflower seed oil, high oleic sunflower seed oil and a combination of habitual diets. The predictive equations for TC, LDL and HDL were modified and therefore TC:HDL ratio was introduced.

It was noted that all the predictive statements or equations were based on the total fatty acid compositions of dietary fats. Therefore, different approach has been carried out to evaluate the effects of dietary fats on cholesterol levels in 2016[10]. Recalculation of serum cholesterol levels using predictive equations based on fatty acids at the *sn*-2 position was introduced in order to account for the discrepancies in response to various types of fats with similar total fatty acid composition but with different fatty acids at the *sn*-2 position such as stearic acid-rich diets (cocoa butter), lard and palm olein. Results indicated that fatty acids at the *sn*-2 position gave significant effects on serum cholesterol levels rather than the overall fatty acid compositions of the TG[10].

6 Other studies

Rand *et al.* (1988)[41] reported that palm oil does not behave like saturated fats, which tend to promote arterial thrombus formation. Conversely, it possesses antithrombitic effect by reducing thromboxane A2 (TXA2) formation, as well as inhibits platelet thromboxane formation. Besides, Renaud *et al.* (1995)[14] investigated the biological effects of native palm oil, interesterified palm oil, lard and interesterified lard in rats. The interesterified oils have similar fatty acid composition with native oils but different saturation levels at the *sn*-1, -2 and -3 positions. The *in vivo* studies confirmed that the fatty acids at the *sn*-2 position in dietary fats significantly influenced the biological effects such as lipemia, platelet aggregation and plasma fatty acids in rats rather than the total fatty acid composition of the dietary fats.

Jaarsveld *et al.* (2002)[42] performed an *in vivo* study to assess the effects of palm oil, sunflower seed oil and lard diets on aortic atherosclerosis in vervet monkeys. It is remarkable to find out that palm oil diet has significantly lowered the risk of developing early lesions in peripheral arteries compared to sunflower seed oil- and lard-fed groups and in aortas of vervet monkeys compared to lard-fed group. The difference between palm oil and sunflower seed oil is deduced to the high content of tocotrienols in palm oil.

7 Conclusions

Previous findings denoted that palm olein is a saturated fat due to its similar fatty acid composition with lard. However, many evidences indicated that the palm olein behaves like olive oil instead of animal fat. This review discovered that palm olein diet has comparable effects on serum cholesterol levels with various vegetable oils such as olive, soybean, peanut, sunflower seed and canola oils whereas the effects of palm olein and lard on serum lipids are different. It was found that palm olein and other vegetable oils contain predominantly UFA at the *sn*-2 positions in TG ranging from 89.6 to 100 % thereby consistent with *sn*-2 hypothesis. Meanwhile, lard has 11.2% of UFA located at the *sn*-2 position of TG distinguishes it from palm olein with a difference of up to 78.4% of UFA at the *sn*-2 position. The stereo-specific structures give a big difference on serum cholesterols contributed by palm olein and lard, attributed to the metabolic pathway of TG in the body. Fatty acids at the *sn*-2 position of TG in dietary fat play a crucial role in regulating serum cholesterol level because these fatty acids are prone to be absorbed and subsequently circulated in the form of lipoproteins through various lipase specific activities. To conclude, there are strong evidences consistent with *sn*-2 hypothesis presented in this review, indicating that plasma lipids are strongly associated with the *sn*-2 position of fatty acid in TG rather than the overall fatty acid composition of dietary fats. Thus, any misconception on the overall fatty acid profile of a vegetable oil in increasing human serum cholesterol levels should be re-examined and verified.

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