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EFFECTS OF FATTY ACIDS AT DIFFERENT POSITIONS IN THE TRIGLYCERIDES ON CHOLESTEROL LEVELS

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ABSTRACT

Previous studies established a series of regression equations for predicting the risk factor effects from serum cholesterol concentrations. However, the degree of saturation was solely based on total fatty acid composition in triglycerides. Our article is focused on the relationships between the published human nutrition studies and predicted values of serum cholesterol levels based on total fatty acid compositions and at sn-2 *position in triglycerides. Twenty-two published human nutrition studies were chosen to assess the effects of palm olein, olive oil, cocoa butter, sunflower seed oil, corn oil, soyabean oil, grapeseed oil, groundnut oil and rice bran oil diets on serum cholesterol levels. There were no statistically significant differences between the predicted values of serum cholesterol levels based on fatty acids at* sn-2 *position and the published human nutrition studies as proven by the statistical analyses with* p *values more than 0.05. In contrast, there were statistically significant differences between the predicted values of serum cholesterol levels based on total fatty acids and the published human nutritional studies with* p *values less than 0.05. Fatty acids at* sn-2 *position appear to influence the cholesterol levels rather than total fatty acids of the triglyceride.*

Keywords: fatty acid compositions, *sn*-2 position, serum cholesterol concentrations, experimental value, predicted value.

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INTRODUCTION

Saturated fatty acids are often perceived to be associated 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 elevate serum cholesterol levels and consequently increase the risk of coronary heart diseases (CHD) (American Heart Association, 1990). In contrast, it was reported by Reiser (1973) that cocoa butter, a highly saturated fat (approximately 64.2% saturated) has neutral effects on serum cholesterol level in human subjects. For the case of palm olein, it is often categorised as a saturated fat as it contains approximately 47.2% of saturated fatty acid, 52.8% of unsaturated fatty acid (Wahid *et al*., 2011; Omar *et al*., 2015) (*Table 1*) which has fatty acid composition similar to lard. However, based on the available studies in human (Choudhury *et al*., 1995; Voon *et al*., 2011; Ng *et al*., 1992; Tholstrup *et al*., 2011), palm olein does not elevate blood cholesterol levels as compared to mono-unsaturated oils.

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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 olein IV56			
Saturated	67.1	10.4	47.2
Mono-unsaturated	26.8	65.8	40.5
Poly-unsaturated	6.1	23.8	12.3
Palm olein IV64			
Saturated	60.7	8.5	43.3
Mono-unsaturated	34.5	63.9	44.3
Poly-unsaturated	4.8	27.6	12.4
Palm olein IV72			
Saturated	51.3	4.3	35.8
Mono-unsaturated	41.9	67.1	50.2
Poly-unsaturated	6.8	28.6	14.0
Olive oil			
Saturated	22.1	0.0	14.9
Mono-unsaturated	71.5	88.0	76.8
Poly-unsaturated	6.4	12.0	8.3
Cocoa butter			
Saturated	95.9	0.0	64.2
Mono-unsaturated	4.1	87.0	31.7
Poly-unsaturated	0.0	13.0	4.1
Sunflower seed oil			
Saturated	16.0	0.0	10.7
Mono-unsaturated	27.0	23.3	25.8
Poly-unsaturated	57.0	76.7	63.5
Corn oil			
Saturated	22.0	0.0	14.5
Mono-unsaturated	27.7	26.0	27.1
Poly-unsaturated	50.3	74.0	58.4
Soyabean oil			
Saturated	20.1	0.0	12.9
Mono-unsaturated	26.6	21.4	24.8
Poly-unsaturated	53.3	78.6	62.0
Grapeseed oil			
Saturated	15.1	0.0	10.0
Mono-unsaturated	18.5	22.3	19.8
Poly-unsaturated	66.4	77.7	70.2
Groundnut oil			
Saturated	28.0	0.0	18.9
Mono-unsaturated	53.4	59.5	55.3
Poly-unsaturated	18.6	40.5	25.8
Rice bran oil			
Saturated	33.7	0.0	23.0
Mono-unsaturated	38.2	38.7	38.4
Poly-unsaturated	28.1	61.3	38.7
Lard			
Saturated	32.5	88.8	49.0
Mono-unsaturated	55.9	11.2	42.8
Poly-unsaturated	11.6	0.0	8.2

These neutral effects may be due to the digestibility which is influenced by the fatty acid distribution (Apgar *et al*., 1987). According to Padley *et al*. (1994) and World Health Organisation (WHO), the *sn*-position in the triglyceride (TG) molecule determines the physical characteristic of fats, affects the absorption of fatty acids, lipid metabolism and fat distribution in tissues. The different digestibility and absorption of fatty acids at different positions in the TG backbone might explain why cocoa butter does not increase serum cholesterol levels although it has high saturated fat content. Nevertheless, no scientific study to date can prove that all fats contain saturated fatty acids lead to the cardiovascular diseases because the metabolic and serum cholesterol responses to all saturated fatty acids are not similar (Mitchell *et al*., 1989).

TG (*Figure 1*) is hydrolysed by the pancreatic lipase into two units of free fatty acids, which are *sn*-1 and *sn*-3 together with one unit of 2-monoglyceride. Once across the enterocyte barriers by diffusion, 2-monoglyceride and free fatty acids will be re-synthesised into TG in endoplasmic reticulum. The 2-monoglyceride and fatty acids released from digestion of TG will be then diffused into cells. Intestinal cholesterol absorption is crucial due to the clinical relevance of cholesterol. There is merely half of the total cholesterol that diffuses into small intestine is ordinarily absorbed while the rest is removed in the faeces. Fatty acids at *sn*-2 position may be preferentially transported to the liver rather than adipose tissue due to the positional specificity of lipoprotein lipase for the *sn*-1 and *sn*-3 positions of TG (Morley and Kuksis, 1972; Berry, 2009). In other words, the glyceride that will be absorbed in the intestine is 2-monoglyceride. Hepatocyte is the major site of action of fatty acids on LDL metabolism, therefore, saturated fatty acids in the *sn*-2 position of dietary TG may elevate LDL concentrations more than the same fatty acids in the *sn*-1 or *sn*-3 positions. In the case of palm olein, the *sn*-2 fatty acids are mainly unsaturated while the fatty acids at *sn*-1 and *sn*-3 are mainly saturated. The percentage of unsaturated fatty acids in the *sn*-2 position is as high as 89.6%, although the saturated fatty acids content in the TG is 47.2% as mentioned earlier (*Table 1*). As a result, the contribution of cholesterol levels is not solely based on the overall composition of the fatty acids, but the 2-monoglyceride fatty acids. Although the total fatty acid compositions of palm olein and lard are similar, there is a big difference when comparing both the *sn*-2 fatty acids compositions. In fact, the *sn*-2 fatty acid compositions of palm olein is similar to that of cocoa butter fat and olive oils (*Table 1*). Previous assumptions which proposed that palm olein elevates the cholesterol level due to its saturated fatty acid content are inappropriate (Chong and Ng, 1991). Even though palm olein contains saturated fatty acids, yet, it behaves more like a mono-unsaturated oil such as olive oil.

With the intention of calculating the risk of CHD from a diet, it is possible to determine risk factor effects from serum cholesterol concentrations using equations where the results are derived from the amounts of saturated, mono-unsaturated and poly-unsaturated fatty acids in the diet. The Mensink and

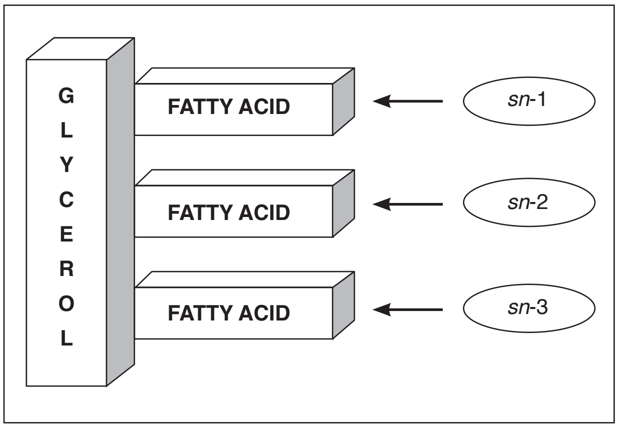

Figure 1. Stereospecific numbering (sn) *of fatty acids in triglyceride structure.*

Katan equation is based on the assumption that total dietary saturated fatty acid raise serum cholesterol, and mono-unsaturated and poly-unsaturated fatty acids reduce serum cholesterol in the bloodstream (Mensink and Katan, 1992).

Our article focuses on the relationships between the published human nutrition studies and predicted values of serum cholesterol levels where the amounts of different fatty acids for the predicted values are based on both total (*sn*-1,2,3) and *sn*-2 fatty acids in triglycerides. The predicted effects of palm olein *vs*. olive oil and cocoa butter with predominantly oleic acid at the *sn*-2 position on serum cholesterol levels were also compared.

EXPERIMENTAL

Twenty-two human nutrition studies designed to evaluate the effects of vegetable oils on serum cholesterol levels were included. All chemically and enzymatically modified fats were excluded from the study as the positional distribution of fatty acids at the triglyceride backbone have been altered that may deviate the objectives of the study. Iodine value (IV) is used to determine the amount of unsaturation in fatty acids. Further grouping of palm olein was also sorted out based on the IV of palm olein (IV 56, 64 and 72). The word 'experimental' is referred to the published human nutrition studies throughout the manuscript.

TRIGLYCERIDE ANALYSIS

Regiospecific analysis of the dietary oils was performed as described by Gouk *et al.* (2012) with some modifications. The quantitative spectra of dietary oils were measured using 100 mg which were dissolved in 0.50 ml of deuterated chloroform ($CDCl_3$). The ^{13}C NMR spectra were recorded on JEOL ECX500 FT NMR Spectrometer system operating at 11.7 T. The fatty acid positional composition of triacylglycerols of dietary oils were acquired under a relaxation delay of 15.0 s, 8192 data points, a 90° pulse angle and a spectral width of 1500 Hz at which the acyl chain carbonyl carbons resonate.

STATISTICAL ANALYSIS

The experimental mean changes of serum cholesterol levels between two oils were calculated referring to the reported human clinical-trial data using RevMan5.3. The predicted mean changes of serum lipid concentrations were calculated using the Mensink and Katan's equation (Mensink *et al*., 2003). Calculations were made by inserting *sn*-1,2,3 (total fatty acid composition) or *sn*-2 fatty acid composition of the test fats into the Mensink and Katan's equations. All *sn*-1,2,3 and *sn*-2 fatty acid compositions were obtained from the latest ^{13}C NMR method for standardisation purposes due to most of the cited references did not report on the *sn*-2 fatty acid composition. Mean Squared Error (MSE), two-samples t-test and R-squared between predicted values based on *sn*-1,2,3 and experimental values as well as predicted values based on *sn*-2 and experimental values were obtained using Minitab 17.

RESULTS AND DISCUSSION

The positional fatty acid compositions of the dietary oils were quantitatively determined using ^{13}C NMR spectroscopy. The comparison groups for the 22 studies were palm olein IV56 *vs*. olive oil (Choudhury *et al*., 1995; Ng *et al*., 1992), palm olein IV56 *vs*. corn oil (Laine *et al*., 1982), palm olein IV56 *vs*. soyabean oil (Laine *et al*., 1982; Marzuki *et al*., 1991), palm olein IV56 *vs*. sunflower seed oil (Wood *et al*., 1993), palm olein IV56 *vs*. groundnut oil (Ghafoorunissa *et al*., 1995), palm olein IV64 *vs*. olive oil (Voon *et al*., 2011; Tholstrup *et al*., 2011), palm olein IV64 *vs*. soyabean oil (Zhang *et al*., 1997), palm olein IV64 *vs*. groundnut oil (Zhang *et al*., 1997), palm olein IV72 *vs*. olive oil (Sun *et al*., 2015), cocoa butter *vs*. olive oil (Denke and Grundy, 1991; Kris-Etherton *et al*., 1993), cocoa butter *vs*. soyabean oil (Kris-Etherton *et al*., 1993), corn oil *vs*. olive oil (Lichtenstein *et al*., 1994), corn oil *vs*. soyabean oil (Laine *et al*., 1982), olive oil *vs* soyabean oil (Kris-Etherton *et al*., 1993), olive oil *vs*. grapeseed oil (Bonanome *et al*., 1992), rice bran oil *vs*. olive oil (Lichtenstein *et al*., 1994) and rice bran oil *vs*. corn oil (Lichtenstein *et al*., 1994).

MSE, two-samples t-test and R-squared between predicted values based on *sn*-1,2,3 and experimental values as well as predicted values based on *sn*-2 and experimental values were obtained using Minitab

17. The predicted mean changes of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein (HDL-C) and TC:HDL based on fatty acids in the *sn*-2 position are valid as they passed the two normality tests (*p*-value > 0.05) which were Anderson-Darling and Ryan-Joiner. There are no outliers at the 5% level of significance in Grubbs' test and Dixon's Q test with *p*-values greater than 0.05.

MSE were run on the predicted values based on *sn*-1,2,3 and experimental values as well as predicted values based on *sn*-2 and experimental values to evaluate the model error variation. The smaller the MSE, the closer the fit is to the data. The MSE for the model of predicted values based on *sn*-2 *vs*. experimental values were 0.047, 0.064, 0.004, 0.124 for TC (N=22), LDL-C (N=22), HDL-C (N=21) and TC:HDL (N=8), respectively. The MSE for the model of predicted values based on *sn*-1,2,3 *vs*. experimental values were 0.049, 0.069, 0.005, 0.137 for TC (N=22), LDL-C (N=22), HDL-C (N=21) and TC:HDL (N=8), respectively. The results indicated that the model of predicted values based on *sn*-2 gave lower error variation.

In addition, two-sample t-tests were run on predicted values based on *sn*-1,2,3 and experimental values as well as predicted values based on *sn*-2 and experimental values to determine whether there were statistically significant mean differences between the two independent test groups. It can be concluded that there is no statistically significant mean difference between predicted values based on *sn*-2 and experimental values as the *p*-values are greater than 0.05 with *p*-values of 0.788, 0.663, 0.070 for TC (N=22), LDL-C (N=22), TC:HDL (N=8), respectively. In contrast, there is a statistically significant difference between predicted values based on *sn*-1,2,3 and experimental values as the *p*-values are less than 0.05 for TC (N=22), LDL-C (N=22) and TC:HDL (N=8).

There is no statistically significant difference in the predicted values based on *sn*-1,2,3, predicted values based on *sn*-2 and experimental values in HDL-C (N=21). Hence, R-squared was performed to evaluate the goodness of fit of the model and the larger the R-squared, the closer the fit is to the data. The result showed that R-squared between predicted values based on *sn*-2 and experimental values is larger than R-squared between predicted values based on *sn*-1,2,3 and experimental values.

According to the commonly applied regression equations of Mensink *et al*. (2003), saturated fatty acids strongly elevate total cholesterol, LDL cholesterol and mildly raise HDL cholesterol and total to HDL cholesterol ratio. In contrast, both mono-unsaturated and poly-unsaturated fatty acids decrease total cholesterol, LDL cholesterol and total to HDL cholesterol ratio in different extents but also moderately raise HDL cholesterol.

Tables 2 to *2.17* illustrates the experimental and mean change of serum cholesterol levels based on the total fatty acid compositions of each triglyceride. There are certain discrepancy between the experimental and calculated results of all the comparisons.

Therefore, a re-analysis of the Mensink and Katan equations by replacing total fatty acids with *sn*-2 fatty acids was performed. Mean absolute differences between experimental and predicted values based on total fatty acid composition and mean absolute differences between experimental and predicted values based on *sn*-2 fatty acids for all the comparisons were also calculated. A remarkable agreement in cholesterol levels can be seen from the mean absolute differences and these calculated values are apparently closer to the experimental data.

Despite the higher percentage of saturated fatty acid (SFA) in palm olein (20.9%-32.3% based on total fatty acid compositions) (*Table 1*) than olive oil, the ability of palm olein and olive oil to regulate serum cholesterol levels in humans is essentially similar (Choudhury *et al*., 1995; Ng *et al*., 1992; Voon *et al*., 2011; Sun *et al*., 2015; Tholstrup *et al*., 2011). The predicted values based on fatty acids at *sn*-2 position were consistent with the published data. The mean absolute differences varied in the range between 0.004 and 0.152. There is a great difference between experimental and predicted values based on total fatty acid composition where the values are ranging from 0.002 to 0.471 (*Tables 2*, *2.5* and *2.9*).

The predicted mean changes in total, LDL, HDL cholesterol concentrations of palm olein *vs*. mono-unsaturated and poly-unsaturated vegetable oils including soyabean oil, sunflower seed oil, groundnut oil with Mensink's equation based on *sn*-2 fatty acids which agreed well with that of the experimental values in human clinical trials (Laine *et al*., 1982; Wood *et al*., 1993; Ghafoorunissa *et al*., 1995). Thus, the predicted values obtained using the formula based on total fatty acid composition were considerably higher (*Tables* 2.2 to 2.4, 2.6 and 2.7).

Previous findings on the effects of dietary cocoa butter *vs*. olive oil (Denke and Grundy, 1991; Kris-Etherton *et al*., 1993), cocoa butter and soyabean oil (Kris-Etherton *et al*., 1993), corn oil *vs*. olive oil (Lichtenstein *et al*., 1994), corn oil *vs*. soybean oil (Laine *et al*., 1982), olive oil *vs*. grapeseed oil (Bonanome *et al*., 1992), rice bran oil *vs*. olive oil (Lichtenstein *et al*., 1994) as well as rice bran oil *vs*. corn oil (Lichtenstein *et al*., 1994) on serum cholesterol profiles are comparable with the predictive formula based on fatty acids in the *sn*-2 position. The predicted values based on *sn*-2 fatty acids are much more closer with the experimental values compared to the predicted values based on total fatty acid composition and these can be seen from the differences of both mean absolute

TABLE 2. EXPERIMENTAL *vs*. PREDICTED MEAN CHANGES AND THEIR MEAN ABSOLUTE DIFFERENCES IN SERUM CHOLESTEROL LEVELS - PALM OLEIN IV56 *vs*. OLIVE OIL

mmol litre^{-1}	ΔTC	ΔLDL	ΔHDL
Experimental			
Choudhury *et al*. (1995)	0.020	-0.080	0.110
Ng *et al*. 1992 (male)	-0.050	-0.030	0.000
Ng *et al*. 1992 (female)	0.000	-0.050	0.000
Average different observed	-0.020	-0.050	0.030
Predicted (average)			
Based on *sn*-1,2,3 (P_1)	0.425	0.421	0.018
Based on *sn*-2 (P_2)	0.086	0.102	-0.002
Mean absolute differences			
Experimental and P_1	0.445	0.471	0.012
Experimental and P_2	0.106	0.152	0.032

Note: TC – total cholesterol. LDL – low-density lipoprotein. HDL – high-density lipoprotein.

TABLE 2.1. EXPERIMENTAL *vs*. PREDICTED MEAN CHANGES AND THEIR MEAN ABSOLUTE DIFFERENCES IN SERUM CHOLESTEROL LEVELS - PALM OLEIN IV56 *vs*. CORN OIL

mmol litre^{-1}	ΔTC	ΔLDL	ΔHDL
Experimental			
Laine *et al*. (1982)	0.690	0.810	-0.060
Predicted			
Based on *sn*-1,2,3 (P_1)	0.748	0.653	0.056
Based on *sn*-2 (P_2)	0.432	0.338	0.043
Mean absolute differences			
Experimental and P_1	0.058	0.157	0.116
Experimental and P_2	0.258	0.472	0.103

Note: TC – total cholesterol. LDL – low-density lipoprotein. HDL – high-density lipoprotein.

TABLE 2.2. EXPERIMENTAL *vs*. PREDICTED MEAN CHANGES AND THEIR MEAN ABSOLUTE DIFFERENCES IN SERUM CHOLESTEROL LEVELS - PALM OLEIN IV56 *vs*. SOYABEAN OIL

mmol litre^{-1}	ΔTC	ΔLDL	ΔHDL	ΔTC:HDL
Experimental				
Laine *et al*. (1982)	0.620	0.800	0.040	Nil
Marzuki *et al*. (1991)	-0.090	-0.040	0.010	-0.110
Average different observed	0.010	0.130	0.010	-0.110
Predicted (average)				
Based on *sn*-1,2,3 (P_1)	0.785	0.683	0.065	0.452
Based on *sn*-2 (P_2)	0.445	0.342	0.051	0.211
Mean absolute differences				
Experimental and P_1	0.775	0.553	0.055	0.562
Experimental and P_2	0.435	0.212	0.041	0.321

Note: TC – total cholesterol. LDL – low-density lipoprotein. HDL – high-density lipoprotein.

TABLE 2.3. EXPERIMENTAL *vs*. PREDICTED MEAN CHANGES AND THEIR MEAN ABSOLUTE DIFFERENCES IN SERUM CHOLESTEROL LEVELS - PALM OLEIN IV56 *vs*. SUNFLOWER SEED OIL

mmol litre^{-1}	ΔTC	ΔLDL	ΔHDL
Experimental			
Wood *et al*. (1993)	0.310	0.130	0.030
Predicted			
Based on *sn*-1,2,3 (P_1)	0.920	0.803	0.070
Based on *sn*-2 (P_2)	0.492	0.382	0.051
Mean absolute differences			
Experimental and P_1	0.610	0.673	0.040
Experimental and P_2	0.182	0.252	0.021

Note: TC – total cholesterol. LDL – low-density lipoprotein. HDL – high-density lipoprotein.

TABLE 2.4. EXPERIMENTAL *vs.* PREDICTED MEAN CHANGES AND THEIR MEAN ABSOLUTE DIFFERENCES IN SERUM CHOLESTEROL LEVELS - PALM OLEIN IV56 *vs.* GROUNDNUT OIL

mmol litre^{-1}	ΔTC	ΔLDL	ΔHDL
Experimental			
Ghafoorunissa *et al.* (1995)	0.130	-0.200	0.050
Predicted			
Based on *sn*-1,2,3 (P_1)	0.376	0.350	0.023
Based on *sn*-2 (P_2)	0.186	0.160	0.015
Mean absolute differences			
Experimental and P_1	0.246	0.550	0.027
Experimental and P_2	0.056	0.360	0.035

Note: TC – total cholesterol. LDL – low-density lipoprotein. HDL – high-density lipoprotein.

TABLE 2.5. EXPERIMENTAL *vs.* PREDICTED MEAN CHANGES AND THEIR MEAN ABSOLUTE DIFFERENCES IN SERUM CHOLESTEROL LEVELS - PALM OLEIN IV64 *vs.* OLIVE OIL

mmol litre^{-1}	ΔTC	ΔLDL	ΔHDL	ΔTC:HDL
Experimental				
Voon *et al.* (2011)	0.160	0.140	0.030	0.060
Tholstrup *et al.* (2011)	0.240	0.220	0.020	0.110
Average different observed	0.220	0.200	0.020	0.090
Predicted (average)				
Based on *sn*-1,2,3 (P_1)	0.375	0.372	0.018	0.261
Based on *sn*-2 (P_2)	0.039	0.062	-0.003	0.045
Mean absolute differences				
Experimental and P_1	0.155	0.172	0.002	0.171
Experimental and P_2	0.181	0.138	0.023	0.045

Note: TC – total cholesterol. LDL – low-density lipoprotein. HDL – high-density lipoprotein.

TABLE 2.6. EXPERIMENTAL *vs.* PREDICTED MEAN CHANGES AND THEIR MEAN ABSOLUTE DIFFERENCES IN SERUM CHOLESTEROL LEVELS - PALM OLEIN IV64 *vs.* SOYABEAN OIL

mmol litre^{-1}	ΔTC	ΔLDL	ΔHDL	ΔTC:HDL
Experimental				
Zhang *et al.* (1997)	0.000	-0.090	0.100	-0.420
Predicted				
Based on *sn*-1,2,3 (P_1)	0.605	0.521	0.050	0.347
Based on *sn*-2 (P_2)	0.331	0.252	0.038	0.157
Mean absolute differences				
Experimental and P_1	0.605	0.611	0.050	0.767
Experimental and P_2	0.331	0.342	0.062	0.577

Note: TC – total cholesterol. LDL – low-density lipoprotein. HDL – high-density lipoprotein.

TABLE 2.7. EXPERIMENTAL *vs.* PREDICTED MEAN CHANGES AND THEIR MEAN ABSOLUTE DIFFERENCES IN SERUM CHOLESTEROL LEVELS - PALM OLEIN IV64 *vs.* GROUNDNUT OIL

mmol litre^{-1}	ΔTC	ΔLDL	ΔHDL	ΔTC:HDL
Experimental				
Zhang *et al.* (1997)	-0.120	-0.280	0.180	-0.830
Predicted				
Based on *sn*-1,2,3 (P_1)	0.368	0.341	0.020	0.243
Based on *sn*-2 (P_2)	0.169	0.147	0.010	0.106
Mean absolute differences				
Experimental and P_1	0.488	0.621	0.160	1.073
Experimental and P_2	0.289	0.427	0.170	0.936

Note: TC – total cholesterol. LDL – low-density lipoprotein. HDL – high-density lipoprotein.

TABLE 2.8. EXPERIMENTAL *vs.* PREDICTED MEAN CHANGES AND THEIR MEAN ABSOLUTE DIFFERENCES IN SERUM CHOLESTEROL LEVELS - PALM OLEIN IV64 *vs.* LARD

mmol litre^{-1}	ΔTC	ΔLDL	ΔHDL	ΔTC:HDL
Experimental				
Zhang *et al*. (1997)	-0.630	-0.720	0.040	-0.820
Predicted				
Based on *sn*-1,2,3 (P_1)	-0.092	-0.084	-0.007	-0.056
Based on *sn*-2 (P_2)	-1.139	-1.073	-0.066	-0.748
Mean absolute differences				
Experimental and P_1	0.538	0.636	0.047	0.764
Experimental and P_2	0.509	0.353	0.106	0.072

Note: TC – total cholesterol. LDL – low-density lipoprotein. HDL – high-density lipoprotein.

TABLE 2.9. EXPERIMENTAL *vs.* PREDICTED MEAN CHANGES AND THEIR MEAN ABSOLUTE DIFFERENCES IN SERUM CHOLESTEROL LEVELS - PALM OLEIN IV72 *vs.* OLIVE OIL

mmol litre^{-1}	ΔTC	ΔLDL	ΔHDL
Experimental			
Sun *et al*. (2015)	-0.020	-0.030	-0.010
Predicted			
Based on *sn*-1,2,3 (P_1)	0.232	0.233	0.013
Based on *sn*-2 (P_2)	-0.024	-0.002	-0.003
Mean absolute differences			
Experimental and P_1	0.252	0.263	0.023
Experimental and P_2	0.004	0.028	0.007

Note: TC – total cholesterol. LDL – low-density lipoprotein. HDL – high-density lipoprotein.

TABLE 2.10. EXPERIMENTAL *vs.* PREDICTED MEAN CHANGES AND THEIR MEAN ABSOLUTE DIFFERENCES IN SERUM CHOLESTEROL LEVELS - COCOA BUTTER *vs.* OLIVE OIL

mmol litre^{-1}	ΔTC	ΔLDL	ΔHDL
Experimental			
Denke *et al*. (1991)	0.260	0.200	Nil
Kris-Etherton *et al*. (1993)	0.340	0.280	-0.100
Average different observed	0.290	0.230	-0.100
Predicted (average)			
Based on *sn*-1,2,3 (P_1)	0.821	0.794	0.042
Based on *sn*-2 (P_2)	-0.006	-0.004	-0.001
absolute differences			
Experimental and P_1	0.531	0.564	0.142
Experimental and P_2	0.296	0.234	0.099

Note: TC – total cholesterol. LDL – low-density lipoprotein. HDL – high-density lipoprotein.

TABLE 2.11. EXPERIMENTAL *vs.* PREDICTED MEAN CHANGES AND THEIR MEAN ABSOLUTE DIFFERENCES IN SERUM CHOLESTEROL LEVELS - COCOA BUTTER *vs.* SOYABEAN OIL

mmol litre^{-1}	ΔTC	ΔLDL	ΔHDL
Experimental			
Kris-Etherton *et al*. (1993)	0.680	0.510	-0.020
Predicted			
Based on *sn*-1,2,3 (P_1)	1.120	0.994	0.081
Based on *sn*-2 (P_2)	0.364	0.243	0.049
Mean absolute differences			
Experimental and P_1	0.440	0.484	0.101
Experimental and P_2	0.316	0.267	0.069

Note: TC – total cholesterol. LDL – low-density lipoprotein. HDL – high-density lipoprotein.

TABLE 2.12. EXPERIMENTAL *vs.* PREDICTED MEAN CHANGES AND THEIR MEAN ABSOLUTE DIFFERENCES IN SERUM CHOLESTEROL LEVELS - CORN OIL *vs.* OLIVE OIL

mmol litre^{-1}	ΔTC	ΔLDL	ΔHDL	ΔTC:HDL
Experimental				
Lichtenstein *et al.* (1994)	-0.280	-0.180	-0.050	-0.070
Predicted				
Based on *sn*-1,2,3 (P_1)	-0.225	-0.150	-0.035	-0.078
Based on *sn*-2 (P_2)	-0.269	-0.176	-0.041	-0.093
Mean absolute differences				
Experimental and P_1	0.055	0.030	0.015	0.008
Experimental and P_2	0.011	0.004	0.009	0.023

Note: TC – total cholesterol. LDL – low-density lipoprotein. HDL – high-density lipoprotein.

TABLE 2.13. EXPERIMENTAL *vs.* PREDICTED MEAN CHANGES AND THEIR MEAN ABSOLUTE DIFFERENCES IN SERUM CHOLESTEROL LEVELS - CORN OIL *vs.* SOYABEAN OIL

mmol litre^{-1}	ΔTC	ΔLDL	ΔHDL
Experimental			
Laine *et al.* (1982)	-0.080	-0.040	0.100
Predicted			
Based on *sn*-1,2,3 (P_1)	0.054	0.046	-0.002
Based on *sn*-2 (P_2)	0.039	0.030	-0.002
Mean absolute differences			
Experimental and P_1	0.134	0.086	0.102
Experimental and P_2	0.119	0.070	0.102

Note: TC – total cholesterol. LDL – low-density lipoprotein. HDL – high-density lipoprotein.

TABLE 2.14. EXPERIMENTAL *vs.* PREDICTED MEAN CHANGES AND THEIR MEAN ABSOLUTE DIFFERENCES IN SERUM CHOLESTEROL LEVELS - OLIVE OIL *vs.* SOYABEAN OIL

mmol litre^{-1}	ΔTC	ΔLDL	ΔHDL
Experimental			
Kris-Etherton *et al.* (1993)	0.340	0.230	0.080
Predicted			
Based on *sn*-1,2,3 (P_1)	0.331	0.230	0.041
Based on *sn*-2 (P_2)	0.370	0.246	0.049
Mean absolute differences			
Experimental and P_1	0.009	0.000	0.039
Experimental and P_2	0.030	0.016	0.031

Note: TC – total cholesterol. LDL – low-density lipoprotein. HDL – high-density lipoprotein.

TABLE 2.15. EXPERIMENTAL *vs.* PREDICTED MEAN CHANGES AND THEIR MEAN ABSOLUTE DIFFERENCES IN SERUM CHOLESTEROL LEVELS - OLIVE OIL *vs.* GRAPESEED OIL

mmol litre^{-1}	ΔTC	ΔLDL	ΔHDL
Experimental			
Bonanome *et al.* (1992)	0.360	0.260	0.060
Predicted			
Based on *sn*-1,2,3 (P_1)	0.510	0.369	0.060
Based on *sn*-2 (P_2)	0.443	0.296	0.059
Mean absolute differences			
Experimental and P_1	0.150	0.109	0.000
Experimental and P_2	0.083	0.036	0.001

Note: TC – total cholesterol. LDL – low-density lipoprotein. HDL – high-density lipoprotein.

TABLE 2.16. EXPERIMENTAL *vs.* PREDICTED MEAN CHANGES AND THEIR MEAN ABSOLUTE DIFFERENCES IN SERUM CHOLESTEROL LEVELS - RICE BRAN OIL *vs.* OLIVE OIL

mmol litre^{-1}	ΔTC	ΔLDL	ΔHDL
Experimental			
Lichtenstein *et al.* (1994)	-0.330	-0.260	-0.050
Predicted			
Based on *sn*-1,2,3 (P_1)	-0.034	0.010	-0.018
Based on *sn*-2 (P_2)	-0.213	-0.139	-0.034
Mean absolute differences			
Experimental and P_1	0.296	0.270	0.032
Experimental and P_2	0.117	0.121	0.016

Note: TC – total cholesterol. LDL – low-density lipoprotein. HDL – high-density lipoprotein.

TABLE 2.17. EXPERIMENTAL *vs.* PREDICTED MEAN CHANGES AND THEIR MEAN ABSOLUTE DIFFERENCES IN SERUM CHOLESTEROL LEVELS - RICE BRAN OIL *vs.* CORN OIL

mmol litre^{-1}	ΔTC	ΔLDL	ΔHDL
Experimental			
Lichtenstein *et al.* (1994)	-0.050	-0.080	0.000
Predicted			
Based on *sn*-1,2,3 (P_1)	0.192	0.160	0.017
Based on *sn*-2 (P_2)	0.056	0.037	0.007
Mean absolute differences			
Experimental and P_1	0.242	0.240	0.017
Experimental and P_2	0.106	0.117	0.007

Note: TC – total cholesterol. LDL – low-density lipoprotein. HDL – high-density lipoprotein.

TABLE 3. PREDICTED MEAN CHANGES IN SERUM CHOLESTEROL LEVELS - PALM OLEIN IV72 *vs.* COCOA BUTTER

mmol litre^{-1}	ΔTC	ΔLDL	ΔHDL	ΔTC:HDL
Based on *sn*-1,2,3	-0.402	-0.379	-0.023	-0.265
Based on *sn*-2	-0.016	0.006	-0.007	0.009

Note: TC – total cholesterol. LDL – low-density lipoprotein. HDL – high-density lipoprotein.

differences as shown in *Tables 2.10* to *2.13* and *Tables 2.15* to *2.17*.

Mean changes of serum cholesterol levels between palm olein IV72 and two vegetable oils, olive oil (*Table 2.9*) and cocoa butter (*Table 3*), were calculated. These vegetable oils exerted comparable effects on serum cholesterol profiles if calculations are based on *sn*-2 fatty acids. This is due to olive oil and cocoa butter possessing similar fatty acid compositions at the *sn*-2 position in triglycerides (*Table 1*). Meanwhile, the comparable effects of palm olein IV72 is due to a higher amount of poly-unsaturated fatty acids which counterbalance the cholesterol-elevating effect of its 4.3% saturated fatty acid.

In addition, mean changes of serum cholesterol levels between palm olein IV64 and animal fat, lard were also calculated. The plasma lipid profiles of those given palm olein IV64 and lard enriched diets were different at the completion of the treatment in the human clinical trial even though they possess similar total fatty acid composition. The mean absolute differences between experimental and predicted values based on *sn*-2 fatty acid is much smaller than that of mean absolute differences between experimental and predicted values based on total fatty acid composition (*Table 2.8*).

In most cases, mean absolute differences between experimental and predicted values based on total fatty acid composition were relatively larger than that of mean absolute differences between experimental and predicted values based on *sn*-2 fatty acids for almost all the comparisons in the mean changes of serum cholesterol levels except for HDL-C. Although several mean changes of HDL-C based on total fatty acid composition were relatively closer to the experimental values compared to the predicted values based on fatty acid at 2-position. However, the mean absolute changes of experimental and predicted *sn*-2 did not differ greatly. Their effects on the cholesterol levels may be attributed to their individual fatty acids (Mensink, 1993) at 2-position as previous studies indicated that different fatty acids possess different biological effects (Small, 1991).

In general, the predicted mean changes of serum cholesterol levels based on fatty acids in the *sn*-2 position between palm olein IV56 *vs*. olive oil, palm olein IV56 *vs*. corn oil, palm olein IV56 *vs.* soyabean oil, palm olein IV56 *vs.* sunflower seed oil, palm olein IV56 *vs*. groundnut oil, palm olein IV64 *vs*. olive oil, palm olein IV64 *vs*. soyabean oil, palm olein IV64 *vs*. groundnut oil, palm olein IV72 *vs*. olive oil, cocoa butter *vs*. olive oil, cocoa butter *vs*. soyabean oil, corn oil *vs*. olive oil, corn oil *vs*. soyabean oil, olive oil *vs*. soyabean oil, olive oil *vs*. grapeseed oil, rice bran oil *vs*. olive oil and rice bran oil *vs*. corn oil, were in good agreement with the experimental results as proven by the mean absolute differences (*Tables 2* to *2.17*), MSE, two-samples t-test as well as R-squared results.

CONCLUSION

Even though the effects of the total fatty acids in triglycerides on serum cholesterol concentrations agreed well with the Mensink and Katan equations (Mensink and Katan, 1992), the extent of the mean changes showed apparent discrepancy. The calculated values are comparable with experimental values if replacement of total fatty acids by fatty acids in the *sn*-2 position as the mean changes of serum cholesterol levels will be elevated or reduced to the same extent compared to the experimental values especially when palm olein is used as control in the calculation. In summary, fatty acids at *sn*-2 position appear to influence the cholesterol levels rather than overall fatty acids of the triglyceride.

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