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BIOCHEMICAL EFFECT OF PALM OIL FRACTIONS ON RATS

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Abstract

This study aimed to discuss the effect of six different sources of fats (100% corn, 100% butter and100% margarine) and three blends from margarine and butter with different percentages) on plasma lipid profile, lipid risk ratios and rats body weight when add in rat diet through a six weeks experimental period. The free fatty acid (FFA)%, peroxide value (PV) and the fatty acid composition of the six types of fats were determined, 36 male Wistar strain rats were categorized to six groups, 6 rats of each. The food intake and body weight of rats were recorded once a week. At the end of the experiment, total food intake, body weight gain and food efficiency ratio were calculated. Total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and triglyceride (TG) were determined in plasma, it found that diet with the 100% margarine showed the highest FFA% and PV. Rat group fed on the 100% margarine diet showed the highest levels of TC, TG, LDL-C and the risk ratio (TC/HDL-C). The least HDL-C and risk ratio LDL-C/HDL-C values were also noticed in 100% margarine fed group, our article concluded that as the margarine percentage in diet increase this was accompanied by elevation in plasma lipid profile and body weight that is well known to have series of deleterious effects on body heath.

Key words: Palm oil, Margarine, Butter.

Introduction

Hyperlipidemia is one of the most fatal disorder leading to sever diseases and accounts for about one third of total deaths around the world especially atherosclerosis of the most valuable arterial network of the main vital organs (Ayata *et al.,* 2013, Jorgensen *et al*., 2013). Not all blood lipids are included in risk factors but blood cholesterol is the main milestone of high stroke incidence (Menet *et al.,* 2018). High intake of SFAs has been related to hypercholesteremic condition, fatal cardiovascular diseases (CVDs) (Page *et al.,* 1957).The later are fatal diseases of the heart and blood streams that include coronary heart disease (CHD) and cerebrovascular disease. CVDs responsible for 31% of global deaths (Holub, 2002). Chen *et al.,* (2011) reported that in developing countries, the rate of CVDs mortality increase with the increase of Palm oil (PO) consumption, Palm oil is a vegetable oil obtained from the palm tree's fruit (*Elaeisguineensis*). PO is considered to be the second biggest vegetable oil produced and traded all over the world. It is composed of 50% palmitic acid (PA) (16:0), 40% of hypocholesterolaemic monounsaturated oleic acid (18:1, omega-9), and 10% linoleic (18:2, omega-6) polyunsaturated fatty acid (Ayorinde *et al*., 2000), in addition tonegligible values of the hypercholesterolemic saturated fatty acids (SFAs) lauric (12:0) and myristic (14:0) acids. The ratio of SFA to unsaturated fatty acid (UFA) of PO is thus near unity. PO contains minor amount of vitamin E, which are powerful antioxidants, represent a natural inhibitors for cholesterolsynthesis and also permits a longer shelf-life for palm-based food products. Abundance of indigenous source of carotenoids, tocopherols and tocotrienols in PO provided it with high oxidative stability (Chong and Ng, 1991; Muller *et al*., 2010). The previously mentioned phytonutrients are associated with several health promoting properties including the anti-cancer, anti-angiogenesis, cardio-protection, cholesterol inhibition, neuro-protective properties, antioxidative defence mechanisms, and anti-diabetes (Loganathan *et al.,* 2010). PO is ordinarily used in shortening, frying fats and margarines (Imoisi *et al.,* 2015). Although many previous studies reported that PO-based diets induce a state of hypercholesteremia than do other vegetable oils like corn, soybean, and sunflower oils, PO consumption causes reduction in endogenous cholesterol level. Researchers render this phenomenon to its tocotrienols content and its peculiar FAs isomeric position.

The stereospecific distribution of SFA in the triacylglycerol (TAG) molecule of PO, decrease their metabolic effects and absorption rate. International guidelines, stated that SFAs intake should not exceed10% of total energy, within a balanced diet; to avoid harmful effects that may occur on human health (mainly CVDs or cancer risk) (Marangoni *et al*., 2017).

The processed food industry is highly dependent on palm production that makes the PO industry critical. United States Food and Drug Administration's ban on *trans-fatty acids* (TFAs) because of their potential adverse health impacts. Elevated consumption of PO as a potential replacement for TFA in ultra-processed foods could be anticipated. As trans-*fats* are considered unhealthy fractions, this directed all worldwide for PO as the healthy natural subrogation (Kadandale *et al*., 2019).

TFAs are certain class of dietary fatty acids that are produced by biohydrogenation process to fat of ruminant animals or by commercial hydrogenation of vegetable oils (Emken, 1984). Relative to UFAs, TFAs/ hydrogenated fats consumption results in increase the risk ratio that represented by (higher LDL-C in relative to lower HDL-C). (Lichtenstein *et al*., 1999).

Hu *et al.,* (2001) stated that the replacement of SFA and TFA with mono and polyunsaturated fats is beneficial and highly effective in preventing CHD than reducing the net fat consumption. TFA from fried foods elevated TG and the (LDL/HDL) cholesterol ratio more than did margarine/hydrogenated vegetable oil products (Sartika, 2011).

The relation of income and food source directed many scientist to discover margarine in 1869 to be cheap source substituted butter but it is not expensive as butter (Chrysam, 1996). Margarine is a water-in-oil emulsion. The aqueous phase composed of water, salt and some preservatives while the fatty phase, is a blend of oils and fats, emulsifiers and antioxidants. The solid fat content of margarine is a result of hydrogenation process to liquid oils. Hydrogenation results in the formation of TFAs where some cis double bonds are rearranged to Trans bonds (Fomuso and Akoh, 2001).

The main object of our study was to evaluate the extent of PO hazardous effect when formulated as margarine using different percentages of butter/margarine blends. This was followed by evaluation of plasma lipid profile and nutritional parameters in rats.

Materials and Methods

Animals:

36 Male Wister albino rats weighing 50-70g. The rats were kept in stainless steel cages under standard conditions. Food and water were given *ad-libtium*. The animal experiment was carried out adopting the Ethics Committee of Cairo University, Giza, Egypt, on ethical approval Number: CU II F 30 19.

Diet:

The basal diet was formulated according to AOAC (2005). The composition of mineral and vitamin mixtures were also according to AOAC (2005).

Diet Preparation:

Refined, bleached and deodorized corn oil, Commercial Unsalted Creamery Butter product of New Zealand, it consists of (82.9% milk fat, 15.7% moisture, 1.4% solids Non-fat) and commercial margarine that is composed of (50% hydrogenated PO, 50% PO) was obtained from IFFCO Egypt Company for Edible Oils and Fats: Bldg. 28, Road 261, New Maadi, and Cairo, Egypt.

Chemical reagents:

Total cholesterol (TC), High density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and triglyceride (TG) were obtained from (BIODIAGNOSTICS®): 29 Tahreer St., Dokki, and Giza, Egypt.

Methods

Free Fatty acid and Peroxide Value:

Free Fatty acid and Peroxide value of oil and fat are the determined according to AOCS official methods (2009).

Fatty acid composition:

The fatty acid composition was determined by the conversion of oil to fatty acid methyl esters prepared by adding 1.0mL of n-hexane to 15 mg of oil followed by 1.0mL of sodium Wister albinomethoxide (0.4 mol), according to the modified method of (Zahran and Tawfeuk, 2019).

Experimental design:

36 male Wister albino rats were categorized to six groups, 6 rats each. Rats were fed on same basal diets varying only in its type of fat and classified as follows: Group I: Normal control received basal diet plus fat (100%

corn oil) as 10% of total basal diet and served as (basal control diet).Group II: received basal diet plus fat (100% butter) as 10% of total basal diet. The other four groups received the basal diet plus different fat blends classified as follows: Group III: received basal diet plus fat (75% butter+25% margarine) (mixtureI) as 10% of total basal diet. Group: IV: received basal diet plus fat (50% butter+50% margarine) (mixtureII) as 10% of total basal diet, V: received basal diet plus fat (25% butter+75% margarine) (mixtureIII) as 10% of total basal diet and finally, Group VI: received basal diet plus fat (100% margarine) as 10% of total basal diet. The FA composition of dietary fat is shown in Table. 2. The experimental period lasted for six weeks.

Blood collection and biochemical assay:

The food intake and body weight of rats were recorded once a week. At the end of the experiment, total food intake, body weight gain and food efficiency ratio were calculated. Every 2 weeks from the experimental period (6 weeks) blood samples were collected from the medial canthus. At the end of the experiment, rats were anesthetized and blood samples were towed away again from the medial canthus after an overnight feed deprivation (16h). Blood samples were collected in plain tubes, followed by centrifugation at 3000 rpm for 15 min for separation of plasma and determination of lipid profile namely, TC according to Richmond (1973) and Allain *et al.,* (1974), HDL-C according to Burstein *et al.,* (1970), LDL-Caccording to Wieland and Seidel (1983) and TG according to Fassati and Prencipe (1982).

Statistical Analysis:

The results of the experiments were expressed as mean ± SE and were statistically analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple comparisons test using software program Assistat 7.7 beta (version 5.00). Difference was considered significant when ≤0.05 (Silva and Azevedo, 2009).

Results and Discussion

Chemical properties and composition

FFAs and PV of Different Fat Types:

The FFAs percent of all six fat samples namely corn oil, butter, margarine and the three fat blends of butter/ margarine were ranged between 0.12 and 0.25% (Table 1). The result showed that the corn oil possess the lowest FFAs content whereas the margarine possess the highest. It is clearly shown that there is a direct relation between FFAs content and the margarine % in fat blends that is to say, as the margarine % increase, the FFAs % also increase and vice versa. FFAs were reported to catalyze the oxidative dissolution of oils by enzymatic and or chemical hydrolysis to form off volatile components (Anio³owska *et al*., 2016). Moreover, FFA value is an indication of lipase efficacy (Elabd *et al.,* 2017). Dunford (2012) reported that high-quality PO possess high % of the neutral (TAGs, or TGs) and decreased FFAs % and this results coincide with the current study. The inevitable sequels of elevated levels of FFAs are the greater risks of cardiovascular events (Jakobsen *et al*., 2009).

Table 1 also presents the PV of the previously mentioned fat samples. The PV was ranged between 0.19 mEq./kg and1.60 mEq./kg for butter and corn oil respectively. The PV is considered a strong indicator of peroxidation, and thus high PV of the oil is a sign of low oil resistance to wards oxidation process during storage, and a detector to deterioration level (Zahran and Tawfeuk, 2019).This wide variation in PV may render to the high UFAs percent content for corn oil compared to butter. Similarly margarine showed high PV 1.19 mEq./kg and thus may share corn oil in its low storage stability.

Fatty acid composition:

Table 2 showed FA composition of the fat samples under test. Corn oil possesses the highest UFA (67.55%) while butter showed the least (26.82). This is obviously shown in linoleic acid which represents the most abundant UFA in corn oil (35.61%), while butter has the least linoleic acid percentage (1.48%). Corn oil also has high percent of oleic acid (31.94%). Dermaux *et al.,* (1999) reported that the most important FAs naturally occurring in the TGs of vegetable oils and margarine are PA, stearic acid (18:0), oleic acid, linoleic acid and linolenic acid (18:3) and such results coincides with our results seen in Table 2.

Six important fatty acids were shown in margarine sample and its blends, which are C14:0, C16:0 and C18:0 as SFAs, and C18:1, C18:2, and C18:3 as UFAs. PA was present in margarine as the highest SFA percent (45.32%), however, the short chain FAs C4:0 to C14:1 were absent in both corn oil and margarine, except myristic acid (C14:0) was reported in margarine in minor amount (1.03%). In addition, the highest SFA level (73.19%) was recorded in butter. On the contrary from corn oil and margarine, blends of butter and margarine with margarine percentage 25 and 50% exhibit very low short chain FA existence ranged from butyric acid (C4:0) to lauric acid (C12:0).

From the data shown in Table 2, The ratio of SFA to USFA was 1.57 (mixtureIII) by mixing butter with margarine at a ratio of 25:75, respectively, while these values increased by increasing the percentage of butter to be 1.73(mixtureII) in case of butter mixed with margarine at a ratio of 50:50, and to be 2.31(mixtureI) on mixing butter with margarine at a ratio of 75:25, respectively. It's obvious that the fatty acid profile change by changing the margarine percentage in three blends under investigation.

The short chain fatty acids: butyric, caproic, caprylic, capric, and lauric acids decreased upon increasing the % of margarine to butter in the three butter/ margarine blends. These results are in agreement with those reported by El-Hadad and Tikhomirova (2018). Fats and oils are made up of a mixture of TGs, which consists of a glycerol backbone to which three FAs are esterified. The positions of fatty acids attached to the glycerol backbone are referred by stereospecific numbering Sn -1, -2 and -3 Fig. 1. The stereospecific position of fatty acids in TG has a great impact on the properties of oils and fats. Renaud *et al.,* (1995) investigated that both native PO and interesterified PO has similar FA composition but different saturation levels at the Sn-1, -2 and -3 positions. *In vivo* studies confirmed that FAs at the Sn-2 position in dietary fats significantly influenced biological effects such as lipemia and platelet aggregation rather than the total FA composition of the dietary fats. The positional distribution of FAs in the TG molecule does give thus an influence of the properties of fats and oils, and should not be classified based on the overall fatty acid composition (Teh *et al*., 2018).

Effects of feed ingrates different fat types for 6 weeks on body weight, food intake and food efficiency ratio:

The results shown in Table 3 represent that increasing the percent of margarine in diet is accompanied by significant elevation in body weight much more clearly seen in 100% margarine diet compared to rat fed diet containing 100 % butter. Such elevation in body weight may be attributed to its dietary SFA content mainly PA. Fig. 2 demonstrated that the rat body weight increase with time with non-significant difference among the experimental groups.

According to (Padley *et al*., 1994) the stereospecific position of FAs in the TG molecule determines the physical characteristic of fats, affects the absorption of fatty acids, lipid metabolism and fat distribution in tissues in addition it plays a major role in serum cholesterol level. PA, the most abundant circulating SFA, The mechanism engaged by SFAs appears to be through TLR4, triggering acute and chronic inflammation in-vivo and in-vitro experimental models (Tian *et al.,* 2015; Wang *et al.,* 2017). TLR4 is an essential modulator of innate immunity, and links it with metabolic disorders like obesity. Ubhyasekera *et al.,* (2013) reported that palmitate is one of the most abundant SFA recorded in obese children.

Mice fed with the chemically interesterified

Table 1: Free Fatty Acid % and Peroxide Value of Different Fat Types.

Fat type	Parameter	
	Free fatty acid	**Peroxide Value (mEq./Kg oil)**
Corn oil	0.12%	1.6
100% Butter	0.14%	0.19
75% Butter+25% Margarine (mixture I)	0.16%	0.68
50% Butter+50% Margarine (mixture II)	0.18%	0.84
25% Butter+75% Margarine (mixture II)	0.20%	0.96
100% Margarine	0.25%	1.19

Table 2: Fatty acid composition of corn oil, margarine, butter and butter/margarine blends

Fatty acids	Area %					
	Corn oil	**Butter**	**(mixture I)**	**(mixture II)**	**(mixture III)**	**Margarine**
Butyric acid (C4:0)	ND	0.58	0.47	0.12	ND	ND
Caproic acid (C6:0)	ND	1.22	0.99	0.46	ND	ND
Caprylic acid (C8:0)	ND	1.18	0.89	0.58	ND	ND
Capric acid (C10:0)	ND	3.58	2.59	1.44	1.18	ND
Lauric acid (C12:0)	ND	5.92	4.41	2.91	1.97	ND
Myristic acid (C14:0)	ND	14.03	10.74	7.15	4.9	1.03
Myristoleic acid (C14:1)	ND	0.87	0.64	0.4	ND	ND
Palmitic acid (C16:0)	24.83	32.37	35.67	38.54	40.03	45.32
Stearic acid (C18:0)	7.62	12.39	11.51	11.21	11.16	11.01
Oleic acid (C18:1)	31.94	20.26	22.43	25.83	29.69	29.52
Linoleic acid (C18:2)	35.61	1.48	2.8	3.89	5.16	5.47
Linolenic acid (C18:3n3)	ND	0.81	1.45	2.77	4.07	7.55
ã- Linolenic acid C18:3n6)	ND	3.4	2.89	3.56	ND	ND
Arachidic acid (C20:0)	ND	1.92	2.51	0.8	1.83	ND
SFA	32.45	73.19	69.78	63.21	61.07	57.36
UFA	67.55	26.82	30.21	36.45	38.92	42.54
SFA/UFA ratio	0.48	2.73	2.31	1.73	1.57	0.64

ND= not detected

Table 3: Effects of Feeding Rats Different Fat Types for 6 Weeks on Body Weight, Total Food Intake and Food Efficiency Ratio.

Diets containing 10%fats	Initial Body Weight (gm)	Body Weight (gm)after Week 1	Body Weight (gm)after Week 2	Body Weight (gm)after Week 3	Body Weight (gm)after Week 4	Body Weight (gm)after Week 5	Final Body Weight (gm)after Week6	BWG (gm)	Total Food Intake (gm)	Food Efficiency Ratio
Corn oil (basal diet)	61.83 ±1.25^{Aa}	94.03 ±2.35^{Bd}	113.80 ±2.60^{Cd}	133.65 ±1.16^{Dd}	150.47 ±1.39^{Ee}	165.23 ±1.85^{Fd}	182.72 ±1.08^{Gf}	120.88 ±1.75^{d}	500.10 ±48.17^{a}	0.26 ±0.03^{b}
100% Butter	62.83 ±0.91^{Aa}	99.38 ±1.20^{Bc}	116.32 ±2.63^{Ccd}	135.87 ±2.77^{Dd}	155.07 ±1.44^{Ed}	172.52 ±1.06^{Fc}	190.95 ±0.90^{Ge}	128.12 ±1.37^{c}	526.50 ±20.66^{a}	0.25 ±0.01^{b}
75% Butter+25% Margarine(mixture I)	64.33 ±0.95^{Aa}	101.36 ±0.46^{Bc}	121.55 ±0.78^{Cc}	141.58 ±0.61^{Dc}	157.75 ±0.71^{Ed}	175.65 ±0.69^{Fc}	197.77 ±0.53^{Gd}	133.43 ±0.92^{c}	517.10 ±15.36^{a}	0.26 ±0.01^{b}
50% Butter+50% Margarine (mixture II)	59.83 ±1.87^{Aa}	103.14 ±1.50^{Bbc}	128.55 ±1.38^{Cb}	146.50 ±1.58^{Db}	161.75 ±1.22^{Ec}	183.38 ±1.14^{Fb}	201.60 ±0.90^{Gc}	141.77 ±2.15^{b}	515.90 ±17.49^{a}	0.28 ±0.01^{ab}
25% Butter+75% Margarine (mixture III)	61.50 ±1.82^{Aa}	106.75 ±1.19^{Bab}	134.68 ±1.80^{Ca}	148.78 ±1.74^{Dab}	166.90 ±0.75^{Eb}	189.98 ±1.67^{Fa}	206.77 ±0.99^{Gb}	145.27 ±1.87^{ab}	500.20 ±15.79^{a}	0.29 ±0.01^{ab}
100% Margarine	59.83 ±1.60^{Aa}	109.13 ±1.99^{Ba}	135.33 ±1.47^{Ca}	151.45 ±0.52^{Da}	171.40 ±0.52^{Ea}	191.65 ±0.78^{Fa}	210.43 ±1.38^{Ga}	150.60 ±2.91^{a}	485.75 ±23.72^{a}	0.31 ±0.01^{a}

Data were expressed as mean ± SE (n=6). $^{A, B, C, D, E, F, G}$ In each row different superscript letters mean significant differences at 0.05 probabilities.
$^{a, b, c, d, e, f}$ In each column the same superscript letter means non-significant difference while different superscript letters mean significant differences at 0.05 probability.

palm olein (IPOo) diet gained much more fat per food consumed when compared with the POo group, despite their identical total FAs compositions. The authors attributed this fat deposition to the higher content of long chain SFA at the Sn 1, 3 positions of TAG in POo (Gouk *et al*., 2013). Many scientific studies explain that the biological effects of SFAs may depend on the length of their chain. As mentioned previously that the number of carbons in the FAs chain, the degree of saturation and the stereospecific positioning of FAs in TAG molecule highly affects FA absorption, and hence affecting their metabolism and their role in CVD (Karupaiah and Sundram, 2007). TAGs contained in PO are mainly constituted of oleic acid, located at the Sn-2 position predominantly, while PA is mainly located at the Sn-1 and Sn-3 positions. Long chain SFAs impairs intestinal absorption rates if occupying the Sn-1/Sn-3 position of glycerol backbone.

Table 3 in addition represents that food efficiency ratio of both 100% butter and 25% margarine in diet showed non-significant difference compared to corn whereas 50, 75 and 100% margarine in diet showed significant difference much more pronounced in the 100% margarine. Several results reported that chronic consumption of high fat diets (HFDs), rich in SFAs such as the PA, increases body weight and triggers several metabolic diseases, such as insulin resistance and type II diabetes mellitus (T2DM) (Hernández-Cáceres *et al*., 2019). Exposure to PA reduces insulin sensitivity in hypothalamic neuronal cells (Rodriguez-Navas *et al*., 2016). FAs play important roles in feeding behavior by triggering the neurons of the hypothalamus that responsible for lipid and glucose metabolism leading to development of obesity and T2DM (Moulle *et al*., 2014). PO-enriched diet also elicit higher amounts of inflammatory markers in plasma (IL-6) and in white adipose tissue (WAT) IL-1β and TLR4 (Laugerette *et al.,* 2012). WAT produces adipokines that are responsible of chronic inflammation processes and are associated with obesity-related metabolic diseases (Exley *et al*., 2014). There is growing evidence linking obesity to changes in gut microbiota (Musso *et al.,* 2011). PO-rich diet determines weight-gain and hepatic lipid accumulation in C57BL/6J mice (De Wit *et al.,* 2012); these observations support the hypothesis that an excessive consumption of PO in the diet triggers changes in gut microbiota components and causes lipid accumulation. Fernandez *et al.,* (1992) demonstrated that there's no significant effects on body weight gain of guinea pigs that were fed PO as a fat source. (Cheng *et al.,* 2015) reported that high-doses of PA induce pro-inflammatory responses and leptin resistance, similarly to obesogenic-diets. Several studies carried out on animal models indicated that, during lactation, the consumption of normo-lipidemic diets, rich in SFAs derived from PO and/or in partially hydrogenated fats (PHF) induces fat retention in the young offspring (Silva *et al.,* 2006). The role of PO consumption in T2DM is controversial, also other findings mentioned that PO-enriched diet impairs glucose tolerance may be due to the reduction in insulin-sensitivity (Kochikuzhyil *et al.,* 2010) this also in conjugation with increase of serum TAGs which associated with insulin-resistance (Storlien *et al*., 2000). PA was found to markedly impair phosphorylation process and thus, activation of insulin receptor, insulin receptor substrate-1, and Act in several cell types, contributing to the onset of insulin-resistance (Mordier and Iynedjian, 2007).

Table 3 also indicated that there were no significant differences among the rat groups in total food intake.

Table 4: Plasma Total Cholesterol and Triglyceride Levels (mg/dl) in Rats Fed Different fat types for 6 weeks.

Diets containing 10% fats	TC (mg/dl) Feeding period (weeks)				TG(mg/dl) Feeding period (weeks)			
	Zero	2	4	6	Zero	2	4	6
Corn oil (basal diet)	119.21 ±3.71^{Ba}	126.86 ±0.95^{Ad}	127.17 ±0.97^{Ac}	131.14 ±0.59^{Ad}	106.54 ±6.14^{Ba}	109.20 ±2.82^{Ba}	115.50 ±3.47^{ABc}	126.34 ±7.43^{Ab}
100% Butter	117.29 ±1.38^{Da}	129.37 ±0.80^{Ccd}	140.76 ±0.64^{Bb}	146.82 ±0.74^{Acd}	104.54 ±8.79^{Ba}	116.41 ±0.63^{Aba}	120.44 ±0.79^{ABbc}	133.52 ±8.91^{Aab}
75% Butter+25% Margarine (mixture I)	119.13 ±1.58^{Da}	131.14 ±0.59^{Ccd}	140.98 ±2.50^{Bb}	150.49 ±4.72^{Acd}	105.34 ±1.32^{Da}	118.01 ±1.28^{Ca}	127.38 ±1.22^{Bab}	134.19 ±1.51^{Aab}
50% Butter+50% Margarine (mixture II)	117.83 ±4.01^{Ca}	133.54 ±0.92^{BCc}	143.20 ±2.80^{ABb}	160.43 ±11.72^{Abc}	107.34 ±1.08^{Da}	122.26 ±1.65^{Ca}	133.24 ±0.82^{Ba}	138.70 ±2.62^{Aab}
25% Butter+75% Margarine (mixture III)	121.73 ±3.40^{Da}	140.41 ±2.21^{Cb}	157.08 ±1.85^{Ba}	173.46 ±4.53^{Aab}	109.20 ±2.82^{Ca}	124.94 ±10.80^{Bca}	130.06 ±1.30^{ABab}	146.70 ±4.15^{Aa}
100% Margarine	118.53 ±1.31^{Ca}	148.22 ±2.09^{Ba}	158.37 ±0.68^{Ba}	189.21 ±11.10^{Aa}	108.82 ±1.35^{Ba}	128.08 ±10.70^{ABa}	135.37 ±8.54^{Aa}	149.22 ±1.18^{Aa}

Data were expressed as mean ± SE (n=6).

a, b, c, d In each column the same superscript letter means non-significant difference while different superscript letters mean significant differences at 0.05 probability.

A, B, C, D In each row different superscript letters mean significant differences at 0.05 probabilities.

Table 5: Plasma HDL-cholesterol and LDL-cholesterol levels (mg/dl) in Rats Fed Different fat types for 6 weeks.

Diets containing 10% fats	HDL-cholesterol(mg/dl) Feeding period (weeks)				LDL-cholesterol (mg/dl) Feeding period (weeks)			
	Zero	2	4	6	Zero	2	4	6
Corn oil (basal diet)	33.31 ±1.09^{Ca}	34.26 ±0.71^{Ca}	38.80 ±0.26^{Ba}	43.77 ±1.73^{Aa}	60.20 ±2.68^{Ba}	64.73 ±4.40^{ABc}	70.97 ±4.78^{ABc}	75.84 ±1.14^{Ac}
100% butter	35.34 ±1.19^{Aa}	33.63 ±1.17^{ABab}	32.60 ±0.70^{ABb}	31.33 ±1.46^{Bb}	67.51 ±4.25^{Ba}	71.18 ±4.46^{Bbc}	73.02 ±6.47^{Bc}	90.57 ±1.93^{Ab}
75% Butter+25% Margarine (mixture I))	34.05 ±1.16^{Aa}	31.13 ±0.88^{ABab}	30.39 ±1.01^{ABbc}	29.39 ±1.54^{Bbc}	66.42 ±1.08^{Ca}	73.42 ±2.20^{BCbc}	83.21 ±5.18^{Bbc}	96.23 ±5.97^{Ab}
50% Butter+50% Margarine (mixture II)	33.63 ±1.26^{Aa}	30.19 ±0.70^{ABbc}	29.59 ±1.49^{ABbc}	27.90 ±2.37^{Bbc}	62.11 ±2.34^{Da}	76.21 ±3.16^{Cab}	89.09 ±4.85^{Bab}	109.61 ±3.62^{Aa}
25% Butter+75% Margarine (mixture III)	32.91 ±0.36^{Aa}	29.39 ±1.51^{Bc}	27.50 ±1.28^{Bc}	26.40 ±1.16^{Bbc}	64.91 ±4.52^{Ca}	80.41 ±0.90^{Bab}	90.68 ±0.98^{Bab}	115.50 ±5.29^{Aa}
100% Margarine	35.22 ±0.91^{Aa}	29.99 ±1.78^{ABbc}	27.90 ±1.71^{BCc}	25.16 ±1.42^{Cc}	60.75 ±2.60^{Da}	85.21 ±3.42^{Ca}	99.14 ±4.65^{Ba}	117.90 ±4.87^{Aa}

Data were expressed as mean ± SE (n=6).

a, b, c In each column the same superscript letter means non-significant difference while different superscript letters mean significant differences at 0.05 probability.

A, B, C, D In each row different superscript letters mean significant differences at 0.05 probabilities.

Table 6: Effect of feeding rats different fat types for 6 weeks on risk ratio 1 (total cholesterol/HDL-cholesterol) and risk ratio 2 (LDL-cholesterol/HDL-cholesterol)

Diets containing 10% fats	Risk Ratio 1 Feeding period (weeks)				Risk Ratio 1 Feeding period (weeks)			
	Zero	**2**	**4**	**6**	**Zero**	**2**	**4**	**6**
Corn oil (basal diet)	3.59 ±0.14^{Aba}	3.71 ±0.10^{Ac}	3.28 ±0.02^{BCc}	3.02 ±0.12^{Cd}	1.81 ±0.06^{Aa}	1.90 ±0.14^{Ad}	1.83 ±0.11^{Ad}	1.74 ±0.08^{Ad}
100% Butter	3.33 ±0.11^{Ca}	3.87 ±0.14^{Bbc}	4.33 ±0.10^{Ab}	4.73 ±0.21^{Ac}	1.92 ±0.13^{Ba}	2.12 ±0.12^{Bcd}	2.26 ±0.25^{Bcd}	2.92 ±0.16^{Ac}
75% Butter + 25% Margarine (mixture I))	3.52 ±0.13^{Ca}	4.23 ±0.13^{Bbc}	4.66 ±0.19^{ABb}	5.19 ±0.38^{Ac}	1.96 ±0.05^{Ca}	2.36 ±0.08^{BCbc}	2.75 ±0.19^{Bbc}	3.32 ±0.29^{Abc}
50% Butter + 50% Margarine (mixture II)	3.51 ±0.10^{Ca}	4.43 ±0.08^{BCab}	4.88 ±0.22^{ABb}	5.98 ±0.82^{Abc}	1.87 ±0.14^{Ca}	2.53 ±0.12^{BCab}	3.05 ±0.25^{Bab}	4.07 ±0.45^{Aab}
25% Butter+ 75% Margarine (mixture III)	3.70 ±0.12^{Da}	4.83 ±0.27^{Ca}	5.76 ±0.26^{Ba}	6.63 ±0.37^{Aab}	1.97 ±0.13^{Ca}	2.77 ±0.17^{Bab}	3.33 ±0.18^{Bab}	4.43 ±0.35^{Aa}
100% Margarine	3.38 ±0.12^{Ca}	5.02 ±0.32^{Ba}	5.76 ±0.35^{Ba}	7.55 ±1.56^{Aa}	1.73 ±0.10^{Da}	2.88 ±0.18^{Ca}	3.57 ±0.10^{Ba}	4.75 ±0.35^{Aa}

Data were expressed as mean ± SE (n=6).

[a, b, c, d] In each column the same superscript letter means non-significant difference while different superscript letters mean significant differences at 0.05 probability.

[A, B, C, D] In each row different superscript letters mean significant differences at 0.05 probabilities.

Effects of feeding rats on different fat types for 6 weeks on plasma lipid profile

The results shown in Tables 4 and 5 represented that increasing the margarine percentage in diet caused significant elevation in plasma TC, TG and LDL-C levels. The elevation in such lipid parameters represent a direct relation with the margarine % in diet blends compared to butter fed rat groups. Grundy *et al*., (1988) reported that consumption of diets containing 8 to 16 carbon-atom SFAs increases the serum concentration of LDL-C. Such elevation in plasma TC, LDL-C and TG was also approved by Denke and Grundy (1992) that render this to the high concentration of PA. Also Table 5 showed that significant reduction in plasma HDL-C levels as margarine % in diet increase. Certain eating pattern that is described by a high intake of total fat, SFA and cholesterol, but low in fiber and PUFAs content may play a crucial role in the CHD risk (Kratz, 2005). Table 4 and 5 and Figs. 3-8 reported the effect of the six different fat types under test on the plasma lipid profile and the risk ratios 1 and 2 with time. The results shown in Table 6 represented that increasing margarine percentage in diet caused significant elevation in risk ratios 1 and 2. This Table 6 also showed that the highest risk ratios 1 and 2 were found in rat group that was fed on margarine as 100% fat. These results are in the same boat with those reported by Asadi *et al.,* (2010) but these results showed disagreement with those reported by Edem (2009). Several studies have demonstrated that PO was similar to unsaturated oils in its effects on blood lipids. PO provides a healthy alternative to TFA containing hydrogenated fats that have been demonstrated to have serious deleterious effects on health. The similar effects of PO on blood lipids, comparable to other vegetable oils could be due to the structure of the major TGs in PO, which has an UFA in the stereospecific numbers Sn-2 position of the glycerol backbone. Many nutritional studies have reported that PO with high monounsaturation at Sn-2 position is comparable to monounsaturated oils (like olive and canola oils) in its effect on lipid profile. In addition, PO is endowed with a bouquet of phytonutrients beneficial to health, such as tocotrienols, carotenoids, and phytosterols (Choo and Kalanithi, 2014). Qureshi *et al*.,

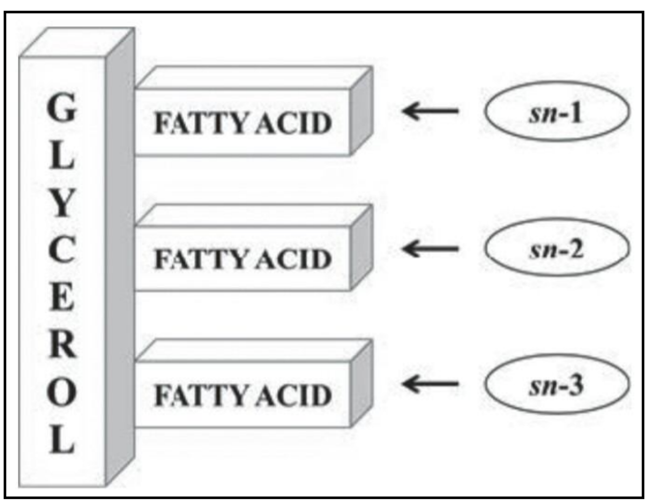

Fig. 1: Stereospecific numbering (Sn) of fatty acid in triglyceride structure (Choo and Kalanithi, 2014).

(1991) reported that tocotrienols in PO exerted a hypocholesterolemic effect in humans aged 30 to 60 years probably through the inhibition of cholesterol synthesis.

Hayes *et al.,* (1991) have reported that PA appeared normocholesterolemic when the supply of linoleic acid was above 6 energy %. It is noticeably that diet contain the 25 and 50% margarine showed non-significant difference in TG level compared to butter, this may related to their comparable percentage of either the short chain FA namely C4, C6 and C8 or γ-Linolenic acid.

Substitution of saturated fats with non-hydrogenated vegetable oils was associated with a rapid decline in CHD mortality (Zatonski and Willett, 2005). This supports our results, which indicate that as hydrogenated oil % increase this is accompanied by a series of deleterious effects on body heath.

PO contains negligible amounts (less than 1.5%) of the hypercholesterolemic SFAs, namely lauric acid (12:0) and myristic acid (14:0). It possesses moderately rich amounts of the hypocholesterolaemic, monounsaturated oleic acid (18:1, omega-9) and adequate amounts of linoleic acid (18:2, omega-6) (Chong and Ng, 1991).

Trans-fat increases LDL-C and decreases HDL-C (Mozaffarian *et al*., 2006). PO significantly increased TC, LDL-, and HDL-C concentrations compared to vegetable oils low in saturated fat. PO was also found to significantly increase HDL-C compared with *trans*-fat containing partially hydrogenated vegetable oils (Sun *et al*., 2015).

Tinahones *et al.,* (2004) reported that diet rich in PA do not raise plasma TC, but reduce LDL-C or even keep it normal, and in addition it elevate plasma HDL. The researcher may render this to the increase in LDL-receptor activity, such results coincide with those of Lindsey *et al*., (1990).The mechanism for the LDL-C elevating effect of hydrogenated fat appears to be similar to that of saturated fat, primarily determined by decreased catabolism. These findings support current recommendations to reduce saturated and hydrogenated fat consumption. Rats fed with hydrogenated oils containing palm, this was accompanied by exacerbation to the atherosclerotic risk through elevation of both serum TC level and oxLDL and on the other hand reduction in paraoxanase-1 activity (Amini *et al*., 2017).

Harris *et al.,* (1983) demonstrated that SFAs with carbon atoms 12, 14 and 16 are considered to have hypercholesterolemic effect. This result is consistent with ours that show elevation in total plasma cholesterol with values 150,160,173 and 189 mg/dl at $P<0.05$in the sixth week of the experiment in diet containing 25, 50, 75 and 100% of margarine respectively. This indicate a direct relation between the margarine percentage and plasma cholesterol level which may be related to the PA % in diet which was shown to be elevated as the concentration of margarine % in diet increase. Tinahones *et al.,* (2004) also reported low LDL-C/HDL-C risk ratio in plasma of rat fed on PA whereas our results showed high levels of this risk ratio with its highest value seen in diet with 100% margarine when compared with butter rat fed group. In our study, plasma TG level increase as the % of margarine in rat diet increase these results come with that of (Lindsey *et al*., 1990; Tinahones *et al.,* 2004).

Studies have also established that SFAs activate inflammatory and innate immune responses (Huang *et al*., 2012). PA increased serum levels of pro-inflammatory cytokines, tumor necrosis factor-α (TNF-α) and interleukin (IL)-6 (Wang *et al*., 2017). The levels of these cytokines were also elevated in the cardiac tissues. PA is the most common SFA found in the human body and

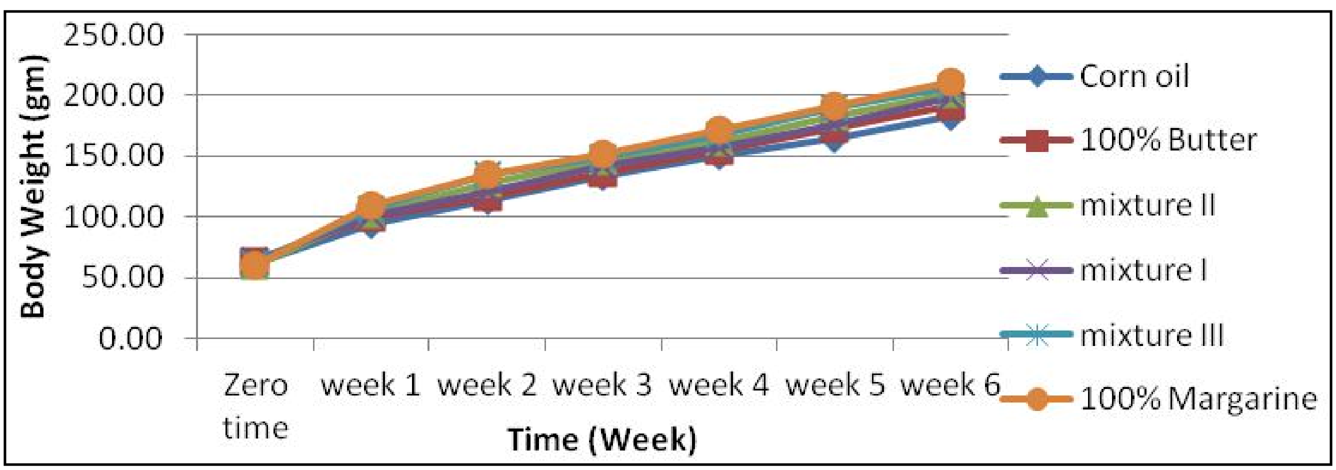

Fig. 2: Effect of feeding rats several fat types (100% corn oil, 100% butter, (75% butter + 25% margarine), (50% butter + 50% margarine), (25% butter + 75% margarine) and 100% margarine) as 10 % of total basal diet for 6 weeks on rat body weight.

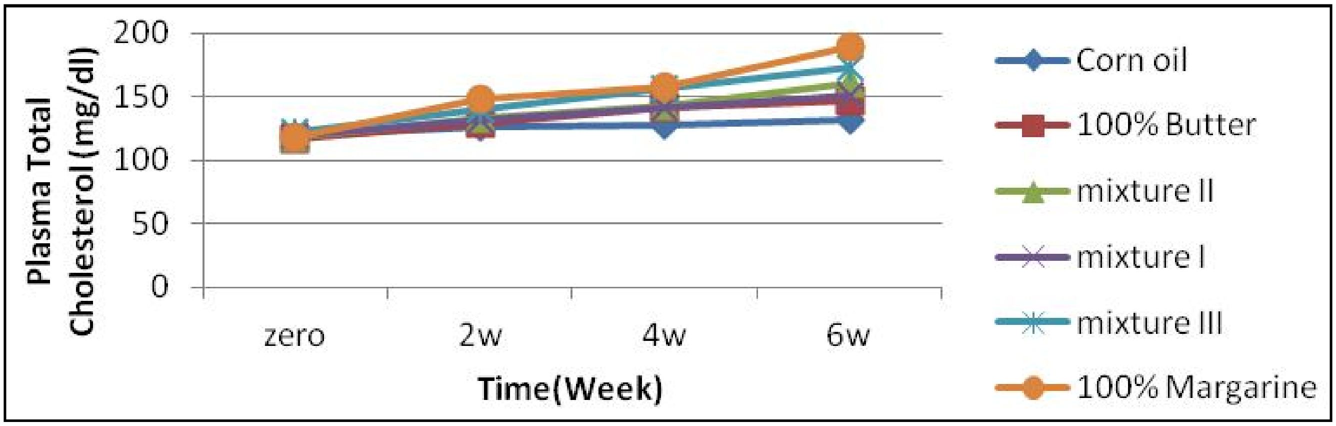

Fig. 3: Effect of feeding rats several fat types (100% corn oil, 100% butter, (75% butter + 25% margarine), (50% butter + 50% margarine), (25% butter + 75% margarine), and 100% margarine) as 10 % of total basal diet for 6 weeks on plasma total-cholesterol level. Plasma cholesterol elevate with time in all rat groups except that of the corn group that showed some fluctuation. A sharp increase in margarine group between week 4 and 6 of the experiment.

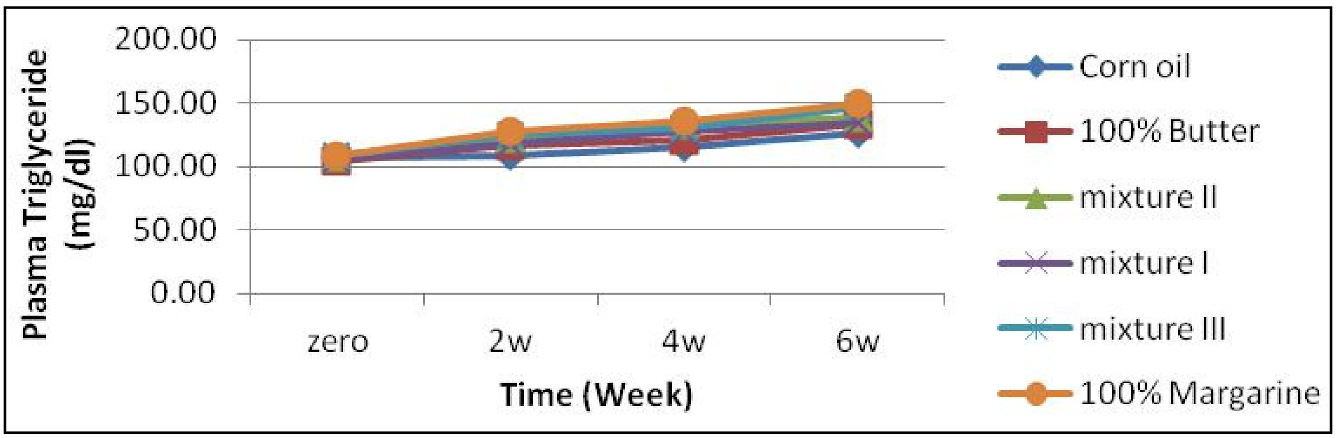

Fig. 4: Effect of feeding rats several fat types (100% corn oil, 100% butter, (75% butter+25% margarine), (50% butter+50% margarine), (25% butter+75% margarine), and 100% margarine) as 10 % of total basal diet for 6 weeks on plasma TG level. TG increase smoothly with time in all groups.

can be provided in the diet or synthesized endogenously from other FAs, carbohydrates and amino acids. PA represents 20–30% of total FAs in membrane phospholipids, and adipose TAG (Carta *et al.,* 2015). In order to maintain membrane phospholipids balance an optimal intake of PA in a certain ratio with UFAs, especially PUFAs of both n-6 and n-3 families are important (Carta *et al*., 2017). PA is a major component of palm oil (44% of total fats), but significant amounts of PA can also be found in meat and dairy products (50–60% of total fats), as well as olive oil (8–20%). Furthermore, PA is also present in breast milk with 20–30% of total fats (Innis, 2016). The average intake of PA is around 20–30 g/d (Sette *et al*., 2011). Changes in PA intake do not influence significantly its tissue concentration (Song *et al*., 2017), as the intake is counterbalanced by PA endogenous biosynthesis via de novo lipogenesis (DNL). Specific nutritional factors and physiopathological conditions may induce DNL, caused elevated tissue content of PA that may lead to disrupted homeostatic control of its tissue concentration (Wilke *et al.,* 2009). Whereas in normal physiological conditions, PA accumulation is prevented by enhanced delta desaturation to palmitoleic acid and/or elongation to stearic acid and followed by delta desaturation to oleic acid (Strable and Ntambi, 2010). PA homeostatic balance disruption is implicated in many pathological conditions such as atherosclerosis, neurodegenerative diseases and cancer.

Conclusion

The current study revealed that the use of margarine substitute of butter in foods should be avoided or partially replaced to prevent its hazardous effects on human health. In addition our study could not establish strong evidence against margarine/palm oil blends consumption relating to non-communicable diseases mortality. Further studies are needed to establish such a relation. Greater awareness should be taken on dealing with unhealthy dietary habits.

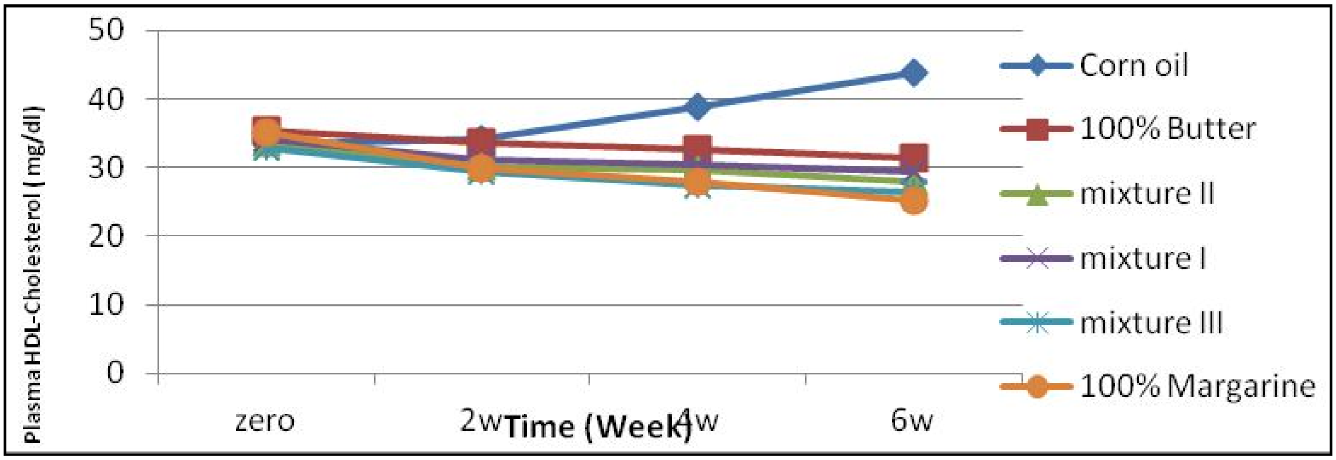

Fig. 5: Effect of feeding rats several fat types (100% corn oil, 100% butter, (75% butter+25% margarine), (50% butter+50% margarine), (25% butter+75% margarine), and 100% margarine) as 10 % of total basal diet for 6 weeks on plasma HDL-C level. Plasma HDL-C level decrease with time in all groups except the corn oil fed group showed sharp elevation started from week 2 to the end of the experiment.

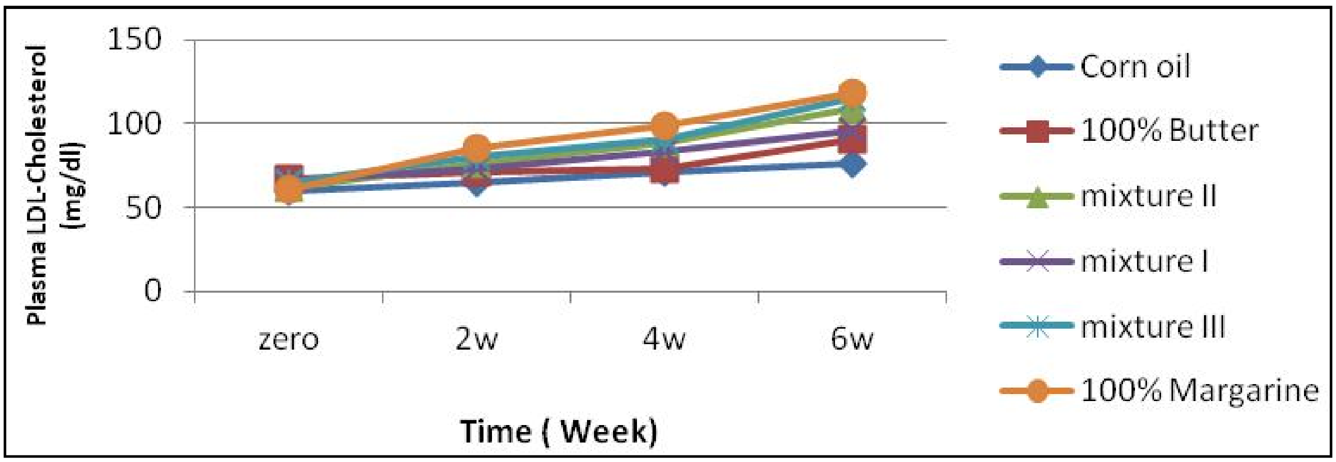

Fig. 6: Effect of feeding rats several fat types (100% corn oil,100% butter, (75% butter+25% margarine),(50% butter+50% margarine), (25% butter+75% margarine), and 100% margarine) as 10 % of total basal diet for 6 weeks on plasma LDL-C level. All rat groups showed significant LDL-C elevation with time except that for both the corn and the 100% butter groups as the former showed weak elevation and the latter showed some fluctuation.

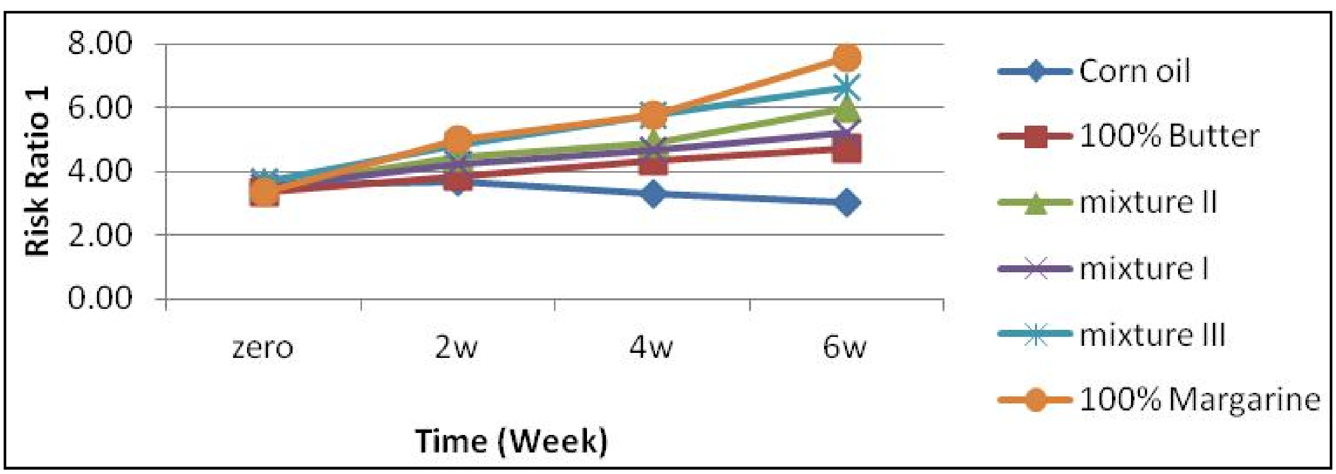

Fig. 7: Effects of feeding rats several fat types (100% corn oil,100% butter, (75% butter+25% margarine),(50% butter+50% margarine), (25% butter+75% margarine), and 100% margarine) as 10 % of total basal diet for 6 weeks on plasma Risk Ratio 1.All rat groups showed elevation in plasma Risk Ratio 1with time except for the corn rat fed group that showed sharp reduction from week 2 to week 6.

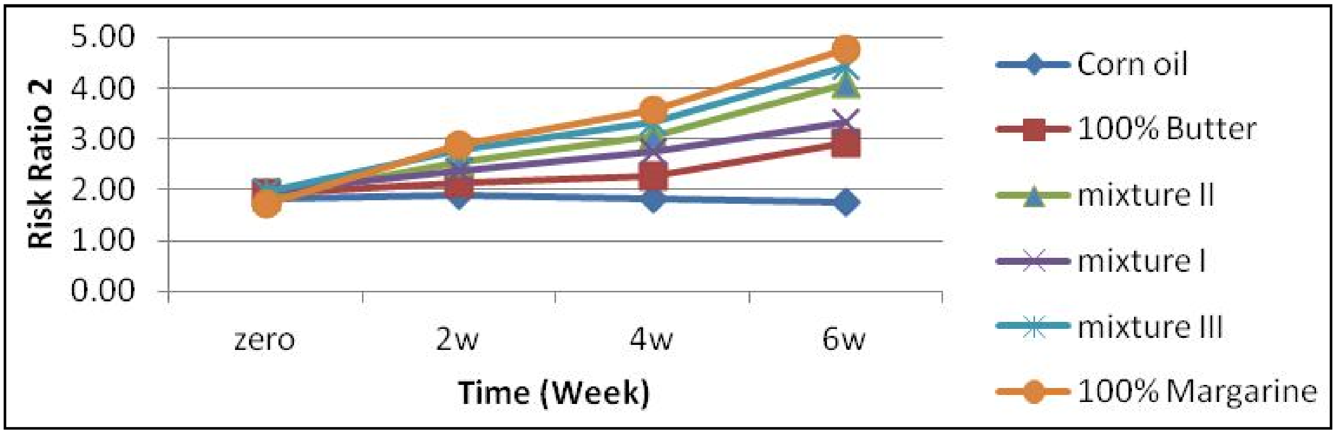

Fig. 8: Effects of feeding rats several fat types (100% corn oil,100% butter, (75% butter+25% margarine),(50% butter+50% margarine), (25% butter+75% margarine), and 100% margarine) as 10 % of total basal diet for 6 weeks on plasma Risk Ratio 2.All rat groups showed elevation in plasma Risk Ratio 2with time whereas the corn rat fed group showed constancy with slight decrease at the end of the experiment.

A healthy overall diet should still be prioritized for good health.

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