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Full Length Research Paper

Low and high ω-3 oils consequences rats' serum lipid fractions

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To study the consequences of some low and high ω-3 edibles oils on fatty acid profile, triacylglycerols and cholesterol levels of rats' serum; males of albino rat were fed on diet containing 5 and 10% from each of linseed, safflower and olive oils individually for 4 weeks. The extracted oils and serum lipids were directed to gas chromatography (GC) analysis. Serum triacylglycerols and total cholesterol were measured. Feeding rats on 5% olive oil for 4 weeks increased ω-6 monounsaturated fatty acid in serum lipid compared with other tested oils. Contrary relationship between oleic and palmetic acids in rat serum was found. Serum ω-6 di-unsaturated and ω-3 tri-unsaturated fatty acids were decreased in all treatments. Feeding rats on diets containing 5% ω-3 rich oil (linseed) was more effective in lowering the increase of serum triacylglycerol (TAG), while feeding on 10% linseed oil decreased total cholesterol levels compared with other oils. Generally, rats fed with ω-3 rich oil recorded the lowest TAG and total cholesterol levels during all feeding periods compared with other tested lower ω-3 oils.

Key words: Omega-3 fatty acids, serum lipids, rats.

INTRODUCTION

Dietary fat is one of three important main sources providing body with energy. Also they supply the body with essential fatty acids that affect serum cholesterol. The dietary unsaturated fatty acids that is, linoleic acid [18:2(n-6)] and linolenic acid [18:3(n-3)] have more effect on serum cholesterol and triacylglycerols levels than saturated fatty acids. 18:2(n-6) represents the major fatty acid in safflower oil; it ranged from 59.7 to 83.7% followed by oleic, palmitic, stearic and palmitoleic acids (Yoshida et al., 1992). Major fatty acid of olive oil is oleic acid (66.4 - 78.3%) followed by 18:2(n-6) (6.1 – 13.37%). Palmitic acid (8.8 – 15.2%), stearic acid (2.4 – 3.4%), palmitoleic acid (0.0 – 1.7%) and other fatty acids represented from (0.0 -1%) (Christie et al., 1991). Effect of diet 18:2(n-6)/ 18:3(n-3) fatty acids ratios on lipid metabolism in growing chicks of 4 weeks age, which fed on diets containing 6%, 1.5% safflower oil (SFO), and 6% linseed oil (LNO). Chicks fed on diets containing mixture of 1.5% SFO and 4.5% LNO had a lower liver triglyceride and cholesterol contents than those of chicks fed on SFO only. Serum triacylglycerols and free cholesterol level decreased markedly in response to dietary LNO. As dietary LNO levels increased, the fatty acid ratios of 18:2(n-6)/ 18:3(n-3) in liver, serum and abdominal fat decreased, but longer chain fatty acids such as eicosapentaenoic acid [20:5(n-3), EPA] and docosahexaenoic acid [22:6(n-3), DHA] increased linearly (Tanaka and Ohtani, 1995). Interestingly, common enzymatic pathways are shared by all fatty acids series, with sometimes competitive interactions between series (Kaushik, 2004). digestion and absorption of saturated

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and monounsaturated fatty acids is inferior to polyunsaturated fatty acids (PUFA), probably due to melting point effects and the tendency for long-chain saturated and monounsaturated fatty acids to form insoluble soaps with divalent cations in the gut, therefore selection of potential vegetable oils in diets must be considered (Bell et al., 2003). Dietary lipids provide essential PUFA for normal growth and development of cells and tissues and cause deposition of 18:2(n-6), retain high levels of 20:5(n-3) and 22:6(n-3) and provide sufficient energy in the form of saturated and monounsaturated fatty acids to maintain high growth rates. Feed supplementation with flaxseed oil contributed greatly to raising the nutritional lipid value (De Souza et al., 2007; Miller et al., 2007). Saturated and monounsaturated fatty acids decreases body weight of rats compared with polyunsaturated fatty acids. However, polyunsaturated fatty acids reduce plasma triacylglycerols and total cholesterol (Hwang et al., 1996). Cholesterol content in the liver and serum of chick's fed on palm oil was significantly higher. Liver triacylglycerols and total cholesterol contents were significantly decreased in chickens fed on diet containing linseed oil or fish oil. Also dietary fat reached with 18:3(n-3) was more effective in lowering serum lipids levels than other fatty acids in tissue lipids (An-Byeong et al., 1997).

Serum, liver, and plasma cholesterol levels were similar in rats fed on polyunsaturated (linseed) oil compared with those fed on monounsaturated (olive) and saturated (coconut) oils (Lu and Huang, 1993). In women and men that received linseed oil or fish oil plus sunflower oil for 4 weeks, 18:3(n-3) from vegetable oils, 20:5(n-3) and 22:6(n-3) from marine source oils affects haemostatic factors and decreases serum cholesterol and triacylglycerols content (Freese and Mutanen, 1997). Dietary with high 18:2(n-6) safflower oil, is not hyper-choleserolemic in aged mice after a long-term feeding comparing with lard and fish oils. Aged mice fed on a standard diet for 10 days, and then fed for 120 days on diets containing 10% lard, safflower seed oil and fish oil, showed a significant increase in whole body cholesterol. Long-term feeding of fish oil decreased serum cholesterol concentrations compared with safflower oil and lard oil without accumulation of cholesterol in the aorta concluding that dietary oils rich in ω-3 fatty acids may be useful to prevent the hyper-cholesterolemia associated diseases (Ishinara et al., 1998). Triacylglycerols and fatty acid esters in rats fed on fish and safflower oils have similar concentrations. In addition, safflower oil caused a significant higher content of total lipids and cholesterol in the liver than fish oil (Chao et al., 2000). Uses of vegetable oils in rats' diets affected serum and tissue concentrations of saturated, monounsaturated, and n-3 polyunsaturated fatty acids (PUFAs) but had no effect on n-6 PUFA (Radcliffe et al., 2004).

This study is carried out to study the effect of some low and high ω-3 edibles oils, with different concentrations (5 and 10%), on serum rats' triacylglycerols and cholesterol levels.

MATERIALS AND METHODS

Experimental materials and chemicals

Linseed (Giza7) and safflower (Giza1) seed were obtained from Oily Crops Department, Agriculture Research Center; Giza, Egypt. Virgin olive oil was obtained from olive fruits (Picua) from Horticulture Department, Agriculture Research Center, Giza, Egypt. Male Wister rats, 60 days old, with an average weight of (100 to 110 g) were obtained from station experimental animals in National Organization for Drug Control and Research (NODCAR) Laboratories, Ministry of Health, Giza, Egypt. Total cholesterol and triacylglycerols serum kits were obtained from Boehringer Mannheim Gmbh, Germany.

Extraction of crude oils

Linseed and safflower seeds were grounded and soaked in pure n-hexane for 24 h. The extracted oils were dried over anhydrous sodium sulphate, filtered, then stored in dark brown bottle and kept at 5^{o}C in refrigerator until analysis (AOCS, 1982).

Physical and chemical properties

Refractive index, specific gravity, acid value, peroxide value, iodine number and saponification value were determined (AOCS, 1982).

Fatty acids composition

The overall fatty acid composition of oils and serum was analyzed by modifications to the procedure of Dahmer et al. (1989). About 1 - 10 mg liver samples were placed into 2 ml of 2% (V/V) H_2SO_4 in methanol. The samples were heated at 80°C until the volume was reduced to approximately 0.5 ml (2 h). One and one-half milliliters of hexane containing 0.001% butylated hydroxytoluene (BHT) was added. The fatty acid methyl esters in the hexane layer were separated and directed to Gas Chromatography (GC) system for fatty acids profile determination (Miquel and Browse, 1992) using 10% SILAR 10C, 100/120 GAS CHROM W-HP column and the initial temperature of 100°C for 2 min followed by increase of 10°C /min to 200°C. The final temperature was maintained for 25 min. The injector and detector temperatures were 150 and 225°C, respectively. Nitrogen was used as the carrier gas with a flow rate of 10 ml/min.

Rats and diets

A total of 96 young male albino rats, 60 days old, with average weight (100 to 110 g) were used. The animals were divided into 8 groups (each group 12 rats) to evaluate the effect of feeding on basal diet (Table 1) containing 5 and 10% from each of fresh linseed, safflower and olive oils respectively compared with control which fed on basal diet. Blood samples were drown from rats eyes every week for four weeks, then centrifuged to separate serum which was kept in deep freezer until analysis.

The basal diet (BD) was formulated according to Reeves et al. (1993). The composition (g / 100 g) of basal diet is shown in Table 1.

Food intake was calculated by subtracting the weights of food

Table 1. Composition of basal diet (g /100 g).

Ingredients	Weight in g/100 g diet
Corn starch	68
Casein	12
Soybean oil (no additives)	10
Fiber	5
Salt mix (AIN-93M-MX)	4
Vitamin mix (AIN-93-VX)	1

Table 2. Body weights, food intake, of animals fed diets containing linseed oil, safflower oil, or olive oil (mean ± SD, *n* = 12 per group).

Parameter	Diet					
	Linseed oil		Safflower oil		Olive oil	
	5%	10%	5%	10%	5%	10%
Initial body weight (g)	142 ± 8	143.1± 6	144 ± 8	145 ± 7	140 ± 8	140.9 ± 4
Final body weight (g)	318 ± 9	319.2 ± 8	320 ± 11	320.7 ± 9	320 ± 10	320.5 ± 6
Food intake (g/rat/d)	19.2 ± 1.2	19.3 ± 1.3	19.4 ± 1.2	19.1± 1.2	19.0 ± 1.1	19.0± 1.2

jars at the end of the feeding period from the weights at the beginning and then correcting for any spillage, which was weighed daily (Table 2).

Determination of serum triacylglycerols

Serum triacylglycerols were determined according to the method described by Wahelefeld (1974). The color was measured at wave length 546 nm.

Determination of serum cholesterol

The colorimetric method of Siedel et al. (1983) was used to determine serum cholesterol. The intensity of the developing color was measured at wavelength 500 nm.

RESULTS AND DISCUSSION

Physical and chemical properties of linseed, safflower and olive oils

Physical and chemical properties of all three edible oils are shown in Table 3. It is clear that no oxidation has taken place during extraction of all oils which were free from any degree of rancidity. These oils were suitable for use in diet for experimental animals.

Fatty acids compositions

Fresh linseed oil characterized with high level of 18:3(n-3) (52.9%) followed by oleic acid (21.7%) while 18:2(n-6) recorded the third order with average value of (15%). On the other hand, safflower oil revealed higher value of 75.5% for 18:2(n-6) followed by 11.8% for oleic acid, while 18:3(n-3) was represented low percent (0.7%). The third vegetable oil sample which represents olive oil had a higher average value of 67.4% for oleic acid followed by 15.1% for palmitic acid and 11.5% for 18:2(n-6) (Table 4).

Effect of 5% linseed, safflower and olive oils on fatty acids of rats' serum after first and fourth weeks is shown in Table 5. Fatty acids of rats' serum were influenced by fat dietary type. Feeding rats with diets containing 5% olive oil for four weeks revealed high percentage of oleic acid (41.49%) when compared with the other tested oils (Hwaing et al., 1996). Data also revealed decrease in the percentages of 18:2(n-6) and 18:3(n-3). After four weeks, the oleic acid content decreased in both animal groups fed 5% linseed and olive oils and vice versa for palmetic acids since it was increased (31.17%) in rat serum fed on linseed oil and in rat serum fed on olive oil (37.38%) after four weeks. Also, from Table 5 it was observed that 18:2(n-6) and 18:3(n-3) in rats serum for all tested oils decreased after the same periods. The decreament of these fatty acids means that they were consumed in formation of other compounds such as prostaglandin. Stearic acid levels were higher in rats serum fed on 5% safflower oil compared with those fed on linseed and olive oils after the first week. Data from Table 5 also showed that, stearic acid recorded 38.38 and 36.08; 14.2 and 2.19 and 8.37 and 3.65% for safflower, olive and linseed oils after the first and fourth weeks, respectively. These results were in agreement with those obtained by Lu and Huang (1993). They reported that the ratio of fatty acids 18:0 and 16:0 were increased in rats' serum during

Table 3. Physical and chemical properties of linseed, safflower and olive oils.

Properties \ Oils	Linseed	Safflower	Olive
Specific gravity at 25^{o}C	0.9378	0.9210	0.9120
Refractive index	1.4835	1.4739	1.4672
Acid value	0.66	0.10	0.4
Saponification value	194.40	183.00	191.70
Iodine number	193.20	143.00	84.00
Peroxide value	0.44	0.50	0.640

Table 4. Relative percentages of fatty acids of fresh linseed, safflower and olive oils.

Fatty acid	Linseed oil	Safflower oil	Olive oil
12:0	0.1	0.1	-
14:0	0.2	0.6	0.5
16:0	6.3	7.9	15.1
18:0	3.3	2.0	2.3
20:0	0.1	1.0	-
*TSFA	10	17.9	11.6
16:1	0.4	0.4	1.9
18:1	21.7	11.8	67.4
18:2	15.0	75.5	11.5
18:3	52.9	0.7	1.3
**TUFA	90	88.4	82.1
***TU/TS	9	7.62	4.59

*TSFA and **T.U.F.A = Total saturated and unsaturated fatty acids. ***TU/TS = Ratio of total unsaturated: total saturated fatty acids.

Table 5. Effect of 5% linseed, safflower and olive oils on fatty acids of rats' serum after first and fourth weeks.

Fatty acids	Oils					
	Linseed		Safflower		Olive	
	First week	Fourth week	First week	Fourth week	First week	Fourth week
6:0	-	-	1.87	0.31	6.75	5.57
8:0	2.78	19.26	2.92	0.72	6.43	2.96
10:0	1.54	5.03	1.92	0.61	3.99	2.33
12:0	0.86	5.47	3.85	2.74	8.15	1.36
14:0	0.83	11.55	6.33	9.13	19.45	4.86
16:0	23.53	31.17	28.58	2.68	21.76	37.38
16:1	5.13	2.66	1.62	0.60	4.91	1.86
18:0	8.37	3.65	38.38	36.08	14.20	2.19
18:1	29.84	19.89	6.27	14.14	14.36	41.49
18:2	10.84	-	8.26	2.99	-	-
18:3	16.28	1.30	-	-	-	-
20:0	-	0.02	-	-	-	-
*TSFA	37.91	67.15	83.85	82.27	80.73	56.65
**TUFA	62.09	23.85	16.15	17.73	19.27	43.35
***TU/TS	1.64	0.36	0.19	0.22	0.24	0.77

*TSFA and **T.U.F.A = Total saturated and unsaturated fatty acids. ***TU/TS = Ratio of total unsaturated: total saturated fatty acids.

Table 6. Influence of feeding with some edible oils (5 and 10%) on serum triacylglycerols.

Concentration and type of oils		Triacylglycerols (mg/dl) (means ± SD)							
		Time of feeding/week							
		1	*Increasing percentage	2	*Increasing percentage	3	*Increasing percentage	4	*Increasing percentage
Linseed oil	5%	39.90 ± 2.32	3.89 ± 0.32	74.78 ± 2.56	9.32 ± 0.32	130.58 ± 1.84	6.77 ± 0.09	136.64 ± 2.51	8.83 ± 0.32
	10%	45.45 ± 1.51	10.36 ± 0.18	106.03 ± 3.46	26.18 ± 0.63	136.58 ± 2.29	9.01 ± 0.17	151.75 ± 3.23	13.66 ± 0.61
Safflower oil	5%	48.33 ± 2.91	13.39 ± 0.66	120.45 ± 1.29	32.02 ± 0.04	145.13 ± 3.29	12.01 ± 0.28	225.55 ± 3.89	32.35 ± 0.68
	10%	75.71 ± 3.45	34.45 ± 0.51	130.35 ± 2.45	35.52 ± 0.31	154.99 ± 1.54	15.23 ± 0.04	234.23 ± 2.19	34.90 ± 0.31
Olive oil	5%	63.49 ± 2.12	26.47 ± .28	103.42 ± 1.96	25.02 ± 0.06	157.48 ± 1.46	16.01 ± 0.03	246.79 ± 3.21	36.32 ± 0.34
	10%	55.08 ± 3.23	19.75 ± 0.36	105.75 ± 2.34	26.06 ± 0.27	162.34 ± 2.41	17.49 ± 0.19	270.42 ± 3.78	40.22 ± 0.63
Control		36.91 ± 1.45	-	62.02 ± 2.21	-	114 ± 1.19	-	115.27 ± 2.49	-

*Incr. %: Increasing percentage = [(corresponding sample - control sample) / (sum of control + sample) X 100].

metabolism of fatty acids.

Influence of different edible oils on serum triacylglycerols levels

Effect of feeding some edible oils (5 and 10%) on serum triacylglycerols content is presented in Table 6. Triacylglycerols levels revealed a gradual increment in control animals, while average values of triacylglycerols in control treatments after one, two, three and four weeks were 36.91, 62.02, 114.00 and 115.27 mg/dl, respectively. Feeding with different levels (5 and 10%) of linseed oil revealed more effectiveness on lowering the increment of triacylglycerols content in rats' serum compared with other two oils. Values of 5% linseed oil were 39.90, 74.78, 130.58 and 136.64 mg/dl for triacylglycerols content in serum after one, two, three and four weeks, respectively. Also, increasing of percentage values to 3.89, 9.32, 6.77 and 8.83 were found for the same treatments respectively (Table 6). These increasing of values were calculated by subtracting control sample from the corresponding sample and the yield was divided on the sum of control + sample. However, feeding on diet containing safflower or olive oils raised the triacylglycerols contents in blood serum after one, two, three and four weeks.

It is quite clear from the same table that 5 and 10% linseed oil caused the lowest increment percent value of 8.83 and 13.66 respectively after four weeks from feeding due to its higher content (52.9%) of 18:3(n-3) (Table 4) compared with the other two oils which contain less amount of this omega-3 fatty acid [18:3(n-3)] (Table 4). 5% safflower oil came in the second order with increasing percentage values of 32.35 in triacylglycerols content after four weeks. However, the highest level of 40.22% for triacylglycerols content of rat serum fed on a diet containing 10% olive oil was observed. These data are in agreement with those mentioned by Rustan et al. (1988); they mentioned that triacylglycerols content remained unchanged using diets rich with 18:3(n-3); also Singer et al. (1990) proved that dietary linseed oil causes a lower increment in rats' serum triacylglycerols level.

The obtained data agreed also with those mentioned by Allen et al. (1996). They found that feeding on diet containing linseed oil rich with 18:3(n-3) is more affective in lowering the increment of serum triacylglycerols level than dietary fat containing safflower oil rich with 18:2(n-6).

Influence of different edible oils samples on serum cholesterol levels

There is a link between elevated triacylglycerols and cholesterol levels and ischemic heart disease.

Table 7. Influence of feeding with different concentrations of some edible oils on content of serum total cholesterol.

Concentration and type of oils		Levels of cholesterol (mg/dl) (means ± SD)			
		Times of feeding/week			
		1	2	3	4
Linseed oil	5%	102.63 ± 1.09	78.99 ± 1.43	69.52 ± 0.81	64.44 ± 0.51
	10%	57.86 ± 1.12	33.56 ± 0.45	15.50 ± 0.29	115.85 ± 0.68
Safflower oil	5%	111.39 ± 0.98	68.70 ± 0.68	23.05 ± 0.39	116.35 ± 0.52
	10%	116.19 ± 1.23	84.92 ± 0.91	29.74 ± 0.48	140.09 ± 0.49
Olive oil	5%	114.50 ± 1.59	44.27 ± 0.86	65.66 ± 0.82	176.93 ± 0.72
	10%	71.64 ± 0.88	58.86 ± 0.43	70.77 ± 0.61	143.60 ± 0.83
Control		123.66 0.79	128.5 ± 0.71	129.72 ± 0.43	132.50 ± 1.02

Changes of serum cholesterol level during feeding experiment for 4 weeks are shown in Table 7. From this table, feeding with 5% linseed oil gave cholesterol levels of 102.63, 78.99, 69.52 and 64.44 mg/dl compared with control values of 123.66, 128.50, 12972 and 132.50 mg/dl after the first, second, third and fourth weeks respectively. This means that using 5% linseed oil in feeding rats lowered the cholesterol content compared with controls. In addition, feeding with 10% linseed oil in the diet caused more decrement in the cholesterol level during the first three weeks, where the results were 57.86, 33.56 and 15.50 mg/dl compared with control respectively. Feeding rats with 10% of each safflower and olive oils achieved higher cholesterol level after four weeks as compared with feeding on 10% linseed oil under the same conditions as shown in Table 7.

Since linseed oil contained a high percent of 18:3(n-3) (52.9%), it caused reduction of cholesterol level in blood serum compared with other oils. These data agree with those mentioned by Grundy and Denke (1990); they stated that polyunsaturated fatty acid (PUFA) especially 18:3(n-3) is effective in lowering blood cholesterol level compared with saturated fatty acid.

Conclusion

This research reveals that 18:3(n-3) has more potent ability on lowering cholesterol and triglyceride in serum than 18:2(n-6). So diets containing high levels of polyunsaturated fatty acids such as linseed oil may prevent the accumulation of triacylglycerols and cholesterol in the serum, where the moderate quantity of these contents help the heart to work very well without exposing it to cardiac disease.

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