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Possible role of lipid oxidation in atherosclerosis : a study of serum polyunsaturated fatty acids and copper

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THE POSSIBLE ROLE OF LIPID OXIDATION IN ATHEROSCLEROSIS

A Study of Serum Polyunsaturated Fatty Acids and Copper

By

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T A B L E O F C O N T E N T S

Introduction

Cardiovascular disease is the leading cause of death in man, accounting for 60% of all deaths. A high percentage of deaths in the cardiovascular category are secondary to underlying atherosclerosis. In 62.5% of sudden deaths caused by coronary heart disease, there was no clinical manifestation of the atherosclerosis prior to the episode which caused death(5). Aside from death, the clinical manifestations of atherosclerosis, such as myocardial infarction and cerebral vascular thrombosis, are associated with a high morbidity. Many of the individuals who develope clinical symptoms of atherosclerosis do so during their most productive years. In order to minimize the mortality and morbidity from underlying atherosclerosis, it would appear to be desirable to try to identify individuals prone to the development of the clinical disease at an age sufficiently early to permit effective preventative measures to be instituted.

A preliminary study(12) has postulated that oxidative-polymerization of lipids in serum and in atheromas may be involved in the etiology of atherosclerosis. This study showed a significant elevation of serum copper levels in individuals with myocardial infarction or angina as compared to the controls; individuals in the control group with a strong family history of clinical atherosclerosis were found to have increased copper levels. Some of the individuals in the group with a history of coronary artery disease had low or normal serum copper levels.

Another factor involved in lipid peroxidation is the degree of unsaturation of lipids in the serum or athromatous lipid deposits for the rate of oxidation by molecular oxygen increases with the degree of unsaturation of the lipids. It was the purpose of the present work to determine if those individuals with the history of coronary artery disease had a higher degree of polyunsaturated acids (PUFA) in their serum as compared to those individuals with a history of coronary artery disease and a low or normal serum to compensate for the low amount of copper.

Serum cholesterol concentrations which has shown a crude correlation to development of atherosclerosis is included in this investigation.

Procedure

Fasting blood samples were drawn from 42 male subjects with ages predominantly between 40 and 50 years. Group I (14 men) have a history of myocardial infarction or of angina pectoris. Group II (28 men) is the control group. The serum samples were stored frozen under nitrogen. Total lipids were extracted from the serum by the method of Sperry(32) using 1:1 methanol-chloroform mixture. Interesterification(33) was accomplished by refluxing the lipid extracts with 5% HCl in superdry methanol, extracting the methyl esters with hexane and purification of the methyl esters by microsublimation (33). The purified methyl esters were then analysed by gas-liquid

chromatography on a diethyglycol succinate column in a Aerograph apparatus utilizing a hydrogen flame detector. Serum total cholesterol was determined by a modified Zak method(28) on an aliquot obtained by extracting serums with a 1:1 mixture of acetone and methyl alcohol. The copper and iron content of each sample was determined simultaneously by a colormetric method developed by Zak(42) utilizing the additive nature of ferrous-bathophenanthroline sulfonate and cuprous-bathocuproine sulfonate complexes.

Results

The mean serum copper levels in group I (14 men with coronary artery disease) is 158 ± 19 micrograms % and in group II (28 men without history of coronary artery disease) is 140 ± 26 (see Table C). This is a statistically significant ($P < 0.05$) elevation of serum copper levels in the group with history of coronary artery disease. Five of the men in group I (see Table A) have low copper levels (below 145 micrograms %); however, two of these men have elevated PUFA (above 31%) and elevated cholesterol (above 200 micrograms %), one has a slightly elevated cholesterol, and two have low cholesterol (below 200 micrograms %) and PUFA (below 31%). Of those members in group I with increased copper levels, (above 140 micrograms %) three have increased PUFA and cholesterol, three have increased PUFA levels only and one has increased cholesterol only.

Twelve of the men in group II (see Table B) have elevated serum copper levels. Five of these men with increased copper levels have increased PUFA and cholesterol, three have increased PUFA only, four have decreased or normal PUFA and normal cholesterol. The remaining 16 men in group II have normal or low copper levels (140 micrograms % or lower). Two of these men (number 10 and 16) have low PUFA, markedly elevated cholesterol and markedly elevated total serum lipids (TSL). Two men have normal PUFA and elevated cholesterol, four have increased PUFA with normal cholesterol, two have increased PUFA and increased cholesterol, one has decreased PUFA and normal cholesterol and five men have normal or low PUFA and cholesterol.

There is no significant difference between the mean serum cholesterol, mean serum PUFA and mean arachadonic acids in the two groups.

The mean serum iron levels in group I is 108 $\pm$ 35 and in group II is 120 $\pm$ 36; however, the difference is not significant.

Discussion

Atherosclerosis is primarily a disease of the intima involving larger conducting arteries such as the aorta, coronary arteries and cerebral vessels. A normal artery consists of intima, media and aventitia. The large arteries such as the aorta contain large amounts of elastic tissue in the media while smaller arteries and arteriols have relatively increased amount of smooth muscle in the intima. The earliest demonstrable

morphologic alteration occurs as splitting and degeneration of the internal elastic membrances of the aorta in new born infants(23). This is followed by deposition of acid mucopolysaccharide, fibroblastic proliferation and regeneration of elastic tissue with residual thickening of the intima and deposition of fat droplets in the intima.

The first gross manifestations of atherosclerosis appear in the first 1 or 2 years of life and are fatty streaks or nodules in the intima of the root, arch and posterior wall of the aorta, especially around the ostia of vessels. Proliferation of fiberous tissue in the fatty deposits with extention into the media form discrete plaques. Atheromas are formed when the plaques eventually necrose, leaving a soft center containing a mixture of lipids and necrotic tissue debris. The hyaline fibrous covering of the atheroma may break, forming a atheromatous ulcer and/or calcification between the intima and media may occur. The contents of a ruptured atheroma may become a embolus and occlude a vessel such as sometimes occurs in myocardial infarction. Atheromas and calcified plaques may narrow the lining of a artery and produce insufficiency such as in coronary artery disease. Injury to the lining of arteries by atherosclerosis encourages thrombosis.

The degree of atherosclerosis is not related linearly to age but increases rapidly in the 30 to 49 year age period, reaches a maximum in the 6th decade and then increases no further with advancing age(39). The phospholipid content of the aortic wall and coronary arteries increases more rapidly

than the other lipid fraction in the early decades (18-33 years) whereas cholesterol and cholesterol esters accumulate more rapidly than the phospholipids in later life. Increased lipid content of coronary arteries begins at least two decades earlier than usual in men with cardiac artery disease(40). There is an increase in cholesterol, cholesterol esters and phospholipids in the coronary arteries of men with coronary artery disease(41) but there is no difference between lipid fraction in aortas of normal persons and persons with coronary artery disease. Mineral deposit (mostly calcium) in the aortas of normal and men with coronary artery disease is the same. There is a sharp increase in mineral deposits in coronary arteries at middle age; however, the increase occurs earlier in those persons with coronary heart disease(41).

Analysis of the lipid content of atheromas have revealed 4.2% saturated fatty acids, 61.5% oleic acid and 23.6% linoleic acid(20). Free sterols amount to about 70% of total sterols present. Analysis of the lipid fractions of aortic intima and media reveal phospholipids carrying mainly saturated acids (palmitic 35-45% and stearic 20-25%) and 20-30% unsaturated fatty acids (with oleic acid being the largest fraction). The saturated fatty acids in the phospholipid fraction decrease slightly with increased severity of the atherosclerosis. The glyceride fraction contains stearic acid (5-10%), palmitic (20-35%), lauric (2-6%), myristic

(3-9%), C_{18} unsaturated acids (35-50%, mostly oleic acid) and a few C_{20} unsaturated acids. Again no relation of saturated fatty acids to the degree of sclerosis. The cholesteryl ester fraction contained palmitic acid (8-25%), stearic (3-10%), palmitic (5-10%), oleic (20-30%) and linoleic (20-30%); the polyunsaturated fatty acids being the predominate fatty acids(3).

The high percentage of unsaturated fatty acids and the decreasing amount of saturated acids with increasing severity of atherosclerosis is of particular interest in the light of certain suggestions which have been made concerning the possible effect of saturated versus unsaturated acids on cholesterol atheromatous deposits. It has been theorized that high saturated acid content of the diet may cause preponderance of cholesteryl esters of saturated acids in blood(31). These saturated acid sterol esters have a higher melting range and are less soluble in the blood than are the unsaturated esters. Thus, it was thought that they may have a greater tendency to deposit in the arterial wall. The high concentration of unsaturated acids would tend to disprove this theory.

Fasting serum contains about 500 micrograms % lipids, 95% of which is combined with protein to form lipoproteins. The remainder of the serum lipids are present as colloidal chylomicroms which are of greatest concentration following fatty meals(19). There is a increase in the amount of lipid in plasma with increased age and also the ability of the body to handle a post-prandial hyperlipemia seems to decline with increased age as is indicated by the prolonged elevated chylo-

microms which are of greatest concentration following fatty meals(19). There is a increase in the amount of lipid in plasma with increased age and also the ability of the body to handle a post-prandial hyperlipemia seems to decline with increased age as is indicated by the prolonged elevated chylomicron counts seen after fatty meals in older persons(7).

About 25% of the lipoproteins are alpha-lipoproteins and about 75% are beta-lipoproteins(6). The beta-lipoproteins contain the low density lipoproteins which can be divided into 2 groups by ultracentrifugation, the S_f 0-20 and S_f 20-10^5 classes. Alpha lipoproteins are high density ($HDL_{2\&3}$). The low density lipoproteins are relatively constant in amount and in composition from day to day. Their concentration can be decreased by fasting and they tend to increase with age, especially in men.

By means of silicic acid column chromatography the serum lipids may be separated into several fraction, i.e. cholesteryl esters, glycerides and phospholipids(19). There appears to be a relatively characteristic fatty acid pattern for each fatty acid containing serum fraction. The cholesteryl esters are a important means of transport of the more highly unsaturated fatty acids (linoleic 55%, oleic 17%, arachidonic 6% and palmitic 10%). The glyceride fraction carries more of the saturated acids (palmitic 31%, oleic 38%, and linoleic 16%). The phospholipids carry nearly equal amounts of saturated and unsaturated fatty acid (palmitic 39%, linoleic 21%, stearic 14%,

oleic 11% and arachidonic 10%). There is a unesterified fatty acid fraction which is thought to be bound to serum albumin and contains oleic acid 25%, palmitic acid 26%, linoleic acid 15%, and more than 9% of those fatty acids with molecular weight less than palmitic acid(19).

The S_f 0-20 lipoprotein class carries the major share of cholesteryl esters. The S_f 20-10^5 lipoprotein class carries about 70% of the serum glycerides and 20% of the total cholesteryl esters. Part of the S_f 20-10^5 class (S_f 20-400) is the class of lipoproteins most likely to be increased in coronary artery disease. The high density lipoproteins ($HDL_{2\&3}$) carry most of the phospholipids. Cholesteryl esters, glycerides and phospholipids in the S_f 0-20 and $HDL_{2\&3}$ lipoprotein classes appear broadly similar in composition. The phospholipid fraction of the S_f 0-20 class contains the highest level of post-arachidonic acids (i.e. those fatty acids with gas chromatography mobilities slower than that of arachidonic acid) than any other class. The levels of prepalmitic acid are higher in the glyceride fraction of all 3 classes than in any other fraction. The S_f 20-10^5 lipoprotein class does not show a consistent fatty acid pattern. The association of the S_f 20-10^5 class with the early phases of fat absorption and transport may be responsible in part for these observed differences. Thus, cholesteryl esters in the early phases of transport in the blood may have a different fatty acid distribution from the cholesteryl esters that are present for longer periods of time in the blood as part of the S_f 0-20 and $HDL_{2\&3}$ lipoproteins(19).

There is a increase in serum total fatty acid (TFA) and a relative decrease in polyunsaturated fatty acids (PUFA) with increasing age, especially in men(24,30,2). The diens and tetraenes are decreased bu the trienes tend to increase. It has been suggested that deficiency of the essential fatty acids EFA is a factor in causation of atherosclerosis(31,21). EFA's increase the rate of cholesterol turnover and cholesterol breakdown(16,14,8). It is believed also that unsaturated cholesteryl esters are needed for lipid transport in blood(15,17). When the serum lipid concentration is high, most of the EFA's available will be bound by the excessive amount of cholesterol for lipid transport which would suggest that relatively less EFA's are remaining for intracellular purposes. By this means a circumstance can be produced in which the EFA's needed for the turnover of cholesterol remain bound to the abundance of cholesterol. In a disordered transport system such as this the overloaded EFA carrier may precipitate together with its cholesterol load in the arterial wall and consequently the EFA content of the plaques exceeds that of the passing blood(24,3). Cholesterol is synthesized by the liver as well as obtained by diet. Blood cholesterol levels may be decreased to a varying degree after a low cholesterol diet for a period of time which would be important in decreasing this cholesterol overload. Since older persons tend to have less PUFA relative to TFA than younger people, their lipid transport system is more suspectable to overloading by extra fat. It would seem logical

to conclude that increasing the PUFA's would lower the incidence of atherosclerosis; however, peroxidation of PUFA's may also be a important factor in the etiology of atherosclerosis.

Lipid peroxides have been found in atheromas(6) in amounts approximately proportional to the severity of the disease. Lipid peroxides are present in fasting serum as determined by the technique of electron spin resonance(13). Normal aortas are relatively free of lipid peroxides. Unsaturated fatty acids are readily oxidized by oxygen and from the known chemistry of their reactions it might be expected that some of the lipids in the serum lipoproteins would undergo oxidative polymerization to higher molecular weight materials(11) containing one or more such groups as hydroxyl, peroxy, carbonyl, carboxyl and a variety of free radicals resulting from scission of the unsaturated fatty acid chain. Hydrolysis of ester groups of oxidized polymerized materials would yield compounds containing hydroxyl and carboxyl groups. The polymerization reactions occur at random with other compounds through hydrogen removal and a variety of addition reactions.

Lipid peroxides are extremely toxic and are thought to act as tissue irritants in the etiology of atherosclerosis. Mitochondria contain about 25% lipids consisting mainly of unsaturated fatty acids(34) which are in close molecular proximity to the cytochromes of the electron-transport chain and to oxygen. Cytochromes are among the most potent lipid peroxidation catalysts known(9,35,20). They catalyze the scission of peroxide into free radicals which initiate further

reaction chains.

Perhaps the development of atherosclerosis depends on the rate of conversion of fatty streaks to fibrous plaques(26,22). The rate of fibrous plaque formation may be dependent on tissue irritation produced by increased concentration lipid peroxide as a result of increased oxidative polymerization of serum and lipid streak fatty acids(4). Cholesterol concentration is high in atheromas and cholesterol is also easily oxidized. As mentioned before, there is a good correlation between the serum levels of the S_f 0-400 lipoprotein class and the extent of atherosclerosis. "Hence, if oxidized-polymerized lipids are the initiating agents in atherosclerosis, their steady state concentration must parallel that of these low density lipoproteins. This does not mean that lipid oxidation-polymerization does not occur elsewhere than in members of the S_f 0-400 group, but implies that if it does, the oxidized material is formed in relatively small amounts or eventually comes to be carried by lipoproteins of the S_f 0-400 range(11)".

The rate of lipid oxidative-polymerization reactions would be expected to depend on the concentration of oxidative catalysts such as copper and iron and on the concentration of PUFA.

Serum copper may be increased by increasing the dietary intake of copper(10). There appears to be a increased incidence of coronary artery disease in population using soft water for drinking purposes(29) possibly because the copper content of water tendsto increase with the softness of the water.

Oxidation of lipids in vitro using copper as a catalyst is inhibited by binding the copper with a chelating agent such as Versene(40). In biological systems, such antioxidants as pyridoxine(27) act as chelating agents which bind copper and iron thereby inhibiting lipid peroxidation. There is evidence that other compounds such as tocopherol(44,1,37), fat soluable vitamins(38) and seleniunn compounds(43) also have antioxidant properties.

In this study, the mean serum copper levels in a group of 14 men with history of coronary artery disease was significantly higher than that of a group of 28 men without a history of coronary artery disease which agrees with a previous study(12). There was no correlation between the mean PUFA concentration in these two groups which is also in agreement with previous studies(24,19). The mean total cholesterol and mean arachidonic acid levels did not show a correlation with coronary artery disease. The mean serum iron levels were lower in men with coronary artery disease but this is not significant. Iron has less catalytic ability for lipid peroxidation as compared to that of copper(25).

On examination of the individual results, there appears to be a tendency toward increased PUFA and/or cholesterol in association with elevated copper levels in those persons with coronary artery disease. Of the men with coronary artery disease and normal or decreased serum copper levels, two had increased PUFA and increased cholesterol, one had increased cholesterol only and two had normal or below normal PUFA and cholesterol;

thus, giving no definite correlation of these factors. It must be considered, however, that some of these men may have altered their diet considerably since the time of their heart attack which could possibly change their lipid distribution and copper levels. In evaluation of this data consideration must be given to the fact that this is a small series and that the people who volunteered to participate in this study were possibly motivated and not a true random sample of our population.

If the tendency of increased serumcopper, PUFA and/or cholesterol in relation to coronary artery disease holds true as is observed in this study, several of the members in group II with increased PUFA, increased copper and increased cholesterol would be expected to develope clinical atherosclerosis within the next few years. A follow up study of this group would be of value.

Note that number 10 and 16 of group II have markedly elevated cholesterol and total serum lipids (TSL). In keeping with the present theory of lipid oxidation-polymerization, the low copper levels and low PUFA levels may possibly be responsible for these men not developing clinical atherosclerosis as yet as might be expected from their high lipid values.

Another factor to be considered is that of antioxidants such as tocopherol in blood. Some individuals with high serum copper levels may also have high blood levels of antioxidants which wouldtend to decrease the rate of oxidative-polymerization of lipids. The converse may be true in those persons with low serum copper who may also have low levels of antioxidants.

Although there appear to be a number of factors involved in the pathogenesis of atherosclerosis, serum copper levels may well prove to be a additional useful aid in predicting the susceptability to subsequent occurance of clinical atherosclerosis.

Summary

In order to minimize the mortality and morbidity from underlying atherosclerosis in cardiovascular disease, it would appear to be desirable to try to identify individuals prone to the development of the clinical disease at an age sufficiently early to permit effective preventative measures to be instituted.

The products of oxidative-polymerization of polyunsaturated fatty acids (PUFA) in serum and in atheromas as catalyzed by copper may act as tissue irritants in the etiology of atherosclerosis. Elevated copper and PUFA levels may indicate a susceptability to this disease.

In a previous study of a group of 42 men with ages predominantly between 40 and 50 years, the mean serum copper levels in 14 men with history of coronary artery disease was significantly higher than that of 28 men without a history of coronary artery disease. No correlation was made between the total cholesterol or serum iron in the 2 groups of that study.

In this study, there is no significant difference between the mean total serum PUFA and arachidonic acids of the two groups. On review of the individual data, those men with normal or low copper levels did not show a significant elevation or change in the serum PUFA.

There appears to be a tendancy toward increased serum PUFA and/or cholesterol levels in association with elevated copper levels in those persons with coronary artery disease.

TABLE A - GROUP I: History of Myocardial Infarction or Angina Pectoris

	Age	TSL mg %	Lauric Acid %	Myristic Acid %	Palmitic Acid %	Palmoleic Acid %	Stearic Acid %	Oleic Acid %	Linoleic Acid %	Linolenic Acid %	Arachidonic Acid %	PUFA %	Copper Micrograms %	Iron Micrograms %	Total Cholesterol mg %
1.	66	908	.68	----	21.60	3.41	5.44	29.70	33.90	----	5.10	39.00	137	82	220
2.	45	1578	1.44	----	23.70	1.44	7.90	18.20	41.25	----	5.99	47.24	153	115	290
3.	43	928	1.60	.23	26.40	2.56	8.72	26.70	29.70	----	4.41	34.11	202	57	180
4.	47	1200	1.95	.10	26.60	2.54	7.63	26.00	31.10	----	3.91	35.01	124	64	250
5.	57		1.85	----	27.50	3.21	6.76	34.85	23.60	----	2.87	26.47	160	150	220
6.	54		.78	.36	27.00	3.40	7.22	22.10	34.15	.54	4.50	39.19	158	188	220
7.	48		.74	.33	27.50	2.15	7.13	21.90	35.50	----	4.64	40.14	185	127	170
8.	53		1.90	.36	33.60	4.67	7.60	28.60	20.00	----	3.06	23.06	140	59	150
9.	48	1078	2.29	.69	41.80	7.94	4.43	19.40	20.15	----	3.21	23.36	135	95	180
10.	45		2.74	.84	46.50	8.40	3.80	23.75	12.67	1.05	.21	13.93	163	130	180
11.	43	1594	3.84	.40	38.90	6.38	4.63	25.50	19.15	.96	.16	20.27	167	102	200
12.	52		.27	.27	31.25	3.91	4.88	21.80	34.40	.98	2.28	37.66	158	147	200
13.	45	982	.99	.45	32.40	4.68	5.40	27.12	27.00	.36	1.62	28.98	136	94	220
14.	42	1228	.94	.19	30.45	3.01	6.58	25.40	29.90	----	3.57	33.47	156	86	240

TABLE B - GROUP II: No History of Coronary Artery Disease

	Age	TSL mg %	Lauric Acid %	Myristic Acid %	Palmitic Acid %	Palmoleic Acid %	Stearic Acid %	Oleic Acid %	Linoleic Acid %	Linolenic Acid %	Arachidonic Acid %	PUFA %	Copper Micrograms %	Iron Micrograms %	Total Cholesterol mg %
1.	46	----	1.33	.47	29.44	4.18	8.78	31.05	24.70	----	----	24.70	110	73	180
2.	49	978	1.68	.19	27.65	3.95	5.70	29.32	26.15	----	5.14	31.29	135	160	190
3.	41	----	.97	----	25.55	3.75	7.33	27.66	26.60	----	7.65	34.25	154	154	210
4.	42	----	1.49	----	24.40	2.68	8.56	27.00	30.05	----	5.36	35.41	145	112	220
5.	44	1052	.13	----	25.20	3.47	6.44	31.65	30.90	----	2.19	33.09	146	142	220
6.	43	902	1.26	----	23.20	2.63	8.74	28.20	29.90	----	5.99	35.89	172	117	210
7.	50	----	1.40	----	28.40	1.74	7.63	34.20	23.70	----	3.03	26.73	170	108	220
8.	40	----	2.12	----	35.50	4.88	5.10	27.20	25.25	----	----	25.25	160	142	210
9.	41	----	1.39	.32	26.50	2.45	7.50	30.01	26.10	1.38	4.49	31.97	133	145	190
10.	44	2800	4.86	1.36	41.55	6.41	4.66	24.00	15.35	1.65	.80	17.80	165	85	250
11.	43	848	.51	.25	27.70	2.56	4.35	27.90	32.10	.38	4.23	36.71	152	144	170
12.	44	1010	1.35	.27	30.70	2.70	5.75	31.10	25.50	----	2.52	28.02	130	100	210
13.	44	940	.55	.14	26.90	2.34	5.64	24.60	33.80	1.10	4.81	39.71	175	142	230
14.	48	1310	1.69	1.02	33.35	2.98	4.34	25.75	28.75	----	2.17	30.92	167	128	220

TABLE B - GROUP II: No History of Coronary Artery Disease (Continued)

	Age	TSL mg %	Lauric Acid %	Myristic Acid %	Palmitic Acid %	Palmoleic Acid %	Stearic Acid %	Oleic Acid %	Linoleic Acid %	Linolenic Acid %	Arachidonic Acid %	PUFA %	Copper Micrograms %	Iron Micrograms %	Total Cholesterol mg %
15.	46	----	.70	.42	26.60	2.66	6.88	24.75	34.80	.42	3.50	38.72	167	114	190
16.	36	2500	4.55	2.24	32.80	8.40	6.87	28.50	14.25	1.25	1.25	16.75	119	85	370
17.	38	924	6.06	1.78	40.30	8.70	2.90	22.90	15.80	.13	1.31	17.06	190	154	200
18.	46	816	.48	.48	27.30	3.85	5.96	27.10	30.10	1.34	2.50	33.94	135	114	190
19.	45	788	.63	.31	31.70	2.76	5.42	25.00	31.60	1.04	1.25	33.89	104	90	180
20.	47	----	.81	.18	31.10	3.97	5.60	23.90	30.70	.90	2.89	34.49	124	138	200
21.	42	932	.56	.14	31.60	4.23	5.92	24.50	27.90	----	5.08	32.98	119	258	210
22.	42	986	2.04	.34	32.00	4.60	5.28	23.90	29.80	1.19	.85	31.84	124	104	260
23.	43	----	1.73	.48	32.40	5.38	4.99	25.00	26.10	.58	3.27	29.95	110	74	180
24.	48	1138	3.25	.35	32.50	5.80	6.33	27.90	22.65	.35	.81	23.81	124	94	210
25.	44	860	.69	----	33.10	3.47	3.93	18.50	36.50	.69	3.00	40.19	100	92	170
26.	51	----	1.32	.69	24.51	4.71	6.10	26.60	33.51	.38	2.15	36.04	102	126	210
27.	46	----	1.91	.84	31.45	3.66	5.50	29.90	24.60	.68	1.45	26.73	70	56	160
28.	43	908	.75	.30	27.70	1.81	6.64	22.60	37.70	.45	1.96	40.11	132	114	180

TABLE C - Mean Values

	PUFA %	Arachidonic Acid %	Copper Micrograms %	Iron Micrograms %	Total Cholesterol mg %
Group I	31.56	3.25	158 ± 19	108 ± 35	206 ± 34
Group II	30.73	2.85	140 ± 26	120 ± 36	201 ± 38

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