PLATELET FATTY ACIDS IN CORONARY HEART DISEASE, DYSLIPIDEMIA, HYPERTENSION AND HEALTHY CONTROLS

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Abstract. A cross-sectional study was performed to investigate 250 volunteers from Pramongkutklao Hospital, Samphanthawong district, Wat Chaiyapreukmala and Wat Pradoo in Taling Chan district. They were divided into groups of 35 apparently healthy males, 16 males with coronary heart disease, 37 males with dyslipidemia and 9 males with hypertension with age ranges of 24-62, 56-69, 25-69 and 26-75 years, respectively. The female groups were composed of 55 apparently healthy females, 10 females with coronary heart disease, 73 females with dyslipidemia and 15 females with hypertension with age ranges of 27-65, 33-67, 22-73 and 38-70 years, respectively. Platelet fatty acids levels were found to have no significant difference between the different male groups. In the female groups, the α-linolenic acid (ALA) level in hypertension was significantly higher than in coronary heart disease (CHD) ($p < 0.05$), whereas the arachidonic acid (AA) level in hypertension was significantly higher than in the apparently healthy females ($p<0.05$). No correlation was found between platelet fatty acids and age or anthropometric parameters, which indicate that platelet fatty acids may not depend on either age or anthropometric parameters. Positive correlations were shown between ALA and eicosapentaenoic acid (EPA), AA and docosahexaenoic acid (DHA), ALA and the diastolic blood pressure, DHA and total cholesterol (TC), and between low density lipoprotein choleoterol (LDL-C) and plasma glucose. Negative correlations were shown between LA and EPA, AA and EPA, EPA and DHA, EPA and the systolic blood pressure, and AA and the diastolic blood pressure.

INTRODUCTION

Coronary heart disease (CHD) remains the leading cause of death in many countries including Thailand (Lochaya, 1983). In the year 1996, there were 46,286 deaths due to heart disease per every 100,000 deaths. In the year 2000 the ratio was reported as 19,708: 100,000 (Ministry of Interior, 1996-2000).

The Framingham Heart Study has contributed significantly to the understanding of the causes of CHD, stroke and other cardiovascular diseases, they are called "cardiovascular risk factors" (Kannel, 1998). The major risk factors include high serum cholesterol, high level of low-density lipoprotein cholesterol (LDL-C), and low level of high-density lipoprotein cholesterol (HDL-C). Other factors than those risk factors include obesity, physical inactivity, hypertriglyceridemia, hypertension, and increased lipoprotein (a) (Grundy, *et al.* 1998).

Cardiovascular disease (CVD) is the general term for all diseases of the heart and blood vessels. CHD is a blood vessel disease of the heart, which results in coronary ischemia which is commonly called a heart attack. Coronary artery disease (CAD) and ischemic heart disease are other names for CHD.

CHD is closely associated with advanced atherosclerosis which reflects several deteriorative phenomena (involving an interaction between plasma lipids, lipoproteins, monocytes, platelets and the endothelium and smooth muscle of the arterial wall), that gradually result in narrowing of coronary arteries, terminating in thrombosis and coronary infarction (Steinberg, 1983; Ross, 1986; Munro and Cotran, 1988).

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Arachidonic acid (AA) is the predominant ω-6 polyunsaturated fatty acid esterified at the second position of the platelet membrane phospholipids. Activated platelets produce prostaglandin endoperoxide intermediate to prostaglandin G_2 (PGG_2) and prostaglandin H_2 (PGH_2) by enzyme cyclooxygenase (COX) and is subsequently converted to thromboxane A_2 (TXA_2). TXA_2 and endoperoxides then exit from the platelet to function as potent agonists and vasoconstrictors.

In addition to AA, COX will oxygenate other polyunsaturated fatty acids. Eicosapentaenoic acid (EPA), ω-3 polyunsaturated fatty acid, is also converted by cyclooxygenase via thromboxane synthetase to TXA_3, TXA_3 is inactive as platelet agonist (Harris, 1997). When the diet is high in EPA and docosahexaenoic acid (DHA), they replace AA in the membrane phospholipids of platelets. Liberation of AA is reduced, EPA and DHA also inhibit the activity of COX, which results in reduced production of TXA_2 leading to reduced platelet aggregation, making thrombus formation less likely (Holub, 2002). When a diet is low in EPA and DHA, activated platelets liberate AA from membrane phospholipids. COX catalyzes the AA conversion to TXA_2, leading to increased platelet aggregation, making thrombus formation more likely (Holub, 2002).

The objective of this study was to examine the profile of relative concentrations in platelets of ω-3 and ω-6 polyunsaturated fatty acids in coronary heart disease, dyslipidemia, and hypertension compared with apparently healthy controls. The other objective was to verify the relation between concentrations of ω-3, ω-6 polyunsaturated fatty acids and various cardiovascular risk factors.

MATERIALS AND METHODS

Volunteer subjects were randomly recruited from Pramongkutklao Hospital, a health center in Samphanthawong district and health centers at Wat Chaiyapreukmala and Wat Pradoo in Taling Chan district. Male groups were divided into groups of: 35 apparently healthy males, 16 males with coronary heart disease, 37 males with dyslipidemia and 9 males with hypertension, with age ranges of 24-62, 56-69, 25-69 and 26-75 years, respectively. The female groups composed of :55 apparently healthy females, 10 females with coronary heart disease, 73 females with dyslipidemia and 15 females with hypertension, with age ranges of 27-65, 33-67, 22-73 and 38-70 years, respectively.

Participants were asked to fast for 12 hours before taken blood samples. Ten milliters of blood was centrifuged to separate serum for lipid profile, plasma glucose, and for other determinations. Platelet isolation for fatty acid analysis was taken from 8 ml of EDTA blood. Platelet fatty acid analysis was adapted from the method of Li (1998).

Total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) were determined by the CHOD-PAP method (Friedewald *et al*, 1972; Gordon *et al*, 1977). Triglyceride (TG) was determined by the GPO-PAP method (Koditschek and Umbriet, 1969). Low-density lipoprotein cholesterol (LDL-C) was calculated from (TC)-(HDL-C) – (TG/5) expressed in mg/dl. Plasma glucose was measured by the GOD-PAP method.

Data analysis

Non-parametric statistics were used, since the majority of the variables had a non-normal distribution. The median, range and 95% confidence interval (95% CI) were calculated instead of mean and standard deviation. Mann-Whitney U- and Wilcoxon rank sum W-tests (two tailed) at 0.05 level of significance were used. Spearman's rank correlation method was analyzed by the SPSS (Statistical Package for Social Science) version 9.5 for WindowsTM computer software (SPSS Inc, 1998). Multivariate analysis was performed to study the association between dependent and independent variables.

RESULTS

Table 1 shows a significantly higher TC/HDL-C ratio in males with dyslipidemia than in apparently healthy males ($p < 0.05$). Plasma glucose in males with CHD was significant higher than in apparently healthy males ($p < 0.05$). No significant difference was found between the cardiovascular risk factors of the female groups (Table 2).

Table 1
Median, range and 95% CI of cardiovascular risk factors in healthy males,and males with coronary heart disease, dyslipidemia and hypertension.

Variables	Healthy (N=35)		CHD (N=16)		Dyslipidemia (N=37)		Hypertension (N=9)	
	Median (range)	95% CI	Median (range)	95%CI	Median (range)	95%CI	Median (range)	95%CI
Systolic BP (mmHg)	120.0 [a] (90-172)	118 -132.2	125.0 [a] (100-140)	115.8 -132.5	130.0 [a] (100-200)	127.2 -141.8	130.0 [a] (110-160)	123.2 -143.5
Diastolic BP (mmHg)	80.0 [a] (60-120)	78.3 -88.1	70.0 [a] (70-90)	70.7 -79.3	87.0 [a] (70-111)	81.9 -89.5	80.0 [a] (50-100)	69.9 -92.3
TC (mg/dl)	204.0 [a] (148-310)	190.3 -219.5	217.5 [a] (151-245)	196.1 -226.8	215.0 [a] (165-326)	208.7 -234.2	205.0 [a] (145-247)	176.5 -232.4
HDL-C (mg/dl)	51.0 [a] (32-82)	47.0 -55.7	44.0 [a] (31-70)	39.1 -52.9	46.0 [a] (22-72)	42.9 -49.3	51.0 [a] (37-70)	42.6 -58.7
LDL-C (mg/dl)	115.7 [a] (60.6-208.2)	109.3 -134.0	154.6[a] (102-179.4)	141.0 -165.9	134.6 [a] (27-224.2)	123.6 -154.1	132.4 [a] (71-170.2)	104.3 -157.7
TC/HDL-C ratio	3.9 [a] (0.57-7.0)	3.58 -4.58	5.2 [a,b] (3.06-7.03)	4.35 -5.69	5.0 [b] (2.7-7.7)	4.6 -5.3	4.6 [a,b] (3.1-5.2)	3.5 -4.8
LDL-C/HDL-C ratio	2.56 [a] (1.25-4.76)	2.18 -2.87	3.7 [b] (1.89-5.17)	2.96 -4.09	3.16 [a,b] (1.2-4.8)	2.6 -3.4	3.15 [a,b] (1.8-3.3)	2.1 -3.2
TG (mg/dl)	122.0 [a] (56-627)	113.6 -188.0	102.0 [a] (55-152)	88.4 -115.5	158.0 [a] (55-870)	140.6 -232.7	92.0 [a] (50-230)	67.6 -160.0
Plasma glucose (mg/dl)	88.0 [a] (63-143)	84.1 -93.0	100.0 [b] (79-262)	89.8 -136.2	95.0 [a,b] (75-185)	91.2 -104.2	94.0 [a,b] (83-106)	88.8 -103.4

TC = Total cholesterol, HDL-C = High density lipoprotein cholesterol, LDL-C = Low density lipoprotein cholesterol, TG = Triglyceride, [a,b,c,d] = Any difference in index letters along the same horizontal line indicate difference between the values $p<0.05$ using Kruskal-Wallis analysis of variance and multiple comparison, except the association with the common index letters.

Platelet fatty acid levels were found to have no significant differences among the male groups (Table 3), whereas a significantly higher level of ALA was found in females with dyslipidemia than in females with CHD ($p < 0.05$) (Table 4). In Table 4, a significantly higher level of AA was seen in females with hypertension than in apparently healthy females ($p < 0.05$).

Correlation coefficients of cardiovascular risk factors and platelet fatty acids are shown in Table 5, ALA was positively correlated and AA was negatively correlated with diastolic blood pressure; EPA was negatively correlated with systolic blood pressure. DHA was found to be positively correlated with TC, LDL-C and plasma glucose.

Table 6 shows the correlation coefficients among platelet fatty acids. LA was negatively correlated with EPA but positively correlated with DHA. ALA was found to be positively correlated with EPA, whereas AA was negatively correlated with EPA and positively correlated with DHA. EPA was also shown to be negatively correlated with DHA.

DISCUSSION

The TC/HDL-C ratio was recommended to be better discriminator of cardiovascular risk (Obermann, 2000), and was somewhat better than the LDL-C/HDL-C ratio. In our study, the TC/HDL-C in CHD and dyslipidemia were higher than in apparently healthy controls, and the ratios were ≥5.0 which showed a higher risk than in the control group. A similar result was also shown in the plasma glucose of the CHD group.

Table 2
Median, range and 95% CI of cardiovascular risk factors in healthy females,and females with coronary heart disease, dyslipidemia and hypertension.

Variables	Healthy (N=55)		CHD (N=10)		Dyslipidemia (N=73)		Hypertension (N=15)	
	Median (range)	95% CI	Median (range)	95%CI	Median (range)	95%CI	Median (range)	95%CI
Systolic BP (mmHg)	120.0 [a] (90-160)	113.2 -121.7	115.0 [a] (90-140)	104.3 -130.7	130.0 [a] (80-190)	121.2 -132.6	130.0 [a] (100-190)	117.9 -146.0
Diastolic BP (mmHg)	80.0 [a] (54-120)	74.6 -81.1	70.0 [a] (60-90)	63.0 -79.5	80.0 [a] (70-111)	77.8 -854	80.0 [a] (60-116)	73.7 -91.0
TC (mg/dl)	215.5 [a] (150-304)	207.1 -228.2	195.0 [a] (165-241)	182.9 -221.6	238.0 [a] (51-155)	219.8 -247.3	225.0 [a] (151-324)	206.8 -225.6
HDL-C (mg/dl)	52.5 [a] (21-82)	52.1 -59.3	61.0 [a] (36-69)	45.2 -64.1	55.0 [a] (29-95)	50.7 -56.8	50.0 [a] (36-86)	46.1 -63.1
LDL-C (mg/dl)	139.7 [a] (77.8-229)	129.7 -149.6	134.0 [a] (86.8-166.4)	110.4 -154.0	149.5 [a] (15.4-394)	140.2 -165.6	150.6 [a] (81.2-214.8)	132.0 -171.4
TC/HDL-C ratio	4.0 [a] (2.2-8.5)	3.8 -4.5	3.8 [a] (2.7-5.7)	3.1 -4.7	4.4 [a] (2.1-8.5)	4.2 -4.9	4.3 [a] (3.0-5.9)	3.9 -4.9
LDL-C/HDL-C ratio	2.5 [a] (1.25-4.76)	2.4 -3.0	2.6 [a] (1.4-3.9)	1.8 -3.3	3.0 [a] (0.53-6.79)	2.7 -3.3	2.9 [a] (1.6-4.1)	2.5 -3.3
TG (mg/dl)	96.0 [a] (50-352)	94.6 -128.2	70.0 [a] (50-136)	57.7 -96.1	118.0 [a] (54-340)	114.9 -146.0	121.0 [a] (65-204)	98.5 -149.6
Plasma glucose (mg/dl)	85.0 [a] (65-103)	83.7 -87.9	88.0 [a] (71-151)	76.0 -120.9	94.0 [a] (54-265)	90.6 -104.1	88.0 [a] (3-106)	71.6 -98.0

TC = Total cholesterol, HDL-C = High density lipoprotein cholesterol, LDL-C = Low density lipoprotein cholesterol, TG = Triglyceride, [a,b,c,d] = Any difference in index letters along the same horizontal line indicate difference between the values $p<0.05$ using Kruskal-Wallis analysis of variance and multiple comparison, except the association with the common index letters.

Table 3
Median, range and 95% CI of platelet fatty acids in healthy males, and males with coronary heart disease, dyslipidemia and hypertension.

Variables	Healthy (N=35)		CHD (N=16)		Dyslipidemia (N=37)		Hypertension (N=9)	
	Median (range)	95% CI	Median (range)	95%CI	Median (range)	95%CI	Median (range)	95%CI
LA (%)[1]	5.2 [a] (3.3-8.6)	4.8 -5.6	5.1[a] (4.2-8.9)	4.8 -6.8	4.7 [a] (2.8-7.6)	4.6 -5.4	5.6 [a] (3.3-8.5)	4.3 -6.9
ALA (%)	0.4 [a] (0-3.2)	0.5 -1.4	0.3 [a] (0-1.4)	1.1 -1.7	0.3 [a] (0-3.3)	0.5 -1.8	0.3 [a] (0-0.8)	0.3 -1.8
AA (%)	16.1[a] (3.0-25)	14.2 -17.2	16.3 [a] (0.3-6.0)	14.6 -19.6	16.6 [a] (0-26)	14.4 -18.2	18.9 [a] (8.8-26)	13.1 -21.4
EPA (%)	0.4 [a] (0.3-7.1)	0.6 -1.7	0.3 [a] (0.3-6.0)	0.1 - 1.9	0.3 [a] (0.3-5.3)	0.5 -1.2	0.3 [a] (0.3-11)	0.5 -5.0
DHA (%)	9.9 [a] (4.1-13.0)	8.8 -10.3	10.1[a] (3.0-14.0)	7.6 -11.3	9.4 [a] (3.6-13)	8.4 -9.9	8.1[a] (15.8-12.0)	7.0 -11.3

LA = Linoleic acid, ALA = α-Linolenic acid, AA = Arachidonic acid, EPA = Eicosapentaenoic acid, DHA = Docosahexaenoic acid, [1] = Percentage of total fatty acid, [a,b,c,d] = Any difference in index letters along the same horizontal line indicate difference between the values $p<0.05$ using Kruskal-Wallis analysis of variance and multiple comparison, except the association with the common index letters.

Table 4
Median, range and 95% CI of platelet fatty acids in healthy females, and females with coronary heart disease, dyslipidemia and hypertension.

Variables	Healthy (N=35)		CHD (N=16)		Dyslipidemia (N=37)		Hypertension (N=9)	
	Median (range)	95% CI	Median (range)	95%CI	Median (range)	95%CI	Median (range)	95%CI
LA (%)[1]	4.9 [a] (2.7-8.5)	4.6 -5.3	5.9 [a] (4.2-8.9)	5.3 -6.4	5.1[a] (2.6-19)	4.7 -5.7	5.3 [a] (4.1-7.3)	4.9 -6.1
ALA (%)	0.5 [a] (0-3.2)	0.8 -1.9	0.1 [a,b] (0-1.5)	0.7 -2.2	0.4 [a,b] (0.4-4.4)	0.2 -0.6	0.3 [a,c] (0-1.3)	0 -0.7
AA (%)	15.1[a] (4.6-24)	13.3 -15.8	19.2 [a,b] (14-23)	16.9 -21.1	15.9 [a,b] (0-25)	14.2 -16.6	18.8 [b] (14-27)	16.3 -21.1
EPA (%)	0.4 [a] (0.3-7.1)	0.6 -1.7	0.3 [a] (0.3-6.0)	0.1 - 1.9	0.3 [a] (0.3-5.3)	0.5 -1.2	0.3 [a] (0.3-11)	0.5 -5.0
DHA (%)	9.0 [a] (1.5-4.0)	8.2 -9.5	9.6 [a] (4.7-14.0)	6.8 -11.3	9.2 [a] (2.4-17)	8.5 -9.9	8.6 [a] (1.3-13)	6.3 -10.5

LA = Linoleic acid, ALA = α-Linolenic acid, AA = Arachidonic acid, EPA = Eicosapentaenoic acid, DHA = Docosahexaenoic acid, [1] = Percentage of total fatty acid, [abcd] = Any difference in index letters along the same horizontal line indicate difference between the values $p<0.05$ using Kruskal-Wallis analysis of variance and multiple comparison, except the association with the common index letters.

Table 5
Correlation coefficient of cardiovascular risk factors and platelet fatty acids.

Variables	LA	ALA	AA	EPA	DHA
TC	-0.039	0.127	-0.015	0.006	0.165[a]
HDL-C	-0.033	-0.009	-0.055	0.020	0.036
LDL-C	-0.004	0.158	-0.026	-0.010	0.181[a]
TC/HDL-C	0.000	0.067	0.076	-0.065	0.091
LDL-C/HDL-C	0.021	0.134	0.044	-0.027	0.130
TG	-0.065	-0.049	0.073	0.000	-0.009
Plasma glucose	0.110	-0.093	0.053	-0.037	0.146[a]
Systolic BP	-0.003	0.059	-0.009	-0.155[a]	0.021
Diastolic BP	-0.073	0.258[a]	-0.146[a]	-0.051	-0.046

[a]$p<0.05$
TC = Total cholesterol, HDL-C = High density lipoprotein cholesterol, LDL-C = Low density lipoprotein cholesterol, TG = Triglyceride, BP = Blood Pressure, LA = Linoleic acid, ALA = α-linolenic acid, AA = Arachidonic acid, EPA = Eicosapentaenoic acid, DHA = Docosahexaenoic acid.

The concentrations of platelet fatty acids in our study were similar to the other previous studies (Dyerberg *et al* 1975; Dyerberg, 1986; Agren *et al* 1995; Li,1998), except DHA, in our study was higher than in those studies. ALA, which is ω-3 PUFA, can convert to EPA and DHA, and DHA can retroconvert to EPA (Fischer *et al*, 1987; Hirai *et al*, 1989). The high DHA in our study may be because of some factors that interfere with the retroconversion or may be because of the high amount of DHA in the diet of the subjects.

In our study, we found LA was higher than

Table 6
Correlation coefficient among platelet fatty acids.

Variables	LA	ALA	AA	EPA	DHA
LA	1.000	-0.116	0.110	-0.243[a]	0.283[a]
ALA	0.116	1.000	-0.213	0.470[a]	-0.234
AA	0.110	-0.213	1.000	-0.213[a]	0.352[a]
EPA	-0.243[a]	0.470[a]	-0.213[a]	1.000	-0.393[a]
DHA	0.283[a]	-0.234	0.352[a]	-0.393[a]	1.000

[a]$p<0.05$
LA = Linoleic acid, ALA = α-linolenic acid, AA = Arachidonic acid, EPA = Eicosapentaenoic acid, DHA = Docosahexaenoic acid.

ALA in every group of subjects. Higher amounts of LA will suppress the conversion of ALA to EPA and DHA because both LA and ALA use the same enzyme set for elongation and desaturation to AA, EPA and DHA. In the controls, however, LA was lower and ALA was higher than in the cases. LA was still higher than ALA and resulted in a high AA and in a low EPA and DHA. From our study, every group of subjects showed a higher AA than EPA and DHA. Perhaps the lack of EPA contributed to the increased platelet aggregation through the absence of competition with AA for the cyclooxygenase pathway in which AA will liberate more TXA, which can increase platelet aggregation, making a blood clot at the site of the plaque, leading to myocardial infarction and death (Holub, 2002).

Usually, the amount of LA in the diet is higher than ALA (Budowsky, 1988), and conversion of ALA to EPA and DHA occurs to a lesser extent about 10% to 15% in the adult human body (Emken *et al*, 1994), Pawlosky *et al* (2001) published a radically different estimate of 0.2%. Further work is clearly needed to determine how much ALA is converted to EPA and DHA in adults *in vivo*. Consumption of EPA and DHA rich foods can maximize the concentration of EPA and DHA and may prevent many diseases such as cardiovascular disease, hypertension, Crohn's disease and some autoimmune diseases. Fish, especially marine fish, and some seafood are a rich source of EPA and DHA. To have fish in the diet may be useful to prevent thrombogenesis and other pathology.

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