Increased Lipid Binding to Thrombi in Coronary Artery Disease

Findings in Patients Without Premedication in Native (Not Anticoagulated) Test Systems

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Thrombi and clots were produced from native (i.e., not anticoagulated) platelet-rich and platelet-poor plasma from patients with coronary artery disease and control subjects who had not taken any medication known to influence plasma lipids, coagulation, or platelet aggregation. The clotting times were recorded, and the lipid content of clots, thrombi, platelets, plasma, and high density lipoprotein was analyzed. Thrombi produced from native platelet-rich plasma were 46% heavier in coronary artery disease patients and contained about 20% more phospholipids and free cholesterol and about twice the amount of triglycerides and esterified cholesterol in both absolute and relative amounts with respect to the corresponding lipids of plasma plus platelets. The elevated content of lipids not only increases the size of the thrombi but also changes their quality because of an increased content in plasmatic lipids, as platelets contain only trace amounts of triglycerides and cholesterol esters. In agreement herewith, fibrinogen and maximal amplitude on the thrombelastogram were increased in coronary artery disease patients, whereas the thrombus-forming time and clotting times of platelet-poor and platelet-rich plasma were shortened, indicating accelerated coagulation and activation of platelets. Analysis of these results suggests a disturbed interrelation in coronary artery disease between lipids and hemostasis, in which platelets, high density lipoprotein, and lipoproteins rich in triglycerides and cholesterol esters may play a role. (*Arteriosclerosis and Thrombosis* 1992;12:1516–1521)

KEY WORDS • **coronary artery disease** • **coagulation** • **thrombus lipids** • **high density lipoprotein cholesterol** • **high density lipoprotein phospholipids** • **native blood**

Thrombosis contributes significantly to the progression of arteriosclerosis,[1,2] and the most deleterious complication of coronary artery disease (CAD), acute myocardial infarction, is caused by thrombi in at least 85% of cases.[3] Although these thrombi consist of not only fibrin but also platelets and plasma lipoproteins,[4,5] their lipid content has not been examined quantitatively. Since the patterns of plasma and platelet lipid contents are very different, such an analysis could give insights into the participation of and/or interaction between plasma lipoproteins, platelets, and fibrin in thrombus formation. In addition, examination of the composition of thrombi might contribute to an understanding of why 25% of coronary thrombi are resistant to lysis.

Whereas a great number of investigations, in particular those on plasma lipids, lipoproteins, platelet activation, and coagulation factors, have been carried out, there are comparatively few studies on the interrelations between these parameters (e.g., see References 6–12).

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Received July 4, 1992; accepted August 14, 1992.

Our approach in previous investigations was to examine the main product of these interactions, the thrombi, in diseases with enhanced hemostasis. In such diseases, e.g., severe septic conditions[10] and acute respiratory distress,[11] we found a considerably augmented lipid content in thrombi produced from platelet-poor plasma (PPP) and a platelet substitute. In CAD with chronic and more localized thrombus formation, no significant changes were observed[12] with this method.

As viable platelets play an important role in thrombus formation, we produced thrombi from citrated platelet-rich plasma (PRP) and observed in CAD an increased content of triglycerides in these thrombi.[12] To examine whether this was a reproducible finding under more physiologically similar experimental conditions, we developed a method for preparing and examining thrombi from native (i.e., not anticoagulated) PPP and PRP to avoid nonphysiological influences of citrate on platelet function,[13,14] coagulation,[15] and their interactions.[16] In addition, we had to take into account the fact that the test system and the number of additional examinations were limited by the amount of blood that could be taken from the patients.

In preliminary and accompanying investigations, we found that β-blockers, anticoagulants, lipid-lowering drugs, and inhibitors of platelet aggregation altered the results. Therefore, we included only persons who were not taking any interfering medications, although we

knew that this would exclude the vast majority of coronary disease patients. The number of eligible patients was further reduced by our additional goal of investigating only patients who were at a comparatively young age, at which time the changes leading to enhanced thrombosis are pronounced. These extensive investigations of lipids and hemostasis and their possible interactions were expected to reflect the influence of coagulation, platelets, and plasma lipids on the formation of thrombi in vivo as closely as possible. Thereby we hoped to obtain more insight into the development of the thrombotic component of CAD.

Methods

Patients and Control Subjects

The study included only subjects who did not suffer from any infectious or metabolic disease and who had not taken any medication known to influence coagulation, platelet function, or plasma lipids. None of the study subjects had received any of the following: 1) heparin for at least 12 hours, 2) acenocoumarol for at least 4 days, 3) other anticoagulants, 4) acetylsalicylic acid at a dose of more than 250 mg/day for at least 3 weeks, 5) other inhibitors of cyclooxygenase for at least 24 hours, 6) other inhibitors of platelet function for at least 2 days, 7) α- or β-blockers for at least 4 days, 8) lipid-lowering drugs for at least 10 days, 9) hormones (e.g., contraceptives) for at least 2 months, and 10) nitrates for at least 12 hours. Informed consent was obtained from all participants.

Our hospital is the only public hospital for about 120,000 people and the only one with the facilities to carry out coronary angiography for about 500,000 people. We were informed of nearly all patients who could possibly fulfill our criteria, and all of these patients were examined.

Despite this, only 37 patients with clinical symptoms of ischemic heart disease admitted to our hospital between 1984 and 1991 fulfilled the criteria mentioned above and were entered into the study. In 26 patients, coronary angiography confirmed the clinical diagnosis. Eleven patients with previous myocardial infarction, which had occurred at least 2 months before blood was taken, were included in the CAD group. Thirty-nine healthy age-matched volunteers without signs of ischemic heart disease served as control subjects. Subjects with negative angiograms but with symptoms of ischemic heart disease were excluded from the calculations. Physical activity was classified as follows: 1, hardly any; 2, very little; 3, little; 4, moderate; 5, much; and 6, very much.

Preparation of Plasma, Clots, Thrombi, Platelets, and HDL

Blood could be taken only once because interfering treatment usually started on the same day. Fifty milliliters of blood was collected by puncture of an antecubital vein by using a 1.2-mm needle and polyethylene syringes. No anticoagulant was added. Until the end of sample collection the blood was cooled in an ice-water bath and then immediately centrifuged at 400*g* and 10°C for 5 minutes. The supernatant PRP was gently pipetted into plastic tubes. Alternatively, the sample was centrifuged for 10 minutes at 10°C and 2,000*g* to yield PPP. Platelets were counted in PPP and PRP, which were then mixed to give 3 ml plasma with a final concentration of 250,000 platelets per microliter (i.e., equivalent to PRP).

Plastic tubes with 3 ml PPP and PRP were put into a 37°C water bath, and the time of appearance of the first visible clot was recorded. Coagulation was completed after 15 more minutes (addition of thrombin to the serum, from which the clot/thrombus had been removed, did not result in any additional clot). The clots/thrombi were centrifuged at 2,000*g* and 10°C for 10 minutes and then washed twice with 3 ml of 0.67% NaF at 10°C and 2,000*g* for 10 minutes. After the second supernatant had been discarded, the clots/thrombi were weighed (wet) and homogenized in a glass homogenizer. The dry weight could not be estimated because partial degradation of lipids occurred during drying.

To examine platelet lipids, 3 ml of the supernatant native PRP was centrifuged at 2,000*g* for 10 minutes, washed with a Ca^{2+}-poor buffer,[17] and centrifuged again. The procedure was repeated twice. Finally the platelets were resuspended in buffer and homogenized. They were slightly aggregated at this stage and therefore could only be used for lipid analysis. High density lipoproteins (HDLs) were prepared according to Burstein et al.[18]

Lipid Extraction, Chromatography, and Estimation

Lipids from clots, thrombi, platelets, plasma PPP, and HDL were extracted according to Folch et al.[19] All extraction procedures were started immediately after preparation because standing of HDL, platelets, and thrombi for more than 1 hour resulted in partial degradation, as revealed by thin-layer chromatography and loss of phospholipids. Two milliliters of the lipid phase of the extracts was taken for estimation of total phospholipids by a modification[20] of the original method.[21]

Thin-layer chromatography and elution of neutral lipids were carried out by a modification[12] of the original method.[22] The eluates were dried, and analyses of cholesterol,[23] glycerides,[24] and free fatty acids[25] were performed in the respective fractions.

In addition, the total cholesterol content of plasma and the HDL and triglyceride content of plasma were measured by enzymatic colorimetric procedures (MA-Kit 100 and MA-Kit 30, respectively, Roche, Basel, Switzerland) on an autoanalyzer (Cobas Mira, Roche, Basel, Switzerland). Low density lipoprotein cholesterol content was calculated according to the Friedewald formula. Reproducibility was tested as described.[12]

Coagulation Tests

Platelets were counted with a Coulter counter. Fibrinogen was estimated by the method of Clauss, and thrombelastography was carried out according to the method of Hartert. Prothrombin time (Boehringer), partial thromboplastin time (Cephaloplastin Dade), and antithrombin III (chromogenic substrate, Boehringer) tests were carried out according to the instructions of the manufacturers.

Statistical Analyses

Calculations were performed with the BMDP statistical software package (BMDP Statistical Software, Inc., Los Angeles). Since most of the data were not normally

TABLE 1. Coagulation Tests, Clotting Times of Platelet-Poor Plasma and Platelet-Rich Plasma in Native Plasma, and Weights of Clots and Thrombi in Normal Control and Coronary Artery Disease Subjects

Test/parameter	Control subjects	CAD subjects	*p*
Fibrinogen (mg/dl)	259.4±54.9	310.6±77.0	0.003
Prothrombin time (%)	100.9±15.9	96.5±13.1	NS
aPTT (seconds)	28.3±2.5	27.9±2.9	NS
Platelet count (g/l)	252.9±78.7	275.1±79.6	NS
Antithrombin III (%)	98.1±11.5	102.7±15.2	NS
r time of TEG (minutes)	8.0±2.1	7.7±2.0	NS
k time of TEG (minutes)	7.4±2.2	6.5±2.7	0.05
MA of TEG (mm)	45.6±8.9	51.7±10.5	0.016
Clotting time of PPP (seconds)	1,223±476	808±492	0.005
Clot wet weight (mg)	181±58	231±83	NS
Clotting time of PRP (seconds)	729±361	551±239	0.021
Thrombus wet weight (mg)	175±49	257±73	0.003

CAD, coronary artery disease; aPTT, activated partial thromboplastin time; r time, reaction time; TEG, thrombelastography; k time, thrombus-forming time; MA, maximal amplitude; PPP, platelet-poor plasma; PRP, platelet-rich plasma.

distributed, the Mann-Whitney *U* test was applied throughout for comparison between the two groups. Significance levels for Pearson correlation coefficients were calculated with standard formulas.[26]

On account of the relatively high number of statistical tests, probability values were also calculated according to Bonferroni.[26] However, this and other procedures used to correct for multiple significance testing reduce the probability of a type I error (incorrect rejection of a true null hypothesis) but increase type II errors, thus reducing the power to detect individual results as significant. This approach is not always the most suitable, particularly for exploratory studies that are designed to identify the results of interest for future confirmatory studies,[27] as in the case of our present investigation. Therefore, probability values are listed without correction.

Correlations were calculated between total phospholipid content, total cholesterol content, and triglyceride content of clots/thrombi and 1) the corresponding lipid fractions of plasma and fibrinogen; 2) the clotting times of PPP and PRP plasma and r, k, and MA of the thrombelastogram; and 3) thrombus wet weight. Only correlations with probability values lower or equal to 0.001 are listed. These retained statistical significance even after Bonferroni correction.

Results

Comparisons

The groups did not differ in age, weight, height, gender distribution, physical activity, or present smoking habits. Body mass index (24.9±2.8 versus 23.7±2.3 kg/m^2, $p<0.05$) and the number of exsmokers (17 versus seven, $p<0.05$) were significantly greater in CAD patients.

Fibrinogen (311±77 versus 259±55 mg/dl, $p<0.01$) and the maximal amplitude of the thrombelastogram (51.7±10.5 versus 45.6±8.9 mm, $p=0.01$) were increased; the clotting times of both native PRP (551±239 versus 729±361 seconds, $p<0.05$) and PPP (808±492 versus 1,223±476 seconds, $p<0.01$) and the thrombus-forming time in the thrombelastogram (6.5±2.7 versus 7.4±2.2 seconds, $p=0.05$) were shorter in CAD patients (Table 1).

HDL cholesterol content (48.3±14.0 versus 60.4±17.7 mg/dl, $p=0.001$) was diminished and plasma triglyceride content (141.0±51.8 versus 93.9±51.5 mg/dl, $p<0.001$) were elevated in the CAD group (Figure 1). Thrombi produced from native PRP had a greater wet weight (257±73 versus 175±49 mg, $p<0.01$; Table 1) and an increased content of phospholipids (11.51±4.23 versus 9.26±3.20 mg/dl, $p=0.01$), triglycerides (4.65±3.50 versus 1.25±0.51 mg/dl, $p<0.001$), free cholesterol (3.44±1.56 versus 2.45±0.76 mg/dl, $p<0.01$), and esterified cholesterol (5.32±3.81 versus 2.17±0.91 mg/dl, $p<0.001$) in CAD patients (Figure 2). Accordingly, the percentage of these lipids bound to thrombi in relation to the corresponding amounts in plasma plus platelets was also significantly higher than in control subjects (therefore, they are not listed separately).

The same lipid fractions in clots produced from native PPP were elevated in the CAD group (Figure 3), although to a lesser extent than in PRP thrombi (total phospholipids, 5.52±2.75 versus 4.05±2.60 mg/dl, NS; triglycerides, 3.66±2.91 versus 1.83±1.71 mg/dl, $p=0.001$; free cholesterol, 1.97±1.47 versus 1.17±0.76 mg/dl, $p=0.01$; cholesterol esters, 5.08±3.60 versus 3.14±1.77 mg/dl, $p=0.02$; total cholesterol, 7.19±5.07 versus 4.29±2.51 mg/dl, $p=0.01$; and free fatty acids, 26.30±17.11 versus 19.70±7.97 μmol/l, NS). The relative amounts of total phospholipids (2.8±1.6% versus 2.0±1.2%, $p=0.05$) and free (3.4±3.3% versus 1.8±0.9%, $p=0.01$), esterified (3.0±1.9% versus 2.1±1.1%, $p=0.05$), and total (3.1±2.1% versus 2.0±0.9%, $p=0.02$) cholesterol in clots as a percentage of the respective plasma lipids were also increased in the CAD group.

Platelet lipid amounts did not differ between CAD and control subjects. Platelets contain only small amounts of triglycerides and cholesterol esters (4.5% each of platelet total lipids versus 65% phospholipids). The main lipid fractions of platelets are phospholipids, which contribute about 65% to platelet lipids but only about 30% to plasma lipids (Figure 1) and 45% to lipids

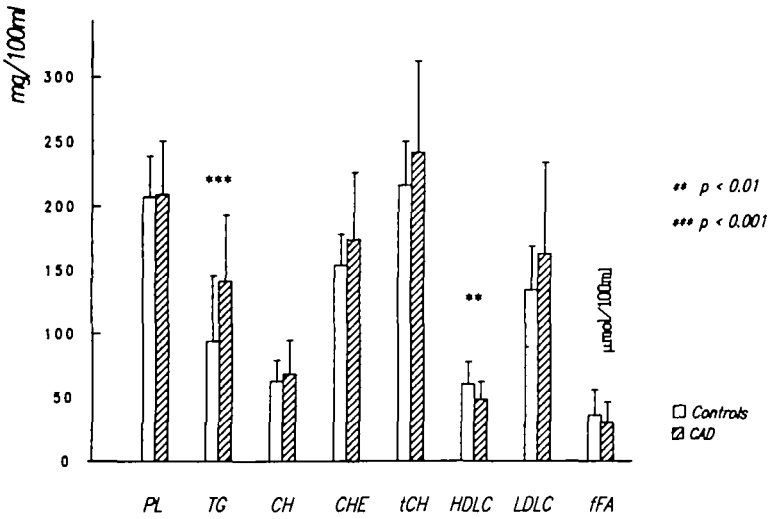

FIGURE 1. *Bar graph of plasma lipid values in control and coronary artery disease (CAD) subjects. Bars represent mean±SD, expressed as milligrams per 100 milliliters, except for free fatty acids, which are given as micromoles per 100 milliliters. PL, phospholipids; TG, triglycerides; CH, cholesterol; CHE, cholesterol esters; tCH, total cholesterol; HDLC, high density lipoprotein total cholesterol; LDLC, low density lipoprotein total cholesterol; fFA, free fatty acids.*

of native PRP thrombi (Figure 2). Apart from an increase of triglyceride content in citrated PRP thrombi (data not listed because they were not different from those published[13]), the changes found in native thrombi were not observed in thrombi produced from recalcified citrated PPP or PRP.

Correlations

In both groups the cholesterol and triglyceride content of plasma was correlated with the absolute but not the relative amounts of the corresponding fractions of PRP thrombi (Table 2). In control subjects HDL total cholesterol was directly correlated with thrombus-forming time and inversely with fibrinogen. Phospholipid contents of plasma correlated with those of thrombi. In the CAD group, wet weights of thrombi and clots were correlated with their contents of phospholipids, triglycerides, and free and esterified cholesterol but not with the corresponding plasma lipids. Phospholipid and triglyceride contents of plasma correlated with the corresponding fractions of clots.

No correlations were found between fibrinogen and lipids or weight of thrombi, plasma triglycerides and any of the coagulation times, plasma free fatty acids and thrombus free fatty acids or thrombus weight, or thrombus free fatty acids and thrombus weight.

Discussion

It was even more difficult than we anticipated to find patients whose average age was less than 50 years who were not taking any interfering medication. Only a few of the many patients admitted with CAD fulfilled these criteria, although the investigation was carried out over more than 6 years. However, exclusion of patients with interfering medication regimens apparently facilitated the clarity of the results. Whereas elevated fibrinogen levels in CAD have been reported before,[28,29] the shortened thrombus-forming time and the higher maximal amplitude of the thrombelastogram have not been described. Fibrinogen and most likely a higher content in lipoproteins interacting with activated platelets contribute to this elevation of maximal amplitude.

Activation and increased release of platelet constituents as well as enhanced coagulation in CAD are also suggested by the shortening of the clotting times of native PRP and PPP as well as thrombus-forming time

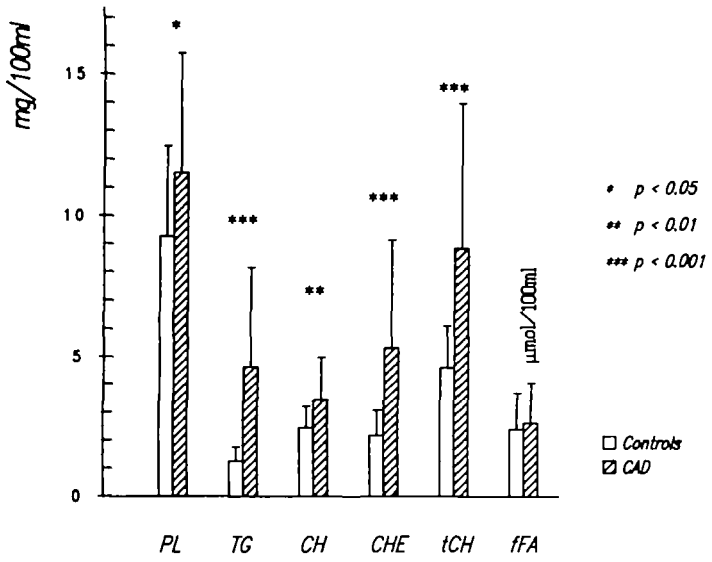

FIGURE 2. *Bar graph of lipid values in thrombi produced from platelet-rich plasma in control and coronary artery disease (CAD) subjects in milligrams per 100 milliliters of platelet-rich plasma used for thrombus generation. Free fatty acid values are given in microliters per 100 milliliters of plasma used for thrombus production. Note the considerable differences in TG and CHE in contrast to fFA. PL, phospholipids; TG, triglycerides; CH, cholesterol; CHE, cholesterol esters; tCH, total cholesterol; fFA, free fatty acids.*

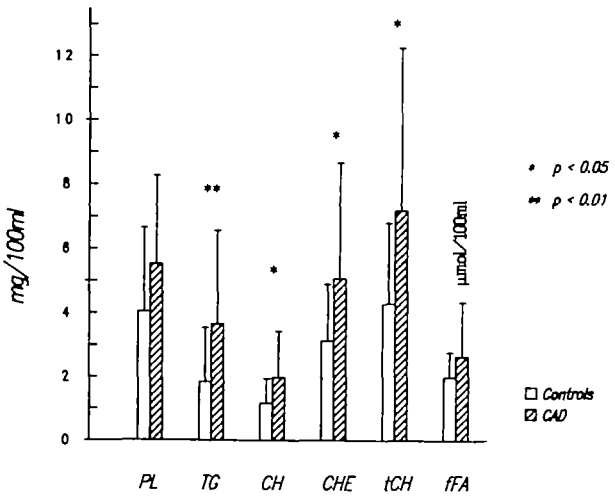

FIGURE 3. *Bar graph of lipid levels in clots produced from platelet-poor plasma of control and coronary artery disease (CAD) subjects given in milligrams per 100 milliliters of plasma used for clot production. Free fatty acids are given in micromoles per 100 milliliters of plasma used for clot production. PL, phospholipids; TG, triglycerides; CH, cholesterol; CHE, cholesterol esters; tCH, total cholesterol; fFA, free fatty acids.*

on the thrombelastogram. This faster clotting appears to be unrelated to the elevated plasma triglyceride levels, as they were not correlated with the various clotting times. Similarly, most other investigators have observed no influence of lipemia on the usual coagulation tests.[6,7] In contrast to body mass index and triglyceride content, the plasma total cholesterol level was not increased in our CAD group because many hypercholesterolemic patients took lipid-lowering medications and therefore had to be excluded. However, this exclusion did emphasize the decreased HDL cholesterol levels in CAD.

Considerably more pronounced than these changes in fibrinogen and plasma lipids was the content of triglycerides and cholesterol esters in thrombi. These must originate mainly from plasma lipids because platelets contain only small amounts of these fractions. Possible reasons for this augmented binding in CAD are changes in lipoprotein subfractions as well as in platelets interacting differently with lipoproteins.[8,9,30] During thrombus formation in CAD, platelets appear to bind more lipoprotein subfractions rich in cholesterol esters and particularly in triglycerides. Their increased binding in CAD appears to contribute to the increased weight of thrombi, as is also suggested by the direct correlations between the cholesterol ester and triglyceride content of thrombi and their weight. In addition the weight of thrombi was correlated with their content of phospholipids and free cholesterol, and it is most likely that more platelet material is also incorporated into thrombi. In agreement herewith, greater availability of platelet phospholipids has been observed in CAD.[31,32] These lipids bound to thrombi not only increase their size but also may make their fibrin less accessible to degradation by fibrinolytic enzymes.[33] This would result in diminished fibrinolysis, which has been observed in CAD.[34]

Apart from the different binding of lipids to thrombi there are also other indications of changed interactions between lipids and coagulation in CAD. Fibrinogen correlated directly with the phospholipid and triglyceride content of clots in CAD but was inversely related to HDL cholesterol in control subjects. These differences between control and CAD subjects make the simple inclusion of lipids in these thrombi unlikely. In addition, lipid trapping can be excluded for the following reasons: 1) Cholesterol and triglycerides bound to thrombi were increased twofold to threefold in CAD at unchanged and only 25% higher levels, respectively, of these fractions in plasma. 2) Thrombus phospholipid levels were 25% higher in CAD at unchanged plasma levels. 3) Plasma phospholipids were correlated in control but not in CAD subjects with thrombus phospholipids. 4) The percent content of triglycerides and cholesterol in thrombi and the respective plasma lipid fractions did not correlate. 5) Fibrinogen did not correlate with either the weight of thrombi or with any of their lipid fractions. 6) Only in CAD did thrombus lipids (except free fatty acids) but not plasma lipids correlate with thrombus weight. 7) In contrast to the other main lipid fractions, free fatty acids were neither elevated in CAD thrombi nor correlated with plasma levels or thrombus weight. As free fatty acids are bound to albumin, increased inclusion of this most abundant plasma protein in thrombi can be ruled out.

Because lipid trapping as a major mechanism for increased lipid binding to thrombi can be excluded, our results suggest that during thrombus formation, there are specific interactions between lipids and hemostasis. These appear to be altered in CAD regardless of plasma lipid levels and result in accelerated coagulation and augmented binding of mainly plasmatic lipids to thrombi. Both may be interrelated pathogenetic mechanisms in CAD that can be particularly revealed by studies with native blood.

TABLE 2. Correlation Coefficients *(r)* and Significance Levels *(p)* of the Most Important Correlations

	CAD subjects		Control subjects	
	r	*p*	*r*	*p*
Thrombus phospholipids versus				
Plasma phospholipids	0.26	NS	0.52	0.000
Thrombus weight	0.75	0.0000	−0.12	NS
Thrombus triglycerides versus				
Plasma triglycerides	0.73	0.0000	0.65	0.0000
Thrombus weight	0.58	0.0002	−0.07	NS
Thrombus total cholesterol versus				
Plasma total cholesterol	0.72	0.0000	0.47	0.0000
Thrombus weight	0.77	0.0000	0.19	NS
Fibrinogen versus				
HDL cholesterol	−0.03	NS	0.54	0.0000
Clot phospholipids	0.44	0.000	0.29	NS
Clot triglycerides	0.46	0.000	0.29	NS

CAD, coronary artery disease; HDL, high density lipoprotein. NS, not significant.

Acknowledgments

The authors are deeply indebted to Miss Sedighe Ahrabian for excellent technical assistance and to Dr. Arnulf Lochs for expert advice on computational problems.

References

1. von Rokitansky: *Handbuch der Pathologischen Anatomie.* Vienna, Braunmüller & Seidel, 1844
2. DeWood MA, Spores J, Notske R, Mouser T, Burroughs R, Golden MS, Lang HT: Prevalence of total coronary occlusion during the early hours of transmural myocardial infarction. *N Engl J Med* 1980;303:897–902
3. Stary HC: Evolution and progression of atherosclerotic lesions in coronary arteries of children and young adults. *Arteriosclerosis* 1989;9(suppl I):I-19–I-32
4. Woolf N, Pilkington TRE, Carstairs KC: The occurrence of lipoprotein in thrombi. *J Pathol Bacteriol* 1966;91:383–387
5. Sinapius D: Zur morphologie und histochemie der fette in coronarthromben. *Virchows Arch Pathol Anat* 1967;342:144–153
6. Merskey C, Marcus AJ: Lipids, blood coagulation and fibrinolysis. *Annu Rev Med* 1963;14:323–338
7. Meuwissen OJ, Hart HC, van Hemel-Rupert SE: The influence of fats on blood coagulation and fibrinolysis. *Thromb Diathes Haemorrh* 1968;19:267–278
8. Hassall DG, Owen JS, Bruckdorfer KR: The aggregation of isolated human platelets in the presence of lipoproteins and prostacyclin. *Biochem J* 1983;216:43–49
9. Curtiss LK, Plow EF: Interaction of plasma lipoproteins with human platelets. *Blood* 1984;64:365–374
10. Kunz F, Hörtnagl H, Kroesen G, Schennach W, Egg D, Rumpl E, Aschauer R, Holzknecht F: Studies on hypo- and hypercoagulability: II. Coagulation and fibrin analyses in severe infectious and toxic conditions. *Blut* 1974;28:360–369
11. Kunz F, Hörtnagl H, Kroesen G, Rumpl E: Plasma lipids, coagulation factors and fibrin formation after severe multiple trauma and in adult respiratory distress syndrome. *J Trauma* 1978;18: 115–120
12. Kunz F, Zwierzina W-D, Hörtnagl H: Clot lipids in ischaemic heart disease. *Atherosclerosis* 1983;49:195–202
13. Zwierzina W-D, Kunz F: A method of testing platelet aggregation in native whole blood. *Thromb Res* 1985;38:91–100
14. Bowry SK, Müller-Berghaus G: Aggregation of washed platelets from non-anticoagulated human blood is not reversible. *Thromb Haemost* 1986;56:172–177
15. Rock G, Titley P, Fuller V: Effect of citrate anticoagulants on factor VIII levels in plasma. *Transfusion* 1988;28:248–252
16. Bowry SK, Selmayr E, Müller-Berghaus G: Evidence of increased association of fibrinogen with platelets caused by sodium citrate. *J Lab Clin Med* 1988;111:315–325
17. Huzoor-Akbar, Ardlie NG: Further evidence for the role of thrombin in the platelet release reaction caused by various agents, and the nature of biphasic platelet aggregation. *Br J Haematol* 1978;38: 381–390
18. Burstein M, Scholnick HR, Morfin R: Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. *J Lipid Res* 1970;11:583–595
19. Folch J, Lees M, Sloane-Stanley GH: A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 1957;226:497–509
20. Kunz F, Hörtnagl H, Kroesen G, Rumpl E, Holzknecht F: Studies on hyper- and hypocoagulability: Part 1. Evidence for occurrence of incomplete consumption coagulopathy. *Blut* 1974;28:56–65
21. Bartlett GR: Phosphorus assay in column chromatography. *J Biol Chem* 1959;234:466–468
22. Kunz F: Separation of "neutral" lipids, particularly of all classes of partial glycerides by one-dimensional thin-layer chromatography. *Biochim Biophys Acta* 1973;296:331–334
23. Searcy RL, Bergquist LM, Jung RC: Rapid ultramicro estimation of serum total cholesterol. *J Lipid Res* 1960;1:349–352
24. Wahlefeld AW: Triglyceride, Bestimmung nach enzymatischer Verseifung, in Bergmeyer HU (ed): *Methoden der Enzymatischen Analyse, Band II.* Weinheim, Verlag Chemie, 1974, pp 1878–1882
25. Duncombe WG: The colorimetric microdetermination of long chain fatty acids. *Biochem J* 1963;88:7–10
26. Sachs L: *Angewandte Statistik, 6 Auflage.* Berlin, Springer Verlag, 1984, pp 331–370
27. Parker RA, Rothenberg RB: Identifying important results from multiple statistical tests. *Stat Med* 1988;7:1031–1043
28. Ogston CM, Ogston D: Plasma fibrinogen and plasminogen levels in health and in ischaemic heart disease. *J Clin Pathol* 1966;19: 352–356
29. Meade TW, Mellows S, Brozovic M, Miller GJ, Chakrabarti RR, North WRS, Haines AP, Stirling Y, Imeson JD, Thompson SG: Haemostatic function and ischaemic heart disease: Principal results of the Northwick Park heart study. *Lancet* 1986;2:533–537
30. Koller E: Lipoprotein-binding proteins in the human platelet plasma membrane. *FEBS Lett* 1986;200:97–102
31. Nordöy A, Rodset JM: Platelet phospholipids and their function in patients with ischemic heart disease. *Acta Med Scand* 1970;188: 133–137
32. Renaud S, Gautheron P, Arbogast R, Dumont E: Platelet factor 3 activity and platelet aggregation in patients submitted to coronarography. *Scand J Haematol* 1974;12:85–92
33. Bang NU: Physiology and biochemistry of fibrinolysis, in Bang NU, Beller FK, Deutsch E (eds): *Thrombosis and Bleeding Disorders.* Stuttgart, FRG, Georg Thieme Verlag, 1971, pp 292–327
34. James DCO, Drysdale J, Billimoria JD, Wheatley D, Gavey CJ, MacLagan NF: Lipaemia and blood-coagulation defects in relation to ischaemic heart disease. *Lancet* 1961;2:798–802

Arteriosclerosis, Thrombosis, and Vascular Biology

JOURNAL OF THE AMERICAN HEART ASSOCIATION

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Arterioscler Thromb Vasc Biol. 1992;12:1516-1521
doi: 10.1161/01.ATV.12.12.1516
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 1079-5642. Online ISSN: 1524-4636