Effect of feeding fat on fibrinolysis, Stypven time, and platelet aggregation in Africans, Asians, and Europeans¹

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SYNOPSIS The effects of acute fat feeding on fibrinolytic activity, platelet aggregation, and Stypven time in 10 Africans and 10 Asians are presented and compared with the results previously obtained in 10 Europeans.

These indicated that the inhibition of fibrinolytic activity seen in Europeans does not occur in either Africans or Asians although the Stypven time was shortened in all three groups.

Platelet aggregation, as measured by the Chandler's tube technique, was inhibited by fat feeding in Europeans but was unchanged in Africans and Asians.

The results also indicate that the fibrinolytic activity of Africans and Asians is greater than that of Europeans.

A relationship between thrombogenic mechanisms and atherosclerosis was originally suggested by Rokitsansky (1852) and revised by Duguid (1946), who demonstrated that intravascular thrombus may be incorporated into the arterial wall leading eventually to the formation of an atheromatous plaque. On the basis of this hypothesis factors favouring intravascular coagulation might be expected to promote atherosclerosis whereas an increase in blood fibrinolytic activity might be expected to exercise a contrary influence.

A previous investigation (Dubber, Rifkind, Gale, McNicol, and Douglas, 1967) has confirmed that in Europeans acute fat feeding leads to a shortening of the Stypven time and also to inhibition of plasma fibrinolytic activity and platelet aggregation.

This investigation was designed to compare the effects of a high-fat meal on the plasma fibrinolytic activity, blood coagulation, and platelet aggregation in Africans and Asians. The findings have been compared with those already obtained in Europeans. The three ethnic groups had a similar socio-economic status.

Subjects, Materials, and Methods

The European group previously studied (Dubber *et al.*, 1967) consisted of nine males and one female, aged 26 to 36 years. The two other groups consisted of 10 healthy male Africans, aged 22 to 29 years, and 10 healthy male Asians, aged 22 to 26 years. The Europeans were all members of the hospital staff of the Royal Infirmary, Glasgow, and the Africans and Asians were medical students living in the students' hostel at the Kenyatta National Hospital, Nairobi.

The diet of the African and Asian students was essentially that of the urbanized European, being high in protein and animal fat. Each subject was investigated on two occasions, once after a low-fat breakfast, the control situation, and again after the same breakfast to which had been added 270 g of double cream. The details of the breakfast are provided in Table I.

Before the breakfast each student had fasted and refrained from smoking from the previous evening. After the breakfast a further three-hour fast was observed and venous blood samples were then taken. Before venepuncture each subject was rested. The order in which the breakfasts were taken was randomized.

After the subject had rested for 15 minutes, and

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Content	Breakfast	
	Low Fat	High Fat
Carbohydrate (g)	62.9	71.0
Protein (g)	4.0	10.2
Fat (g)	0.6	95.1

Table I Content of both types of breakfast

with minimal occlusion of the circulation, 50 ml of venous blood was obtained in plastic syringes after a clean venepuncture using a needle of gauge no. 19. The blood was allocated as follows: 27 ml in 9-ml amounts was mixed in three chilled siliconized centrifuge tubes, each containing 1 ml of 3.8% sodium citrate for fibrinolytic, coagulation, and platelet aggregation studies; 2.5 ml was mixed in a sequestrene container (Stayne Laboratories Limited, High Wycombe) for platelet counts; the remainder was collected as serum for lipid assays.

The citrated silicone specimens were kept in ice and centrifuged 10 minutes after collection (600 *g* for five minutes at 4°C) to obtain platelet-rich plasma.

Methods Used to Assess the Fibrinolytic System

EUGLOBULIN CLOT LYSIS TIME

This was measured by the method of Nilsson and Olow (1962). Since fibrinolytic activity is proportional to the reciprocal of the lysis time (Sherry and Alkjaersig, 1957), the results were expressed as units of activity, the reciprocal of a lysis time of 300 minutes being taken as 1 unit (Sherry, Lindemeyer, Fletcher, and Alkjaersig, 1959). This test was designed to measure plasma plasminogen activator level.

UROKINASE SENSITIVITY TEST

This was performed according to the method of McNicol, Gale, and Douglas (1963); 0.32 ml of urokinase (500 Ploug units per ml) (Leo Laboratories, Copenhagen) was added to 0.2 ml of plasma which was clotted with 0.1 ml thrombin (20 NIH units per ml) and the lysis time measured. The results are expressed in units, a unit being defined as the reciprocal of a lysis time of 10 minutes. Urokinase sensitivity in a population is a measure of variations in the levels of fibrinolytic inhibitors.

PLASMINOGEN ASSAY

This was performed by a modification of the caseinolytic method of Remmert and Cohen (1949) as described by Alkjaersig, Fletcher, and Sherry (1959).

FIBRINOGEN ASSAY

This was performed by a modification of the method of Ratnoff and Menzie (1951) as described by McNicol and Douglas (1964).

PLATELET AGGREGATION

This was measured by a Chandler's tube technique, modified to use platelet-rich plasma rather than whole blood. In the European study the method was to add 5 ml platelet-rich plasma to a Chandler's tube (gauge N/17, Portland Plastics, Kent) and make this up to 15 ml with 0.9% NaCl. The platelet-rich plasma was recalcified with one-tenth volume 0.25 M CaCl₂ exactly 30 minutes after venepuncture and the tube was then placed on a turntable of a blood cell suspension mixture rotating at 25 rpm. The time between recalcification and the appearance of the 'snowstorm' effect was taken as the time for platelet aggregation.

In the studies on the Africans and the Asians the basic technique was the same but the volumes were smaller and the tubing used was narrower (gauge N/13, Portland Plastics, Kent). One ml of platelet-rich plasma was diluted with 2.5 ml of 0.9% NaCl and recalcified with 1 ml 0.25 M CaCl₂.

PLATELET COUNT

This was performed by the method of Dacie (1956).

STYPVEN (RUSSELL'S VIPER VENOM) TIME

This was measured by a modification of the method of MacLagan, Billimoria, and Curtis (1958). The plasma used was that collected in siliconized tubes and centrifuged at 600 *g* for five minutes at 4°C. It was further centrifuged at 2,000 *g* for 15 minutes at 4°C to render it platelet poor. To 0.1 ml of this plasma in a glass test tube at 37°C, were added simultaneously 0.1 ml Stypven (Burroughs Wellcome & Co.) and 0.1 ml 0.025 M CaCl₂, and the clotting time was recorded.

BLOOD LIPID ASSAYS

Triglyceride levels were measured by the method of Van Handel and Zilversmit (1957).

Results

EUGLOBULIN CLOT LYSIS TIME

The results are shown in Figure 1. In the Europeans the mean euglobulin lysis activity after the high-fat (1.10 units) breakfast is significantly less than the mean activity after a low-fat (1.72 units) breakfast ($t = 3.57$, $P < 0.01$). This difference

Fig. 1 Euglobulin clot lysis time expressed as units of activity (mean and standard deviation) after both types of breakfast in each group.

Fig. 2 Urokinase sensitivity expressed in units (mean and standard deviation) after both types of breakfast in each group.

was not seen in the Africans ($t = 0.52$, $P > 0.70$) or in the Asians ($t = 0.42$, $P > 0.1$).

UROKINASE SENSITIVITY TEST
These results are shown in Figure 2. In the European group, a reduction in lytic activity was observed after the fat-supplemented breakfast ($t = 3.09$, $P < 0.02$). This was not the case in the Africans ($t = 0.26$, $P > 0.1$) or in the Asians ($t = 1.23$, $P > 0.1$).

PLASMINOGEN AND FIBRINOGEN LEVELS
These results are shown in Table II. In all three groups no significant difference was noted in the levels of plasminogen and fibrinogen after the fatty breakfast as compared with the control breakfast.

PLATELET AGGREGATION IN CHANDLER'S TUBE
The results are listed in Table III. In the Europeans the time for platelet aggregation was significantly prolonged by the fat-supplemented meal.

		Breakfast		Significance
		Low Fat	High Fat	
Plasminogen (casein units)	Europeans	2.4 ± 0.3	2.3 ± 0.2	t = 0.375, P < 0.8
	Asians	2.7 ± 0.4	2.8 ± 0.5	t = 0.53, P < 0.7
	Africans	3.2 ± 0.6	3.1 ± 0.5	t = 1.00, P < 0.4
Fibrinogen (mg/100 ml)	Europeans	253 ± 97	253 ± 94	t = 0.098, P < 0.95
	Asians	298 ± 64	306 ± 73	t = 0.39, P < 0.8
	Africans	286 ± 65	305 ± 80	t = 0.85, P < 0.5

Table II Plasminogen and fibrinogen levels after both types of breakfast in each group

Group	Breakfast	
	Low Fat	High Fat
Europeans	521 ± 87	603 ± 68
Asians	681 ± 132	843 ± 299
Africans	720 ± 245	642 ± 253

Table III Time for platelet aggregation (seconds) after both types of breakfast in each group

($t = 4.26, P < 0.01$). This effect was not observed in either the Asian ($t = 2.11, P > 0.10$) or African groups ($t = 0.46, P > 0.1$).

PLATELET COUNT

These results are shown in Table IV. No significant difference was noted in the platelet counts after the control breakfast as compared with the test breakfast in either of the three groups.

STYPVEN TIME

These results are shown in Figure 3. In all groups this was significantly reduced after the fatty breakfast as compared with the control. The fall was of similar proportion in all groups ($P < 0.001$ in each case).

Fig. 3.

BLOOD TRIGLYCERIDE LEVELS

The results are shown in Table V. Significant increases occurred in the levels of triglyceride (Europeans $t = 4.43, P < 0.005$; Africans $t = 6.09, P < 0.001$; Asians $t = 5.64, P < 0.001$) demonstrating adequate absorption of the ingested fat.

COMPARISON BETWEEN GROUPS

Apart from the observation made in each group with regard to the effect of the fatty meal, certain other comparisons were made. In Fig. 4 is shown the basal euglobulin clot lysis activity for each group, ie, after the control breakfast. The Europeans have the lowest level of activity, the Africans the highest, and the Asians occupy an intermediate position. The difference in mean

Fig. 4.

Fig. 3 Stypven time in seconds (mean and standard deviation) after both types of breakfast in each group.

Fig. 4 Euglobulin clot lysis activity (mean and standard deviation) for each group after low fat breakfast.

Group	Breakfast		Significance
	Low Fat	High Fat	
Europeans	219.7 ± 58.6	232.4 ± 50.2	t = 1.18, P < 0.3
Asians	184 ± 136.1	187 ± 26.2	t = 0.20, P < 0.9
Africans	188 ± 33.7	190 ± 24.9	t = 0.04, P < 0.97

Table IV Platelet counts (thousands/mm³) after both types of breakfast in each group

Group	Breakfast	
	Low Fat	High Fat
Europeans	81 ± 34	164 ± 82
Asians	140 ± 60	421 ± 183
Africans	84 ± 27	212 ± 73

Table V Serum triglyceride levels after both types of breakfast in each group

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Fig. 5 Plasminogen levels (casein units—mean and standard deviation) for each group after the low fat breakfast.

euglobulin clot lysis activity between Europeans and Africans ($t = 7.06$, $P < 0.001$) or Asians ($t = 2.52$, $P < 0.05$) is significant. The difference, however, between Africans and Asians ($t = 2.05$, $P > 0.10$) is not significant.

The same pattern can again be seen in Fig. 5 where the basal plasminogen levels are plotted. The differences between the Europeans and the Africans ($t = 3.71$, $P < 0.005$) or Asians ($t = 2.34$, $P < 0.05$) are significant. The difference between the Africans and the Asians, however, is not significant ($t = 2.02$, $P > 0.10$).

Discussion

The evidence presented demonstrated that a high fat meal which produced striking hyperlipidaemia in every subject influenced fibrinolytic activity in a different manner in Europeans as compared with Africans and Asians. The fat meal caused a significant depression of euglobulin clot lysis activity and increased resistance of plasma clots to lysis by urokinase in Europeans but was without such influence in Africans and Asians. In all three racial groups plasminogen and fibrinogen levels were unchanged after the fatty meal, suggesting that the changes in lytic activity in the Europeans could not therefore be attributed to change in these levels. The fat load appeared to influence platelet aggregation in the Europeans by prolonging the time taken to aggregation in the Chandler's tube but this effect did not occur in either the Asians or the Africans.

The results show that in Europeans only, of the three ethnic groups studied, hyperlipidaemia both accelerates blood coagulation as measured by the Stypven time and inhibits fibrinolytic activity. If, as has been postulated, persistence of fibrin deposits on the endothelium is important in the genesis of atheroma, the shortening of the Stypven time by increasing the tendency to fibrin formation and the impairment of fibrinolytic activity by interfering with the removal of fibrin

might tend to facilitate the development of atheroma. It is also of interest that the control level of the euglobulin clot lysis activity, ie, after a low fat breakfast was substantially different in each group, the Africans showing much greater lytic activity than Europeans or Asians and the Asians showing significantly greater activity than Europeans. A similar pattern was also seen in the levels of plasminogen after the control breakfast.

Wainwright (1961), comparing the incidence of atherosclerosis in Europeans, Africans, and Asians in Natal, found that the Europeans were most affected, the Africans least so, and that the Asians occupied an intermediate position. The pattern of differences in basal euglobulin clot lysis activity and basal plasminogen levels found in the present study in the different racial groups is compatible with the theory that fibrinolytic activity may have a role in the genesis of the varying incidence of atheroma in these groups. The failure of the high fat breakfast to depress fibrinolytic activity in Africans and Asians may have some bearing on the observed racial differences in the incidence of atheroma. Increased euglobulin clot lysis activity in Africans and Asians as compared with Europeans has also been shown by Menon (1967). However, he was unable to show any difference between Europeans and Asians resident in Britain and he did not report plasminogen levels.

The work presented here in which Europeans, Africans, and Asians from comparable socio-economic groups were studied suggests that there may be genetic differences between Europeans, Africans, and Asians with respect to the levels of activity of their fibrinolytic enzyme system which may play a part in determining the differing incidences of atherosclerosis which have been observed in these three racial groups.

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