Lipolysis generates platelet dysfunction after in vivo heparin administration

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ABSTRACT

Heparin, when administered to patients undergoing operations using cardiopulmonary bypass, induces plasma changes that gradually impair platelet macroaggregation, but heparinization of whole blood in vitro does not have this effect. The plasma changes induced by heparin in vivo continue to progress in whole blood ex vivo. Heparin releases several endothelial proteins, including lipoprotein lipase, hepatic lipase, platelet factor-4 and superoxide dismutase. These enzymes, which remain active in plasma ex vivo, may impair platelet macroaggregation after in vivo heparinization and during cardiopulmonary bypass. In the present study, proteins were added in vitro to hirudin (200 units ml⁻¹)-anticoagulated blood from healthy volunteers, and the platelet macroaggregatory responses to ex vivo stimulation with collagen (0.6 μg ml⁻¹) were assessed by whole-blood impedance aggregometry. Over a 4 h period, human lipoprotein lipase and human hepatic lipase reduced the platelet macroaggregatory response from 17.0±2.3 to 1.5±1.3 and 1.2±0.6 X respectively (means±S.D.) (both P<0.01; n=6). Other lipoprotein lipases also impaired platelet macroaggregation, but platelet factor-4 and superoxide dismutase did not. Platelet macroaggregation showed an inverse linear correlation with plasma concentrations of non-esterified fatty acids (r²=0.69; two-sided P<0.0001; n=8), suggesting that heparin-induced lipolysis inhibits platelet macroaggregation. Lipoprotein degradation products may cause this inhibition by interfering with eicosanoids and other lipid mediators of metabolism.

INTRODUCTION

Platelet dysfunction is a major contributor to the bleeding diathesis that increases transfusion requirements in up to 29% of patients undergoing operations using cardiopulmonary bypass [1]. The platelet function defect that develops during cardiopulmonary bypass is an inability to form large stable aggregates (macroaggregates) [2–4], while the formation of small aggregates (microaggregates) is not impaired [2–4].

While heparin has pro-aggregatory effects that augment platelet microaggregation [5,6], we and others have shown that heparin impairs platelet function before the start of extracorporeal circulation [4,7]. It has also been shown that, while intravenous heparin markedly inhibits platelet macroaggregation, this inhibition is not reproduced by in vitro heparinization of whole blood [4,8,9]. Platelet secretion of 5-hydroxytryptamine is similarly impaired by heparin in vivo, but not in vitro [10]. These contrasting effects of heparin in vivo and in vitro suggest possible endothelial involvement in heparin–platelet interactions.

In earlier studies we showed that in vivo heparinization gradually induced platelet dysfunction by causing plasma changes [9]; these plasma changes continued to develop ex vivo after heparinization in vivo [9]. We also observed that this action of heparin was not dose-related when heparin doses of 30 units kg⁻¹ or more were given [9]. When fully developed, the effect almost completely abolished platelet macroaggregation [9]. In contrast, the
plasma obtained from blood in which platelets were dysfunctional, after heparinization in vivo, inhibited platelet macroaggregation in vitro without delay [9]. These observations also support the suggestion by previous workers that the platelet defect associated with cardiopulmonary bypass is extrinsic to the platelet [11], and suggest that intermediaries may act on plasma components to produce inhibitory substances, rather than inhibiting platelets directly. Micro- and macroaggregation in blood from unheparinized subjects was not affected by delay [12,13].

Heparin releases several endothelial proteins into the plasma [14], including lipoprotein lipase and hepatic lipase. These enzymes continue to hydrolyze plasma lipoproteins ex vivo after heparinization in vivo [15,16]. Several products of lipoprotein hydrolysis inhibit platelet macroaggregation; these include non-esterified fatty acids (with unsaturated non-esterified fatty acids having a more pronounced effect) [17,18], apolipoprotein E, [19], high-density lipoprotein [20] and lysophospholipids [21]. Other heparin-releasable endothelial proteins include platelet factor-4 and superoxide dismutase [14]. We hypothesized that heparin-induced lipolysis may cause platelet dysfunction, and therefore investigated the effects of these proteins on platelet macroaggregation in whole blood.

METHODS

We carried out a series of four in vitro studies. In Study 1, the effects of human post-heparin lipases on platelet macroaggregation were studied using blood from six volunteers. In Study 2, the effects of lipoprotein lipase, extracted from bovine milk, on platelet macroaggregation was studied using blood from 12 volunteers. In Study 3 the effects of lipoprotein lipase from Pseudomonas spp. on non-esterified fatty acid concentrations and platelet macroaggregation were studied using blood from eight volunteers, and those of human post-heparin hepatic lipase were studied using blood from five volunteers. In Study 4, the effects of human platelet factor-4 and of superoxide dismutase of bovine erythrocyte origin on platelet macroaggregation were studied in blood from three volunteers.

All studies were carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association and with the approval of the Royal Infirmary of Glasgow Research Ethics Committee [Project No. 97SC001 (896) on May 7th 1997]. Informed consent was obtained from all participants.

Blood sampling

Venous blood was taken, using a 19 G needle, from the antecubital fossa of healthy volunteers, who had not taken non-steroidal anti-inflammatory drugs or other antiplatelet medication for at least 7 days. Blood was anticoagulated with r-hirudin (200 units · ml⁻¹) in siliconized glass containers, as previously described [13]. In earlier studies we demonstrated that both platelet aggregatory responses [12] and existing platelet microaggregates [13] are stable under these conditions for up to 24 h.

We elected to study platelet aggregation in hirudin-anticoagulated whole blood, as hirudin maintains normocalcaemia and has no known direct action on platelets. Platelet aggregation has classically been studied in citrate-anticoagulated blood or platelet-rich plasma. Chelating agents, such as EDTA and citrate, cause hypocalcaemia and therefore may not give a true picture of platelet responses [22,23]. Hypocalcaemia favours thromboxane A₂ production and thromboxane A₂-dependent secretion during aggregation induced by weak agonists, such as ADP or adrenaline [24]. Hirudin specifically inhibits thrombin, but has little or no effect on platelet activation induced by other agonists, such as ADP and collagen in vitro [25]; hirudin also does not inhibit the in vivo propagation of platelet-rich thrombus [26]. For these reasons, hirudin is suitable as an in vitro anticoagulant for studying platelet aggregation [27,28]. We have previously studied platelet dysfunction occurring during cardiopulmonary bypass using hirudin anticoagulation [2,4,9].

Protein addition

Four heparin-releasable proteins were studied: lipoprotein lipase and hepatic lipase (for reasons discussed in the Introduction), superoxide dismutase because of its known enzymic activity in blood, and platelet factor-4 because of its known association with the platelet. These proteins were added to achieve concentrations similar to those observed previously after in vivo heparinization. The concentrations were 50 units · ml⁻¹ platelet factor-4 [29], 100 units · ml⁻¹ superoxide dismutase [30] and 100 units · ml⁻¹ total lipase activity [31]. The blood was then placed in a water bath at 37 °C and aliquots were sampled at set time points for aggregometry and for non-esterified fatty acid assays.

Impedance aggregometry was performed on 0.5 ml aliquots of blood immediately after sampling. For assay of non-esterified fatty acids, in Study 3, 2.5 µg of paraoxon (a cholinesterase inhibitor that also has non-specific inhibitory effects on lipases [16]) was added to 1 ml of the blood immediately after sampling. These samples were then centrifuged at 1500 g and 4 °C for 15 min, and the supernatant plasma was stored at −70 °C. The samples were analysed in bulk at the end of the studies.

Protein sources

Human hepatic lipase and lipoprotein lipase were extracted from post-heparin plasma, as described below. Superoxide dismutase and lipoprotein lipase enzymes, the
former of bovine erythrocyte origin and the latter extracted from bovine milk or from *Pseudomonas* spp. cultures, were purchased from Sigma-Aldrich Co. Ltd (Poole, Dorset, U.K.). Human platelet factor-4 was kindly donated by the National Institute for Biological Standards and Control (London, U.K.). Our preliminary studies showed that the Tris buffers, hypertonc saline and ammonium sulphate in which the bovine and human enzymes were preserved impaired platelet macroaggregation in some individuals. We therefore dialysed these enzymes against normal saline before adding them to blood.

**Post-heparin lipase extraction**

**Patients**

Supplies of human post-heparin hepatic lipase and lipoprotein lipase for laboratory use were not commercially available; therefore two patients undergoing elective coronary artery bypass grafting using cardiopulmonary bypass were recruited for extraction of post-heparin lipases. Informed consent was obtained from these patients. Platelet function was not studied in these patients; their heparinized blood was used solely as a source of lipolytic enzymes. These patients were not on aspirin, other non-steroidal anti-inflammatory drugs, platelet suppressants, steroids, warfarin, intravenous nitrates or heparin. Anaesthetic premedication was with temazepam, and induction was by propofol. Anaesthesia was maintained with propofol infusion and opiates. Heparinization was with pig heparin (Leo Laboratories, Risborough, Bucks., U.K.; 300 units · kg⁻¹) given through a central venous cannula just before cannulation of the aorta. A 50 ml sample of blood was obtained 10 min after heparinization, but before the start of extracorporeal circulation.

**Lipase extraction**

Hepatic lipase and lipoprotein lipase were extracted by stepwise elution through a heparin–Sepharose column as previously described [32]. Briefly, post-heparin blood was anticoagulated with 200 units ml⁻¹ r-hirudin and placed on ice immediately after sampling. The separation process was carried out in a refrigerated room at 4 °C. Half of the heparin content, as estimated by a protamine titration using the Hepcon* system (Medtronic Ltd, Watford, Herts., U.K.), was neutralized ex vivo with protamine. This neutralization lowered the heparin concentration to levels closer to those in the original description of this extraction procedure [32], as the heparin in solution may compete with that in the column for binding of the lipases and reduce the efficiency of extraction. Plasma was separated after centrifugation at 1500 g for 15 min.

Heparin–Sepharose columns were equilibrated with 5 mM sodium barbital buffer, pH 7.4, containing 0.15 M NaCl. Aliquots of 4 ml of post-heparin plasma were diluted 1:1 (v/v) with 5 mM sodium barbital buffer, pH 7.4, containing 0.45 M NaCl. The separation columns were loaded with 8 ml of diluted plasma. Elution through the columns was by washing with 8 ml fractions. The first fraction was eluted with 5 mM sodium barbital, pH 7.4, containing 0.3 M NaCl, the next two with 5 mM sodium barbital, pH 7.4, containing 0.72 M NaCl, and the final two with 5 mM sodium barbital, pH 7.4, containing 1.5 M NaCl.

The fractions were then dialysed against 3.8 M ammonium sulphate and then against 0.1 M phosphate buffer, pH 7.4. This method of extraction gives high yields of hepatic lipase in fraction 3 and of lipoprotein lipase in fraction 5 [32].

**Impedance aggregometry in whole blood**

An impedance aggregometer (500-VS; Chronolog Corp., Havertown, PA, U.S.A.) measured electrical impedance changes in whole blood. Aliquots of 500 μl of whole blood were diluted with the same volume of NaCl (0.9%) in plastic cuvettes and equilibrated at 37 °C before measurement. The macroaggregatory response to 0.6 μg of collagen (Hormon Chemie, Munich, Germany) was read as the scale deflection in cm at 5 min. The aggregometer was calibrated according to the manufacturer’s instructions, so that a 20 Ω change in electrical impedance would result in a deflection of 14 cm, giving a conversion factor of 1.43 Ω per cm.

Collagen was chosen as the *in vitro* agonist for aggregometry, because it is the principle agonist the platelet encounters in damaged vessel walls during primary haemostasis. Later, during secondary haemostasis, thrombin is also an important platelet agonist. Furthermore, platelet macroaggregation in response to low concentrations of collagen is largely the result of platelet secretion of thromboxane A₂, and platelet release of ADP and 5-hydroxytryptamine [33,34]; the involvement of these three other important endogenous platelet agonists makes the study of responses to low-dose collagen stimulation *in vitro* a useful global means of assessing platelet responses to physiologically relevant stimulation [2,4,9,12,34]. High concentrations of collagen can induce a full platelet aggregatory response independently of this autocrine positive feedback; however, this may be the result of excessive and therefore probably non-physiological stimulation. To ensure that stimulation remained within the physiological range, a collagen concentration of 0.6 μg · ml⁻¹ was used; we had previously determined that this was just below the minimum concentration that elicited a maximal macroaggregatory response [4].

**Assays of non-esterified fatty acids**

Levels of non-esterified fatty acids were serially determined as a measure of lipase enzyme activity. As most
enzyme assays use the rate of release of breakdown products, under specific conditions, to generate an index of enzyme activity, the titres of these breakdown products may more accurately reflect enzyme activity. These assays were performed using NEFA C test kits (Wako Chemicals, Neuss, Germany). Briefly, this is a two-reaction assay, performed on 96-well microtitre plates. The first reaction is the acylation of CoA by fatty acids in the presence of acyl-CoA synthetase. This was achieved by incubating 5 l of plasma with 100 l of Reagent A, which contains acyl-CoA synthetase, ascorbate oxidase, CoA, ATP and 4-aminoantipyrine. In the second reaction, acyl-CoA is oxidized by acyl-CoA oxidase to produce hydrogen peroxide, which in the presence of peroxidase causes oxidative condensation of 3-methyl-N-ethyl-(β-hydroxyethyl)aniline with 4-aminoantipyrine to form a purple pigment. This was effected by adding 200 l of Colour Reagent B to the wells; this reagent contains acetyl-CoA oxidase and peroxidase. The absorbance of the resultant solution was then read at 550 nm using a Dynatech MR 5000 platelet reader (Dynatech Corp., Burlington, MA, U.S.A.). The concentrations of non-esterified fatty acids were read directly from a calibration curve that was prepared using the standards that were supplied with the kit.

Materials
Heparin–Sepharose CL-6B was purchased from Amersham Pharmacia Biotech AB (Uppsala, Sweden); sodium barbital, HCl and ammonium sulphate were from BDH Laboratory Supplies (Poole, Dorset, U.K.); sodium dihydrogen phosphate was from Formachem (Research International) Ltd (Strathaven, Lanarkshire, U.K.); disodium hydrogen phosphate was from Fisons Scientific Equipment (Loughborough, Leics., U.K.); dialysis tubing (pore size 12000–14000 Da) was from Medicell International Ltd (London, U.K.); paraoxon was from Sigma-Aldrich.

Statistical analysis
Data are expressed as means±S.D. Comparisons were made using two-level ANOVA with the Bonferroni correction for multiple comparisons. Analyses were performed using Arcus Quickstat Biomedical software (Addison Wesley Longman; trading as Research Solutions, Cambridge, U.K.).

RESULTS
Study 1

**Human post-heparin hepatic lipase**
The human post-heparin hepatic lipase extract lowered the platelet macroaggregatory response to 0.6 µg·ml⁻¹ collagen from 17.0±2.3 Ω to 4.8±3.7 Ω after 2 h and to 1.2±0.6 Ω after 4 h (P < 0.0001 and P = 0.01 respectively; n = 6) (Figure 1).

**Study 2: lipoprotein lipase from bovine milk**
Lipoprotein lipase from bovine milk lowered the platelet macroaggregatory response less markedly than the human enzymes, from 17.6±3.1 Ω to 12.8±7.3 Ω after 2 h and to 5.8±5.3 Ω after 4 h (P = 0.06 and P = 0.02 respectively; n = 12) (Figure 2). The subjects studied appeared to comprise two distinct groups: a quick-
than either the human or the bovine isoenzymes, low-
lipase inhibited platelet macroaggregation more rapidly

(i) Platelet macroaggregation. This bacterial lipoprotein
lipoprotein lipase from Pseudomonas spp.

Study 3

Data are means ± S.E.M. (n = 8). ■, Non-esterified fatty acids (NEFA);
○, impedance changes. Significance of differences: * P < 0.05 for NEFA
compared with baseline; † P < 0.05 for impedance change compared with
baseline. For correlations, see the text.

responding group and a slow-responding group (Figure
2). In the quick responders, the platelet macroaggregatory
response fell from 16.4 ± 1.6 Ω to 7.7 ± 6.6 Ω over the
first 2 h and to 4.3 ± 3.4 Ω over the next 2 h (P = 0.06 and
P = 0.02 respectively; n = 6). However, in the slow
responders, the platelet macroaggregatory response of
18.8 ± 3.8 Ω was essentially unchanged over the first 2 h,
but then fell to 7.3 ± 6.7 Ω over the next 2 h (P = 0.3 and
P = 0.05 respectively; n = 6) (Figure 2).

Lipoprotein lipase from Pseudomonas spp.

(i) Platelet macroaggregation. This bacterial lipoprotein
lipase inhibited platelet macroaggregation more rapidly
than either the human or the bovine isoenzymes, low-
ering the response to 0.6 µg · ml⁻¹ collagen from 16.4 ±
3.3 Ω to 1.3 ± 1.1 Ω within 1 h (P < 0.0001; n = 8)
(Figure 3). The bacterial lipoprotein lipase, which was
supplied as a freeze-dried powder and therefore did not
require dialysis, appeared to be the most potent in both
inducing lipolysis and inhibiting platelets. Dialysing out
preservatives may have impaired the activity of the other
enzymes studied.

(ii) Release of non-esterified fatty acids. The generation
of non-esterified fatty acids was measured in samples to
which Pseudomonas spp. lipoprotein lipase had been
added. The release of non-esterified fatty acids was most
marked in the first 15 min, increasing from 0.13 ±
0.33 mmol · l⁻¹ to 2.29 ± 0.86 mmol · l⁻¹ (P = 0.004;
n = 8). The apparent increase over the last 15 min, from
3.26 ± 1.04 mmol · l⁻¹ to 3.59 ± 1.04 mmol · l⁻¹, failed to
reach statistical significance (P = 0.06; n = 8), suggesting
that an equilibrium level or maximal generation was
being approached (Figure 3). There was a significant
inverse linear correlation between the concentration of
non-esterified fatty acids and the platelet macroaggregatory
response (r² = 0.69; two sided P < 0.0001; n = 8)
(Figure 3).

Human hepatic lipase

(i) The effects of hepatic lipase on levels of non-esterified
fatty acids and platelet macroaggregation were also
studied. In blood from this additional group of volun-
teers, human hepatic lipase reduced the platelet macro-
aggregatory response less markedly than in the samples
from previous subjects. The response fell from 13.7 ±
1.2 Ω to 9.2 ± 1.5 Ω after 2 h and to 7.6 ± 2.1 Ω after 4 h
(P = 0.06 and P = 0.2 respectively; n = 5) (Figure 4).

(ii) Over the same period the levels of non-esterified
fatty acids rose from 0.26 ± 0.08 to 0.41 ± 0.01 mmol · l⁻¹
and then to 0.53 ± 0.08 mmol · l⁻¹ respectively (P =
0.08 and P = 0.2 respectively; n = 5) (Figure 4). There
was once again a significant inverse linear correlation
between non-esterified fatty acid levels and the platelet
macroaggregatory response (r² = 0.81; two-sided P =
0.03; n = 5) (Figure 4).

Study 4: platelet factor-4 and superoxide
dismutase

Platelet factor-4 and superoxide dismutase did not affect
platelet macroaggregation in whole blood from healthy
volunteers in vitro, even after incubation at 37 °C for 3 h
(Table 1). No synergistic actions of these enzymes which
inhibited platelet macroaggregation were detected, even
in the presence of heparin (Table 2).
Table 1  Effects of heparin, platelet factor-4 and superoxide dismutase on whole-blood impedance aggregometry

<table>
<thead>
<tr>
<th>Protein</th>
<th>Time</th>
<th>Control</th>
<th>Immediate</th>
<th>13.1</th>
<th>25.0</th>
<th>20.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Immediate</td>
<td>13.1</td>
<td>25.0</td>
<td>20.0</td>
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<td></td>
</tr>
<tr>
<td>Heparin (4 units·ml⁻¹)</td>
<td>Immediate</td>
<td>13.6</td>
<td>24.3</td>
<td>18.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF4 (100 units·ml⁻¹)</td>
<td>Immediate</td>
<td>16.4</td>
<td>22.0</td>
<td>17.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD (100 units·ml⁻¹)</td>
<td>Immediate</td>
<td>16.6</td>
<td>25.1</td>
<td>26.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2  Effects of various combinations of heparin and other platelet factor-4 and superoxide dismutase on whole-blood impedance aggregometry

<table>
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<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
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<td>H + P</td>
<td>18.6</td>
<td>23.4</td>
<td>20.3</td>
<td></td>
</tr>
<tr>
<td>Heparin (4 units·ml⁻¹)</td>
<td>3 h</td>
<td>H + S</td>
<td>17.9</td>
<td>26.6</td>
<td>27.3</td>
<td></td>
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<tr>
<td>PF4 (100 units·ml⁻¹)</td>
<td>3 h</td>
<td>P + S</td>
<td>14.9</td>
<td>27.0</td>
<td>19.4</td>
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<tr>
<td>SOD (100 units·ml⁻¹)</td>
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<td>17.1</td>
<td>26.0</td>
<td>24.6</td>
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</tbody>
</table>

DISCUSSION

Lipolytic enzymes are released from the endothelium into the plasma by heparinization in vivo. These lipases, and similar enzymes derived from other sources, impaired platelet macroaggregation when added to whole blood in vitro. This inhibition was similar to that observed previously after in vivo heparinization [9]. Platelet dysfunction was correlated strongly with plasma non-esterified fatty acid levels, suggesting that products of plasma lipoprotein hydrolysis impaired platelet macroaggregation; the correlation between plasma lipase activity and bleeding times previously observed in heparinized rabbits [35] supports this hypothesis. The other heparin-releasable proteins studied did not affect platelet macroaggregation.

Heparin and lipid homoeostasis

Lipoprotein lipase is bound to the endothelial surfaces of arteries and capillaries by heparan sulphate. Hepatic lipase is similarly anchored on the luminal aspect of liver sinusoids. The location of these enzymes couples lipoprotein hydrolysis with the uptake of lipids and apolipoproteins. This helps keep plasma concentrations of these lipoprotein breakdown products low, favouring further lipoprotein degradation. Dislocation of these enzymes, by heparin, uncouples lipoprotein hydrolysis from product uptake, thus increasing plasma concentrations of lipoprotein breakdown products, such as non-esterified fatty acids [31,36]. Accumulation of lipoprotein breakdown products inhibits further lipid breakdown [37]; this inhibition may occur in both the extracellular and the intracellular compartments, as small non-polar molecules rapidly cross cell membrane lipid bilayers.

Mechanisms by which lipolysis may affect platelet aggregation

Platelet agonists are classified as weak or strong, depending on their ability to stimulate platelet secretion and aggregation independently of autocrine positive feedback [38]. Thromboxane A₂, an eicosanoid (lipid mediator derived from arachidonic acid), is a strong platelet agonist that is secreted during the autocrine positive feedback induced by weak agonists. The alterations in plasma lipids caused by lipolysis may interfere with the metabolism of thromboxane and its precursors in several ways. Arachidonic acid is stored in cell membranes; it is the most abundant fatty acid in platelet membranes [38]. Integrin-controlled phospholipases [38] mobilize large quantities of arachidonic acid in response to stimulation; this occurs in two stages during platelet activation [39]. Arachidonic acid is either obtained from dietary sources or synthesized in the liver and transported to the tissues that utilize it, as a constituent of plasma lipoproteins; only trace quantities are found in the free form. Hepatic lipase and lipoprotein lipase release arachidonic acid from plasma lipoproteins [40]. Arachidonic acid released from plasma lipoproteins during intravascular lipolysis will cross cell membranes, thus bypassing the rate-limiting (integrin-controlled) step in eicosanoid synthesis. Lipolysis also increases the concentrations of lysophospholipids and non-esterified fatty acids, which will inhibit the hydrolysis of membrane phospholipids by mechanisms described previously [37]. Lipolysis may therefore increase basal or resting thromboxane production, while retarding its generation in response to stimuli. Increased thromboxane production occurs after in vivo heparin administration [41,42]. Thromboxane concentrations were correlated with non-esterified fatty acid concentrations in one of these studies [41]. The previously observed decreased secretion of

Lipolysis may also interfere with platelet macroaggregation, because other fatty acids normally present in lipoproteins, such as eicosapentaenoic acid, compete with arachidonic acid for enzyme binding sites [43,44]. Eicosanoid receptor responses may also be affected by metabolites of arachidonic acid [45] and other fatty acids [43,44].

**Implications for cardiopulmonary bypass**

During cardiopulmonary bypass, plasma concentrations of lipoprotein breakdown products rise markedly after heparinization and remain elevated throughout the period of extracorporeal circulation [46,47]. Haemodilution, which occurs during cardiopulmonary bypass, may amplify the effects of lipolysis by increasing the amounts of lipids that are aqueous (or free) as opposed to protein bound. Thus the metabolic effects of heparin-induced lipolysis may be more pronounced during cardiopulmonary bypass than they are in other clinical situations.

**Limitations of study**

We did not specifically control for the effects of time on platelet macroaggregation; however, in previous studies we noted that platelet macroaggregatory responses were preserved in hirudin-anticoagulated whole blood for up to 24 h [12]. In hirudin-anticoagulated whole blood to which heparin was added in vitro, these responses were stable for at least 3 h [9]. The finding in the present study that platelet responses were stable over 3 h after addition of heparin, platelet factor-4 or superoxide dismutase also suggests that deterioration in platelet responses was not caused by the delay or the enzyme vehicles.

We did not investigate specific lipoprotein breakdown products for their inhibitory effects on platelet macroaggregation; however, previous studies have shown that several of these products inhibit platelets in a dose-dependent manner [17–20]. It is therefore likely that multiple inhibition occurs simultaneously. The generation of other lipoprotein breakdown products is likely to correlate with that of non-esterified fatty acids, and hence their levels would also correlate with platelet dysfunction.

We are uncertain as to why platelet macroaggregation deteriorated more quickly in blood from some individuals than others. This may have been because these blood samples had fewer available lipid binding sites on their plasma proteins, or alternatively the faster response may be related to the lipoprotein constituents.

**Conclusion**

Heparin releases lipolytic enzymes from the endothelium, and these enzymes hydrolyse plasma lipoproteins. Lipolysis in whole blood caused platelet dysfunction that was correlated with the concentration of lipoprotein degradation products. This suggests that lipoprotein degradation products may cause the platelet dysfunction that occurs in heparinized subjects, including those undergoing operations using cardiopulmonary bypass.

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