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Surface expression of fatty acid translocase (FAT/CD36) on platelets in myeloproliferative disorders and non-insulin dependent diabetes mellitus: Effect on arachidonic acid uptake

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Abstract

Increased platelet reactivity has been implicated in the vascular complications of myeloproliferative diseases and diabetes mellitus. The mechanisms of platelet hyperresponsiveness have not been fully explained. Expression of CD36 or fatty acid translocase (FAT) and its role in arachidonic acid (AA) uptake by platelets were examined in subjects with myeloproliferative disorders (MPD), those with non-insulin-dependent diabetes mellitus (NIDDM), and in normal, healthy, age-matched controls. Surface expression of CD36 on platelet membranes was increased in MPD (10.94 ± 0.76 pmol/mg protein) compared with normal controls (6.94 ± 0.48 pmol/mg protein), $p < 0.001$. Total platelet content of CD36 was also significantly higher (32.1 ± 0.61 pmol/mg protein, $p < 0.01$) compared with those in sex and age matched normal controls (25.7 ± 1.09 pmol/mg protein). In contrast, platelet surface expression of CD36 in NIDDM (6.5 ± 0.56 pmol/mg protein) was not significantly different from those of normal controls despite higher total content of CD36 (32.8 ± 1.2, pmol/mg protein, $p < 0$ .01). Intact MPD platelets bound significantly more arachidonic acid (AA) (1.53 ± 0.16 nmol/mg protein, $p < 0.05$), compared with controls (1.12 ± 0.07 nmol/mg protein) or NIDDM subjects (1.16 ± 0.16 nmol/mg protein). The capacity of MPD platelet membranes to bind ^{14}C-AA was also increased (1.72 ± 0.25 nmol/mg protein, $p < 0.05$) compared with that of controls (1.62 ± 0.05 nmol/mg protein) and of NIDDM (1.22 ± 0.08 nmol/mg protein). This is consistent with higher surface expression of CD36 in MPD platelets. Membrane fatty acid analysis indicated that the % of AA in platelet phospholipids was significantly lower in MPD (3.15 ± 0.81%) compared with the controls (5.62 ± 1.7%, $p < 0.05$. The AA content of diabetic platelets (4.82 ± 1.1%) was not significantly different from normal controls. In summary, both total and surface expression of CD36 are increased in MPD, consistent with an enhanced capacity for uptake of AA by platelets. Increased expression of CD36 in platelets may play a role in the vaso-occlusive manifestations of MPD. (Mol Cell Biochem **239**: 203–211, 2002)

Key words: FAT, CD36, fatty acid translocase, arachidonic acid, platelets, diabetes, myeloproliferative disorders, fatty acid uptake, thromboxane A2, membrane phospholipids

Introduction

Vascular occlusions are a major cause of morbidity and death in myeloproliferative disorders (MPD) [1] and diabetes mellitus [2]. In MPD, multiple platelet abnormalities result from clonally derived megakaryocytopoiesis [3]. The abnormalities leading to thrombosis have not been fully characterised, but may include an increased thromboxane A_2 (TxA_2) synthesis, increased granule secretion and spontaneous platelet aggregation [4, 5]. Abnormalities in platelet

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membrane glycoproteins (GP) and altered platelet aggregation were also observed in MPD platelets [5–7]. Variable abnormalities in membrane GP content of non-stimulated platelets have been described in MPD [8], however the pattern of GP redistribution in these patients has rarely been addressed [9, 10]. Platelet abnormalities with increased platelet reactivity, are also a feature of diabetes mellitus. They may contribute to the pathogenesis of microvascular complications [11]. Platelet membrane GPs serve a variety of functions including as receptors for ligands necessary for platelet adhesion to leukocytes, fibrin, endothelial cells and extracellular matrix. For example, the GPIIb/IIIa complex possesses activation dependent receptors for fibrinogen, fibronectin and von Willebrand factor (vWf) essential for platelet aggregation and adherence [12]. In contrast, GPIV (CD36) which is present on human platelets and other cells including monocytes/macrophages, and placenta [13] mediates platelet adhesion to monocytes via bound thrombospondin [14–16]. CD36 is also known as fatty acid translocase (FAT) and is composed of a highly glycosylated polypeptide chain with an apparent molecular mass of 88 kDa [17]. It is a multifunctional protein and has a number of putative ligands including oxidised LDL, and fatty acids [13]. A series of studies have shown that CD36/FAT is involved in free fatty acid uptake [13]. It is highly induced during adipocyte differentiation and in diabetes, and the induction is paralleled with an increase in free fatty acid (FFA) uptake by the cells [18]. Furthermore, we have shown recently that CD36/FAT is involved in arachidonic acid uptake by human platelets [19]. This observation was later confirmed by others through lack of arachidonic acid-induced aggregation in the presence of anti-CD36 antibody [20]. Arachidonic acid is the substrate for synthesis of TxA_2, a potent platelet agonist and vasoconstrictor [21, 22]. To serve as the substrate for synthesis of TXA_2, arachidonic acid is liberated from membrane phospholipids by phospholipases [21]. However, exogenous arachidonic acid is readily taken up by platelets and consequently the need for release of arachidonic acid from membrane phospholipids may be bypassed. Absorbed arachidonic acid is available to platelets and is converted rapidly to TxA_2 [21]. The extent to which this process occurs depends largely on the concentration of exogenous arachidonic acid as well as the arachidonic acid uptake activity of the platelets. Arachidonic acid uptake has also been reported to be significantly higher in platelets from patients with diabetes mellitus [2, 23–25]. However very little information is available regarding platelet arachidonic acid uptake and metabolism in MPD. We postulated that altered expression of CD36/FAT could lead to hyperactivation of platelets in diabetes and MPD through increased uptake and metabolism of arachidonic acid. The aim of the present study was therefore, to investigate both CD36/FAT expression and arachidonic acid uptake by platelets from subjects with MPD and NIDDM.

Materials and methods

Materials

[^{14}C]Arachidonic acid (58 mCi/mmol) was obtained from Amersham, UK. Na^{125}I was obtained from ICN Biomedicals, UK. The monoclonal antibodies to CD36 (131.1, 131.2 and 131.7), supplied by Dr. N.N. Tandon (Otsuka America Pharmaceutical). These monoclonal antibodies were obtained from mouse ascites fluid and prepared using a hybridoma cell line and characterised [26–29]. Triton X-114, protease inhibitors, essentially fatty acid free bovine serum albumin and unlabelled arachidonic acid were obtained from Sigma, UK. All other reagents used were of analytical grade.

Subjects and study protocol

Blood samples were obtained from healthy donors and patients (MPD and NIDDM) upon informed consent and in accordance with the Declaration of Helsinki. The age range of patients suffering from MPD and NIDDM was between 65 and 86 years of age. Control subjects were comparable for age and sex. This study protocol was approved by the Joint Ethical Committee of the Grampian Health Board and the University of Aberdeen (Scotland, UK).

Purification of CD36 from human platelets

Outdated platelet concentrates were obtained from the North East Scotland Blood Transfusion Service, Aberdeen. Purification of CD36 was carried out according to the method previously described [30]. Platelets (55×10^9) were suspended in 5 mM EGTA and centrifuged at 3200 × g for 3.5 min at 22°C to sediment red blood cells. The supernatant was then removed and further centrifuged at 3200 × g for 15 min to pellet platelets which were then resuspended and washed twice with buffer (96.5 mM NaCl, 85.7 mM glucose, 10 mM Tris.HC1, 1 mM EDTA, and 5 mM EGTA, pH 7.4). The platelets were then suspended in the above buffer without EDTA and EGTA. CD36 was then purified to an electrophoretic homogeneity using these platelets as starting material. The purified protein was stored at –80°C for further use.

Electrophoretic separation and Western blot analysis of membrane proteins

Polyclonal antisera against the purified CD36 was used [31]. Western blot analysis of solubilised platelet membrane proteins and purified CD36 was carried out as described by Campbell and Dutta-Roy [32]. After electrophoresis, proteins

were transferred onto PVDF membrane at 70°C for 2 h. The membrane was probed for the presence of CD36 by incubating with rabbit polyclonal antiserum to CD36. Antibody-antigen complexes were then detected with the secondary antibody (horseradish peroxidase-linked anti-rabbit IgG fraction of donkey polyclonal antiserum, Scottish Antibody Production Unit, UK), as described [33].

Protein assay

Protein concentration of samples was determined using the Bio-Rad Protein Microassay [34].

Iodination of monoclonal antibodies (131.1 and 131.7) against CD36. Antibodies were iodinated using the chloramine T procedure as described by Legrand *et al.* [35]. Typically, 500 μCi of stock $Na^{125}I$ was added to 250 μg of antibodies (131.7) in a total vol. of 625 μl of 0.5 M sodium phosphate (pH7.5) in a 1.5 ml tube, followed by the addition of 5 μl of chloramine T (10 mg/ml in phosphate buffered saline (PBS), pH7.4). After incubation at room temperature for 90 sec, 50 μl of chloramine T stop reagent (2.4 mg/ml $Na_2S_2O_5$, 10 mg/ml tyrosine (saturated), 10% glycerol, 0.1% xylene cylanol in PBS), kept on ice was added. Iodinated protein was then purified using a Sephadex G-25 (5 ml bed volume) column (Pharmacia-LKB Biotechnology, Sweden). If the incorporation exceeded 95% the antibody was stored in small aliquots 100–200 μl /vial at –80°C.

Platelet isolation from freshly drawn blood

Blood (60 ml) from normal, or patients with MPD and NIDDM was collected into acid citrate anticoagulant (final concentration 15 mM). Platelet-rich plasma (PRP) was obtained by centrifugation at 180 × g for 10 min at 20°C. Platelets were washed by repeated centrifugations at 670 × g for 10 min in a modified Tyrode's buffer (0.137 M NaCl, 2.68 mM KCl, 11.9 mM $NaHCO_3$, 0.146 mM NaH_2PO_4, 1 mM $MgCl_2$, pH 6.5) containing 3.5 mg/ml bovine serum albumin (BSA), 100 nM PGE_1 and 1 U/ml apyrase [35]. Platelets were resuspended (~1.25×10^8 platelets/ml) in modified Tyrode's buffer. pH 7.4, containing 2 mM $CaCl_2$, 1 mM $MgCl_2$ and 3.5 mg/ml of BSA [36]. Platelet suspensions contained less than 0.005% monocytes, assessed by microscopic examination after methanol/ methylene blue/ eosin staining (Bayer Diagnostics, USA).

Platelet lysate preparation

Platelets (~2×10^9 platelets/ml) as isolated above were resuspended in Tris-buffered saline, TBS (10 mM Tris-HCI, 150 mM NaCl, pH 7.4) containing protease inhibitors. Membrane proteins were solubilised in 1% (v/v) Triton X-100 by gentle agitation for 30 min at 4°C. Soluble proteins were separated by ultracentrifugation for 30 min at 100,000 × g and 4°C, and immediately frozen at –80°C.

Platelet membrane preparation

Platelets as isolated above, washed in a buffer containing 1 mM EDTA, 0.15 M NaCl and 20 mM Tris.HCl, pH 7.5 was used instead of modified Tyrode's buffer to wash platelets. Platelets were then resuspended in the above buffer (~1×10^9 platelets/ml). Crude membranes were prepared by lysing washed platelets by repeated freeze/thaw using liquid N_2 followed by centrifugation at 10,000 × g for 10 min at 4°C to remove cell debris and unbroken platelets and then at 100,000 × g for 30 min to sediment the platelet membrane fraction, as described [37].

Radioimmunoassay for total CD36

Radioimmunoassay for total CD36 in platelets was carried out as described [8]. The immunocapture of CD36 by a solid-phase adsorbed monoclonal antibody, MoAb 131.2 was carried out. Polystyrene tubes were coated overnight at room temperature with 100 μl of MoAb 131.2 at a concentration of 10 μg/ml in TBS containing 2 mM Ca^{2+}. After four washes with TBS containing 2 mM Ca^{2+}, 1 mM Mg^{2+}, 0.05% (v/v) Tween 20 and 0.02% (w/v) sodium azide (TBS-Tween), the tubes were incubated for 60 min with 100 μl of a 1.5% (w/v) solution of BSA in TBS in order to block unreacted sites. Tubes were then washed four times with TBS-Tween and incubated for 150 min with 100 μl of purified CD36 or platelet lysate, both diluted in TBS containing 0.1% (v/v) Triton X-100. After four washes, the tubes were incubated for 150 min with 100 μl of radioiodinated monoclonal antibody 131.1 (~500,000 cpm/well) at a concentration of 10 μg/ml in TBS-Tween. After four final washes, the radioactivity of each tube was counted in a Cobra 5003 gamma counter (Canberra-Packard, UK). In control experiments, tubes were coated with monoclonal antibody, 131.2 and incubated with TBS-Tween lacking platelet lysate. Background radioactivity (less than 10%) was subtracted from the total radioactivity and the amount of monoclonal antibody, 131.1 bound was expressed in nanograms of ^{125}I-monoclonal antibody, 131.1 bound per well. Results obtained with tested samples were related to the standard curve obtained on the same day with purified CD36 and expressed as micrograms of CD36 per 100 μg of platelet proteins. Identical standard curves were obtained when purified CD36 was diluted in platelet lysate from a Nak^a-negative donor [38], showing that under our experimental conditions, no GPIV alteration occurred in the platelet lysate.

Binding of radioiodinated monoclonal antibody (131.7) to platelets

In order to investigate the expression of CD36 on platelet surface, direct binding experiments using ^{125}I-monoclonal antibody (MAB), 131.7, were carried out. Typically, platelet suspensions were incubated with various concentrations of the ^{125}I-monoclonal antibody, 131.7 (1, 2, 4, 6, 8 and 10 μg/ml) for 30 min at room temperature, in order to determine the saturating concentration of antibody in patients' and normal platelets. Aliquots (100 μl) were then layered over 500 μl of silicone oil and centrifuged at 12,000 × g for 5 min. The radioactivity associated with the pellets was counted in a Cobra 5003 gamma counter (Canberra-Packard, UK). Background radioactivity, determined by centrifuging each concentration of antibody in buffer without platelets, was subtracted from the radioactivity in the platelet pellets. Platelet suspensions (10^9 cells/ml) incubated with saturating concentrations of the ^{125}I-MoAb 131.7 were carried out to determine the surface expression of CD36 in platelets of individual group of patients and normal controls. Results are expressed as CD36/mg of platelet protein. Saturating concentrations of monoclonal antibody for control and NIDDM platelets was 4 μg/ml whereas the value for MPD was 6 μg/ml.

Non-specific binding at respective saturating binding concentrations was assessed through competition binding between radiolabelled antibody and a 500-fold excess of unlabelled antibody.

[14C] Arachidonic acid binding assay

Binding [^{14}C]arachidonic acid to platelets was determined as described by Dutta-Roy *et al.* [19]. [^{14}C]Arachidonic acid was mixed with BSA (fatty acid free) to obtain molar ratio of [^{14}C]arachidonic acid to BSA of 1:1 in PBS pH 7.4. Platelet membranes (75 μg) were incubated with [^{14}C]arachidonic acid bound to BSA in a 1:1 molar ratio for 20 min at 37°C in PBS pH 7.4, in a total volume of 500 μl. The non-specific binding was determined from parallel experiments adding a 500-fold excess of unlabelled arachidonic acid to the incubations. At the end of the incubations the unbound ligand was separated from the bound ligand by rapid vacuum filtration of 400 μl aliquots of the mixtures through Whatman GF/C glass fibre microfilters (Whatman, UK) which had been pre-soaked in ice-cold PBS. Each filter was washed with 3 × 5 ml of ice-cold 2 μM BSA in PBS. The filters were then dried and suspended in 5 ml of scintillation fluid (Ultima Gold, Packard, UK) before scintillation counting (Tricarb 1900CA, Packard, UK).

The saturating concentration of AA was determined using various concentrations (0.4, 0.6, 0.8, 1.0, 1.2 and 1.4 μM) of radiolabelled AA. Saturating concentration of radiolabelled AA for patients and normal controls was 1 μM.

Lipid analysis

Platelet and erythrocyte lipids were extracted by the method of Bligh and Dyer [39]. Lipids fractions were then separated by thin layer chromatography (TLC) using thin layer silica plates (Silica Gel, LK5), into cholesterol ester, triacylglycerol, FFA, diacylglycerol, and phospholipids. Total phospholipids were scraped off the plate, extracted 3 times with chloroform:methanol (2:1, v/v) and separated by TLC (LK5 plates) into phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol using the solvent system chloroform/ethanol/water/triethylamine (30:34:8:35, v/v/v/v) [40]. Lipid fractions were identified by co-chromatography with suitable standards and visualised under UV light after spraying with 0.2% dichlorofluorecene in ethanol. Appropriate bands were scrapped off and fatty acid compositional analysis was performed.

Fatty acid composition was then determined after their derivatisation with pentafluorobenzyl bromide [41]. The derivatised fatty acids were then analysed by gas liquid chromatography using electron-capture detection in a Hewlett Packard 6890 gas chromatography system. Data acquisition and analysis were performed using Hewlett Packard Chem Station software. Pentaflurobenzyl esters (PFBE) of the fatty acid samples were analysed on a 30 m × 0.258 mm, 0.25 μm DB-23 column (Jones Chromatography, UK) using high purity helium as the carrier gas. The ^{63}Ni electron-capture detector used nitrogen gas and was maintained at a temperature of 300°C throughout. The oven temperature was held at 85°C for 2 min, increased to 165°C at a rate of 40°C/ min, then increased to 250°C, at a rate of 3.5°C *l* min and held for 1 min at 250°C. The PFBE of the fatty acids were quantified relative to the internal standard heptadecanoylpentaflurobenzyl ester.

Statistical analysis

Results were analysed using the Student's *t*-test. The results are presented as the mean ± S.D.

Results

The total CD36/FAT content in platelets isolated from age and sex matched normal controls as well as from MPD and NIDDM patients were measured using a radioimmuno assay. The platelets of MPD patients exhibited a mean level of 30.13 ± 0.61 pmol of CD36/mg protein, whereas NIDDM platelets had 32.88 ± 1.24 pmol of CD36/mg protein compared with that of age and sex matched controls (25.70 ± 1.09 pmol of CD36/mg protein). Despite a broad inter individual variability (Fig. 1), there was a significant difference between the val-

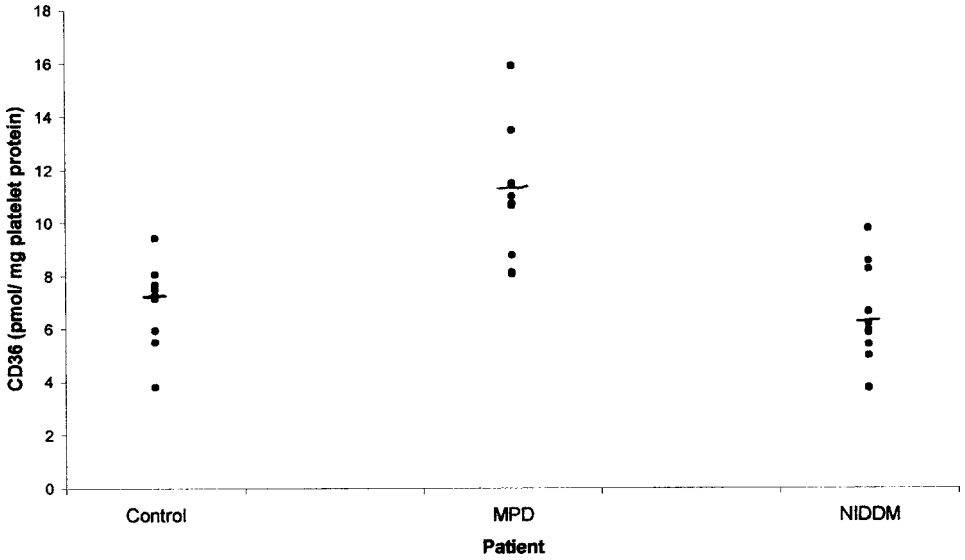

Fig. 1. Surface expression of CD36 on platelets. Each point represents a different subjects, and the mean value of each group is indicated (–). For details please see the Materials and methods section.

ues of patients (MPD and NIDDM) and normal platelets. There was no significant difference between the MPD and NIDDM platelets.

To determine whether this increase in total CD36/FAT content in patients' platelets was correlated with an enhanced surface expression of the molecule, we measured the binding of ^{125}I labelled monoclonal antibody (131.7) to intact platelets from MPD and NIDDM patients. Expression of CD36 on the platelet membrane was increased in MPD (10.94 ± 0.76 pmol/ mg protein) compared with normal controls (6.94 ± 0.48 pmol/mg protein), $p < 0.001$. However, despite increase in total CD36 molecules in NIDDM, surface expression of CD36 in platelets of NIDDM patients (6.5 ± 0.56 pmol/mg protein) was not significantly different from those of normal controls (Fig. 2).

The fatty acid composition of platelet phospholipids was given in Table 1. The level of arachidonic acid in MPD platelets (3.1 ± 0.1% of total fatty acids present in platelet membrane phospholipids) appeared to be significantly lower than in age-sex matched controls (5.6 ± 1.7% of total fatty acids present in platelet membrane phospholipids, $p < 0.001$). Whereas no significant difference was observed in arachi-

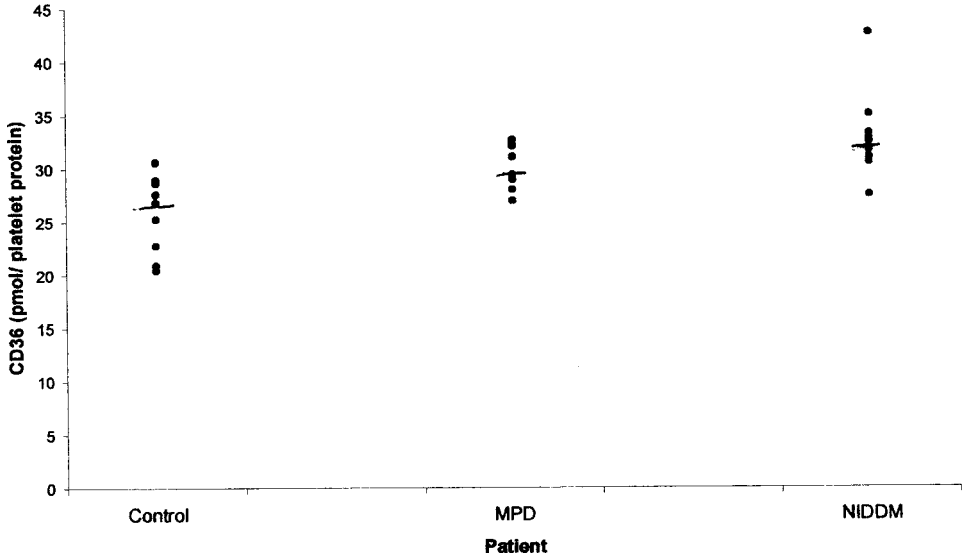

Fig. 2. Total CD36 content of platelets. Each point represents a different subjects and the mean value of each group is indicated (–). For details please see the Materials and methods section.

Table 1. Distribution of fatty acids in platelet phospholipids

% Fatty acid (FA)	Control Mean ± S.E.	MPD Mean ± S.E.	NIDDM Mean± S.E.
14:0	12.1 ± 1.2	10.2 ± 1.0†	10.6 ± 1.4
16:0	26.7 ± 2.1	32.3 ± 2.4‡	30.4 ± 2.3
18:0	14.2 ± 1.7	13.7 ± 1.5	13.2 ± 1.7
18:1 (n-9)	23.8 ± 1.5	23.1 ± 2.3	21.7 ± 2.1
18:2 (n-6)	9.5 ± 1.1	6.4 ± 0.8	7.8 ± 0.8†
18:3 (n-6)	0.9 ± 0.9	0.0 ± 0.0	0.2 ± 0.0
20:4 (n-6)	5.6 ± 1.7	3.1 ± 0.1¶	4.8 ± 1.1
20:5 (n-3)	0.7 ± 1.3	0.3 ± 0.2	1.0 ± 0.7
22:6 (n-3)	0.6 ± 0.2	0.4 ± 0.1	0.8 ± 0.4

Values are expressed as mean ± S.E.M. *p < 0.05; †p < 0.01; ‡p < 0.005; ¶p < 0.001 vs. control.

donic acid content between control and NIDDM (4.8 ± 1.1%). Similarly, myristic (14:0) and linoleic (18:2 n-6) acids were lower in MPD compared with control. Whereas palimitic (16:0) and arachidic (20:0) acids were both increased in MPD platelets (p < 0.005; p < 0.001, respectively) compared with control. No such difference was found in levels of arachidonic acid in erythrocytes between patients with MPD and controls (data not shown). The fatty acid composition of platelet membrane phospholipids is given in Table 2. Distribution of arachidonic acid in platelet membrane phospholipid classes indicated that arachidonic acid content in combined phosphatidylinositol and phosphatidylserine classes were found to be significantly lower in MPD than in controls (p < 0.001).

Arachidonic acid uptake was then determined in platelet membranes and intact cells using radiolabelled arachidonic

Table 2. Levels of arachidonic acid in platelet phospholipid fractions

	ARA (ng/mg total lipid)		
	PC	PE	PI + PS
Control	0.24 ± 0.14	0.94 ± 0.78	0.83 ± 1.03
MPD	0.17 ± 0.17	0.38 ± 0.31	0.26 ± 0.36**
NIDDM	0.40 ± 0.61	0.79 ± 0.76	0.84 ± 1.07

PC – phosphatidylcholine; PI + PS – phosphatidylinositol and phosphatidylserine; PE – phosphatidylethanolamine. Values are expressed as mean ± S.D. **p < 0.001 vs. control.

acid. Consistent with the increased surface expression of CD36, intact platelets from MPD patients bound significantly more arachidonic acid (1.53 ± 0.16 nmol/mg protein), compared with controls (1.12 ± 0.07 nmol/mg protein) or NIDDM subjects (1.16 ± 0.16 nmol/mg protein) p < 0.05. The capacity of MPD platelet membranes to bind ^{14}C-arachidonic acid was also increased (1.72 ± 0.25 nmol of ^{14}C-arachidonic acid bound per mg of protein in MPD (p < 0.05); 1.62 ± 0.05 nmol/mg protein in controls; 1.22 ± 0.08 nmol/mg protein in NIDDM) (Fig. 3).

Scatchard analysis of binding data indicate that Kd values for arachidonic acid binding to these platelets did not differ between the normal and patients platelets (0.47 ± 0.09 μM for normal platelets; 0.43 ± 0.09 μM for NIDDM platelets, and 0.57 ± 0.09 μM for MPD platelets, p > 0.5). However, B_{max} value for MPD platelets (3.08 ± 0.20 nmol/mg protein) was much higher than those of normal platelets (1.89 ± 0.13 nmol/mg protein) or NIDDM platelets (1.87 ± 0.13 nmol/mg protein, p < 0.005).

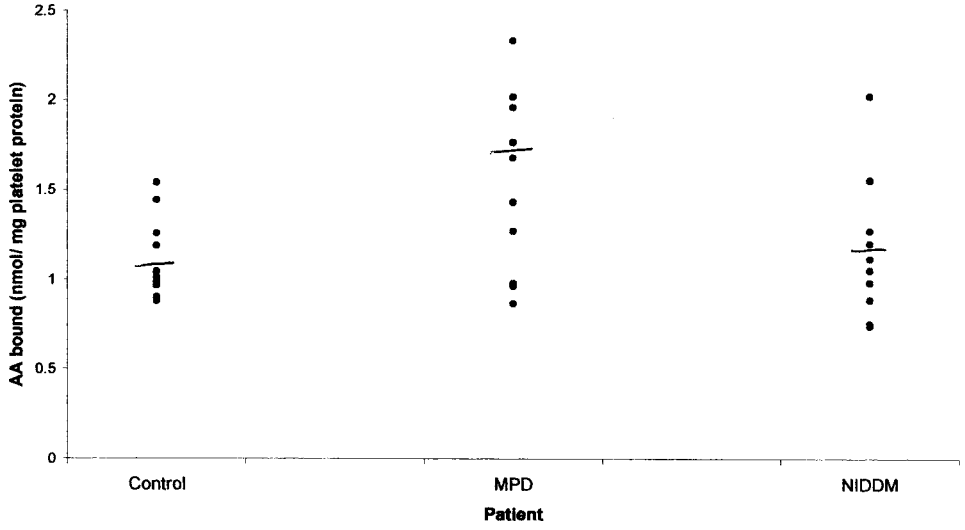

Fig. 3. Arachidonic acid uptake by platelets. Each point represents a different subjects and the mean value of each group is indicated (–). For details please see the Materials and methods section.

Discussion

Here we report that both surface expression and total content of CD36 are increased in MPD platelets compared with those of age and sex matched normal controls. In contrast total, but not surface CD36 is increased in platelets from subjects with type II diabetes. Furthermore, arachidonic acid uptake is also increased in MPD platelets, but not in diabetic platelets, in keeping with the hypothesis that the increased surface expression of CD36/FAT may be responsible for the increased uptake. The increase in CD36 surface expression in MPD platelets previously been reported by Thiebert *et al.* [8]. The extent of surface expression of CD36 may be called into question in the aforementioned study as the antibody, FA6-152, may induce platelet activation (Tandon, 1999, personal communication), which may lead to increased CD36 surface expression from internal pools [10]. The antibody used in this study (MoAb 131.7) does not activate platelets but inhibits platelet adhesion to type 1 and insoluble collagen [28, 29]. The use of MoAb 131.7 in this study, allows a more accurate measurement of CD36. Cellular fatty acid uptake is mediated in part by multiple membrane-associated transport or binding proteins (FABPpm, FAT/CD36, FATP) [13]. Some cell types such as cardiomyocyte, adipocyte or placental trophoblast have multiple membrane-associated transport/binding proteins, and these membrane proteins are proposed to work in tandem for effective extraction of free fatty acids from plasma [13]. In contrast, human platelets contain only one type of membrane fatty acid transporter (CD36/FAT), therefore any alteration in the expression of CD36/FAT in human platelets may have significant effect on fatty acid uptake. We have shown earlier that CD36/FAT is involved, at least in part, in the uptake of exogenously added arachidonic acid by human platelets [19]. Recent studies by Kerkhoff *et al.* [42] have also provided some evidence that the uptake of exogenous arachidonic acid by human umbilical vein endothelial cells was predominantly mediated by FAT/CD36. Irreversible aggregation of platelets depends on intake of arachidonic acid once liberated from platelets and production of its metabolite TxA_2 [20]. Therefore, the involvement of CD36 in arachidonic acid-induced platelet aggregation may be highly significant in diseases characterized by enhanced expression of CD36/FAT as well as increased arachidonic acid uptake by platelets. In a calcium-deficient state, CD36-deficient platelets exhibited a delay and decline of irreversible aggregation following agonist stimulation. Defective aggregation of CD36-deficient platelets in a calcium-deficient state seemed to be due to defective uptake of arachidonic acid [20]. In addition, uptake of oxidised LDL by platelets is also mediated by CD36 [43], and this may enhance TxA_2 synthesis by these platelets [44], as generally observed in MPD platelets. CD36 has been shown to be up-regulated in various cells via induction of a nuclear transcription factor, peroxisome proliferator activated receptor (PPAR) gamma [45]. The mechanisms involved in increased expression of CD36/FAT in platelets (de-nucleated cells) of MPD is not known at present. However, it is possible that this up-regulation of CD36 may occur during megakaryocytopoiesis. Although increased arachidonic acid uptake has been reported in diabetic platelets by several workers [23–25], we did not observe any such increase in arachidonic acid uptake, nor in the surface expression of CD36. This situation is in clear contrast to that of MPD. Whether the degree of glycaemic control could influence the results obtained for arachidonic acid uptake in our diabetic population is unknown, although intake of antioxidants and certain drugs decrease CD36/FAT levels in human macrophages [45, 46]. In NDDM platelet membrane phospholipids , no changes in the levels of arachidonic acid were observed compared with that of controls. It still remains unclear whether there is any alteration of incorporation of endogenous arachidonic acid into platelet membrane phospholipids of NIDDM. Increased [47], reduced [48] and unaltered [49] arachidonic acid levels in membrane phospholipids in NIDDM subjects have been reported.

Despite the increased uptake by MPD platelets, arachidonic acid levels in these platelets are lower. Increased consumption of arachidonic acid to support TxA_2 synthesis in MPD may have contributed to the depression of arachidonic acid in MPD platelets. Our findings of normal arachidonic acid content of corresponding erythrocyte membranes is consistent with this, as our earlier observation that normal aggregation to exogenous arachidonic acid may be accompanied by reduced aggregability and thromboxane synthesis in response to collagen in MPD [50].

Increased levels of CD36 and with increased uptake of arachidonic acid could lead to overproduction of TxA_2 in MPD. Such a mechanism might contribute to the vaso-occlusive manifestations which form part of the natural history. Increased platelet thromboxane synthesis *in vivo* in polycythemia vera and thromboxane dependent platelet activation preceding thrombosis in thrombocythaemia are consistent with putative pathogenic mechanism [4, 5]. Since CD36 is also involved in platelet aggregation and adhesion to collagen via several mechanisms in addition to its role as uptake protein for arachidonic acid increased surface expression and enhance platelet reactivity through thromboxane-independent pathways. These findings could have implications in relation to choice of platelet inhibitory medications in MPD.

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References

1. Cortelazzo S, Viero P, Barbui T: Platelets activation in myeloproliferative disorders. Thromb Haemost 45: 211–213, 1981
2. Duttaroy AK: Insulin mediated processes in platelets, monocytes/ macrophages and erythrocytes: Effects of essential fatty acid metabolism. Prost Leuk Essntl Fatty Acids 51: 385–389, 1994
3. Wehmeier A, Sudhoff T, Meierkord F: Relation of platelet abnormalities to thrombosis and hemorrhage in chronic course of myeloproliferative disorders. Eur J Haematol 45: 191–197, 1997
4. Landolfi R, Ciabattoni G, Patrignani P, Castellana MAL, Pogliani E, Bizzi B, Patrono C: Increased thromboxane biosynthesis in patients with polycythemia vera: Evidence for aspirin-suppressible platelet activation *in vivo*. Blood 80: 1965–1971, 1992
5. Van Genderen PJJ, Prins FJ, Michiels JJ, Schror K: Thromboxane-dependent platelet activation *in vivo* precedes arterial thrombosis in thrombocythaemia: As an antithrombotic agent. Br J Haematol 104: 438–441, 1999
6. Balduini CL, Bertolino G, Noris P, Piletta GC: Platelet aggregation in platelet rich plasma and whole blood in 120 patients with myeloproliferative disorders. Am J Clin Pathol 95: 82–86, 1991
7. Kaywin P, McDonough M, Insel PA, Shattil SJ: Platelet function in essential thrombocythemia. Decreased epinephrine responsiveness associated with a deficiency of platelet alfa-adrenergic receptors. N Engl J Med 299: 505–509, 1978
8. Thibert V, Bellucci S, Cristofari M, Gluckman E, Legrand C: Increased platelet CD36 constitutes a common marker in myleoproliferative disorders. Br J Haematol 91: 618–624, 1995
9. Le Blanc K, Lindahl T, Rosendahl K, Samuelsson J: Impaired platelet binding of fibrinogen due to a lower number of GPIIb/IIIa receptors in polycythemia vera. Thromb Res 91: 287–295, 1998
10. Michelson AD, Wencel-Drake JD, Kestin AS, Barnard MR: Platelet activation results in a redistribution of glycoprotein IV (CD36). Arterioscler Thromb 14: 1193–1201, 1994
11. Ford I, Malik RA, Newrick PG, Preston FE, Ward JD, Greaves M: Thromb Haemost 68: 628–633, 1992
12. Phillips DR, Charo IF, Parise LV, Fitzerglad LA:The platelet membrane glycoprotein IIb-IIIa complex. Blood 71: 831–843, 1988
13. Duttaroy AK: Cellular uptake of long-chain fatty acids: Role of membrane-associated fatty acid-binding/transport proteins. Cell Mol Life Sci 57: 1360–1372, 2000
14. Ruggeri ZM: The platelet glycoprotein Ib-IX complex. Prog Hemost Thromb 10: 35–68, 1991
15. Silverstein RL, Asch AS, Nachman RL: Glycoprotein IV mediates thrombospondin-dependent platelet-monocyte and platelet-U937 cell adhesion. J Clin Invest 84: 546–552, 1989
16. Greenwalt DE, Lipskey RH, Ockenhouse CF, Ikeda H, Tandon NN, Jamieson GA: Membrane glycoprotein CD36: A review of its role in adherence, signal transduction, and transfusion medicine. Blood 80: 1105–1115, 1992
17. Abumrad NA, El-Maghrabi MR, Amri EZ, Lopez E, Grimaldi PA: Cloning of a rat adipocyte membrane-protein implicated in binding or transport of long chain fatty acids that is induced during preadipocyte differentiation: Homology with human CD36. J Biol Chem 268: 17665–17668, 1993
18. Greenwalt DE, Scheck SH, Rhinehart T: Heart CD36 expression is increased in murine models of diabetes and in mice fed a high fat diet. J Clin Invest 96: 1382–1388, 1995
19. Duttaroy AK, Gordon MJ, Campbell FM, Crosbie L: Arachidonic acid uptake by human platelets is mediated by CD36. Platelets 7: 291–295, 1996
20. Ikeda H: Platelet membrane CD36. Hokkaido Igaku Zasshi 74: 99–104, 1999
21. Arita H, Nakano T, Hanasaki K: Thromboxane A_2 – its generation and role in platelet activation. Prog Lipid Res 28: 273–301, 1989
22. Duttaroy AK, Ray TK, Sinha AK: Prostacyclin stimulation of the activation of blood coagulation factor X by platelets. Science 231: 385–388, 1986
23. Perez C, Ramiro JM, Campillo JE: Increased arachidonic acid uptake by platelets from insulin-dependent diabetics and diabetic rats. Diabet Res Clin Prac 7: 69–77, 1989
24. Mikhailidis DP, Jeremy JY, Barradas MA, Dandona P: Increased arachidonic acid incorporation into platelet phospholipids in type-2 (non-insulin-dependent) diabetes. Diabetologia 27: 242–248, 1984
25. Prisco D, Rogasi PG, Pannicia R, Abbate R, Gensini GF, Pinto S, Vanni D, Neri Seneri GG: Altered membrane fatty acid composition and increased thromboxane A_2 generation in platelets from patients with diabetes. Prost Leuk Essen Fatty Acids 35: 15–33, 1989
26. Kieffer N, Bettaieb A, Legrand C, Coulombel L, Vainchenker W, Edelman L, Breton-Gorius J: Developmentally regulated expression of a 78 Kda erythoblast membrane glycoprotein immunologically related to the platelet thrombospondin receptor. Biochem J 262: 835–842, 1988
27. Beiso P, Pidard D, Fournier D, Dubernard V, Legrand C: Studies on the interaction of platelet glycoprotein IIb-IIIa and glycoprotein IV with fibrinogen and thrombospondin; a new immunochemical approach. Biochim Biophys Acta 1033: 7–12, 1990
28. Matsuno K, Diaz-Ricart M, Montgomery RR, Aster RH, Jamieson GA, Tandon NN: Inhibition of platelet adhesion to collagen by monoclonal anti-CD36 antibodies. Br J Haematol 92: 960–967, 1996
29. Diaz-Ricart M, Tandon NN, Gomezoritz G, Carretero M, Escolar G, Ordinas A, Jamieson GA : Antibodies to CD36 (GPIV) inhibit platelet-adhesion to sub-endothelial surfaces under flow conditions. Arterioscler Thromb Vasc Biol 16: 883–888, 1996
30. Tandon NN, Kralisz U, Jamieson GA: Identification of glycoprotein IV (CD36) as a primary receptor for platelet collagen adhesion. J Biol Chem 264: 7576–7583, 1989
31. Campbell FM, Bush P, Veerkamp JH, Duttaroy AK: Distribution of membrane associated and cytoplasmic fatty acid-binding proteins in human placenta. Placenta 19: 409–415, 1998
32. Campbell FM, Duttaroy AK: Plasma membrane fatty acid-binding protein (FABPpm) is exclusively located in the maternal facing membranes of the human placenta. FEBS Letts 375: 227–230, 1995
33. Campbell FM, Gordon MJ, Duttaroy AK: Plasma membrane fatty acid binding protein from human placenta: Identification and characterization. Biochem Biophys Res Commun 209: 1011–1017, 1995
34. Bradford MM: A rapid and sensitive method for the quantitation of microgramme quantitites of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248–254, 1976
35. Legrand C, Dubernard V, Kieffer N, Nurden AT: Use of a monoclonal antibody to measure the surface expression of thrombospondin following platelet activation. Eur J Biochem. 171: 393–399, 1988
36. Legrand C, Pidart D, Beiso P, Tenza D, Edelman L: Interaction of monoclonal antibody to glycoprotein IV (CD36) with human platelets and its effect on platelet function. Platelets 2: 99–105, 1991
37. Duttaroy AK, Sinha AK: Purification and properties of prostaglandin E_1/prostacyclin receptors of human blood platelets. J Biol Chem 262: 12685–12891, 1987
38. Yamamoto N, Ikeda H, Tandon NN, Herman J, Tomiyama Y, Mitani T, Sekiguchi S, Lipskey R, Kralisz U, Jamieson GA: A platelet membrane glycoprotein (GP) deficiency in healthy blood donors: NAK[a] platelets lack detectable GPIV (CD36). Blood 76: 1698–1703, 1990
39. Bligh EG, Dyer WJ: A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37: 911–917, 1959
40. Crabtree J, Gordon MJ, Campbell FM, Duttaroy AK: Differential distribution and metabolism of arachidonic acid and docosahexaenoic

acids by human placental cells. Mol Cell Biochem 185: 205–212, 1998
41. Major C, Wolf BA: Quantitation of the fatty acids composition of phosphatidic acid by capillary gas chromatography electron capture detection with picomole sensitivity. J Chromat B 658: 233–240, 1994
42. Kerkhoff C, Sorg C, Tandon NN, Nacker W: Interaction of S100A8/S100A9-arachidonic acid complexes with the scavenger receptor CD36 may facilitate fatty acid uptake by endothelial cells. Biochem 40: 241–248, 2001
43. Volf I, Moeslinger T, Cooper J, Schmid W, Koller E: Human platelets exclusively bind oxidised low density lipoprotein showing no specificity for acetylated low density lipoprotein. FEBS Lett 449: 141–145, 1999
44. Mahfouz MM, Kummerow FA: Oxidised low-density lipoprotein (LDL) enhances thromboxane A2 synthesis by platelets, but lysolecithin as a product of LDL oxidation has an inhibitory effect. Prost Other Lipid Mediat 62: 183–200, 2000
45. Chawla A, Boisvert WA, Lee CH, Laffitte BA, Barak Y, Joseph SB, Lia D, Nagy L, Edwards PA, Curtiss LK, Evans RM, Tontonoz P: A PPAR gamma-LXR-ABCA1 pathway in macrophages is involved in cholesterol efflux and atherogenesis. Mol Cell 7: 161–171, 2001
46. Devraj S, Hugou I, Jialal I: α-Tocopherol decreases CD36 expression in human monocyte-derived macrophages. J Lipid Res 42: 521–527, 2001
47. Kalofoutis A, Lekakis J: Changes of platelet phospholipids in diabetes mellitus. Diabetologia 21: 540–543, 1981
48. Jones DB, Carter RD, Haitas B, Mann JI: Low phospholipid arachidonic acid values in diabetic platelets. Br Med J 286: 173–175, 1983
49. Nordy A, Rodset JM: Platelet phospholipids and their function in patients with juvenile diabetes and maturity onset diabetes. Diabetes 19: 698–702, 1970
50. Castaldi PA, Brendt MC, Booth W, Bull H, Greaves M: Evidence for a platelet membrane defect in the myeloproliferative syndromes. Thromb Res 27: 601–609, 1982