

# Blood Glucose and Platelet-Dependent Thrombosis in Patients With Coronary Artery Disease

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**OBJECTIVES** To investigate the influence of blood glucose on platelet-dependent thrombosis (PDT).

**BACKGROUND** Elevated blood glucose is a predictor of adverse cardiovascular risk independent of a diagnosis of diabetes, possibly due to adverse effects promoting thrombosis. The effects of blood glucose on PDT have not been characterized.

**METHODS** An ex vivo extracorporeal perfusion protocol was used to measure PDT in 42 patients with stable coronary artery disease (CAD). The Badimon chamber was perfused with unanticoagulated venous blood and PDT evaluated using computerized morphometry. Whole blood impedance aggregometry and flow cytometry evaluated platelet aggregation and P-selectin expression, respectively.

**RESULTS** Using a multivariate stepwise regression model, blood glucose was the best independent predictor of PDT ( $R^2 = 0.19$ ,  $p < 0.008$ ), followed by apolipoprotein B ( $R^2 = 0.18$ ,  $p = 0.002$ ) and intracellular magnesium levels ( $R^2 = 0.12$ ,  $p = 0.02$ ). Platelet-dependent thrombosis was significantly greater in patients with blood glucose  $>$ , compared with  $\leq$ , the median value of 4.9 mmol/l ( $159 \pm 141$  vs.  $67 \pm 69 \mu\text{m}^2/\text{mm}$ ,  $p < 0.01$ ). Neither platelet aggregation nor P-selectin expression was significantly different between the two groups. Insulin levels correlated with blood glucose ( $r = 0.56$ ,  $p = 0.0003$ ), but were not independently associated with either PDT, platelet aggregation or P-selectin expression. A two-way analysis of variance demonstrated an interaction between insulin ( $>126 \text{ pmol/l}$ ) and blood glucose ( $>4.9 \text{ mmol/l}$ ) in modulating PDT ( $F [1,38] = 8.5$ ,  $p < 0.006$ ).

**CONCLUSIONS** Blood glucose is an independent predictor of PDT in stable CAD patients. The relationship is evident even in the range of blood glucose levels considered normal, indicating that the risk associated with blood glucose may be continuous and graded. These findings suggest that the increased CAD risk associated with elevated blood glucose may be, in part, related to enhanced platelet-mediated thrombogenesis. (J Am Coll Cardiol 2000;35:300–7) © 2000 by the American College of Cardiology

Diabetes mellitus is associated with increased incidence of cardiovascular events (1–3), with atherosclerotic coronary artery disease (CAD) accounting for most of the associated morbidity and mortality (4). The enhanced CAD risk in diabetes appears to be related to elevated blood glucose levels (5) or increased plasma insulin levels (6). Recent epidemiologic evidence suggests that, similar to CAD risk associated with increasing cholesterol concentrations or

systolic blood pressure, risk associated with blood glucose may also be continuous and graded, with increased risk evident even in the normal “nondiabetic” range (5).

Biological mechanisms to explain the enhanced CAD risk in diabetes are less well explored. In particular, the hypothesis that diabetes may increase CAD risk due to an enhanced tendency to intravascular thrombosis is untested (7–9). Because platelets play a key role in acute vascular thrombosis, the increased risk of cardiovascular events among diabetic patients may be, in part, due to altered platelet function. Evidence for increased platelet adhesion and aggregation (10–12), platelet-dependent thrombin generation (13,14) and impaired platelet-mediated vasodilation (15) has been demonstrated in diabetics. Alteration of platelet function in diabetes appears to be mediated by elevated blood glucose (16).

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Manuscript received July 27, 1998; revised manuscript received August 16, 1999, accepted October 18, 1999.

#### Abbreviations and Acronyms

ADP	= adenosine diphosphate
ANOVA	= analysis of variance
BMI	= body mass index
CAD	= coronary artery disease
CHD	= coronary heart disease
FBG	= fasting blood glucose
HDL-C	= high-density lipoprotein cholesterol
LDL-C	= low-density lipoprotein cholesterol
NYHA	= New York Heart Association
PDT	= platelet-dependent thrombosis

Despite the evidence for the link between elevated blood glucose and altered platelet function, little is known about the role of blood glucose and plasma insulin levels in platelet-mediated thrombogenesis. Thus, the aim of this study was to explore the relationship between platelet-dependent thrombosis (PDT) and blood glucose. We used an ex vivo model to examine platelet-thrombus formation in stable CAD patients. We hypothesized that blood glucose was positively and independently correlated with PDT and that this association was likely to be evident even at levels considered within the normal "nondiabetic" range.

## METHODS

**Study design and population.** Patients were recruited from a supervised cardiac exercise program at Cedars-Sinai Medical Center for a randomized trial of oral magnesium treatment. None of the patients had received treatment with magnesium at the time of collection of these data. Study inclusion criteria included men and women age  $>20$  years, with CAD documented by prior myocardial infarction, coronary artery bypass surgery operation or coronary angiography or angioplasty. Exclusion criteria included unstable angina, contraindication to exercise testing, congestive heart failure  $>$  New York Heart Association (NYHA) class IV, chronic diarrhea, renal failure (serum creatinine  $> 265 \mu\text{mol/l}$ ), acute myocardial infarction  $<3$  months, hyper/hypothyroidism, type I diabetes mellitus, clinically-evident peripheral vascular disease, history of drug or alcohol abuse, chronic liver disease or refusal to sign the informed consent. The institutional review board approved the study, and all participants gave written informed consent.

After an overnight fast, the patients underwent a physical examination, blood tests for measurement of PDT, platelet aggregation, flow-cytometry, blood glucose, insulin, lipids, blood cell count, electrolytes, apolipoprotein A-I, apolipoprotein B and fibrinogen, as part of their baseline assessment prior to entry into the randomized trial.

**Ex vivo assay for platelet-dependent thrombosis.** A 19-gauge butterfly catheter was inserted atraumatically without a tourniquet into an antecubital vein. Flowing unanticoagu-

lated venous blood from the patient was drawn over a segment of prepared porcine aortic media held in a tubular superfusion flow chamber, by a peristaltic pump placed distal to the chamber. The chamber was designed to mimic the cylindrical shape of blood vessels and contained a window that permitted direct exposure of the aortic media to the flowing venous blood (17,18). A perfusion chamber with an internal diameter of 1.0 mm was selected to generate shear rates of  $800 \text{ s}^{-1}$ , at a flow rate of 5 ml/min for 5 min. This shear rate corresponds to that encountered in mild to moderately stenosed arteries. The aortic media used in the superfusion chamber was obtained from normal pigs by opening the aorta longitudinally and peeling off and discarding the intima and a thin portion of the subjacent media. The remaining aortic media was then divided into  $35 \times 15$  mm segments to be placed inside the superfusion flow chamber to be exposed to flowing blood in the chamber. Exposure of the arterial media simulates a deep arterial wall injury with a thrombogenic response similar to that encountered during plaque rupture.

After the perfusion, the aortic media strips were removed from the chambers, fixed in 2% glutaraldehyde in 2 M sodium cacodylate and processed for morphological analysis. The tissues were stained with hematoxylin-phloxine-safranin. The stained histological tissue was then analyzed under a light microscope, and platelet thrombus formation on the aortic media was quantified morphometrically by computer-assisted morphometry, using image analysis software (Bioscan, Optimas, Washington). All measurements were made by two different observers who were blinded to the patients' blood glucose, and intraassay variability was  $5.5 \pm 5.6\%$ . Thrombus size measurements were expressed as the average of six analyzed sections per tissue (two in the proximal, two in the middle and two in the distal section) expressed as the surface area in square micrometers and normalized to the cross-sectional diameter of the exposed media (in millimeters). This morphometric method has been previously validated and shows a strong correlation ( $r = 0.84$ ,  $p = 0.0001$ ) between the amount of indium-labeled platelets deposited on the media and the morphometrically assessed thrombus size (19).

**Platelet aggregation and P-selectin flow cytometry.** Citrated whole blood samples (4.5 ml) were collected from the patients before starting the ex vivo thrombosis experiment, and these were diluted with an equal volume of isotonic saline. Collagen (2  $\mu\text{g/ml}$ )-induced whole blood platelet aggregation was measured by impedance aggregometry (20). Platelet aggregation was measured as the maximal change in impedance produced 6 min after the addition of collagen and expressed in ohms and the rate (slope) expressed in ohms/min.

Platelet alpha-granule release, detected by expression of P-selectin (the CD 62P antigen), was measured by whole blood flow cytometry. Blood samples were prepared for flow-cytometric analysis using the whole blood method

**Table 1.** Baseline Characteristics of Study Population (n = 42)

CAD Risk Factors	n (%)	Concomitant Medication	n (%)
Diabetes mellitus	2 (5)	Beta-blocking agents	15 (36)
Hypertension	21 (50)	Calcium antagonists	14 (36)
Current smokers	0	Hypoglycemic agents	2 (5)
Hypercholesterolemia	27 (64)	Lasix	5 (12)
		Digoxin	4 (26)
		Aspirin	42 (100)
		Long-acting nitrates	4 (26)
		ACE inhibitors	12 (29)
		Lipid lowering agents	27 (64)

ACE = angiotensin converting enzyme; CAD = coronary artery disease.

described by Janes et al. (21). Five microliters of citrated blood was added to tubes containing 50  $\mu$ l of Hepes-buffered saline (NaCl 0.145 mol/l; KCl 5 mmol/l; MgSO<sub>4</sub> 1 mmol/l; Hepes 10 mmol/l; pH 7.4) plus 5  $\mu$ l of fluorescein isothiocyanate- and phycoerythrin-conjugated anti-human monoclonal antibodies (Becton Dickinson, Immunocytometry Systems, San Jose, California). After gentle mixing, the samples were incubated for 20 minutes then diluted with 0.5 ml of 0.2% formyl saline to inhibit further activation. A sample incubated with FITC-conjugated irrelevant mouse IgG<sub>1</sub> at identical concentration served as negative isotype control. Incubations were carried out at room temperature (22°C to 26°C). All samples were analyzed within 1 h of collection in a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, California) with LYSIS II software. The platelet population was identified based on their forward- (size) and side-(granularity) scatter characteristics and the expression of GPIIIa (FITC-CD61 profile), a membrane protein present exclusively on all resting and activated platelets. Activated platelets were identified based on the expression of P-selectin (PE-CD62 profile), a platelet alpha-granule protein which is only expressed on the platelet surface following activation and degranulation. Platelet CD62P expression was measured at baseline and following stimulation with adenosine diphosphate (ADP) (5  $\mu$ mol/l) (Chronolog Corp., Havertown, Pennsylvania). For each sample, 5,000 platelets were gated and platelet activation expressed as the number of CD-62 + ve platelets as a percentage of CD-61 + ve platelets after adjusting for nonspecific fluorescence.

**Fasting blood glucose, insulin, blood cell count and electrolytes.** Overnight fasting blood samples were taken for hemoglobin, total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), very low-density lipoprotein cholesterol, triglycerides, apolipoprotein A-I, apolipoprotein B and fibrinogen using standard autoanalyzer techniques. Platelet count was measured from the whole blood by autonalyzer, using GENS coulter counter. Fasting plasma glucose was measured with a hexokinase reagent kit (A-gent glucose test,

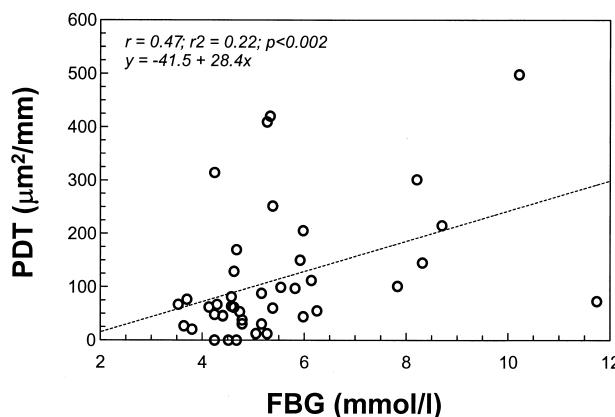
Abbott, South Pasadena, California). Glucose assays were run in duplicate, and the intraassay coefficient of variation ranged from 2% to 3%, depending on the assayed glucose level. Fasting insulin was measured in plasma as total immunoreactive insulin (Coat-A-Cout Insulin, Diagnostic Products Corp., Los Angeles, California) and calibrated to serum levels for reporting purposes (normal level <126 pmol/l). Cross-reactivity of this assay with proinsulin at midcurve is approximately 40%, the intraassay and interassay coefficients of variation ranged from 5.0% to 10.0% for insulin concentrations reported here and the lower limit of sensitivity was 8 pmol/l.

**Intracellular magnesium levels.** Mononuclear cells were isolated from the whole blood by modified Elin's method (22). The intracellular levels of magnesium in isolated mononuclear cells were measured by atomic absorption spectrophotometry (normal value: 1.23  $\pm$  0.02  $\mu$ g/mg protein) (23). Intraassay variability for intracellular magnesium was 5.1  $\pm$  7.2%, interassay variability was 7  $\pm$  5%.

**Statistical analysis.** Group data are expressed as mean  $\pm$  SD. Comparison of biochemical measurements was performed using the unpaired Student *t* test and Wilcoxon signed-rank test. The relationship between baseline characteristics and blood glucose concentration with PDT was performed using the Spearman's Rank correlation, as appropriate. Log transformations were used to normalize data for regression analysis. Predictors of PDT were first determined using univariate linear regression and only statistically significant univariate predictors of PDT were entered into multiple stepwise regression analysis. A *p* value  $<0.05$  was required to reject the null hypothesis.

## RESULTS

Our study population was comprised of 42 stable coronary patients (37 men and 5 women), with a mean age of 68  $\pm$  9 years (range 48–83) and mean body mass index (BMI) of 25  $\pm$  4 kg/m<sup>2</sup> (range 20–37). All patients had stable CAD (Table 1) documented by absence of rest pain, crescendo or new onset angina or unstable angina. Coronary artery disease was evidenced by a previous myocardial infarction



**Figure 1.** Correlation of fasting blood glucose and platelet-dependent thrombosis demonstrating a linear correlation ( $n = 42$ ). The correlation was unchanged when the two outlier points (glucose = 9.9 and 12.2 mmol/l) or the two diabetic patients were excluded. FBG = fasting blood glucose; PDT = platelet-dependent thrombosis.

( $n = 23$ ), coronary artery bypass surgery ( $n = 26$ ) or coronary angioplasty ( $n = 23$ ) (25 patients had multiple diagnoses). Among the patients, 2/42 (5%) were diabetic on oral agents (glyburide and metformin hydrochloride), 8/42 (19%) had blood glucose above the "normal" range ( $>6.1$  mmol/l) and 16/42 (38%) had elevated insulin levels ( $>126$  pmol/l). Overall, 7/42 (17%) demonstrated the dysmetabolic triad of hypertension, obesity (BMI  $> 27$  kg/m<sup>2</sup>) and insulin levels  $>126$  pmol/l.

Correlation analysis demonstrated that blood glucose was significantly and positively correlated with PDT (Fig. 1). This correlation remained significant following exclusion of the two diabetic patients ( $r = 0.41$ ,  $p = 0.01$ ). Platelet-dependent thrombosis also correlated inversely with intracellular magnesium ( $r = -0.46$ ,  $p = 0.003$ ) and positively with total cholesterol ( $r = 0.34$ ,  $p = 0.04$ ). Insulin was positively correlated with blood glucose ( $r = 0.56$ ,  $p < 0.001$ ) but not with PDT ( $r = 0.19$ ,  $p = 0.26$ ). Neither platelet aggregation nor CD 62P expression correlated with either blood glucose or PDT (data not shown).

A univariate linear regression analysis assessed the effects of various covariates on PDT. The variables tested included glucose, insulin, apolipoprotein B, LDL-C, total cholesterol, triglycerides, HDL-C, platelet count, platelet aggregation, P-selectin expression (with and without ADP stimulation) and intracellular magnesium levels. Only blood glucose, intracellular magnesium levels, apolipoprotein B, LDL-C and total cholesterol were statistically significant predictors of PDT. Following adjustment in a multivariate stepwise regression model, blood glucose was the best predictor of PDT ( $R^2 = 0.19$ ,  $p < 0.008$ ), followed by apolipoprotein B ( $R^2 = 0.18$ ,  $p = 0.002$ ) and intracellular magnesium levels ( $R^2 = 0.12$ ,  $p = 0.02$ ). Following exclusion of the two diabetic patients, blood glucose re-

mained a significant predictor of PDT in a stepwise regression model ( $R^2 = 0.09$ ,  $p = 0.03$ ).

When stratified according to those equal or below versus above the median blood glucose level of 4.9 mmol/l, the two groups were similar with respect to male gender, age, BMI, use of medications and the presence of risk factors for CAD. The two groups were also similar with regard to the baseline lipid values as well as fibrinogen and platelet count (Table 2) although apolipoprotein B and insulin were significantly higher in the group with higher fasting glucose levels. Platelet-dependent thrombosis was significantly greater in patients with blood glucose  $>$  compared with  $\leq 4.9$  mmol/l ( $159 \pm 141$  vs.  $67 \pm 69$   $\mu\text{m}^2/\text{mm}$ ,  $p < 0.01$ , respectively) (Fig. 2 and 3). Only 3/42 (7%) of patients with blood glucose  $\leq 4.9$  mmol/l had PDT above the mean value of  $111 \pm 118$   $\mu\text{m}^2/\text{mm}$ . Neither platelet aggregation, nor platelet CD62P expression, was significantly different between the two groups (Fig. 2). Assessment of the platelet parameters according to fasting blood glucose  $\geq 6.1$  mmol/l ( $n = 8$ ) also demonstrated no significant differences.

The data were further analyzed by arranging the blood glucose values in quartiles and determining their relationship with PDT values that were normalized by log-transformation. The results in Table 3 show a continuous and graded relationship between the two variables that was nearly statistically significant ( $F$  value of 2.66,  $p = 0.06$ , analysis of variance [ANOVA]). Subjects in the lowest blood glucose quartile demonstrated the least amount of PDT. Conversely, subjects in the highest blood glucose quartile had the highest PDT.

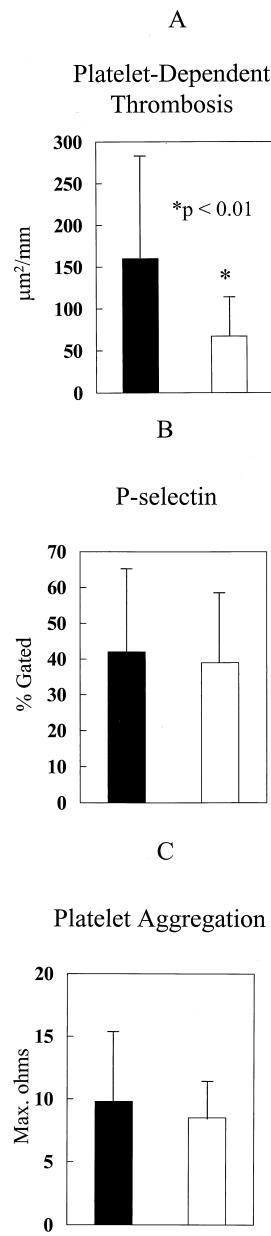
To examine a potential interaction between blood glucose and insulin levels in modifying PDT, we performed a two-way ANOVA, where patients were divided into four groups according to the median fasting blood glucose (FBG) value of 4.9 mmol/l and normal plasma insulin value

**Table 2.** Laboratory Variables Stratified by Fasting Blood Glucose

Variable	Fasting Blood Glucose (mmol/l)		p Value
	$\leq 4.9$ (n = 21)	$> 4.9$ (n = 21)	
T-Cholesterol (mmol/l)	$4.29 \pm 0.72$	$4.55 \pm 0.64$	0.25
LDL-C (mmol/l)	$2.50 \pm 0.67$	$2.79 \pm 0.64$	0.18
HDL-C (mmol/l)	$1.10 \pm 0.20$	$1.0 \pm 0.20$	0.19
TG (mmol/l)	$3.43 \pm 2.12$	$4.21 \pm 3.36$	0.23
Apo A-I (mmol/l)	$3.36 \pm 0.49$	$3.15 \pm 0.28$	0.22
Apo B (mmol/l)	$2.22 \pm 0.72$	$2.40 \pm 0.49$	$< 0.05$
Fibrinogen (mmol/l)	$7.42 \pm 0.90$	$7.88 \pm 1.99$	0.49
PLT count ( $\times 10^3/\mu\text{L}$ )	$198 \pm 50$	$195 \pm 50$	0.79
Glucose (mmol/l)	$4.4 \pm 0.3$	$6.7 \pm 1.8$	$< 0.0001$
Insulin (pmol/l)	$108 \pm 66$	$168 \pm 66$	0.01

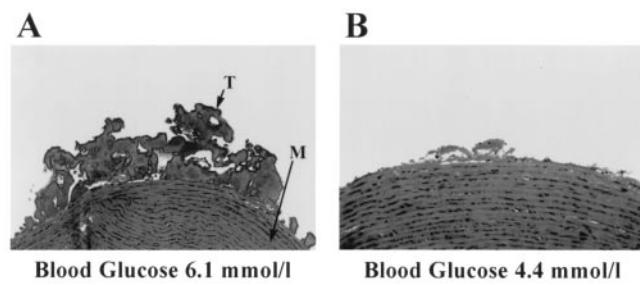
Values are expressed as mean  $\pm$  SD.

Apo = apolipoprotein; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; PLT = platelets; T-Cholesterol = total cholesterol; TG = triglycerides.



**Figure 2.** Bar graphs showing (A) platelet-dependent thrombosis, (B) P-selectin (CD62 antigen) expression and (C) platelet aggregation in patients with fasting blood glucose  $\leq$  (open bar) and  $>$  (closed bar) 4.9 mmol/l. Data are expressed as mean  $\pm$  SD.

of 126 pmol/l. A significant interaction between insulin and blood glucose ( $F = 8.5$ ,  $p < 0.006$ ) was demonstrated. The PDT values were the highest in the group with FBG  $>$  4.9 mmol/l plus insulin levels  $> 126$  pmol/l (Fig. 4). Additional analyses provide support in favor of this interaction. Comparison of regression lines (between PDT and FBG) revealed a significant relationship only in patients with insulin levels above normal ( $r^2 = 0.33$ ,  $p = 0.005$ ) but not in patients with insulin levels below normal where the



**Figure 3.** Representative histological sections (hematoxylin-phloxine-safranin stain) showing the platelet-dependent thrombosis deposited (T) on porcine aortic media (M) taken from two patients: (A) a patient with blood glucose of 6.1 mmol/l, with a large thrombus area, and (B) a patient with blood glucose of 4.4 mmol/l and a smaller thrombus area.

line was virtually flat (Fig. 5A). The ratio of plasma insulin to FBG was found to have the strongest correlation with PDT ( $r^2 = 0.77$ ,  $p < 0.0001$ ) among all variables tested (Fig. 5B). Thus, these data indicate a significant interaction between insulin and blood glucose in modulating PDT.

## DISCUSSION

Our study is the first to explore the relationship of blood glucose in PDT in an ex vivo model of thrombogenesis and test this relationship across a range of blood glucose values, including a normal “nondiabetic” range. The three principal findings of this study are:

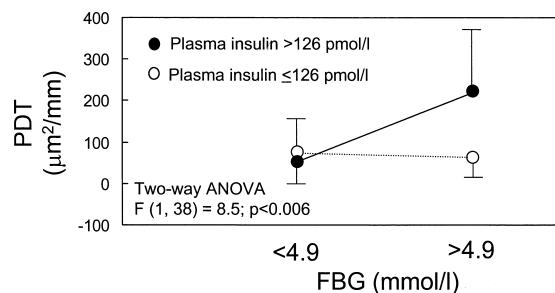
- 1) FBG is an independent predictor of PDT in stable CAD patients,
- 2) the relationship between blood glucose and PDT is continuous and graded and evident even in the range of blood glucose levels considered normal, and
- 3) plasma insulin levels do not independently predict PDT but may interact with blood glucose in modulating PDT.

Angiographic and necropsy studies show that patients with diabetes, especially type II diabetes mellitus, have more extensive and severe CAD than those without diabetes (7–9). Plaque rupture with intracoronary thrombosis is increased, as are subsequent cardiac events. Evidence for increased platelet adhesion and aggregation (10–12), platelet-dependent thrombin generation (13,14) as well as

**Table 3.** Relationship Between Blood Glucose and PDT Arranged in Quartiles

Quartile	Blood Glucose (mmol/l)	Log PDT
1 <sup>st</sup> (n = 10)	< 4.4	1.75 $\pm$ 0.32
2 <sup>nd</sup> (n = 8)	4.4–4.8	1.83 $\pm$ 0.25
3 <sup>rd</sup> (n = 11)	4.9–5.8	1.91 $\pm$ 0.33
4 <sup>th</sup> (n = 10)	5.9–11.7	2.14 $\pm$ 0.31

Analysis of variance,  $F = 2.66$ ,  $p = 0.06$ . PDT = platelet-dependent thrombosis.



**Figure 4.** Plot showing interaction between FBG and plasma insulin in modifying platelet-dependent thrombosis. Patients were divided into four groups:

- 1) FBG  $\leq$  4.9 mmol/l plus insulin levels of  $\leq$  126 pmol/l (n = 12);
- 2) FBG  $\leq$  4.9 mmol/l plus insulin levels of  $>$  126 pmol/l (n = 9);
- 3) FBG  $>$  4.9 mmol/l plus insulin levels of  $\leq$  126 pmol/l (n = 8); and
- 4) FBG  $>$  4.9 mmol/l plus insulin levels of  $>$  126 pmol/l (n = 13).

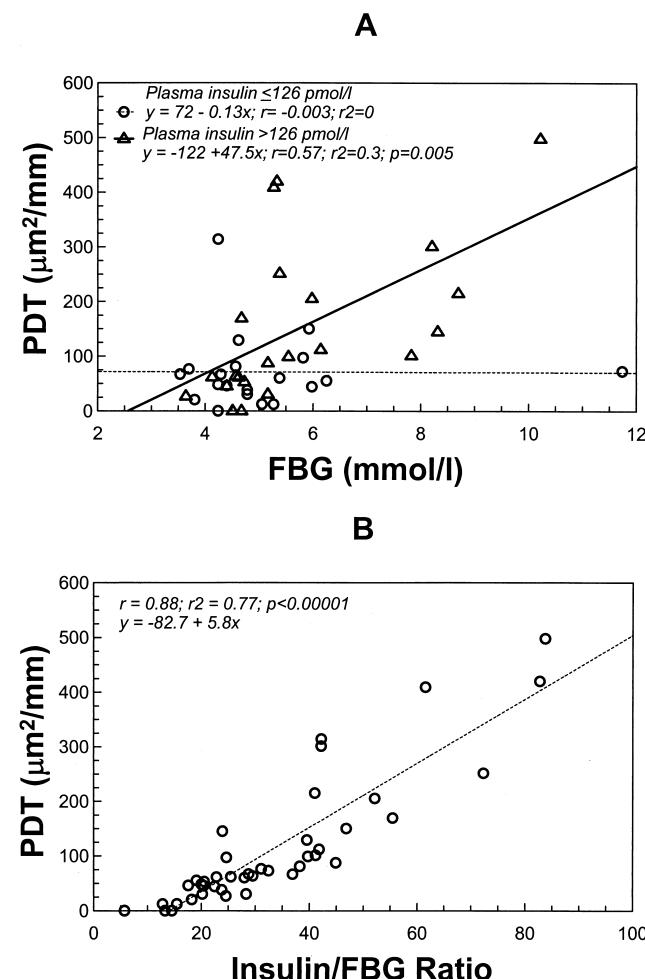
Data are expressed as mean  $\pm$  SD (one-sided error bars). FBG = fasting blood glucose; PDT = platelet-dependent thrombosis.

impaired platelet-mediated vasodilation (15) has been demonstrated in diabetics. Platelet-dependent thrombin generation and coagulation factors including von Willebrand factor, factor VII, factor VIII and fibrinogen are significantly enhanced in diabetes mellitus (24). Furthermore, plasma concentrations of plasminogen activator inhibitor 1 are increased in hyperinsulinemia, insulin-resistant states and type II diabetes mellitus and may account for the decrease in fibrinolysis (25). Alteration of platelet and coagulation function appears to correlate with elevated blood glucose concentration in these patients (16).

Although elevated blood glucose, the hallmark of diabetes, has been incriminated in the adverse cardiovascular events, recent evidence is emerging that suggests that the association between elevated blood glucose and coronary heart disease (CHD) risk may not be confined to the discrete categories of impaired glucose tolerance or type II diabetes mellitus. In the Framingham Offspring Study (26), blood glucose was correlated with the risk of CHD in 2,853 patients not previously diagnosed with diabetes. In addition, fasting blood glucose was found to be an independent predictor for cardiovascular mortality in 2,014 healthy men 40–49 years of age (5). Our results are also consistent with these observations and suggest that risk associated with blood glucose and CHD risk may be continuous and graded. As suggested by the interaction data, this relationship is evident even at levels considered within the normal range, especially if associated with elevated plasma insulin levels.

Previous work has also demonstrated that platelets from diabetic patients exhibit an enhanced adhesiveness and hyperaggregability in response to both strong (thromboxane A<sub>2</sub>, collagen, epinephrine) and weak (adenosine diphosphate) agonists (10,11). Platelet hyperaggregability has also

been shown to correlate with increased cardiovascular events in diabetic patients (16). Platelet hyperaggregability has been observed in newly diagnosed as well as chronic diabetics, suggesting that the altered platelet function may be a consequence of metabolic changes rather than the diabetes per se (10,12). In this study, platelet aggregation was not significantly different when patients were stratified by above and below the median glucose of 4.9 mmol/l, a threshold glucose of  $\geq$  6.1 mmol/l or grouped according to the presence of diabetes. Several factors may have contributed to these conflicting results. First, all of the current study patients were on aspirin therapy, which is known to suppress platelet aggregation but not platelet adhesion (27). Second, our PDT studies were performed ex vivo with unanticoagulated blood, while the previously cited aggregation studies (10–12,16) were performed in vitro using citrated blood. Thus, the enhanced platelet adhesion effects of elevated glucose may have been detectable by our ex vivo



**Figure 5.** (A) Comparison of regression lines in patients with plasma insulin levels below or above normal (126 pmol/l). (B) Correlation between the ratio of plasma insulin and FBG and PDT. FBG = fasting blood glucose; PDT = platelet-dependent thrombosis.

PDT formation measurement, whereas no effect was detectable using in vitro platelet aggregation and alpha-granule release reaction (P-selectin expression) measurements.

Measuring P-selectin expression in response to ADP is an indicator of platelet reactivity and does not provide direct information on the circulating activated state of platelets. However, our data on the surface expression of P-selectin without the addition of agonists did not show any direct relationship of glucose levels with platelet degranulation.

Elevated insulin levels may be an independent risk factor for CAD (6). The validity of this relationship and possible explanatory mechanism(s), however, remain speculative. In our current study, insulin levels did not predict PDT independent of glucose levels. In patients with elevated insulin levels ( $>126$  pmol/l), PDT was increased only if blood glucose levels were also increased, suggesting that the increased thrombogenicity demonstrated in this ex vivo model may be related to an interaction between insulin and blood glucose, especially at relatively higher levels.

Apolipoprotein B, the major carrier of LDL-C, was a significant and positive predictor of PDT in the regression analysis. Our demonstration of an independent predictive value for apolipoprotein B in the multivariate analysis argues against this relationship being simply a marker for insulin resistance. Indeed, the constellation of dyslipidemia, hypertension and insulin resistance first described by Reaven et al. (28) has more recently been found to be related to a single gene locus at or near the lipoprotein lipase on the short arm of chromosome 8 (29). These findings, combined with our current study results, suggest that this genetic constellation of dyslipidemia, hypertension and insulin resistance may also include prothrombosis.

**Study limitations.** We studied in a cross-sectional design a relatively small number of patients with stable CAD receiving aspirin and lipid lowering therapy and participating in a supervised cardiac exercise program. It is possible that this relatively low risk CAD population may have minimized the observed effects related to blood glucose and PDT. The mean baseline LDL-C of 2.68 mmol/l in our patients is close to the ideal goal of the National Cholesterol Education Program guidelines for patients with established CAD of  $<2.58$  mmol/l (30). Because cholesterol lowering therapy has been shown to rapidly reduce PDT (31), it is possible that the near-optimal lipid levels observed in our patients (the majority of whom were on lipid lowering therapy) may have minimized the platelet-thrombus burden, thereby reducing our ability to detect an even more pronounced effect of blood glucose.

An additional limitation is the ex vivo model used in our study in which predominantly shear-dependent acute (5 min) platelet-dependent thrombus formation was examined. Other factors that modulate thrombogenesis in vivo conditions such as coagulation factors and pro- and anti-fibrinolytic factors do not play a critical role in this model. Nonetheless, this validated model allows us to study platelet

vessel wall interactions under controlled and well-defined conditions. Although the clinical relevance of thrombus formation in this experimental model has not been clearly defined, the reproducibility and simplicity of this ex vivo system makes it a sensitive tool for study of the interaction of blood elements with thrombogenic surfaces and assessment of antithrombotic therapeutic interventions.

**Conclusions.** In summary, elevated FBG is associated with greater PDT in patients with CAD. This finding appears to be independent of the presence of diabetes or elevated insulin levels and is evident in previously considered "normal" fasting blood glucose levels. These findings suggest that the increased CAD risk with hyperglycemia may be, in part, related to enhanced thrombogenesis.

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