Inhibition of Platelet-Collagen Interaction
An In Vivo Action of Insulin Abolished by Insulin Resistance in Obesity

Jukka Westerbacka, Hannele Yki-Järvinen, Anu Turpeinen, Aila Rissanen, Satu Vehkavaara, Martti Syrjälä, Riitta Lassila

Abstract—Insulin resistance is associated with an increased risk of atherothrombotic vascular disease, but the mechanisms are poorly understood. We determined how insulin in vivo regulates platelet activation in nonobese and obese subjects by using methods mimicking thrombus formation. Twelve nonobese (aged 42 ± 2 years, body mass index 24.0 ± 0.4 kg/m²) and 14 obese (aged 43 ± 1 years, body mass index 37.2 ± 1.5 kg/m²) subjects were studied under euglycemic hyperinsulinemic (3-hour insulin infusion of 1 mU · kg⁻¹ · min⁻¹) conditions. Before and at the end of hyperinsulinemia, the following were determined: (1) platelet-related early hemostasis (shear rate of ≈4000 s⁻¹) by platelet function analysis; (2) platelet deposition to collagen during whole-blood perfusion (shear rate of 1600 s⁻¹); (3) aggregation responses to collagen, thrombin receptor–activating peptide, ADP, and epinephrine; and (4) platelet cGMP concentrations. Insulin action on glucose metabolism was 69% lower in the obese subjects (1.6 ± 0.2 mg · kg⁻¹ · min⁻¹) than in the nonobese subjects (5.4 ± 0.4 mg · kg⁻¹ · min⁻¹, P < 0.0001). The in vivo insulin infusion inhibited platelet deposition to collagen from 4.3 ± 0.6 × 10⁶ to 3.5 ± 0.4 × 10⁶ per square centimeter in the nonobese subjects (P < 0.05) but failed to do so in the obese subjects (5.2 ± 0.8 × 10⁶ versus 5.5 ± 0.7 × 10⁶ per square centimeter, P = NS; P < 0.01 versus nonobese subjects). Epinephrine- and ADP-primed closure times by platelet function analysis were prolonged by insulin in the nonobese but not the obese subjects (P < 0.05 for between-group difference). In the nonobese subjects, insulin decreased aggregation to all agonists and significantly increased platelet cGMP concentrations (2.5 ± 0.3 versus 3.2 ± 0.5 pmol/10⁶ for before versus after insulin, respectively; P < 0.01). In the obese subjects, insulin did not alter collagen-induced aggregation or cGMP concentrations (1.9 ± 0.2 versus 1.8 ± 0.1 pmol/10⁶ for before versus the end of in vivo hyperinsulinemia, respectively; P = NS). These data demonstrate that normal in vivo insulin action inhibits platelet interaction with collagen under conditions mimicking thrombus formation and reduces aggregation to several agonists. These platelet-inhibitory actions of insulin are blunted or absent in obese subjects and could provide a mechanism linking insulin resistance to atherothrombotic disease. (Arterioscler Thromb Vasc Biol. 2002;22:167-172.)

Key Words: insulin • collagen • nitric oxide • obesity • cGMP

Insulin resistance predisposes individuals to atherothrombotic vascular disease, but the mechanisms are incompletely understood. Human platelets have insulin receptors that participate in the regulation of platelet functions. In vitro and in vivo studies have demonstrated that insulin inhibits platelet aggregation in healthy nonobese subjects. In obese and hypertensive subjects, this antiaggregating effect of in vitro insulin has been shown to be blunted. These data suggest that insulin resistance also involves platelets, but it is currently unknown whether the resistance of platelets to insulin occurs in vivo and whether insulin also regulates the adhesion and activation of platelets on subendothelial matrix proteins, such as collagen, which are exposed after intravascular injury.

The interaction of platelets with collagen under flowing whole-blood conditions mimics the early steps of arterial thrombus formation in vivo and has allowed studies of the crucial adhesive platelet receptors and their activation routes. Collagen offers a natural immobilized ligand for platelets and high (>800 s⁻¹) shear rates provide additional flow-related stimulation of platelets. In the present study, we wished to examine whether insulin normally regulates adhesion-triggered platelet functions under high shear rate conditions. We determined the effect of insulin on platelet deposition to collagen during whole-blood perfusion and on primary hemostasis in epinephrine- and ADP-primed collagen cartridges. To test whether insulin influenced platelet aggregation in response to soluble agonists, we stimulated platelets with collagen, thrombin receptor–activating peptide (TRAP), ADP, and epinephrine. Furthermore, the effect of insulin on intraplatelet cGMP concentrations has been determined, inasmuch as a blunted in vitro ability of insulin to...
Baseline Characteristics of the Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nonobese Subjects</th>
<th>Obese Subjects</th>
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<tr>
<td>Male/female, n</td>
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<td>13/1</td>
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<td>Age, y</td>
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<td>BMI, kg/m²</td>
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<td>Body fat, %</td>
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<td>33 ± 2*</td>
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<tr>
<td>Waist/hip ratio</td>
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<td>1.07 ± 0.02*</td>
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<td>Systolic blood pressure, mm Hg</td>
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<td>134 ± 4†</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>74 ± 2</td>
<td>86 ± 2†</td>
</tr>
</tbody>
</table>

Data are mean ± SEM.

*P<0.001 and †P<0.01 for nonobese vs obese subjects.

Methods

Subjects and Study Design

A total of 26 healthy subjects were recruited for the present study. Twelve were nonobese with a body mass index (BMI) <25 kg/m², and 14 were obese with a BMI >30 kg/m². The physical and biochemical characteristics of the study subjects are shown in the Table. The subjects were healthy, as judged by history, physical examination, ECG, and routine laboratory tests. They were not taking any regular medications, and they denied having taken any medication during the prior 10 days. Exercise and alcohol consumption were prohibited 2 days before the study. Also, for 2 days before the study, the subjects consumed a weight-maintaining diet containing at least 200 g carbohydrate per day. There was 1 smoker in both groups, and they were advised not to smoke for 12 hours before the study. In each subject, in vivo insulin action was measured by using the euglycemic insulin clamp technique. Platelet aggregation responses and cGMP concentrations were measured in all study subjects. Whole-blood perfusion studies and platelet function analysis were performed in 11 nonobese and 8 obese subjects. This was because it was not always feasible to perform simultaneous in vivo and in vitro studies. The characteristics of the nonobese or obese subjects were similar to those of the whole group. The insulin-clamp study was performed after an overnight fast starting at 7:30 AM. Before and at the end of the insulin infusion, blood samples were taken for platelet function testing, as described in detail below. Written informed consent was obtained after the purpose, characteristics, and potential risks of the experiments had been explained to the subjects. The study protocol was approved by the Ethics Committee of the Department of Medicine, Helsinki University Central Hospital.

Insulin Action on Glucose Metabolism

Insulin action on whole-body glucose metabolism was determined under normoglycemic hyperinsulinemic conditions, which were created by using the insulin clamp technique.10 The rate of the continuous insulin infusion was 1 mU·kg⁻¹·min⁻¹. At baseline, free-flowing blood for platelet studies was collected via an 18-gauge catheter (Venflon, Viggo-Spectramed) inserted in the left antecubital vein. After blood sampling, the catheter was used for glucose and insulin infusions, and another catheter was inserted in a heated dorsal hand vein for sampling of arterialized venous blood for measurement of glucose and insulin concentrations, as previously described.11 The amount of glucose infused to maintain normoglycemia during hyperinsulinemia, which was corrected for changes in the glucose space,10 was used to measure whole-body insulin sensitivity and was expressed per kilogram of body weight, square meter of body surface area, and kilogram of fat-free mass.10 At the end of the 3-hour insulin infusion, a third catheter was freshly inserted in an intact median antecubital vein on the opposite side for a second blood sampling for platelet studies. All platelet analyses were performed within 90 minutes of blood sampling.

Whole-Blood Perfusion

Blood Preparation and Labeling of Platelets

Blood for the platelet perfusion study was collected in tubes containing 40 µmol/L (final concentration) D-phenylalanyl-1-propyl-1-arginine chloromethyl ketone (PPACK, Calbiochem, Calbiochem-Novabiochem Corp). Platelet-rich plasma (PRP) was separated from blood by centrifugation (180g, 12 minutes), and platelets in PRP were then labeled with 0.45 µL [¹²⁵I]serotonin per milliliter PRP (10 mmol/L) by incubation at 37°C for 15 minutes. Before the perfusions, labeled PRP and the remaining blood cells were recombined and incubated at 22°C for 30 minutes to stabilize the conditions.8,12

Preparation of the Collagen-Coated Coverslip Perfusion Procedure

Plastic coverslips (Thermanox, NUNC) were coated with collagen fibrils (equine type I, Horm-collagen, Nycomed Arzneimittel; 0.36 mg/mL of 5 mmol/L acetic acid), stored in a moist atmosphere, and used on the same day. The perfusion chamber of Turitto’s group (Hall et al13), with a slit height of 100 µm and a shear rate of 1600 s⁻¹, was used. Before the whole-blood perfusion, the chamber was rinsed with PBS for 60 seconds to ensure airtightness. The surface area of the collagen-coated coverslip exposed to blood in the chamber was 8.1 cm². Blood was divided into aliquots and warmed at 37°C for 5 minutes before the experiment, and aliquots were perfused through the chamber in a single passage for 2 minutes. After the perfusion, the chamber was rinsed with PBS for 30 seconds to clean the coverslips of excessive blood.

Duplicates of coated coverslips were perfused before and after the insulin infusion. After the perfusion, the ¹²⁵I scintillation activities of the coverslips were analyzed by using a liquid scintillation counter (WinSpectral, Wallac). Before and after the perfusions, platelet counts and whole-blood ¹²⁵I scintillation activities were determined as previously described.12 The data are expressed as the number of ¹²⁵I-positive platelets deposited per square centimeter of the coverslip. The platelet-specific activity was calculated from the scintillation activity in the blood with the determined platelet count (Coulter Electronics).

Platelet Function Analyses

Samples of citrated plasma were analyzed with a platelet function analyzer (PFA-100 system, Dade Behring AG) that measures platelet-related primary hemostasis under high shear rate conditions.14 The cartridges contain, in addition to collagen fibrils of the same origin as used in perfusion studies, ADP or epinephrine incorporated in the collagen mesh, which blood encounters in a capillary at a shear rate of ~4000 s⁻¹. Duplicates of samples were loaded in collagen/ADP and collagen/epinephrine cartridges, and the mean value of the closure time was determined.

Platelet Aggregation

Blood for platelet aggregation measurements was collected into tubes containing 0.129 mol/L sodium citrate (9:1) and centrifuged to obtain PRP. Platelet-poor plasma was prepared by centrifugation of the rest of the blood at 2000g for 10 minutes. The concentration of platelets in PRP was determined by a cell counter (Cell-Dyn 1600, Abbott Diagnostics) and adjusted to 250×10⁶/mL with autologous platelet-poor plasma.
In vitro platelet aggregation in PRP was measured by using a 4-channel turbidimetric aggregometer (Aggrecorder IIPA-3220, Kyoto Daichi Kagaku). The aggregating agents were collagen (final concentrations 0.5 and 1.0 \mu g/mL, Hormon Chemie), TRAP (final concentrations 5 and 10 \mu g/L, Bachem), ADP (final concentrations 0.5 and 1.5 \mu mol/L, Boehringer-Mannheim), and epinephrine (final concentrations 5 and 10 \mu mol/L, Sigma Chemical Co). The aggregation response was expressed as the maximal rate (slope of the aggregation curve) of primary aggregation (percentage per minute). Samples were kept at 22°C, whereas reagents were kept on ice.

**Platelet cGMP Concentrations**

Citrated PRP was mixed with an equal volume of ice-cold HEPES buffer (0.128 mol/L NaCl, 2.7 mmol/L KCl, 0.5 mmol/L MgCl2, 0.36 mmol/L NaH2PO4, 12 mmol/L NaHCO3, and 10 mmol/L HEPES) and centrifuged at 700g for 10 minutes at 4°C. The platelet pellet was resuspended in HEPES, quickly frozen, and stored at −70°C. Platelet cGMP concentrations were measured after sonication by using an ELISA (cGMP Immunoassay [low pH], R&D Systems). Results were expressed as picomoles per 10^9 platelets.

**Other Measurements**

Fat-free mass and the percentage of body fat were determined by using bioelectrical impedance analysis (BioElectrical Impedance Analyzer System model BIA-101A, RJL Systems). Serum-free insulin concentrations were measured before and at 30-minute intervals during hyperinsulinemia by double-antibody radioimmunoassay (Pharmacia Insulin RIA kit) after precipitation with polyethylene glycol. Plasma glucose concentrations were measured in duplicate by the glucose oxidase method with the use of a Beckman Glucose Analyzer II (Beckman Instruments). Hemoglobin A1c was measured by high-performance liquid chromatography with the use of a fully automated Glycosylated Hemoglobin Analyzer System (Bio-Rad). Plasminogen activator inhibitor (PAI)-1 antigen was measured by ELISA (TintEliza PAI-1, BioPool).

**Statistical Analysis**

Analyses of insulin effects between lean and obese subjects were made by ANOVA for repeated measures with use of the SYSTAT statistical package. Correlation analyses were performed by the Spearman nonparametric correlation coefficient. The results are expressed as mean±SEM. A value of \( P<0.05 \) was considered statistically significant.

**Results**

**Metabolic Parameters**

The obese subjects exhibited several features of the insulin resistance syndrome, including fasting hyperinsulinemia, slightly increased glucose concentrations, hypertriglyceridemia, and low HDL cholesterol concentrations, as shown in the Table. The plasma PAI-1 concentration was significantly lower in the nonobese subjects (8±1 ng/mL) than in the obese subjects (30±3 ng/mL, \( P<0.001 \)). During the insulin infusion, serum-free insulin concentrations averaged 66±2 mU/L in the nonobese subjects and 86±2 mU/L in the obese subjects (\( P<0.01 \)). Plasma glucose averaged 5.1±0.1 and 5.0±0.1 mmol/L during hyperinsulinemia in the nonobese and obese subjects, respectively. Insulin action on whole-body glucose metabolism was 69% lower in the obese subjects (1.6±0.2 mg · kg⁻¹ · min⁻¹) than in the nonobese subjects (5.4±0.4 mg · kg⁻¹ · min⁻¹, \( P<0.0001 \)). When expressed per body surface area, the difference was 68% (74±29 versus 231±21 mg · m⁻² · min⁻¹) for obese versus nonobese subjects, respectively; \( P<0.0001 \) and 67% when expressed per fat-free mass (2.4±0.3 versus 7.2±0.5 mg/kg).

![Figure 1. Platelet deposition to collagen during the 2-minute whole-blood perfusion (top) and intraplatelet cGMP concentrations (bottom) in nonobese and obese subjects at baseline (open bars) and after 3 hours of hyperinsulinemia (solid bars) created with the use of euglycemic hyperinsulinemic clamp technique (insulin infusion rate of 1 mU · kg⁻¹ · min⁻¹). *\( P<0.05 \) and **\( P<0.02 \) for insulin vs basal values; \( \times \times \)\( P<0.02 \) for change by in vivo insulin between the groups.](image)

**Effect of In Vivo Insulin on Platelet-Collagen Interaction and Intraplatelet cGMP Concentrations**

Blood platelet counts were similar in the nonobese and obese subjects and were not altered by hyperinsulinemia (189±9×10^9 and 182±10×10^9 per liter in the nonobese and obese subjects, respectively, at baseline; 178±10×10^9 and 180±8×10^9 per liter in the nonobese and obese subjects, respectively, at the end of the insulin infusion). In the nonobese subjects, platelet deposition to collagen in whole-blood perfusion decreased from 4.3±0.6×10^6 per square centimeter before to 3.5±0.4×10^6 per square centimeter at the end of hyperinsulinemia (\( P<0.05 \) versus basal values, Figure 1). In contrast, in the obese subjects, insulin failed to reduce platelet deposition to collagen (5.2±0.8 versus 5.5±0.7×10^6 per square centimeter, \( P<0.01 \) for change between nonobese versus obese subjects).

At baseline, before the start of the insulin infusion, the intraplatelet cGMP concentration tended to be higher in the nonobese than in the obese subjects (2.5±0.3 versus 1.9±0.2 pmol per 10^9 platelets, respectively; \( P=0.08 \), Figure 1). Compatibile with inhibited platelet function, insulin increased platelet cGMP concentrations by 28% to 3.2±0.5 pmol/10^9 platelets in the nonobese subjects (\( P<0.01 \), Figure 1). Again, in the obese subjects, cGMP concentrations remained unchanged (1.8±0.1 pmol/10^9 platelets) and were 44% lower than cGMP concentrations in the nonobese subjects (\( P<0.01 \) at the end of the insulin infusion (Figure 1).
Insulin action on whole-body glucose metabolism was correlated, regardless of the way it was expressed, with the change in platelet deposition to collagen \( (r = -0.58 \ [P < 0.01], \ r = -0.60 \ [P < 0.01], \) and \( r = -0.67 \ [P < 0.01] \) for glucose metabolism expressed per kilogram body weight, body surface area, and kilogram fat-free mass; Figure 2). Also, triglyceride and PAI-1 antigen concentrations were correlated with the insulin-induced change in platelet deposition to collagen \( (r = 0.52 \ [P < 0.05] \) and \( r = 0.55 \ [P < 0.02] \), respectively).

**Platelet Function Analysis**

The closure time in platelet function analysis was prolonged by 12±7 seconds by insulin in the nonobese subjects but was shortened by 13±8 seconds in the obese subjects when the epinephrine/collagen cartridge was used \( (P < 0.05 \) for change in nonobese versus obese subjects). Insulin infusion prolonged the closure time by 3±3 seconds by using ADP/collagen cartridge in the nonobese subjects, whereas it was shortened by 12±6 seconds in the obese \( (P < 0.05 \) for change in nonobese versus obese subjects). The insulin-induced change in platelet deposition to collagen was inversely correlated with the change in closure time in platelet function analysis when the epinephrine/collagen \( (r = -0.64, \ P = 0.01) \) and the ADP/collagen \( (r = -0.56, \ P < 0.05) \) cartridges were used. Thus, the less platelets deposited, the more prolonged the closure time in platelet function analysis, irrespective of the cartridge.

**Effect of In Vivo Insulin on Platelet Aggregation**

In the nonobese subjects, insulin decreased platelet sensitivity to aggregation at the lower concentrations of soluble collagen \( (0.5 \ \mu g/mL, \ P = 0.017 \) versus basal values), TRAP \( (5 \ \mu m/L, \ P < 0.001 \) versus basal values), and ADP \( (0.5 \ \mu m/L, \ P < 0.001 \) versus basal values; Figure 2). Insulin had less marked antiaggregating effects at the higher concentrations of the aggregating agents (data not shown). When epinephrine was used as the aggregating agent, insulin significantly prevented the aggregatory response at the higher \( (10 \ \mu m/L) \) concentration \( (P = 0.014 \) versus basal values) and almost significantly at the 5 \( \mu m/L \) concentration \( (P = 0.08) \).

In the obese subjects, in vivo hyperinsulinemia also decreased platelet aggregation in response to the lower concentrations of TRAP \( (P = 0.015 \) versus basal values), ADP \( (P = 0.015), \) and epinephrine \( (P = 0.011) \). However, the insulin-induced decrease was significantly greater in the nonobese than in the obese subjects for TRAP (Figure 3). Again, collagen was a remarkable agonist, inasmuch as insulin completely failed to decrease its aggregation response in the obese subjects (Figure 3). In the obese subjects, except for ADP \( (at 1.5 \ \mu m/L, \ P = 0.011), \) insulin did not decrease platelet aggregation at the higher concentrations of collagen, TRAP, or epinephrine (data not shown).

**Discussion**

In the present study, we used 3 different approaches to determine how insulin normally regulates platelet function and whether platelets are insulin resistant. In healthy nonobese subjects, in vivo insulin reduced platelet deposition to
collagen in flowing whole blood and impaired the platelet-related primary hemostasis under high shear rate conditions. Insulin also uniformly inhibited platelet aggregation responses to multiple agonists. These seemingly beneficial in vivo effects of insulin were associated with an increase in intraplatelet cGMP concentrations. In obese insulin-resistant subjects, insulin completely failed to inhibit platelet-collagen interaction, as judged from preserved platelet deposition to immobilized collagen during whole-blood perfusion and aggregation induced by soluble collagen. Insulin also inhibited TRAP-, ADP-, and epinephrine-induced aggregation less in the insulin-resistant than in the insulin-sensitive subjects. In parallel, insulin failed to increase intraplatelet cGMP concentrations in the obese subjects.

The following findings are novel: (1) normal in vivo action of insulin inhibits platelet-collagen interaction, and (2) platelets from obese subjects are resistant to this action of insulin. During thrombus formation after vessel wall injury, exposure of collagen to flowing blood initiates platelet adhesion and subsequent aggregation.\textsuperscript{6–8} Collagen is a unique agonist by simultaneously being an adhesive surface and a potent activator for platelets. Platelet receptors, especially glycoproteins I\textsubscript{a}/II\textsubscript{a} and VI, have specific and synergistic roles in adhesion and further activation on type I collagen.\textsuperscript{7,8,19} We used a thrombin inhibitor, PPACK, instead of citrate, as an anticoagulant to preserve physiological concentrations of cations, which platelets need for normal adhesive and platelet activation functions.\textsuperscript{24} Moreover, in these obese subjects, defective inactivation of platelets by insulin was also documented in platelet-mediated primary hemostasis, in which collagen similar to that used in the perfusion studies interacts with blood under capillary flow.

Previous studies have demonstrated insulin to prevent platelet aggregation in in vitro\textsuperscript{3} and in vivo\textsuperscript{3,4} studies in normal subjects, a finding that has been reproduced in the present study. It has also been reported that obesity blunts the ability of insulin to diminish aggregation when platelets are exposed to insulin in vitro.\textsuperscript{2} However, the present study is the first to quantify in vivo insulin actions on glucose metabolism and platelet function simultaneously and to demonstrate a platelet defect in insulin action in vivo in insulin-resistant obese subjects. The obese subjects were also characterized by other consequences of insulin resistance, such as an increase in serum triglycerides, which might reflect hepatic insulin resistance of VLDL production,\textsuperscript{20} and they had increased concentrations of plasma PAI-1. Compared with nonobese subjects, the obese subjects had normal, although slightly higher, blood pressures. Previous studies in hypertension have reported unaltered platelet adhesion to collagen\textsuperscript{21} and either increased\textsuperscript{22} or unaltered\textsuperscript{23} collagen-induced aggregation.

The effects of insulin on blood vessels are at least partly mediated via NO-related mechanisms and appear to be attenuated in insulin-resistant obese subjects.\textsuperscript{34} The effects of insulin on vascular smooth muscle cells and platelets share similarities, inasmuch as insulin increases cAMP and cGMP via NO-dependent mechanisms in both cell types.\textsuperscript{25} In mice, insulin at physiological doses prevents the development of coronary thrombi induced by ADP, collagen, and thrombin.\textsuperscript{26} This protective effect correlates with insulin-induced increases of circulating NO concentrations. Platelets from insulin-resistant obese\textsuperscript{29} and hypertensive\textsuperscript{27} subjects are also resistant to platelet-inhibitory effects of nitrates/NO. Also, NO fails to inhibit platelet aggregation to the same extent in patients with stable angina or acute coronary syndromes compared with nonischemic patients.\textsuperscript{28} Consistent with these data, we found an in vivo insulin infusion to increase cGMP in platelets of insulin-sensitive but not insulin-resistant obese subjects. However, we cannot conclude that this is an exclusively NO-mediated defect, because direct NO donors or NO synthase inhibitors were not used. However, preliminary data suggest that the regulation of cAMP-related signaling (rather than cGMP) may be involved in mediating the antiaggregatory effects of insulin.\textsuperscript{29} Quantification of the effects of NO on platelet function could have provided insight into the mechanism responsible for our observations. Because insulin is unable to increase intraplatelet cGMP concentrations in obese subjects not only in vivo but also in vitro,\textsuperscript{2} it is possible that this defect is due to a direct platelet effect of insulin, although it is not possible to exclude the contribution of indirect mechanisms. The mechanisms underlying insulin inhibition of platelet-collagen interaction have not been determined. It is known that during platelet adhesion to collagen, cGMP and cAMP exhibit a temporary increase,\textsuperscript{30} but the consequences on platelet adhesion are poorly understood. Most likely, the insulin-induced increases in cGMP contribute to the inactivation of platelets after adhesion, but whether adhesion itself is inhibited by cGMP remains to be determined. Insulin also decreases calcium concentrations in vivo\textsuperscript{31,32} and in vitro\textsuperscript{33} in platelets from insulin-sensitive subjects, whereas in insulin-resistant subjects, insulin appears to increase intraplatelet calcium concentrations and, thereby, platelet activation and aggregation.\textsuperscript{31–33} These findings are consistent with the idea that normal insulin actions are antithrombotic and that such actions are impaired in insulin-resistant conditions.

The present data demonstrating insulin resistance in platelets in vivo may provide mechanistic insight for understanding why the triad of hypertension, obesity, and diabetes was the only significant and independent predictor of 6-month target-vessel revascularization in the Evaluation of Platelet IIb/IIIa Inhibitor for Stenting Trial (EPISTENT) substudy.\textsuperscript{34} It is also of interest that an acutely started insulin infusion followed by insulin therapy has been shown to improve long-term survival of diabetic patients with acute myocardial infarction.\textsuperscript{35} Our results also demonstrate great interindividual variation in platelet activation by insulin and raise the possibility that some of the hitherto unexplained individual insensitivity to antplatelet regimens\textsuperscript{16} could be due to differences in insulin sensitivity. Regarding therapeutic implications of the present study, stronger antplatelet medication, ie, clopidogrel, glycoprotein IIb/IIIa antagonists, or prostacyclin (elevating cAMP in platelets and restoring their resting state), could be used to improve platelet inhibition in insulin-resistant subjects.

In conclusion, insulin at physiological doses attenuates crucial steps in arterial thrombus formation, including the inhibition of platelet-collagen and subsequent platelet-platelet interactions in healthy subjects. These antithrombotic effects are defective in obesity and could provide a mechanism linking insulin resistance to atherothrombotic vascular disease. Insulin resistance in platelets could also contribute to
interindividual variation in the efficacy of antiplatelet therapies.

Acknowledgments

This study was supported by grants from the Sigrid Juselius Foundation (H.-Y.-J.), the Academy of Finland (H.-Y.-J.), the Finnish Diabetes Research Society (I. W.), and the Yrjö Jannson and Finnish Cultural Foundations (A.T.). We wish to thank Kati Tuomola, Marja Lemponen, Tuula Järvenpää, and Ulla Tamminen for their excellent technical assistance and the volunteers for their help.

References


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doi: 10.1161/hq0102.101546
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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