Platelet Resistance to Nitrates in Obesity and Obese NIDDM, and Normal Platelet Sensitivity to Both Insulin and Nitrates in Lean NIDDM

GIOVANNI ANFOSSI, MD
ELENA M. MULARONI, MD
SERENELLA BURZACCA, MD
MARIA C. PONZIANI, MD
PAOLA MASSUCCO, MD
LUIGI MATTIELLO, MS
FRANCO CAVALOT, MD
MARIELLA TROVATI, MD

OBJECTIVE — Previous studies in our laboratory showed that the platelet anti-aggregating effect exerted by insulin, mediated by a nitric oxide (NO)-induced increase of guanosine-3′,5′-cyclic monophosphate (cGMP), is lost in the insulin-resistant states of obesity and obese NIDDM. It is not clear *1*) whether the alterations observed in obese NIDDM patients are attributable to the obesity-related insulin resistance or to diabetes per se and 2) whether insulin-resistant states present a normal or a blunted response to NO. This study has been conducted to investigate *1*) the platelet sensitivity to insulin in lean NIDDM and 2) the platelet sensitivity to an NO donor, glyceryl trinitrate (GTN), in obesity and in both lean and obese NIDDM.

RESEARCH DESIGN AND METHODS — We determined *1*) ADP-induced platelet aggregation and platelet cGMP content in platelet-rich plasma (PRP) obtained from 11 lean NIDDM patients, after a 3-min incubation with insulin (0, 240, 480, 960, 1,920 pmol/l) and 2) ADP-induced platelet aggregation and platelet cGMP content in PRP obtained from 9 obese subjects, 11 lean and 8 obese NIDDM patients, and 18 control subjects, after a 3-min incubation with 0, 20, 40, and 100 μmol/l GTN.

RESULTS — Insulin dose-dependently decreased platelet aggregation in lean NIDDM patients ($P = 0.0001$): with 1,920 pmol/l of insulin, ADP ED_{50} was 141.5 ± 6.4% of basal values ($P = 0.0001$). Furthermore, insulin increased platelet cGMP ($P = 0.0001$) from 7.5 ± 0.2 to 21.1 ± 3.7 pmol/10^9 platelets. These results were similar to those previously described in healthy subjects. GTN reduced platelet aggregation in all the groups ($P = 0.0001$) at all the concentrations tested ($P = 0.0001$), but GTN IC_{50} values were much higher in insulin-resistant patients: 36.3 ± 5.0 μmol/l in healthy control subjects, 26.0 ± 6.0 μmol/l in lean NIDDM patients (NS vs. control subjects), 123.6 ± 24.0 μmol/l in obese subjects ($P = 0.0001$ vs. control subjects), and 110.1 ± 19.2 μmol/l in obese NIDDM patients ($P = 0.0001$ vs. control subjects). GTN dose-dependently increased platelet cGMP in all the groups ($P = 0.0001$ in control subjects, lean NIDDM patients, and obese subjects; $P = 0.04$ in obese NIDDM patients).Values reached by obese subjects and obese NIDDM patients, however, were lower than those reached by control subjects (with 100 μmol/l of GTN, $P = 0.001$ and $P = 0.0001$, respectively). In healthy control subjects and in obese subjects, the insulin:glucose ratio, used as an indirect measure of insulin sensitivity, was positively correlated to GTN IC_{50} ($r = 0.530$, $P = 0.008$), further suggesting that the sensitivity to NO is reduced in the presence of insulin resistance.

CONCLUSIONS — The insulin anti-aggregating effect is preserved in lean NIDDM; platelet sensitivity to GTN is preserved in lean NIDDM but is reduced in the insulin-resistant states of obesity and obese NIDDM. Resistance to nitrates, therefore, could be considered another feature of the insulin-resistance syndrome.

From the Diabetes Unit, Department of Clinical and Biological Sciences of the University of Turin, San Luigi Gonzaga Hospital, Orbassano (Turin), Italy.

Address correspondence and reprint requests to Mariella Trovati, MD, Diabetes Unit, Department of Clinical and Biological Sciences of the University of Turin, San Luigi Gonzaga Hospital, 10043 Orbassano (Turin), Italy.

Received for publication 22 May 1997 and accepted in revised form 16 September 1997.

Abbreviations: ANOVA, analysis of variance; cGMP, guanosine-3′,5′-cyclic monophosphate; GTN, glyceryl trinitrate; PPP, platelet-poor plasma; PRP, platelet-rich plasma; RIA, radioimmunoassay.

In healthy subjects, insulin exerts a platelet anti-aggregating effect, both in vitro (1–3) and in vivo (2,4,5); actually, it reduces platelet responses to different agonists even at the physiological concentration of 240 pmol/l (2), commonly occurring in daily life. Insulin, therefore, modulates platelet function, potentially contributing to the reduction of the atherothrombotic risk; actually, platelet hyperactivation plays a role in the pathogenesis of atherosclerosis, both in the general population and in diabetes (6). The anti-aggregating effect of insulin is mediated by platelet guanosine-3′,5′-cyclic monophosphate (cGMP) (7); the insulin-induced cGMP increase is attributable to nitric oxide (NO), since it is blunted by NO synthase inhibition (8). It is known that *1*) an L-arginine/NO pathway is present in human platelets (9), 2) NO enhances cGMP through activation of a soluble heme-containing guanylate cyclase (10), and 3) cGMP reduces the availability of intracellular calcium, which is an essential step in platelet activation (11). Very recently, we described that insulin increases NO synthesis in human platelets (12). Thus, the insulin ability to reduce agonist-stimulated calcium fluxes in platelets (13) could be ascribed to the insulin effects on cGMP (7,8,12).

The physiological insulin-induced platelet anti-aggregating effect is blunted in the classical insulin-resistance conditions of obesity (14), obese NIDDM (14), and arterial hypertension (13). Therefore, the increased vascular risk of insulin-resistant subjects could be attributable, at least in part, to the lack of the beneficial anti-aggregating effect exerted by insulin.

The inability of insulin to increase cGMP and to anti-aggregate platelets in obese subjects and in obese NIDDM patients (14) could be attributed to *1*) a defective insulin-induced increase of NO synthesis (resistance to insulin at the NO-synthase level), 2) a reduced ability of NO to activate platelet guanylate cyclase (resistance to NO at the guanylate cyclase level), or 3) a combination of both phenomena. To verify the second hypothesis, we investi-

Table 1—*Clinical characteristics of the study subjects*

	Healthy subjects	Obese subjects	Obese NIDDM patients	Lean NIDDM patients
n	18	9	8	11
BMI (kg/m²)	22.6 ± 0.5	29.8 ± 1*	32.3 ± 1.7*	23.6 ± 0.4
Diabetes duration (years)	—	—	4.7 ± 1.3	3.6 ± 0.9
Glucose (mmol/l)	5.2 ± 0.1	5.2 ± 0.2	8.1 ± 0.9*	7.4 ± 0.3*
Insulin (pmol/l)	47.5 ± 5.5	90.4 ± 5.8*	118.8 ± 29.4†	35.8 ± 8.3
HbA_{1c} (%)	4.8 ± 0.1	5.1 ± 0.2	7.4 ± 0.2*	6.2 ± 0.3*
Systolic blood pressure (mmHg)	125.6 ± 2.2	126.4 ± 4.9	140.6 ± 6.0‡	131.8 ± 3.9
Diastolic blood pressure (mmHg)	78.5 ± 1.7	79.2 ± 2.9	83.7 ± 2.3	79.1 ± 1.3
Triglycerides (mmol/l)	1.0 ± 0.06	1.5 ± 0.3§	1.7 ± 0.2*	1.0 ± 0.1
Total cholesterol (mmol/l)	4.8 ± 0.2	5.3 ± 0.4	4.9 ± 0.3	5.6 ± 0.4
HDL cholesterol (mmol/l)	1.6 ± 0.06	1.3 ± 0.1§	1.1 ± 0.08*	1.5 ± 0.2

Data are means ± SE. Significance is versus healthy subjects. *P = 0.0001, †P = 0.002, ‡P = 0.007, §P = 0.03.

gated whether obese subjects and obese NIDDM patients present a normal or impaired platelet sensitivity to glyceryl trinitrate (GTN). As is well known, nitrates induce not only vasodilation but also platelet anti-aggregation (15–17), since they act as NO donors (18).

A second aim of our study was to elucidate whether the alterations in the insulin/NO pathway observed in obese NIDDM are also present in lean NIDDM.

As is well known, NIDDM is a very heterogeneous disorder, in which different degrees of insulin resistance and β-cell dysfunction can occur in a precarious balance between insulin action and insulin secretion (19). The large majority of NIDDM patients in the Caucasian populations belong to the obese category, in which insulin resistance is a major determinant for the development of the disease (19); they are hyperinsulinemic in the first phases of the disease, but they can become hypoinsulinemic in later phases owing to β-cell exhaustion or to glucose toxicity (19). There is, however, a minority of patients who show the features of the lean category of NIDDM, in which hyperinsulinemia does not occur even in the early stages, and abnormalities of insulin secretion, more than of insulin sensitivity, are the major determinants for the development of the disease (20). In this light, the second aim of the present study was to clarify whether lean NIDDM patients show a preserved platelet sensitivity to the anti-aggregating effects of insulin; in this case, the blunted response to insulin of obese NIDDM patients would be more attributable to obesity-related insulin resistance than to diabetes per se. Furthermore, also in lean NIDDM patients, we measured platelet sensitivity to GTN.

RESEARCH DESIGN AND METHODS

Subjects

The study was carried out on 5 different subject groups: group A included 9 obese subjects (6 men, 3 women) aged 35.3 ± 3.1 years; group B included 8 diet-treated obese NIDDM patients (6 men, 2 women) aged 53.7 ± 3.3 years; group C included 11 diet-treated lean NIDDM patients (8 men, 3 women) aged 53.4 ± 1.9 years; group D included 9 healthy volunteers (6 men, 3 women) aged 34.0 ± 1.2 years, matched for age with obese subjects; and group E included 9 healthy volunteers (7 men, 2 women) aged 51.7 ± 2.0 years, matched for age with diabetic patients. Because both the clinical characteristics and the platelet responses to GTN were similar in the two groups of control subjects, we pooled them together. NIDDM was defined in accordance with the National Diabetes Data Group criteria (21). Obesity was defined as the presence of a BMI >25. Obese subjects were otherwise healthy and did not present a family history of diabetes. Diabetic patients were free from late diabetic complications, evaluated by means of a medical history, clinical examination, fundoscopy, fluoroangiography, electrocardiography, large vessel Doppler ultrasonography, renal clearances, proteinuria and microalbuminuria, electromiography, and tests for the detection of autonomic neuropathy. Some clinical characteristics are shown in Table 1.

Fasting venous plasma glucose was measured by a Beckman Glucose Analyzer (Beckman, Fullerton, CA). Fasting plasma insulin was measured by radioimmunoassay (RIA) (Kit Ares Serono, Milan, Italy): specificity 100% for human insulin, 15% for human proinsulin, and <0.01% for C-peptide and glucagon. Glycosylated hemoglobin was measured by high-performance liquid chromatography (Bio-Rad, Hercules, CA), and fasting serum cholesterol, HDL cholesterol, and triglycerides were measured by automated chemistry.

Subjects did not take any drug that influences platelet function for 4 weeks before the study and gave an informed consent before the investigation.

As an indirect measure of insulin sensitivity in healthy control subjects and in obese subjects, we calculated the fasting insulin (pmol/l):fasting glucose (mmol/l) ratio (22). The insulin:glucose ratio was 8.97 ± 0.98 in healthy control subjects and 16.87 ± 1.23 in obese subjects (P = 0.0001 vs. control subjects).

Materials and methods

Human recombinant insulin was obtained from Calbiochem (La Jolla, CA), adenosine-5 diphosphate sodium salt (ADP) was obtained from Sigma (St. Louis, MO), and GTN was obtained from Simes (Milan, Italy). Human insulin was dissolved in modified Tyrode's buffer containing bovine serum albumin (144 mmol/l NaCl, 2.7 mmol/l KCl, 11.9 mmol/l $NaHCO_3$, 0.4 mmol/l NaH_2PO_4, and 0.04 mmol/l bovine serum albumin, pH 7.4). Subjects were studied after overnight fasting. A venous blood sample was withdrawn without stasis and anticoagulated with 3.8% sodium citrate, pH 7.4 (vol/vol: 1/9). Platelet-rich plasma (PRP) was obtained by a 20-min centrifugation at 100*g* at room temperature; platelet-poor plasma (PPP) was prepared by a further PRP centrifugation at 2,000*g* for 10 min. Platelet counts were determined on an S-Plus Coulter Counter

Table 2—*Influence of a 3-min PRP incubation with increasing concentrations of GTN on maximal aggregation to ADP and on platelet content of cGMP in the different groups*

	n	GTN (μmol/l) 0	20	40	100
Maximal aggregation to ADP (%)					
Healthy subjects	18	100	59.1 ± 3.2	45.5 ± 3.2	33.1 ± 2.5
Obese subjects	9	100	71.0 ± 1.0**	64.0 ± 3.0†	49.6 ± 4.3‡
Obese NIDDM patients	8	100	75.0 ± 4.2¶	64.6 ± 5.5§	47.9 ± 6.5#
Lean NIDDM patients	11	100	48.4 ± 6.6	37.3 ± 6.8	22.8 ± 4.7††
cGMP values (pmol/10^9 platelets)					
Healthy subjects	18	7.9 ± 0.9	19.0 ± 2.6	22.8 ± 3.0	30.0 ± 3.1
Obese subjects	9	7.7 ± 0.6	10.5 ± 1.2††	11.4 ± 1.5#	13.1 ± 1.7†
Obese NIDDM patients	8	8.2 ± 0.6	8.5 ± 1.4**	8.7 ± 1.4‖	11.2 ± 1.6*
Lean NIDDM patients	11	7.5 ± 0.2	13.1 ± 1.9	16.2 ± 2.7	22.0 ± 2.8

Data are means ± SE. Significance is versus healthy subjects. *P = 0.0001, †P = 0.001, ‡P = 0.002, §P = 0.004, ‖P = 0.005, ¶P = 0.009, #P = 0.015, **P = 0.02, and ††P = 0.04.

(Coulter Electronics, Hertfordshire, U.K.). The platelet number ranged between 250,000 and 300,000/μl in PRP samples and was not adjusted, since the study design consisted of the determination of platelet responses in samples from the same PRP after addition of buffer solutions or different substances for each subject.

Platelet aggregation studies. Platelet aggregation was carried out according to Born's method (23) using a model 500 Chrono Log aggregometer (Havertown, PA) at a constant stirring of 900 rpm. In aggregometric studies, the parameter considered is light absorption, which is evaluated on a scale of 0–100%. Because platelets are randomly dispersed in PRP and virtually absent in PPP, light absorption is very high with PRP and very low with PPP. At the beginning of each aggregometric experiment, light absorption of the PRP examined is conventionally set at 90%, and light absorption of PPP is set at 10%. When platelets aggregate, light absorption is reduced, and the reduction is correlated with the extent of platelet aggregation. The aggregating response to each agonist concentration is therefore evaluated as reduction of light absorption and quantified as maximal aggregation. Maximal aggregation is calculated using the Weiss and Rogers formula (24)

$$\text{Maximal aggregation (\%)} = 100 \times (OD_0 - OD_m) / OD_0$$

where OD_0 is the optical density (OD) before agonist addition and OD_m is the minimum OD reached after agonist addition. Maximal aggregation is calculated for each of the agonist doses used.

In the different subject groups, GTN-induced modulation of platelet aggregation responses to ADP (2.4 μmol/l) was determined after a 3-min PRP preincubation with GTN (0, 20, 40, and 100 μmol/l) or the same volume of buffer alone. IC_{50} for GTN (minimal GTN concentration necessary to reduce the platelet response to ADP by half) was determined by probit analysis.

Insulin-induced modulation of platelet aggregation response to ADP was evaluated in lean NIDDM patients following the same procedure already used for control subjects, obese subjects, and obese NIDDM patients (14). Briefly, different doses of ADP (1–8 μmol/l) were used to determine the ADP concentration able to elicit a maximal aggregation of 50% (ADP ED_{50} values) after a 3-min preincubation with insulin (0, 240, 480, 960, and 1,920 pmol/l). Results are expressed as percentages of the ADP ED_{50} values without preincubation with insulin. In experiments with insulin, ED_{50} was used instead of IC_{50}, which was used in experiments with GTN, to allow a comparison of the results with those of a previous study evaluating the platelet sensitivity to insulin in control subjects, obese subjects, and obese NIDDM patients (14).

Platelet content of cGMP. Platelet content of cGMP was measured in PRP samples (500 μl) incubated at 37°C for 3 min without stirring in the presence of 0, 20, 40, and 100 μmol/l GTN or with 0, 240, 480, 960, and 1,920 pmol/l human recombinant insulin. The platelet reactions were stopped with 30% trichloroacetic acid (100 μl). The precipitated proteins were removed by means of a centrifugation at 2,000g for 20 min at 4°C. After the addition of 1 mol/l HCl (100 μl), the supernatant was submitted to 10 extractions with ethylic ether to remove trichloroacetic acid. The samples were then lyophylized and kept at −70°C until the RIA of cGMP (Kit Advanced Magnetics, Cambridge, MA): specificity of 100% for cGMP, 0.027% for cAMP, and <0.001% for GMP, GDP (guanosine 5′-diphosphate), ATP, and GTP (guanosine 5′-triphosphate); sensitivity of 0.01 pmol/0.1 ml. The intra- and interassay coefficients of variation are 5.2 and 8.6%, respectively. Data are expressed as pmol/10^9 platelets.

Statistical analysis

Data, in the text and in the figures, are expressed as means ± SE. For the statistical evaluation we used, when appropriate, the analysis of variance (ANOVA) for repeated measures and Student's t test for paired and unpaired data; furthermore, the correlation coefficient between the insulin:glucose ratio and the GTN IC_{50} values was calculated.

RESULTS — The effect of GTN on ADP-induced platelet aggregation is shown in Table 2 and Fig. 1. In healthy control subjects, GTN dose-dependently reduced the platelet response to ADP (ANOVA for repeated measures: P = 0.0001). A significant GTN effect was evidenced at all the concentrations tested (P = 0.0001). The GTN IC_{50} value was 36.3 ± 5.0 μmol/l .

Similar effects were observed in lean NIDDM patients (ANOVA for repeated measures: P = 0.0001). Also in lean NIDDM patients, a significant GTN effect was evidenced at all the concentrations tested (P = 0.0001), and the IC_{50} value was

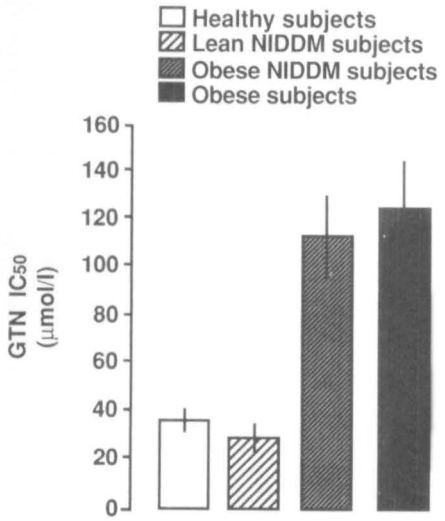

Figure 1—*GTN IC_{50} in 18 healthy control subjects, 11 lean NIDDM subjects, 9 obese subjects, and 8 obese NIDDM subjects. GTN IC_{50} values of healthy subjects and of lean NIDDM patients did not differ, whereas values of obese subjects and of obese NIDDM subjects were significantly higher than those of control subjects* (P = *0.0001*).

26.0 ± 6.0 μmol/l (not significant versus control subjects).

In obese subjects and in obese NIDDM patients, GTN caused a significant decrease of platelet responses to ADP (ANOVA for repeated measures: $P = 0.0001$), with significant effects at all the concentrations tested ($P = 0.0001$). The IC_{50} values, however, were much higher than in control subjects. In particular, they were 123.6 ± 24.0 μmol/l in obese subjects (significance versus healthy subjects: $P = 0.0001$) and 110.1 ± 19.2 μmol/l in obese NIDDM patients (significance versus healthy subjects: $P = 0.0001$). Also, the GTN effects at the different concentrations were lower in obese subjects and in obese NIDDM patients than in control subjects (Table 2).

When GTN IC_{50} was correlated to the insulin:glucose ratio in healthy control subjects and in obese subjects, a positive correlation was found ($r = 0.530$, $P = 0.008$).

The effect of GTN on platelet content of cGMP is shown in Table 2. In control subjects, GTN dose-dependently increased the platelet content of cGMP (ANOVA for repeated measures: $P = 0.0001$). A significant effect was evidenced at all the GTN concentrations tested ($P = 0.0001$).

Similar effects were observed in lean NIDDM patients (ANOVA for repeated measures: $P = 0.0001$). All GTN concentrations were effective (20 μmol/l, $P = 0.016$; 40 μmol/l, $P = 0.01$; 100 μmol/l, $P = 0.001$).

In obese subjects, GTN increased intraplatelet cGMP levels (ANOVA for repeated measures: $P = 0.0001$); all the GTN concentrations tested exerted significant effects (20 μmol/l, $P = 0.04$; 40 μmol/l, $P = 0.05$; 100 μmol/l, $P = 0.004$). The cGMP increase, however, was lower than in control subjects (Table 2).

In obese NIDDM patients, GTN increased cGMP levels (ANOVA for repeated measures: $P = 0.04$), but this increase was significant only at 100 μmol/l ($P = 0.04$). The cGMP increase was lower than that in control subjects (Table 2).

The effects of insulin on ADP-induced platelet aggregation and on platelet cGMP content in lean NIDDM patients are shown in Table 3. Insulin dose-dependently decreased the platelet aggregation to ADP, as indicated by the increase of ADP ED_{50} (ANOVA for repeated measures: $P = 0.0001$). This insulin effect was evidenced at all the concentrations tested (at 240 pmol/l, $P = 0.07$; at 480 pmol/l, $P = 0.004$, and at 960 and 1,920 pmol/l, $P = 0.0001$).

Insulin dose-dependently increased the platelet content of cGMP (ANOVA for repeated measures: $P = 0.0001$). This effect was evidenced at all the insulin concentrations tested (at 240 pmol/l, $P = 0.042$; at 480 pmol/l, $P = 0.025$; at 960 pmol/l, $P = 0.012$; and at 1,920 pmol/l, $P = 0.004$).

CONCLUSIONS — This study shows for the first time that *1*) lean NIDDM patients show a normal platelet sensitivity to both insulin and GTN, 2) obese subjects and obese NIDDM patients show a platelet resistance to the NO-donor GTN, and 3) the fasting insulin:glucose ratio is positively correlated to GTN IC_{50} in healthy control subjects and in nondiabetic obese subjects. Obviously, our present data do not exclude that obesity and obese NIDDM are resistant to the anti-aggregating effect of insulin (14) not only because they are resistant to NO but also because they present a blunted insulin-induced increase of NO synthesis.

Insulin resistance is a multifaceted syndrome (25). Some important facets, not necessarily present all together, are hyperinsulinemia, obesity, glucose intolerance, low HDL cholesterol, and high triglyceride concentrations (25). Our insulin-resistant subjects presented some features of the syndrome: obesity with or without NIDDM, hyperinsulinemia, hypertriglyceridemia, and low HDL cholesterol. This study allows us to speculate that platelet resistance to GTN could be considered another facet of this syndrome. Unpublished data from our laboratory show that obese subjects are also resistant to sodium nitroprusside; the resistance to GTN described in the present study, therefore, should not be considered specific for this substance but rather a marker of platelet resistance to the NO donors. In this study, we employed, as an indirect measure of insulin sensitivity, the fasting insulin:glucose ratio (22). Obviously, this parameter cannot be reliably used in NIDDM patients, since they present, by definition, an impaired insulin secretion with respect to glucose values; their insulin concentrations, therefore, cannot be considered a mirror of the degree of insulin resistance. Even with these limitations in mind, our study clearly shows that, in nondiabetic patients, GTN IC_{50} correlates with the degree of insulin resistance.

The report of other investigators, showing that platelets from patients affected by essential hypertension, which is another condition of insulin resistance (26), present a reduced sensitivity to

Table 3—*Influence of a 3-min PRP incubation with increasing concentrations of insulin on ADP ED_{50} and on platelet content of cGMP in 11 lean NIDDM patients*

	Insulin pmol/l				
	0	240	480	960	1,920
ADP ED_{50} (% of basal values)	100	106.0 ± 2.8	117.0 ± 4.6	126.6 ± 4.9	141.5 ± 6.4
cGMP values (pmol/10^9 platelets)	7.5 ± 0.2	11.8 ± 1.8	14.4 ± 3.0	17.5 ± 3.1	21.1 ± 3.7

Data are means ± SE. ANOVA for repeated measures; $P = 0.0001$ for both ADP ED_{50} and cGMP values.

sodium nitroprusside (27), further supports the link between insulin resistance and platelet resistance to NO.

The resistance to both insulin and NO-donors in insulin-resistant subjects is not surprising. It is known that NO mediates not only platelet anti-aggregation but also vasodilation (10) and that insulin exerts its vasodilating effect through NO (28,29). NIDDM patients present an impaired vascular relaxation in response to insulin (30), to acetylcholine (a classic endothelium-dependent vasodilator, acting by increasing the endothelial production of NO) (31), and to GTN, employed as a model of endothelium-independent vasodilator (31). Furthermore, in arterial hypertension, which is another insulin-resistant state (26), platelets present a reduced response to both insulin (13) and sodium nitroprusside (27).

As far as lean NIDDM patients are concerned, our study shows for the first time that they present normal platelet responses to both insulin and GTN. When the results of the present investigation are compared with those of a previous one—carried out with the same experimental protocol in nondiabetic normotensive subjects, obese subjects, and obese NIDDM patients (14)—one can easily see that the platelet responses elicited by insulin in lean NIDDM patients are perfectly normal and completely different from the blunted responses observed in obese subjects and obese NIDDM patients (14). The fact that lean NIDDM patients have a preserved platelet sensitivity to insulin further supports the conclusion that obesity is responsible for the platelet insulin resistance shown in obese NIDDM; this conclusion was already suggested by the fact that the presence of diabetes did not further increase the platelet resistance to insulin described in obesity (14). Our lean NIDDM patients did not show the classical aspects of the insulin resistance syndrome; for instance, their fasting insulin concentrations were relatively low as an expression of a primitive defect, since their short diabetes duration excludes the presence of β-cell exhaustion and their good blood glucose control excludes the presence of glucose toxicity. When fasting insulin concentrations of our lean NIDDM patients are compared with those of the other groups, one can recognize that they are not significantly different from those of age-matched healthy control subjects but are much lower than those of obese subjects and obese NIDDM patients. Therefore, even if we cannot use the insulin:glucose ratio as a reliable marker of insulin resistance in NIDDM patients for the reasons discussed above and cannot assume that they are perfectly sensitive to insulin, we have to suppose that insulin sensitivity of our lean NIDDM patients is higher than that of obese subjects with or without diabetes. Furthermore, they do not present the lipid markers of insulin resistance; for instance, their HDL cholesterol is not reduced and triglycerides are not increased when compared with healthy control subjects. It has also been observed that insulin-induced vasodilation, which is another NO-mediated phenomenon (28,29), is preserved in lean NIDDM patients (32). The insulin-NO interrelationships, therefore, seem unaltered in these patients. Interestingly, the present study shows that lean NIDDM patients also have preserved platelet responses to GTN. Therefore, a decreased sensitivity to GTN cannot be considered a feature of NIDDM per se but only of the obese insulin-resistant subset of NIDDM, which is the largest one in Caucasian populations (21).

It should be observed that our NIDDM patients presented with good glycemic control; this fact could be important, since hyperglycemia results in the production of advanced glycosylation end products that can inactivate both endogenous and exogenous NO (33) and induce an oxidative stress (34), which can offset NO (35). The importance of insulin resistance per se in our study is further underlined by the fact that obese subjects, though absolutely normoglycemic, presented an impairment of platelet responses to GTN. Because these patients were also perfectly normotensive, it is clear that obesity per se accounts for their resistance to nitrates.

The present study, therefore, further supports the concept that insulin-resistant states (obesity, NIDDM, and arterial hypertension) are characterized by a reduced response to insulin and to NO-donors. Is there a common explanation for these two phenomena? Because insulin interplays with many cellular ionic pumps, it has been proposed that there is an ionic basis in the insulin-resistance syndrome, characterized by an increased intracellular content of calcium and sodium and a decreased intracellular content of magnesium. According to this hypothesis, the different components of syndrome X are different clinical expressions of the same underlining cellular ionic defects, occurring alone or in association, according to genetic and dietary-environmental conditions (36). In light of this intriguing hypothesis, further studies should evaluate whether the platelet resistance to both insulin and NO-donors shown in the different insulin-resistant states has a common ionic basis, contributing to altered intracellular signaling pathways.

In any case, platelet response to NO is essential to modulate platelet activation in vivo and to prevent thrombus formation; a potential reduction of NO action in platelets could play a role in the pathophysiology of atherothrombosis in the insulin-resistant states. Furthermore, because vascular thrombosis is a common occurrence in NIDDM (37), the resistance of these patients to GTN, a widely used cardiovascular drug, could further reduce the success of our therapeutic efforts when patients are affected by ischemic accidents.

References

1. Trovati M, Anfossi G, Cavalot F, Massucco P, Mularoni E, Schinco P, Tamponi G, Emanuelli G: Studies on mechanisms involved in hypoglycemia-induced platelet activation. *Diabetes* 35:818–825, 1986
2. Trovati M, Anfossi G, Cavalot F, Massucco P, Mularoni E, Emanuelli G: Insulin directly reduces platelet sensitivity to aggregating agents: studies in vitro and in vivo. *Diabetes* 37:780–786, 1988
3. Trovati M, Anfossi G, Mularoni E, Massucco P, Bosia A, Emanuelli G: Insulin reduces platelet sensitivity to platelet-activating factor in washed human platelets resuspended in a calcium-free medium. *Diabetes Metab Nutr* 2:151–153, 1989
4. Hiramatsu K, Nozaki H, Arimori A: Reduction of platelet aggregation induced by euglycaemic insulin clamp. *Diabetologia* 30:310–313, 1987
5. Trovati M, Anfossi G, Cavalot F, Massucco P, Mularoni E, Emanuelli G: Physiological insulin concentrations reduce platelet sensitivity to adrenaline in vivo. In *Tissue-Specific Metabolic Alterations in Diabetes. Frontiers in Diabetes*. Vol. 10. Belfiore F, Molinatti GM, Reaven GM, Eds. Basel, Switzerland, Karger, 1990, p. 166–169
6. Colwell JA, Gisinger C, Klein R: Altered platelet function in diabetes mellitus: effect of glycemic regulation and antiplatelet agents. In *Hyperglycemia, Diabetes, and Vascular Diseases*. Ruderman N, Williamson J, Brownlee M, Eds. New York, Oxford University Press, 1992, p. 30–47
7. Trovati M, Massucco P, Mattiello L, Mularoni E, Cavalot F, Anfossi G: Insulin increases guanosine-3′,5′-cyclic monophosphate in

human platelets: a mechanism involved in the insulin anti-aggregating effect. *Diabetes* 43:1015–1019, 1994
8. Trovati M, Massucco P, Mattiello L, Piretto V, Cavalot F, Mularoni E, Anfossi G: The insulin-induced increase of guanosine-3′,5′-cyclic monophosphate in human platelets is mediated by nitric oxide. *Diabetes* 45:768–770, 1996
9. Radomski MW, Palmer RMJ, Moncada S: An L-arginine/nitric oxide pathway present in human platelets regulates aggregation. *Proc Natl Acad Sci USA* 87:5193–5197, 1990
10. Moncada S, Palmer RMJ, Higgs EA: Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol Rev* 43:109–142, 1991
11. Nakashima Y, Tohmatsu T, Hattori H, Okano Y, Nozawa Y: Inhibitory action of cyclic GMP on secretion, polyphosphoinositide hydrolysis, and calcium mobilization in thrombin-stimulated human platelets. *Biochem Biophys Res Commun* 135:1099–1104, 1986
12. Trovati M, Anfossi G, Massucco P, Mattiello L, Costamagna C, Piretto V, Mularoni E, Cavalot F, Bosia A, Ghigo D: Insulin stimulates nitric oxide synthesis in human platelets and, through nitric oxide, increases platelet concentrations of both guanosine-3′,5′-cyclic monophosphate and adenosine-3′,5′-cyclic monophosphate. *Diabetes* 46:742–749, 1997
13. Touyz RM, Schiffrin EL: Blunted inhibition by insulin of agonist-stimulated calcium, pH, and aggregatory responses in platelets from hypertensive patients. *J Hypertens* 12:1255–1263, 1994
14. Trovati M, Mularoni E, Burzacca S, Ponziani MC, Massucco P, Mattiello L, Piretto V, Cavalot F, Anfossi G: Impaired insulin-induced platelet anti-aggregating effect in obesity and in obese non-insulin-dependent diabetes mellitus. *Diabetes* 44:1318–1322, 1995
15. Ring T, Knudsen F, Kristensen SD, Larsen CE: Nitroglycerin prolongs the bleeding time in healthy men. *Thromb Res* 29: 553–559, 1983
16. Karlberg KE, Torfgard K, Ahlner J, Sylven C: Dose-dependent effect of intravenous nitroglycerin on platelet aggregation and correlation with plasma glyceryl dinitrate concentration in healthy men. *Am J Cardiol* 69:802–805, 1991
17. Diodati J, Theroux P, Latour JG, Lacoste L, Lam JYT, Waters D: Effects of nitroglycerin at therapeutic doses on platelet aggregation in unstable angina pectoris and acute myocardial infarction. *Am J Cardiol* 66:683–688, 1990
18. Lam JYT, Chesebro JH, Fuster V: Platelet, vasoconstriction and nitroglycerin during arterial wall injury: a new antithrombotic role for an old drug. *Circulation* 78:194–200, 1988
19. DeFronzo RA, Bonadonna RC, Ferrannini E: Pathogenesis of NIDDM: a precarious balance between insulin action and insulin secretion. In *International Textbook of Diabetes Mellitus*. Alberti KGMM, DeFronzo RA, Keen H, Zimmett P, Eds. Chichester, U.K., Wiley, 1992, p. 568–633
20. Bennett PH, Bogardus C, Tuomilehto J, Zimmett P: Epidemiology and natural history of NIDDM: nonobese and obese. In *International Textbook of Diabetes Mellitus*. Alberti KGMM, DeFronzo RA, Keen H, Zimmett P, Eds. Chichester, U.K., Wiley, 1992, p. 148–176
21. National Diabetes Data Group: Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes* 28:1039–1057, 1979
22. Fulcher GR, Walker M, Alberti KGMM: The assessment of insulin action in vivo. In *International Textbook of Diabetes Mellitus*. Alberti KGMM, DeFronzo RA, Keen H, Zimmett P, Eds. Chichester, U.K., Wiley, 1992, p. 513–529
23. Born GVR: Aggregation of blood platelets by adenosine diphosphate and its reversal. *Nature* 194:927–929, 1962
24. Weiss HJ, Rogers J: Thrombocytopenia due to abnormalities in platelet release reaction. *Blood* 39:2–8, 1972
25. DeFronzo RA, Ferrannini E: Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. *Diabetes Care* 14:173–194, 1991
26. Ferrannini E, Buzzigoli G, Bonadonna R, Giorico MA, Oleggini M, Graziadei L, Pedrinelli R, Brandi L, Bevilacqua S: Insulin resistance in arterial hypertension. *N Engl J Med* 317:350–357, 1987
27. Woods JD, Edwards JS, Ritter JM: Inhibition by nitroprusside of platelet calcium mobilization: evidence for reduced sensitivity to nitric oxide in essential hypertension. *J Hypertens* 11:1369–1373, 1993
28. Steinberg HO, Brechtel G, Johnson A, Fineberg N, Baron A: Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent: a novel action of insulin to increase nitric oxide release. *J Clin Invest* 94:1172–1179, 1994
29. Scherrer U, Randin D, Vollenweider P, Vollenweider L, Nicod P: Nitric oxide release accounts for insulin's vascular effects in humans. *J Clin Invest* 94:2511–2515, 1994
30. Laakso M, Edelman SV, Brechtel G, Baron AD: Impaired insulin-mediated skeletal muscle blood flow in patients with NIDDM. *Diabetes* 41:1076–1083, 1992
31. McVeigh GE, Brennan GM, Johnston GD, McDermott BJ, McGrath LT, Henry WR, Andrews JW, Hayes JR: Impaired endothelium-dependent and -independent vasodilation in patients with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 35:771–776, 1992
32. Tack CJJ, Smits P, Willemsen J, Lenders JWM, Thien T, Lutterman JA: Effects of insulin on vascular tone and sympathetic nervous system in NIDDM. *Diabetes* 45: 15–22, 1996
33. Bucala R, Tracey KJ, Cerami A: Advanced glycosylation products quench nitric oxide and mediate defective endothelium-mediated vasodilation in experimental diabetes. *J Clin Invest* 87:432–438, 1991
34. Ceriello A, Dello Russo P, Amstad P, Cerutti P: High glucose induces antioxidant enzymes in human endothelial cells in culture: evidence linking hyperglycemia and oxidative stress. *Diabetes* 45:471–477, 1996
35. Gryglewski RJ, Palmer RMJ, Moncada S: Superoxide anion is involved in the breakdown of endothelium-derived relaxing factor. *Nature* 320:454–456, 1986
36. Resnick LM: Ionic basis of hypertension, insulin resistance, vascular disease, and related disorders: the mechanism of "Syndrome X." *Am J Hypertens* 6:1235–1345, 1993
37. Colwell JA: Vascular thrombosis in type II diabetes mellitus. *Diabetes* 42:8–11, 1993