In Vivo Effect of Insulin on Intracellular Calcium Concentrations: Relation to Insulin Resistance

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Elevated intracellular calcium concentrations ($[Ca^{2+}]_i$) have been described in essential hypertension and other insulin-resistant states. Our aim was to explore the relationship between insulin resistance and abnormal Ca^{2+} metabolism. In 50 nondiabetic subjects, half of whom had untreated essential hypertension, we simultaneously measured the in vivo effect of insulin on glucose metabolism (by the insulin clamp technique) and on platelet $[Ca^{2+}]_i$ (by the Fura-2 method). In each subject, $[Ca^{2+}]_i$ measurements (both in Ca^{2+}-free medium and, sequentially, following in vitro Ca^{2+} loading) were obtained in the fasting state and after 2 hours of euglycemic hyperinsulinemia. In the fasting state, no association was found between any measure of $[Ca^{2+}]_i$ and gender, age, body mass index (BMI), blood pressure, or insulin sensitivity. In contrast, following in vivo insulin, platelet $[Ca^{2+}]_i$ increased significantly (from 23 ± 1 to 28 ± 1 nmol/L in Ca^{2+}-free medium, $P < .01$) in the whole group, and an insulin-induced increase in $[Ca^{2+}]_i$ was associated with insulin resistance ($r = .35$, $P = .01$) but not with hypertension ($r = .2$, $P = .17$) and with impaired glucose storage (as determined by indirect calorimetry, $r = .39$, $P = .01$) but not with glucose oxidation. Thus, the 12 most insulin-resistant subjects were characterized by a cluster of abnormalities (mild overweight, higher blood pressure and prevalence of hypertension, higher serum triglycerides and insulin response to oral glucose, and reduced glucose storage) that included an insulin-induced increase in $[Ca^{2+}]_i$ (9 ± 2 nmol/L, $P < .001$ v basal). We conclude that insulin resistance, rather than hypertension, is associated with an abnormal in vivo effect of insulin on platelet $[Ca^{2+}]_i$.

CYTOSOLIC FREE CALCIUM concentrations ($[Ca^{2+}]_i$) are a major determinant of tension development in vascular smooth muscle, with increased $[Ca^{2+}]_i$ leading to increased arteriolar resistance.[1] On the other hand, $[Ca^{2+}]_i$ modulates multiple metabolic pathways, some of which are insulin-sensitive.[2] In rat fat cells, for example, there appears to be an optimal range of $[Ca^{2+}]_i$ for insulin stimulation of glucose transport,[3] although it is still disputed as to whether $[Ca^{2+}]_i$ is a bona fide intracellular mediator of insulin action.[4,5] Altered cellular Ca^{2+} metabolism has been shown to be associated with essential hypertension[6-8] and to predict preeclampsia[9] in humans. Following the demonstration that essential hypertension is an insulin-resistant state in man,[10] it has been logical to link both insulin resistance and high blood pressure with abnormal intracellular Ca^{2+} fluxes. The hypothesis is that a primary defect in the regulation of $[Ca^{2+}]_i$ impairs insulin action on the one hand and leads to high vascular resistance and hypertension on the other.[11,12] However, the evidence supporting this hypothesis is scarce and still largely circumstantial.

Our approach was to simultaneously measure insulin's effect on $[Ca^{2+}]_i$ and glucose metabolism in vivo over a wide range of insulin sensitivity in nondiabetic patients. Essential hypertension was chosen as the model insulin-resistant state because of the consistent abnormalities in Ca^{2+} metabolism associated with this disease.[6-9] Platelets were chosen as the probe for changes in $[Ca^{2+}]_i$ not only because they are easily obtained, but also because they carry surface insulin receptors[13] and share some,[14,15] if not all,[16] physiological properties of vascular smooth muscle cells.

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Submitted January 25, 1996; accepted May 28, 1996.

Supported in part by a research grant (Fondi 60%) from the Italian Ministry of University and Scientific Research.

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0026-0495/96/4511-0015$03.00/0

SUBJECTS AND METHODS

Subjects

The study population consisted of 25 subjects with essential hypertension recruited through the Hypertension Clinic and 25 normotensive controls (Table 1). Hypertension was defined as systolic blood pressure greater than 160 mm Hg and/or a diastolic value greater than 95 mm Hg. Secondary forms of hypertension were excluded by a complete clinical evaluation. The hypertensive patients either were untreated or had stopped antihypertensive therapy for 3 to 4 weeks before the study. All study subjects had a body mass index (BMI) less than 30 $kg \cdot m^{-2}$; the hypertensives as a group were significantly heavier than the normotensive group. All study subjects had normal oral glucose tolerance (on a 75-g oral glucose tolerance test [OGTT]) by conventional criteria.[17] No subjects were engaged in competitive sports, and all were recommended to refrain from strenuous physical exercise on the day preceding the study. Body weight and diet were stable. The study protocol was approved by the Institutional Ethics Committee; informed consent was obtained from all subjects before participation in the study.

Protocol

Insulin sensitivity was measured by the euglycemic insulin clamp[18] using an insulin infusion rate of 7 $pmol \cdot min^{-1} \cdot kg^{-1}$ (1 $mU \cdot min^{-1} \cdot kg^{-1}$). Briefly, polyethylene cannulas were inserted into an antecubital vein (for infusion of test substances) and retrogradely into a wrist vein heated at 60°C in a hot box (for intermittent blood sampling of arterialized venous blood). After a 60-minute baseline period, a 2-hour euglycemic-hyperinsulinemic clamp was performed. In 38 subjects (17 normotensive and 21 hypertensive), whole-body substrate oxidation rates were measured by indirect calorimetry using an open-circuit, computerized canopy system (Horizon; SensorMedics, Anaheim, CA). Calorimetric data were recorded for 45 minutes before starting the exogenous insulin infusion, and then throughout the clamp study. In each study subject, platelet $[Ca^{2+}]_i$ measurements were made twice: in the fasting state (~40 minutes before starting insulin infusion and 20 minutes after cannulation) and in the insulinized

Table 1. Characteristics of the Study Subjects

Characteristic	Normotensive	Hypertensive	P*
No.	25	25	
Gender (M/F)	16/9	20/5	NS
Age (yr)	41 ± 2	47 ± 2	<.04
BMI ($kg \cdot m^{-2}$)	23.3 ± 0.4	26.0 ± 0.4	<.0001
Blood pressure (mm Hg)			
Systolic	117 ± 2	149 ± 3	<.0001
Diastolic	76 ± 2	99 ± 2	<.0001
Mean	90 ± 2	116 ± 2	<.0001
Pulse pressure (mm Hg)	41 ± 2	50 ± 3	<.01
M value			
($\mu mol \cdot min^{-1} \cdot kg^{-1}$)	37.1 ± 1.6	27.2 ± 1.6	<.001

NOTE. Values are the mean ± SEM.
*By unpaired *t* test.

state (at the end of 2 hours of euglycemic hyperinsulinemia). Investigators were fully blinded with respect to the matching of platelet $[Ca^{2+}]_i$ results to in vivo insulin sensitivity measures. Arterial blood pressure was measured three times (at 5- to 10-minute intervals) in the fasting state and three more times at the end of the clamp. Each of the two sets of readings were averaged.

Platelet Preparation

Platelet-rich plasma was prepared from 9 mL arterialized venous blood (drawn into tubes containing 3.8% trisodium citrate) by centrifugation at 160 × *g* for 10 minutes at room temperature. The platelet-rich plasma was then diluted 1:2 with 2× Tyrode-HEPES buffer (290 mmol/L NaCl, 10 mmol/L KCl, 1 mmol/L Na_2HPO_4, 20 mmol/L HEPES, 11.2 mmol/L glucose, and 6 mmol/L EGTA, pH 7.4) and centrifuged at 2,000 × *g* for 10 minutes at room temperature. The pellet was resuspended in 2 mL Tyrode-HEPES buffer (145 mmol/L NaCl, 5 mmol/L KCl, 0.5 mmol/L Na_2HPO_4, 20 mmol/L HEPES, 5.6 mmol/L glucose, and 3 mmol/L EGTA, pH 7.4) and incubated with Fura-2/acetoxymethyl ester ([Fura-2/AM] Molecular Probes, Eugene, OR) at a final concentration of 5 μmol/L for 30 minutes at 37°C. To wash out the indicator, the platelet suspension was washed twice with buffer by centrifugation at 600 × *g* for 5 minutes at 4°C. The pellet was finally resuspended in buffer (at an approximate concentration of 1×10^8 cells/mL) and equilibrated for 1 minute at 37°C. Fura-2 fluorescence was read in a thermostat (37°C) cuvette at an excitation wavelength of 340 nm and an emission wavelength of 496 nm (on a model 650-40 spectrofluorometer; Perkin Elmer, Norwalk, CT).

In Ca^{2+}-supplemented media, estimation of $[Ca^{2+}]_i$ is critically dependent on the reincubation time of the platelets.[16] This dependence reflects the time required by Ca^{2+} transport mechanisms to reach equilibrium. However, backward leakage of dye (by exocytosis[19,20]) and transmembrane Ca^{2+} leakage from the Ca^{2+}-rich medium both can contribute to increasing $[Ca^{2+}]_i$ in a nonspecific manner. Therefore, for each set of $[Ca^{2+}]_i$ measurements, readings were first obtained in the absence of external Ca^{2+} (F_0). Immediately afterward, external Ca^{2+} was increased to a final concentration of 1.2 mmol/L by addition of $CaCl_2$, and further readings were made within 30 seconds ($F_{0.5'}$) and 5 minutes ($F_{5'}$) and 10 minutes ($F_{10'}$) later. The Fura-2/AM signal was calibrated as described by Tsien et al[21] according to the formula, $[Ca^{2+}]_i = K_d (F - F_{min})/(F_{max} - F)$, where K_d (224 nmol/L) is the dissociation constant of the Fura-2-Ca^{2+} complex and F corresponds to $F_{0'}$,$F_{0.5'}$, $F_{5'}$, and $F_{10'}$ alternatively. F_{max} was obtained by solubilizing the platelet suspension with 0.2% Triton X-100 and saturating the soluble Fura-2 with Ca^{2+}. F_{min} was obtained by adding an excess of 4.3 mmol/L EGTA (pH > 8.3).

All readings were made in duplicate. Precision was 8.5% for readings taken in Ca^{2+}-free medium and 7.5% in 1.2 mmol/L Ca^{2+}. Platelet responsivity in our serum-free system was checked by measuring the increase in $[Ca^{2+}]_i$ following the in vitro addition of thrombin (0.3 U/mL). On sequential measurements made in the absence of external Ca^{2+}, platelet $[Ca^{2+}]_i$ was found to be stable for up to 60 minutes (data not shown).

Data Analysis

Mean blood pressure was calculated as the sum of diastolic blood pressure and one third of the pulse pressure. Insulin sensitivity was expressed as the M value, ie, whole-body glucose disposal during the last 60 minutes of euglycemic hyperinsulinemia.[18] As previously shown, at the insulin infusion rate used in these studies, endogenous (hepatic) glucose production is fully suppressed during the second hour of the clamp in both normotensive and hypertensive individuals.[10] The M value was therefore calculated from the exogenous glucose infusion rate after correction for changes in glucose concentration in a total distribution volume of 250 $mL \cdot kg^{-1}$.[22] Net rates of carbohydrate oxidation were calculated from standard calorimetric equations, as described in detail elsewhere.[23] Nonoxidative glucose disposal, or glucose storage, was calculated as the difference between the M value and the carbohydrate oxidation rate. On the OGTT, glucose and insulin (in 35 subjects) areas under the curve were calculated by trapezoidal integration.

Data are shown as the mean ± SEM. Comparison of mean group values was made by two-tailed Student's *t* test, paired or unpaired as appropriate. Single and multiple regression analyses were made by standard techniques.

RESULTS

Insulin Sensitivity

Tolerance to oral glucose was maintained at the expense of significant hyperinsulinemia in the hypertensive group in comparison to the normotensive group (Fig 1). There was no significant difference in the glucose area under the curve (15.6 ± 0.6 *v* 16.5 ± 0.5 $mol \cdot l^{-1} \cdot 2\ h^{-1}$, normotensive *v* hypertensive, P = NS), but the plasma insulin area under the curve was 70% higher in hypertensive than in normotensive subjects (5.4 ± 0.5 *v* 3.2 ± 0.3 $nmol \cdot L^{-1} \cdot 2\ h^{-1}$, $P < .001$). Insulin sensitivity (M value) had a fourfold range (14 to 56 $\mu mol \cdot min^{-1} \cdot kg^{-1}$), and was 40% lower in the hypertensive group compared with the normotensive subjects (Table 1). This difference remained significant when statistically adjusted for BMI or age. Insulin sensitivity and the insulin area under the curve were significantly related in an inverse fashion ($r = .43$, $P < .01$). During the clamp, whole-body net carbohydrate oxidation was not different between the two groups (12 ± 2 *v* 11 ± 1 $\mu mol \cdot min^{-1} \cdot kg^{-1}$, normotensive *v* hypertensive, P = NS), whereas glucose storage was 35% lower in hypertensives than in controls (16 ± 2 *v* 24 ± 2 $\mu mol \cdot min^{-1} \cdot kg^{-1}$, $P < .01$). During the clamp, mean blood pressure decreased from 90 ± 2 to 88 ± 2 mm Hg in controls and from 116 ± 2 to 114 ± 2 mm Hg in hypertensives; neither change reached statistical significance.

Platelet $[Ca^{2+}]_i$

In the fasting state, $[Ca^{2+}]_i$, measured in the absence of external Ca^{2+}, was 23 ± 1 nmol/L in the whole group. This

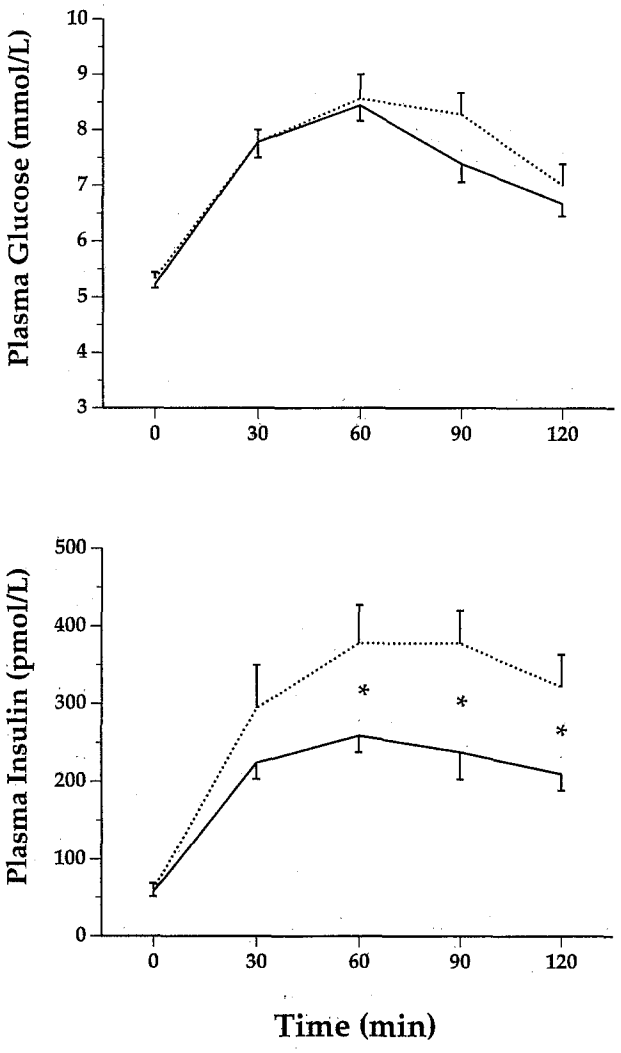

Fig 1. Plasma glucose and insulin concentrations in response to an oral glucose load in normotensive (——) and hypertensive (...) study subjects. *Mean values that are significantly different from one another (at $P \leq .05$) by unpaired t test.

value is similar to that obtained by Jacobs et al[24] in resting human platelets incubated in Ca^{2+}-free media. Following in vitro Ca^{2+} addition, $[Ca^{2+}]_i$ increased to 33 ± 1 nmol/L at 0.5 minutes, 47 ± 2 nmol/L at 5 minutes, and 58 ± 2 nmol/L at 10 minutes (Fig 2). None of these values nor the rate of $[Ca^{2+}]_i$ increase between 0.5 and 10 minutes after Ca^{2+} loading (a mean of 2 nmol · L^{-1} · min^{-1} for the whole group) showed a significant relationship with gender, age, BMI, blood pressure, or insulin sensitivity.

After the clamp, $[Ca^{2+}]_i$ was significantly increased in the whole study group regardless of whether Ca^{2+} was present in the incubation medium ($P \leq .01$ for all four measurements; Fig 2). Interindividual variability of the insulin-induced change in $[Ca^{2+}]_i$ was smaller when measured in the absence than in the presence of in vitro Ca^{2+} (coefficient of variation, 195% v 270%). The insulin-induced change in platelet $[Ca^{2+}]_i$ in the absence of external Ca^{2+} ($\Delta[Ca^{2+}]_i$) was not significantly related to age, gender, or BMI, and showed only weak positive associations with

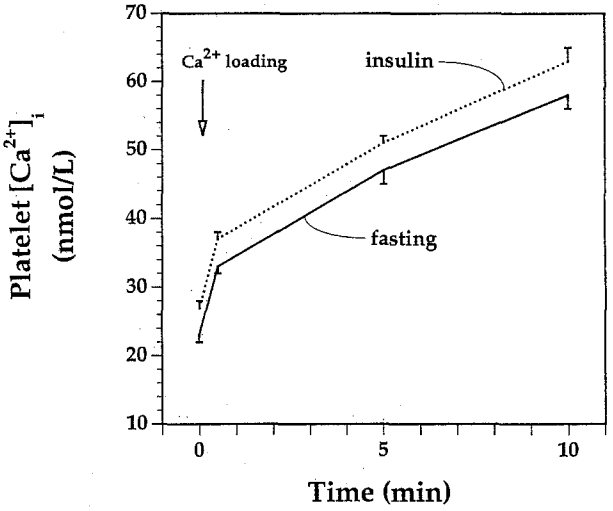

Fig 2. Platelet $[Ca^{2+}]_i$ in the absence of Ca^{2+} in the medium (time 0) and 0.5, 5, and 10 minutes following in vitro Ca^{2+} loading in 50 nondiabetic subjects. In each subject, $[Ca^{2+}]_i$ measurements were obtained in the basal (fasting) state (——) and again following in vivo insulin administration (...).

measures of arterial blood pressure (r = .25 to .30, P = .08 to .04). In contrast, a positive $\Delta[Ca^{2+}]_i$ was consistently associated with insulin resistance (r = .35, P = .01; Fig 3). $\Delta[Ca^{2+}]_i$ and BMI contributed to insulin resistance independently of one another, together accounting for 45% of the observed variability of M values in a multiple linear regression model ($P < .0001$). Furthermore, $\Delta[Ca^{2+}]_i$ was significantly related to glucose storage in an inverse fashion (r = .39, P = .01; Fig 4), whereas it showed no relation to carbohydrate oxidation during fasting or insulin infusion.

Obesity (BMI) was only weakly related to $\Delta[Ca^{2+}]_i$ (r = .25, P = .08). Accordingly, when overweight subjects (BMI > 27 kg · m^{-2}, n = 12) were excluded, a higher $\Delta[Ca^{2+}]_i$ remained associated with lower insulin sensitivity (r = .42, $P < .01$, n = 38) and lower glucose storage (r = .49, $P < .01$, n = 28).

To characterize insulin resistance in our study popula-

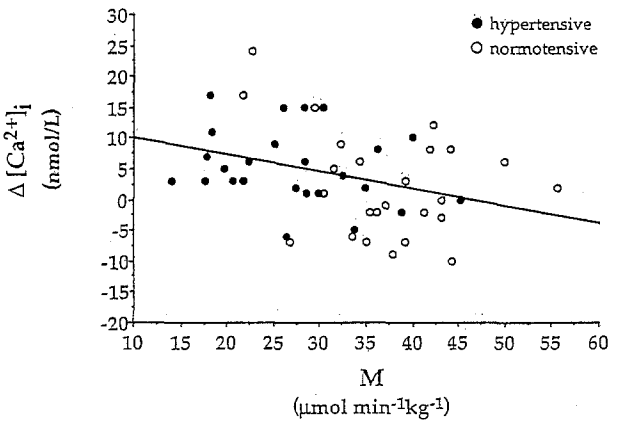

Fig 3. Inverse relationship between insulin sensitivity (M value) and $\Delta[Ca^{2+}]_i$ in normotensive (○) and hypertensive (●) nondiabetic subjects.

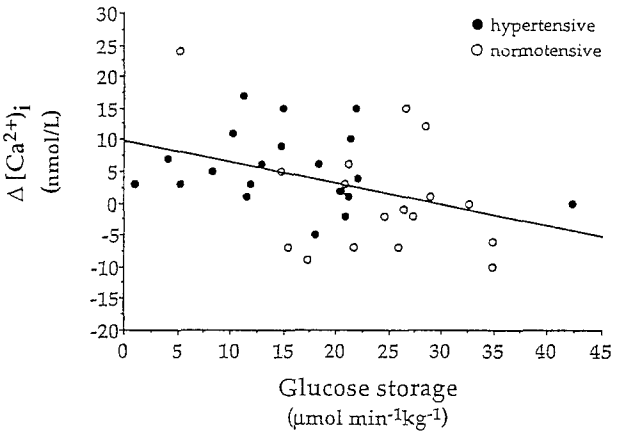

Fig 4. Inverse relationship between insulin-mediated glucose storage and $\Delta[Ca^{2+}]_i$ in normotensive (○) and hypertensive (●) nondiabetic subjects.

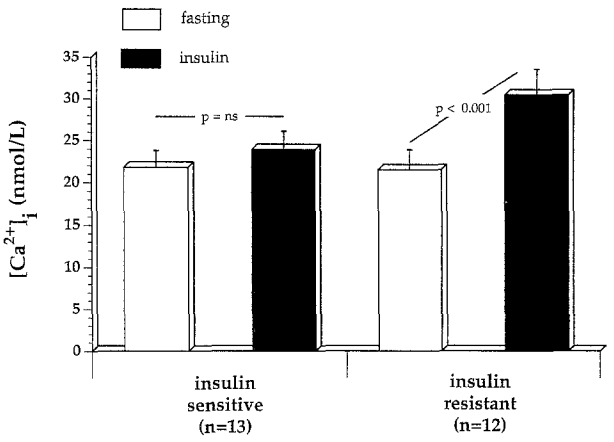

Fig 5. Fasting and insulin-stimulated platelet $[Ca^{2+}]_i$ in subjects in the bottom and top quartiles of insulin sensitivity. *P* refers to group comparisons by paired *t* test.

tion, subjects in the lowest quartile of M values (<26.1 $\mu mol \cdot min^{-1} \cdot kg^{-1}$) were compared with those in the top quartile (>39.1 $\mu mol \cdot min^{-1} \cdot kg^{-1}$) (Table 2). Insulin-resistant subjects were heavier, had higher serum triglyceride but not total cholesterol concentrations, and had higher blood pressure (by standard diagnostic criteria, 85% were hypertensive *v* 15% of insulin-sensitive individuals). With a comparable degree of glucose tolerance (glucose area under the curve), insulin-resistant subjects had a hyperinsulinemic response (insulin area under the curve) and lower rates of glucose storage than insulin-sensitive individuals. In response to in vivo insulin, there was no change in platelet $[Ca^{2+}]_i$ in insulin-sensitive subjects ($\Delta[Ca^{2+}]_i$, 2 ± 2 nmol/L), whereas a significant increase occurred in insulin-resistant individuals (9 ± 2 nmol/L, $P < .02$ *v* insulin-sensitive subjects; Fig 5).

In five healthy volunteers (aged 32 ± 2 years), a time-control study was performed by measuring platelet $[Ca^{2+}]_i$ in the fasting state and again after 3 hours of a sham clamp study in which saline replaced insulin infusion. The two sets of readings were not statistically different (16 ± 3 *v* 17 ± 3 nmol/L in the absence of external Ca^{2+}, 41 ± 7 *v* 35 ± 3 nmol/L at 5 minutes, and 50 ± 8 *v* 46 ± 4 nmol/L at 10 minutes following Ca^{2+} addition, all P = NS).

Table 2. Characteristics of Insulin-Resistant and Insulin-Sensitive Subjects

Characteristic	Resistant	Sensitive	*P**
No.	12	13	
Gender (M/F)	11/1	9/4	NS
Age (yr)	48 ± 2	40 ± 3	NS
BMI ($kg \cdot m^{-2}$)	26.9 ± 0.6	23.3 ± 0.6	.0003
Blood pressure (mm Hg)			
Systolic	143 ± 4	119 ± 4	.0002
Diastolic	96 ± 3	78 ± 3	.0006
Hypertensives (%)	83	15	.0002
Total cholesterol (mmol/L)	4.9 ± 0.3	5.2 ± 0.5	NS
Triglycerides (mmol/L)	1.3 ± 0.2	0.88 ± 0.1	$.1 > P > .05$
Glucose area ($mol \cdot L^{-1} \cdot 2\ h^{-1}$)	16.5 ± 0.7	14.7 ± 0.6	NS
Insulin area ($nmol \cdot L^{-1} \cdot 2\ h^{-1}$)	6.2 ± 0.8	3.4 ± 0.7	<.03
M value ($\mu mol \cdot min^{-1} \cdot kg^{-1}$)	19.9 ± 0.9	43.7 ± 1.3	.0001
Glucose storage ($\mu mol \cdot min^{-1} \cdot kg^{-1}$)	8.5 ± 1.4	29.2 ± 2.4	.0001

NOTE. Results are from subjects in the upper and lower quartiles of log M values (mean ± SEM).

*By unpaired *t* test.

DISCUSSION

In a study population enriched with insulin-resistant hypertensive patients, we found that insulin resistance is associated with abnormalities of in vivo platelet Ca^{2+} handling. In insulin-sensitive subjects insulin administration did not change platelet $[Ca^{2+}]_i$ (measured in Ca^{2+}-free medium), whereas in insulin-resistant individuals insulin increased $[Ca^{2+}]_i$. This differential Ca^{2+} response segregated with insulin resistance more closely than with hypertension or overweight, although insulin-resistant subjects showed the usual cluster of abnormalities (higher blood pressure, body weight, insulin response to oral glucose, and serum triglycerides). Furthermore, the insulin-induced increase in $[Ca^{2+}]_i$ was clearly associated with impaired stimulation of glucose storage (ie, glycogen synthesis). These results are compatible with the conclusion that insulin resistance extends to the regulation of platelet Ca^{2+} metabolism. The implication is that an abnormal $[Ca^{2+}]_i$ response to insulin may be one cellular basis for at least two defects (increased vascular resistance and hyperaggregability) that characterize hypertension, diabetes, and obesity, ie, insulin-resistant states. However, both the conclusion and the implication require careful specification.

Firstly, we measured $[Ca^{2+}]_i$ both in the absence of external Ca^{2+} and, sequentially, after in vitro Ca^{2+} loading, because at 37°C leakage of dye and Ca^{2+} can enhance the fluorescence signal in a nonspecific manner.[16,19,20] Measurements of $[Ca^{2+}]_i$ in Ca^{2+}-free medium were found to be stable over time, and following in vivo insulin administration, the increase in $[Ca^{2+}]_i$ in the whole study group was already seen in platelets incubated in Ca^{2+}-free medium, the gain in $[Ca^{2+}]_i$ being essentially constant upon loading cells with 1.2 mmol/L Ca^{2+} (Fig 2). These results protect against the confounding effect of in vitro dye and/or Ca^{2+}

leakage, as well as reincubation time. More importantly, they indicate that the effect of insulin, whether on Ca^{2+} inward flux, Ca^{2+} efflux, or Ca^{2+} exchange with the dense tubular system, was a true in vivo effect.

Secondly, in our studies the effect of insulin on $[Ca^{2+}]_i$, if consistent, was small (5 nmol/L in the whole study group and 9 nmol/L in insulin-resistant subjects). However, we used unstimulated cells, in which hormonal effects on $[Ca^{2+}]_i$ are normally small. In dispersed vascular smooth muscle cells from canine artery, for example, a short preincubation with physiological amounts of insulin reduced basal $[Ca^{2+}]_i$ only from 78 to 62 nmol/L, but markedly attenuated serotonin-induced $[Ca^{2+}]_i$ spikes and accelerated their dissipation.[25] Other studies—in a7r5 cells,[26] phenylephrine-stimulated aortic strips from Zucker rats,[27] and cultured vascular smooth muscle cells[28]—have found that insulin attenuates $[Ca^{2+}]_i$ increases by stimulating Ca^{2+} efflux. Whether by acting on influx or efflux, the ability of insulin to blunt Ca^{2+} transients in smooth muscle cells in vitro seems to be an established phenomenon. Consequently, in these cells, resistance of intracellular Ca^{2+} metabolism to insulin action could translate into higher $[Ca^{2+}]_i$ responses to the hormone. To the extent that platelets are a model for vascular smooth muscle, our finding—that insulin in vivo is associated with an increase in unstimulated platelet $[Ca^{2+}]_i$ in insulin-resistant subjects—is compatible with this concept. Whether platelet $[Ca^{2+}]_i$ spikes in response to proper agonists (eg, vasopressin) also are larger in insulin-resistant subjects remains to be directly verified.

Thirdly, we did not detect any association between basal platelet $[Ca^{2+}]_i$ and the presence of hypertension or the blood pressure level. This result is in contrast with several studies[29-35] that have reported increased basal $[Ca^{2+}]_i$ in patients with essential hypertension. However, the magnitude of this change has been surprisingly variable, ranging from as high +100% (as compared with normotensive controls)[34] to +14%,[31] with correlation coefficients of basal $[Ca^{2+}]_i$ with blood pressure ranging from .92[30] to .22.[35] Negative studies also are reported in the literature.[36-38] Furthermore, in confirmed preeclamptic women, in whom the $[Ca^{2+}]_i$ response to vasopressin predicted the development of preeclampsia, basal $[Ca^{2+}]_i$ was not elevated in the face of increased blood pressure.[9] A genetic analysis of the association of blood pressure with $[Ca^{2+}]_i$ in a large sample of normotensive twins[37] provided evidence that environmental, but not genetic, factors explain the covariation of these two traits. In the aggregate, it would appear that methodological differences (eg, Quin-2 *v* Fura-2 as the probe, presence or absence of Ca^{2+} in the incubation media, correction for leakage, etc.) and a host of physiological circumstances (age, obesity, severity of hypertension, serum parathyroid hormone and ionized Ca^{2+} concentrations, shear stress, levels of circulating agonists, previous or current drug treatment, etc.) contribute to the variability of reported basal platelet $[Ca^{2+}]_i$ values in human hypertension.

With regard to the mechanism(s) underlying the observed association between insulin's effect on $[Ca^{2+}]_i$ and insulin sensitivity, the following observations can be made. Ca^{2+} metabolism in platelets appears to be directly affected by insulin (in addition to other factors), with a normal in vivo response consisting of decreased $[Ca^{2+}]_i$. In human platelets, ouabain increases both cytosolic Ca^{2+} and Na^+ by blocking Na^+-K^+ countertransport.[39] Insulin is a potent stimulus for the Na^+-K^+ pump—an effect that is canceled by ouabain in vivo.[40] Thus, insulin could enhance Ca^{2+} sequestration within the dense tubular system by promoting Na^+-K^+ countertransport at the cell membrane. Alternative (or concurrent) pathways could involve stimulation of Ca^{2+}-Mg^{2+} adenosine triphosphatase activity or Ca^{2+} efflux.[26,27] One physiological counterpart of this action on $[Ca^{2+}]_i$ is reduced platelet aggregability. In fact, physiological amounts of insulin antagonize human platelet aggregation in response to different agonists,[41,42] an effect dependent on an intact tyrosine kinase activity of the insulin receptor.[43] Therefore, insulin resistance of cellular Ca^{2+} homeostasis should result in increased $[Ca^{2+}]_i$ and hyperaggregability. Recent studies by Touyz and Schiffrin[44] support this prediction. These investigators have reported that angiotensin II– and endothelin-1–stimulated increases in $[Ca^{2+}]_i$ are attenuated by in vitro incubation with physiological doses (70 μU/mL) of insulin. Concomitantly, angiotensin II– and thrombin-induced platelet aggregation were consistently blunted. Both these effects of in vitro insulin were impaired in patients with essential hypertension, in proportion to the degree of hyperinsulinemia.[44] Our current findings appear to be the exact in vivo equivalent of these in vitro results: in insulin-resistant hyperinsulinemic subjects, in vivo insulin administration increases platelet $[Ca^{2+}]_i$.

In patients with non–insulin-dependent diabetes mellitus, who generally are insulin-resistant, altered cellular Ca^{2+} and Na^+ transport has been described in platelets and in a variety of other tissues (reviewed in Levy et al[45]). Our results are compatible with the idea that in vivo insulin resistance of intracellular Ca^{2+} metabolism is one common defect underlying mellitus and hypertension.

ACKNOWLEDGMENT

We wish to thank Karl E. Sussman, MD, for inspiring our interest in calcium metabolism and for providing critical intellectual support.

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