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# Cytosolic Calcium and Insulin Resistance in Elderly Patients With Essential Hypertension

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**We evaluated insulin sensitivity in normotensive (blood pressure, BP, < 135/85 mm Hg) and hypertensive (BP > 160/90 mm Hg) elderly subjects over 65 years old who were stratified as normal weight (body mass index, BMI, < 27) and obese (BMI > 27). Obese hypertensive individuals demonstrated marked hyperinsulinemia ($P < .01$) and significantly reduced ($P < .05$) submaximally stimulated adipocyte 2-deoxyglucose (2-DOG) uptake (abdominal wall fat biopsy). Normal weight hypertensive subjects also demonstrated higher levels of insulinemia and lower insulin-stimulated 2-DOG uptake than nonobese controls. Adipocyte $[Ca^{2+}]_i$ levels were elevated in all elderly subjects compared to young individuals ($P < .01$). Basal and maximally stimulated 2-DOG uptake were similar in all groups.**

**One month of therapy with a calcium channel blocker, 10 mg nitrendipine twice daily, reduced blood pressure in the hypertensive subjects, reduced plasma insulin to control values during an oral glucose tolerance test in obese hypertensive individuals ($P < .01$), and restored adipocyte 2-DOG uptake at submaximally effective insulin concentration to control values in normal weight and obese hypertensive subjects.**

**In summary, older hypertensive, and particularly older obese hypertensive, patients manifest significant insulin resistance accompanied by elevated levels of $[Ca^{2+}]_i$ in their adipocytes. Am J Hypertens 1992;5:459–464**

KEY WORDS: **Hypertension, insulin resistance, cytosolic calcium.**

The presence of insulin resistance in many patients with hypertension has become a well-recognized phenomenon.[1–3] However, the mechanism of this association remains enigmatic. At least three possibilities may explain the association: 1) generalized or selective insulin resistance with ensuing hyperinsulinemia may participate in the pathogenesis of hypertension; 2) alterations in the intracellular milieu induced by or associated with hypertension may cause insulin resistance; or 3) insulin resistance and hypertension may represent two independent consequences of the same metabolic abnormalities which results in insulin resistance in insulin target cells or vasoconstriction in vascular smooth muscle cells.

We hypothesize that abnormal cellular $Ca^{2+}$ handling, particularly elevations in cytosolic free $Ca^{2+}$ concentrations, $[Ca^{2+}]_i$, may represent a common intracellular abnormality, a missing link, responsible for the frequent coexistence of insulin resistance with hypertension.

We have recently shown that sustained elevations of $[Ca^{2+}]_i$ in insulin target cells, such as observed in patients with obesity, non–insulin-dependent diabetes mellitus, and hypertension, may lead to the development of insulin resistance.[4,5] The mechanisms leading to such increases are not yet well understood. They appear to

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include an enhanced influx of calcium via calcium channels. Thus, we found that the presence of the calcium channel blocker verapamil in the incubation media prevented hyperglycemia- and hyperinsulinemia-induced rises in $[Ca^{2+}]_i$ and decreases in the insulin stimulated glucose uptake.[4] Recently, Sheu and coworkers[6] demonstrated that treatment of hypertensive patients with nifedipine decreased their insulin resistance and enhanced insulin-stimulated glucose uptake.

To further evaluate the existence of an association between elevated levels of $[Ca^{2+}]_i$ and diminished cellular sensitivity to insulin in patients with essential hypertension, we studied these factors in adipocytes obtained from older hypertensive nondiabetic subjects.

## MATERIALS AND METHODS

**Materials** Porcine insulin was a gift from Eli Lilly Co. (Indianapolis, IN). Fura-2 and fura-2AM were purchased from Behring Diagnostics (San Diego, CA), collagenase was obtained from Worthington Biochemical Corp. (Freehold, NJ), [$^3$H]2-deoxyglucose from Amersham Corp. (Arlington Heights, IL), L-[$^3$H]glucose from DuPont-New England Nuclear (Boston, MA), and all standard chemicals from Sigma Company (St. Louis, MO). Nitrendipine was provided by Miles Pharmaceutical Inc. (New Haven, CT).

**Subjects** Four groups of subjects were studied. Group I consisted of five normotensive normal weight control subjects (BMI 22.9 ± 0.9) with an average age of 67 ± 0.5. Group II consisted of three normotensive obese control subjects (BMI 32.3 ± 3.7, $P < .01$ *v* Group I) with an average age of 69 ± 1.2. Group III included six normal weight hypertensive patients (BMI 24.4 ± 0.6) with an average age of 68 ± 1.2 years old; and Group IV included five obese hypertensive patients (BMI 30.8 ± 1.1, $P < .01$ *v* Group III) with an average age of 71 ± 1.8. The studies were performed at the Clinical Research Center of the University of Colorado Health Sciences Center.

**Protocol** Caucasian subjects over age 60 were recruited from five contiguous urban counties. Subjects were excluded for the following: complications of hypertension, secondary hypertension, abnormal renal function, diabetes mellitus, systolic blood pressure > 210 mm Hg or diastolic blood pressure > 115 mm Hg, alcoholism, or other chronic illnesses. A complete history and informed consent were obtained and a physical examination performed. Following this, antihypertensive medications were stopped for 2 weeks. Hypertensive subjects then qualified when the average of three casual sitting blood pressures measured with calibrated random zero sphygmomanometers by trained observers averaged > 160/95 mm Hg. The first and fifth Korsakoff sounds were used respectively as the systolic and diastolic blood pressure (BP). An age- (± 3 years) and sex-matched normotensive control subject, BP < 135/85 mm Hg determined by the same method, was selected for each hypertensive subject.

All subjects underwent a 3 h oral glucose tolerance test (OGTT) to measure serum glucose and insulin and low abdominal wall fat biopsy.[5] Adipocytes were isolated from a biopsy specimen and used to determine the levels of $[Ca^{2+}]_i$ and both basal and insulin-stimulated 2-deoxyglucose uptake. Following this procedure, all patients (controls and hypertensive) were given the calcium channel blocker nitrendipine, 10 mg twice daily, for 30 days. Both OGTT and fat biopsy were repeated and the same measurements performed at the end of the treatment period.

Nitrendipine significantly decreased systolic and diastolic blood pressure from a baseline of 161 ± 5/91 ± 3 mm Hg to 141 ± 4/82 ± 4 mm Hg ($P < .003$) in the hypertensive subjects and the normotensive controls decreased from 132 ± 4/82 ± 3 mm Hg to 125 ± 4/81 ± 3 mm Hg ($P$ = NS).

*Measurements of $[Ca^{2+}]_i$ and 2-Deoxyglucose Uptake* The measurments of $[Ca^{2+}]_i$ were performed as previously described[4,5] using a spectrofluorometer (model 340; Turner Designs, Mountain View, CA) and fura-2 $Ca^{2+}$ indicator. During fura-2 loading (45 min at 37°C) and $Ca^{2+}$ measurments, the cells were incubated in 2.4 mL of Krebs-HEPES buffer containing 1 mmol/L $CaCl_2$, 118.4 mmol/L NaCl, 4.69 mmol/L KCl, 1.2 mmol/L $MgCl_2$, 1.18 mmol/L $KH_2PO_4$, 1.25 mmol/L $NaHCO_3$, 20 mmol/L HEPES, 5 mg/mL bovine serum bovine serum albumin, and 30 mg/dL glucose at pH 7.4. The final cell concentration was approximately $2 \times 10^5$ cells/cuvette (or $8 \times 10^4$ cells/mL). To introduce fura-2 intracellularly, 12.5 μg fura-2AM was added to 2 mL of the cell suspension to yield a fura-2 concentration of 3.11 μmol/L. The loading of adipocytes with fura-2 in concentrations ranging between 3 and 8 μmol/L did not affect the final determinations of $[Ca^{2+}]_i$. The fluorescence of the extracellular fura-2 was estimated by adding $MnCl_2$ (50 μmol/L, which quenches extracellular fura-2. $MnCl_2$ was then chelated by the addition of 100 μmol/L pentetic acid. The fluorescence of either the buffers used in these studies or of tissues (without fura-2) was 10 to 13% of that observed with the cells loaded with the probe. For the assessment of 2-deoxyglucose uptake, adipocytes ($2 \times 10^5$ cells) were incubated in the absence and in the presence of insulin (5 and 25 ng/mL) for 30 min at 37°C, as previously described.[4,5]

*Statistics* The results of all experiments are presented as mean ± SEM and compared using a two-way analysis of variance or multiple paired *t* test with Scheffé and Bonferroni corrections. All variables were normally dis-

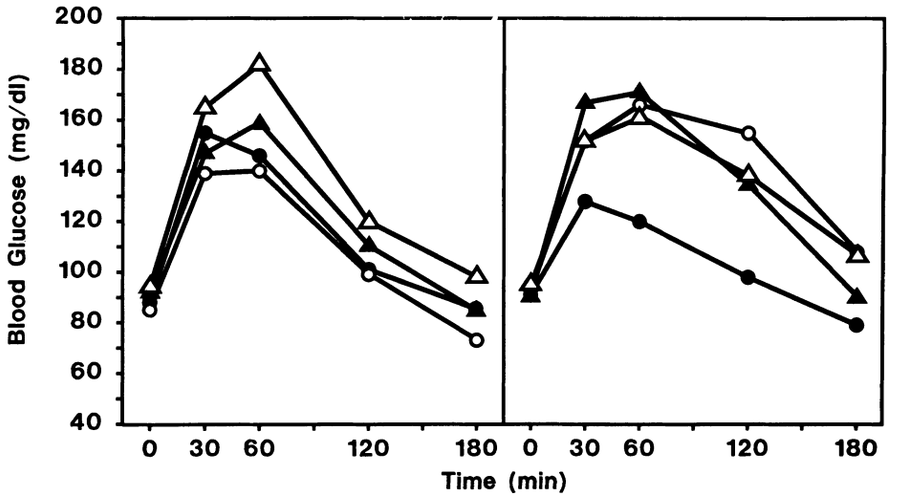


**FIGURE 1.** *The levels of glycemia during oral glucose tolerance test in control (A) and hypertensive (B) patients before (●, ○) and after (▲, △) nitrendipine treatment. ●, ▲ = normal weight; ○, △ = obese.*

tributed and the variances were homogeneous across groups.

## RESULTS

Figure 1 depicts glucose levels during OGTT performed before and after nitrendipine treatment. The data are presented as mean values; standard errors are not shown for clarity. There was no significant difference in either basal or postload levels of glycemia among the four groups of individuals. Nitrendipine treatment did not alter glucose response to oral glucose load in these patients.

Obese patients, both normotensive and hypertensive (Groups II and IV), displayed fasting hyperinsulinemia (12.6 ± 1.5 and 13.8 ± 1.9 μU/mL) as compared with normal weight normotensive and hypertensive subjects (6.8 ± 1.2 and 7.8 ± 0.9 μU/mL, respectively, $P < .05$). Prior to the therapy, obese normotensive individuals (Group II) had higher levels of insulin than normal weight controls (Group I) (Figure 2). Although normal weight hypertensive subjects (Group III) demonstrated higher levels of insulinemia than nonobese normotensive controls (Group I, $P < .05$ at 120 min, 64 ± 14 *v* 35 ± 7 μU/mL), the highest levels of insulinemia were observed in obese hypertensive individuals (Group IV) (Figure 2, $P < .05$ at 60, 120, and 180 min).

One month of treatment with nitrendipine did not appreciably change levels of insulin in the control normotensive, obese normotensive, or normal weight hypertensive patients (Groups I, II, and III). However, nitrendipine treatment resulted in a significant ($P < .01$) reduction in the level of insulin in obese hypertensive patients (Figure 2). The differences among the groups and the effect of nitrendipine therapy were even more evident when the levels of insulinemia were expressed as areas under the curve (Figure 3). The area under the curve in obese hypertensive individuals (1470 ± 150 units) was almost additive to those found in obese (870 ± 62) or hypertensive (830 ± 54) subjects, which in turn were significantly greater than controls (530 ± 42, $P < .05$). Taken together with equal levels of glycemia, the presence of hyperinsulinemia indicates insulin resistance in these subjects.

The measurement of adipocyte $[Ca^{2+}]_i$ is summarized in Table 1. Since all patients in the present study were over the age of 60, we compared these data with our previous observations in younger (35 to 60 years old) individuals.[5] The assays were performed concurrently with the samples reported in the present manuscript.) The levels of $[Ca^{2+}]_i$ in young normal weight people were 176 ± 12 nmol/L. Older subjects, whether normal weight or obese, whether hypertensive or not, all had significantly ($P < .05$) higher levels of $[Ca^{2+}]_i$ in their adipocytes. There was no significant change in adipocyte $[Ca^{2+}]_i$ following 1 month of nitrendipine therapy (Table 1).

Figure 4 demonstrates 2-deoxyglucose uptake in adipocytes isolated from the study patients. Hypertensive patients (Groups III and IV) demonstrated decreased sensitivity to insulin. The percent stimulation of glucose transport with submaximally effective concentrations of insulin (5 ng/mL) in hypertensive patients was approximately half that observed in control individuals ($P < .05$).

Nitrendipine treatment did not appreciably affect glucose transport in either normal weight or obese normotensive patients (Groups I and II). However, nitrendipine improved glucose uptake at submaximally effective insulin concentrations in both hypertensive groups (Groups III and IV, $P < .05$). This improvement was

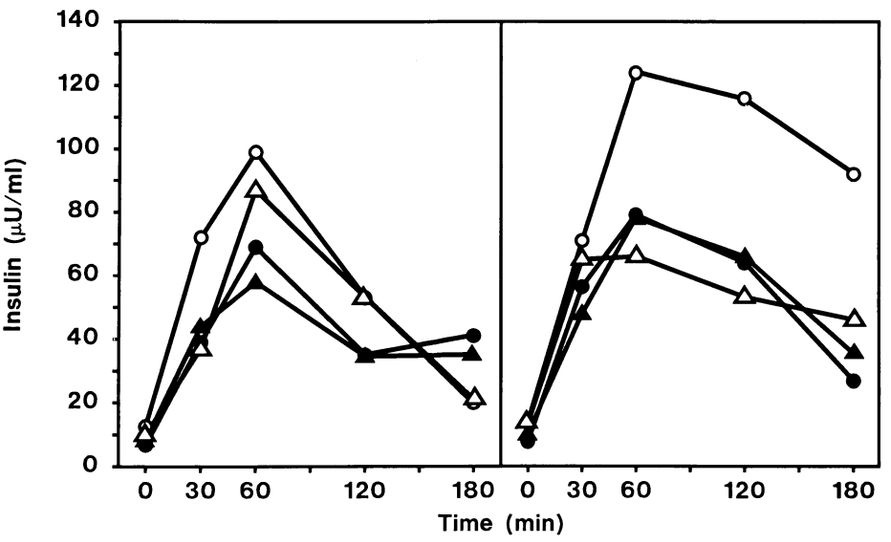


**FIGURE 2.** *The levels of insulinemia during oral glucose tolerance test in control (A) and hypertensive (B) patients before (●, ○) and after (▲, △) nitrendipine therapy. ●, ▲ = normal weight; ○, △ = obese.*

particularly significant ($P < .01$) in hypertensive obese individuals (Figure 4).

## DISCUSSION

The present findings confirm the existence of insulin resistance in hypertensive individuals.[1–3] They also indicate that older obese hypertensive subjects are insulin resistant to a degree greater than can be explained by the presence of either obesity or hypertension separately. The insulin resistance in these patients manifests both in vivo by the presence of hyperinsulinemia (both fasting and postglucose load) and in vitro by the diminished insulin-stimulated glucose uptake in isolated adipocytes.

Although we have observed elevated levels of $[Ca^{2+}]_i$ in all four groups of patients studied, only hypertensive patients and especially obese hypertensive patients demonstrated insulin resistance. Normal weight and obese hypertensive patients exhibited modestly higher levels of adipocyte $[Ca^{2+}]_i$ (10 to 12%) that were not significantly different from older controls. It is conceivable that this trend may become significant with an increase in sample size. The similar lack of one-on-one correlation between $[Ca^{2+}]_i$ and insulin sensitivity was previously observed in patients with obesity and non–insulin-dependent diabetes mellitus (NIDDM).[5] In the previous studies, we found high levels of $[Ca^{2+}]_i$ concentrations in patients with obesity and in patients with NIDDM (treated with either insulin or oral agents), but the degree of insulin resistance among these patients varied widely and did not correlate with the absolute levels of $[Ca^{2+}]_i$. One explanation for the apparent lack of a one-to-one correlation is that the measurements of $[Ca^{2+}]_i$ do not provide an adequate assessment of the

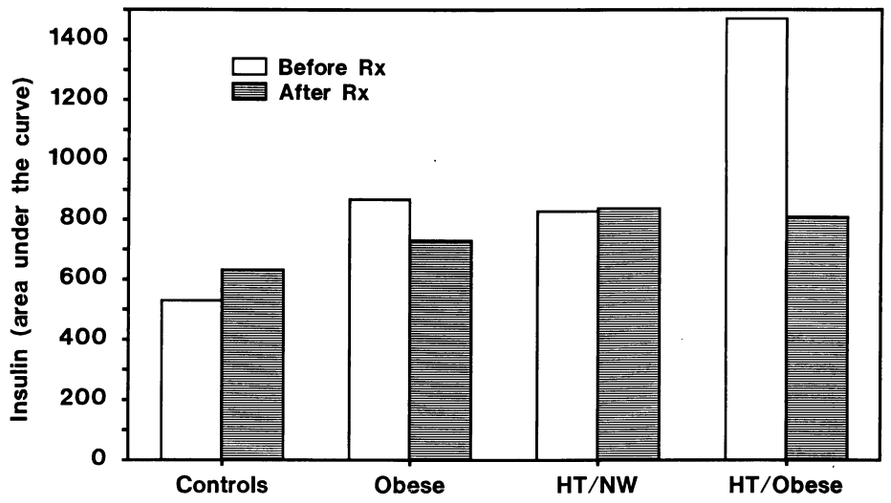


**FIGURE 3.** *The levels of insulinemia expressed as area under the curve in study patients before and after nitrendipine treatment.*

TABLE 1. ADIPOCYTE $[Ca^{2+}]_i$, nmol/L

| | Controls* (35–60 years old) | Controls† (65–77 years old) (Group I) | Normotensive† Obese (Group II) | Hypertensive† Group III) | Hypertensive† Obese (Group IV) |
|---|---|---|---|---|---|
| Before nitrendipine | 176 ± 12 | 229 ± 19 | 214 ± 11 | 248 ± 19 | 236 ± 4 |
| After nitrendipine | | 220 ± 13 | 209 ± 17 | 216 ± 17 | 225 ± 13 |

* *Data from Segal et al,[5] performed concurrently, but reported earlier.*

† $P < .05$ v *controls (35–60 years old).*

$Ca^{2+}$ content in various cellular compartments nor do they reflect $Ca^{2+}$ fluxes or oscillations (or lack of them) in intracellular $Ca^{2+}$. Kelly et al[7] found no obligatory relationship between an acute elevation in $[Ca^{2+}]_i$ and diminished insulin action, but reported that an impairment in the insulin-stimulated glucose uptake in adipocytes correlated ($r = -0.92$) with $Ca^{2+}$ influx. Since measurement of $[Ca^{2+}]_i$ represents only one facet of the cellular $Ca^{2+}$ handling, it may not correlate precisely with the degree of insulin insensitivity.

The mechanism of the elevations of $[Ca^{2+}]_i$ in hypertension is not well understood. Blaustein put forth a hypothesis suggesting that increases in $[Ca^{2+}]_i$ in hypertension are secondary to an inhibition of $Na^+/K^+$-ATPase with accumulation of intracellular sodium.[8] The latter inhibits $Na^+$-$Ca^{2+}$-exchange ($Na^+$ in, $Ca^{2+}$ out), thereby diminishing $Ca^{2+}$ efflux and stimulating $Ca^{2+}$ increases intracellularly. In many instances, an accumulation of intracellular $Na^+$ may force the $Na^+$-$Ca^{2+}$ exchange to operate in "reverse" mode to promote $Ca^{2+}$ influx in exchange for $Na^+$ expulsion.[9] With certain assumptions and modifiers, Blaustein's hypothesis remains one of the best in explaining the increases in $[Ca^{2+}]_i$ so commonly seen in hypertension. The existence of a putative circulating $Na^+/K^+$-ATPase inhibitor in hypertension has been proposed, but its nature has not been yet identified.[10] Nevertheless, if decreased $Na^+/K^+$-ATPase activity in hypertension represents a generalized phenomenon, than it can become an important pathogenetic mechanism for increases in $[Ca^{2+}]_i$ in various tissues, including insulin target cells. Elevations of $[Ca^{2+}]_i$ in platelets and white blood cells of patients with hypertension have been reported previously,[11] but the present study is the first that identifies high levels of $[Ca^{2+}]_i$ in insulin target cells of patients with hypertension.

Regardless of the mechanism leading to the elevations of $[Ca^{2+}]_i$ in insulin target cells, once these levels are elevated, they result in diminished insulin action.[4,5,12] The mechanism of this detrimental effect of sustained levels of $[Ca^{2+}]_i$ is not well understood either. We have previously shown that sustained levels of $[Ca^{2+}]_i$ lead to diminution in insulin-stimulated glucose transport in the presence of normal insulin binding and only minimally decreased tyrosine kinase activity of insulin receptor.[13] The number and cellular distribution of

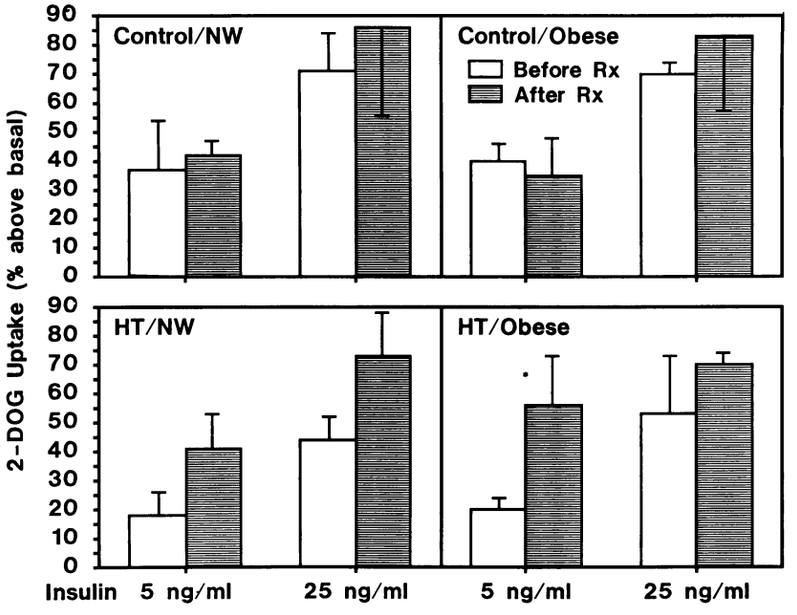


**FIGURE 4.** *2-Deoxyglucose uptake expressed as percent stimulation above basal in control and hypertensive (HT) patients. NW = normal weight, * $P < .01$.*

glucose transporters (assessed by cytochalasin B binding) was also normal in these adipocytes.[13] These results suggested that high levels of $[Ca^{2+}]_i$ induce an impairment in insulin action at the postreceptor steps, which might include a decrease in intrinsic activity of glucose transporters.

The nature of intrinsic activity of glucose transporter has not been identified. Conceivably, the state of phosphorylation of this protein may regulate its intrinsic activity.[14] If this were the case, then the high levels of $[Ca^{2+}]_i$ may alter the state of its phosphorylation. We have recently found that high levels of $[Ca^{2+}]_i$ increase phosphorylation of insulin-regulatable glucose transporter (IRGT) and inhibit phosphoserine phosphatase activity.[14,15] Investigations of the relationship between the state of phosphorylation of IRGT and its intrinsic activity are needed to further detail this possibility.

Our experience with nitrendipine indicates that 1 month of treatment with this calcium channel blocker improved cellular sensitivity to insulin in older obese hypertensive subjects. A lesser degree of improvement was observed in normal weight hypertensive individuals as well. Similar improvement in insulin action has been recently reported when hypertensive subjects were treated with nifedipine,[5] prazosin,[16] or captopril.[17] At the same time, a month of treatment with nitrendipine failed to significantly reduce the levels of adipocyte $[Ca^{2+}]_i$. This, again, points out at the lack of an obligatory one-to-one relationship between the absolute levels of $[Ca^{2+}]_i$ and the magnitude of insulin-induced glucose uptake.

In summary, older hypertensive, and particularly older obese hypertensive, patients manifest significant insulin resistance accompanied by elevated levels of $[Ca^{2+}]_i$ in their adipocytes. These findings support our hypothesis that elevated $[Ca^{2+}]_i$ plays a central role in the mechanism of an association of hypertension with insulin resistance. High $[Ca^{2+}]_i$ in insulin target cells may produce insulin resistance, whereas high $[Ca^{2+}]_i$ in vascular smooth muscle cells may result in hypertension.

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