# Lack of In Vivo Effect of Vanadium on GLUT4 Translocation in White Adipose Tissue of Streptozotocin-Diabetic Rats

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**Vanadium treatment, in vivo, corrects the severe hyperglycemia observed in streptozotocin (STZ)-diabetic rats. A number of metabolic effects of vanadium have been demonstrated in vitro and might contribute importantly to normalization of glucose homeostasis. However, many in vitro effects of vanadium occur at concentrations substantially higher than those achieved in vivo. Effects of vanadium on white adipose tissue have been particularly well characterized in vitro. To examine the relationship between in vitro and in vivo actions of vanadium, we examined the effects of vanadium treatment on acute glucose tolerance and adipose tissue GLUT4 control in vivo. In agreement with previous studies, vanadium treatment of STZ-diabetic rats restored normoglycemia with no appreciable restoration of insulin secretion. GLUT4 expression in white adipose tissue was reduced by 22% in STZ-diabetic rats compared with controls. Vanadium treatment did not significantly alter GLUT4 expression in controls, but completely restored normal expression levels in STZ-diabetic rats. In overnight-fasted control animals, GLUT4 translocation to the plasma membrane (PM) was maximally elevated (by 50%) in adipose tissue within 5 to 10 minutes after an intravenous (IV) glucose challenge. No glucose-induced translocation of GLUT4 was detected in diabetic rats, and peak PM GLUT4 content was 40% lower than in controls. Vanadium treatment did not increase peak PM GLUT4 content in either control or diabetic animals in response to a glucose load. Finally, the suppression of whole-body acute glucose tolerance in diabetic animals was only partially normalized by vanadium treatment. We conclude: (1) that concentrations of vanadium effective for maintaining normoglycemia in vivo (typically below 30 $\mu$mol/L) promote normal GLUT4 expression, but do not influence the subcellular localization of GLUT4 in white adipose tissue and (2) that in vivo effects of vanadium may not necessarily reflect the actions observed in vitro at supraphysiologic concentrations.**


VANADIUM IS A TRACE element shown to have glucose-lowering properties in type 1 and type 2 diabetes.[1,2] The mechanisms of the antihyperglycemic effects of vanadium are as yet unclear, but attention has focused on enhancement of glucose transport[3] and/or the inhibition of hepatic glucose output.[4] Using euglycemic clamp techniques, vanadium has been shown to enhance insulin-mediated glucose disposal, an effect mostly attributed to an increased glycogen synthesis in both partially pancreatectomized diabetic rats[5] and type 2 diabetic humans.[1] In addition, vanadium has been reported to increase glucose transport, either directly or by augmenting the effects of insulin in adipocytes isolated from normal[6,7] or insulin-resistant[8] rats. Vanadium-stimulated glucose transport in vitro has also been demonstrated in rat skeletal muscle[9] and in normal and insulin-resistant human muscle.[10] Insulin-mediated glucose utilization in vivo is critically dependent on the expression and cellular localization of GLUT4. In streptozotocin (STZ)-diabetic rats, whole body insulin-stimulated glucose uptake is decreased, coinciding with a reduced expression of GLUT4 in adipose tissue, skeletal muscle, and heart.[11,12] The mechanisms by which vanadium influences glucose transport in vivo are not completely resolved. In several studies, vanadium treatment of STZ-diabetic rats led to improved GLUT4 expression in skeletal and cardiac muscle.[13,14] However, the effects of vanadium on GLUT4 translocation in vivo have not been reported. In one study, it was concluded that vanadium exerted effects on the intrinsic activity of muscle glucose transporters.[15]

As a number of the metabolic effects of vanadium in vitro are achieved only at concentrations well above those achieved in vivo, it is crucial to examine if a cardinal rapid effect of insulin such as GLUT4 translocation is, in fact, altered by low concentrations of vanadium in vivo. We considered that studies of the effects of vanadium on adipose tissue in vivo would be particularly valuable because the corresponding in vitro effects of vanadium on glucose transport and fat cell metabolism have been especially well documented. The focus on adipose tissue was also considered to be appropriate because both insulin and vanadium induce large absolute changes in several indices of in vitro adipose tissue metabolism, including GLUT4 translocation.[15-17] From a practical perspective, the preparation of subcellular membrane fractions from adipose tissue also offers some advantages over that from skeletal muscle. In this regard, important considerations are the aggressive homogenization conditions required to adequately disrupt muscle tissue, the greater cross-contamination of membrane fractions,[18] and the significant heterogeneity in hormonal regulation of GLUT4 content between muscles of different fiber compositions. In contrast, white adipose tissue provides a more easily disrupted and homogeneous tissue preparation.[19] Our results indicate that although vanadium may induce insulin-like effects on GLUT4 in isolated adipocytes,[15-17] the low concentrations of vanadium generated in vivo have no effect on GLUT4 translocation in adipose tissue, either basally or in response to a physiologic glucose load.

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## MATERIALS AND METHODS

### *Treatment and Maintenance of Animals*

Male Wistar rats (200 to 250 g) were obtained from Charles River (Montreal, Quebec) and maintained on standard laboratory chow. STZ (Sigma, St Louis, MO; 55 mg/kg intravenous [IV]) was administered to the diabetic group, while control rats received vehicle (NaCl, 154 mmol/L, pH 7.2). An oral glucose tolerance test (OGTT) was performed 5 days after STZ. Before vanadium treatment, diabetic rats were divided such that mean body weight, nonfasted glucose, and integrated glucose response (area under the curve, glucose [$AUC_g$]) after an oral glucose load were similar in both treated and untreated groups. At 7 days post-STZ, vanadyl sulfate treatment was initiated at a concentration of 0.75 mg/mL in the drinking water. Vanadium concentrations were increased by 0.25 mg/mL every 1 to 2 weeks in both diabetic treated (DT) and control treated (CT) groups to a maximum of 1.75 mg/mL, and this final concentration was maintained for 3 weeks until the animals were killed at week 10. Plasma samples were collected at 10 AM on a weekly basis for measurement of glucose and insulin. Pancreatic insulin content was measured after acid extraction of the pancreas as previously described.[20]

In a series of OGTT studies, a close correlation ($r = .99$) existed between $AUC_g$ and plasma glucose levels measured 60 minutes after the glucose load. The 60-minute glucose value was therefore used as a measure of glucose tolerance weekly. At 9 weeks, an intravenous glucose tolerance test (IVGTT) was performed by collecting blood samples before (0 minutes) and at 5, 15, 30, and 60 minutes after injection of glucose (0.5 g/kg) into the tail vein. Plasma insulin was measured via radioimmunoassay using rat insulin standards. The glucose disappearance rate constant (Kg) was calculated as the slope of the least squares regression line relating the log-transformed glucose concentration to time from 5 to 30 minutes after the glucose bolus during the IVGTT. Kg is expressed as percent per minute (%/min).

### *Subcellular Fractionation of Adipose Tissue*

Because glucose lowering by vanadium in diabetic animals was evident at low circulating insulin levels, subcellular distribution of GLUT4 was measured after a glucose bolus (to produce physiologic insulin release) rather than after an insulin injection. At 10 weeks of diabetes, overnight fasted rats were anesthetized with sodium pentobarbital (30 mg/kg, intraperitoneal [IP]) and administered IV glucose (0.5 mg/kg). Blood samples were removed from the tail vein 5 minutes after glucose administration, and then the animals were killed 2.5 minutes later. This treatment protocol was found to bring about maximal translocation of GLUT4 in adipose tissue from control rats (data not shown). Subcellular fractionation was performed on epididymal fat pads pooled from 2 rats as previously described.[21] All procedures were performed in HES buffer (20 mmol/L Hepes, 1 mmol/L EGTA, 255 mmol/L sucrose; pH 7.4) at $\leq$ 4°C. Adipose tissue was minced in 10 mL HES using a polytron homogenizer (2 $\times$ 1-second bursts), and centrifuged at 1,000*g* for 5 minutes. The infranatant was removed and centrifuged at 17,500*g* for 30 minutes. The resulting pellet (A) was washed twice and centrifuged at 17,500*g* for 15 minutes. The final pellet was resuspended and layered on top of a discontinuous sucrose gradient made up of 0.8 mol/L and 1.12 mol/L sucrose, centrifuged at 150,000*g* for 40 minutes, and plasma membrane (PM) collected from the 0.8/1.12 mol/L interface. The supernatant from the initial spin (A) was further centrifuged at 50,000*g* for 20 minutes. From this step, the pellet (B) was resuspended and centrifuged at 50,000*g* for 60 minutes to obtain high density microsomal membranes (HDM). The supernatant (B) was centrifuged at 200,000*g* for 60 minutes and the resulting pellet containing low-density microsomes (LDM) layered on a discontinuous gradient of 0.4 mol/L and 1.5 mol/L sucrose, and centrifuged at 150,000*g* for 40 minutes.[22] Enriched LDM was collected at the 0.4/1.5 mol/L interface and further centrifuged at 200,000*g* for 60 minutes. Membrane fractions were stored at -70°C for GLUT4 measurements.

### *Membrane Marker Enzymes*

Enzyme marker analysis was performed on subcellular fractions from fresh adipose tissue obtained over the course of the study. Nicotinamide adenine dinucleotide, reduced form (NADH)-cytochrome c reductase, a marker of endoplasmic reticulum (ER) was measured spectrophotometrically.[23] The specific activity of cytochrome c reductase was highest in the HDM fraction (1.48 $\pm$ 0.03 $\mu$mol/min/mg), 5.7-fold greater than PM (0.26 $\pm$ 0.02), and 1.8-fold greater than LDM (0.83 $\pm$ 0.05). Ouabain-sensitive $Na^+/K^+$-adenosine triphosphatase (ATPase), a marker of PM, was measured as described.[24] Activities in PM, HDM, and LDM were 107 $\pm$ 7, 48 $\pm$ 6, and 10 $\pm$ 3 $\mu$mol $P_i$/mg/h, respectively. Thus, contamination of either PM with ER (17.5%), or of LDM with PM (10%) was consistently low and similar to or less than previously reported.[21] In the absence of a glucose challenge, LDM GLUT4 content was at least 10-fold higher than PM, and LDM GLUT4 comprised approximately 50 % of the total GLUT4 content in adipose tissue in control animals in the basal state.

### *GLUT4 Analysis by Enzyme-Linked Immunosorbent Assay*

GLUT4 content of subcellular membranes (PM and LDM) was measured by competitive enzyme-linked immunosorbent assay (ELISA). Briefly, 96-well plates (Maxisorp; Nunc, Roskilde, Denmark) were coated with GLUT4 C-terminus peptide (Biogenesis, Sandown, NH) in 0.1 mol/L sodium carbonate-bicarbonate buffer (pH 9.5) and incubated overnight at 50°C. The plates were washed with 0.1% Tween in phosphate-buffered saline (PBS) and blocked for 1 hour with PBS containing 10% (vol/vol) goat serum. Solubilized membrane fractions or GLUT4 peptide were serially diluted (1:2) in 1% Triton (vol/vol) in PBS. Monoclonal anti-GLUT4 antibody (1F8, Biogenesis), diluted 1:3,000 in PBS containing ELISA-grade bovine serum albumin (BSA) (0.5%, wt/vol) and skim milk (1%, wt/vol), was mixed with competing antigen, and 50 $\mu$L of the mixture added to the peptide-coated plates and incubated for 6 hours at 37°C in a shaking water bath. After washing, 100 $\mu$L of peroxidase-linked sheep antimouse Ig $F(ab')_2$ fragment (Amersham, Pharmacia Biotech, Piscataway, NJ) diluted 1:1,000 in antibody dilution buffer was added for 2 hours. After washing, substrate (2 mmol/L o-phenylenediamene, 0.01% $H_2O_2$ in 0.1 mol/L citrate buffer, pH 5.0) was added and at 30 minutes, the enzyme reaction was stopped with 40 $\mu$L of 8 N $H_2SO_4$ and absorbance measured at 490 nm. Membrane protein was quantified fluorimetrically.[25] GLUT4 content was expressed as relative amount per milligram protein. In each assay plate, 2 internal standards (adipose tissue homogenates) were assayed concurrently to ensure reproducibility of the ELISA. The intra and interassay percent coefficient of variation (%CV) in all assays for both internal standards was $<$10%. In addition, at least 1 sample from each experimental group was analyzed per ELISA plate to avoid a bias in the data. The ELISA procedure was validated by parallel experiments using conventional Western blot technique, which produced equivalent results (Li and McNeill, unpublished observations).

### *Statistical Analysis*

Two-way analysis of variance (ANOVA) was used followed by the Fisher's least significant difference test. Curve fitting and calculation of data was performed via the Fig. P Scientific Processor (Biosoft, Ferguson, MD). Data are expressed as mean $\pm$ SEM. $P < .05$ was considered significant.

## RESULTS

### *General Characteristics*

Within 5 to 7 days after STZ administration, all animals exhibited significant hyperglycemia. All diabetic animals, whether assigned to the untreated or treated diabetic groups, exhibited a similar degree of diabetes. Hence, initial plasma glucose, 23.8 ± 1.2 versus 22.3 ± 0.5 mmol/L; insulin, 39.4 ± 4.3 versus 34.7 ± 1.9 μU/mL; and glucose tolerance (plasma glucose at 60 minutes), 19.2 ± 0.7 versus 17.8 ± 1.0 mmol/L were not significantly different between the untreated and treated diabetic groups before vanadium treatment (Fig 1). In diabetic animals, plasma glucose was reduced within the first week of vanadium treatment, but complete normoglycemia within the entire group (12 of 12) was achieved only at higher vanadium concentrations (1.25 mg/mL, vanadium dose, 142 ± 7.7 mg/kg/d) administered by week 4 (Fig 1A). Unexpectedly, in the untreated diabetic group, we also observed a gradual decrease in mean plasma glucose from 3 weeks post-STZ. Closer examination of this group showed that spontaneous reversal of the diabetic state had occurred in 6 of 12 animals, and average plasma glucose and insulin levels of these 6 spontaneously "reverted" animals were not different from control at 9 weeks (Fig 1, inset). Because their characteristics more closely paralleled control rather than diabetic animals, spontaneously reverted animals (DR) were analyzed as a subgroup distinct from those that had remained hyperglycemic (D). In both control and diabetic animals, vanadium treatment markedly reduced plasma insulin to levels even lower than the hyperglycemic D group (Fig 1B).

### *OGTT and IVGTT*

Despite further increase of the vanadium concentrations of drinking water to 1.75 mg/mL, well beyond that which effectively maintained normoglycemia, DT animals continued to show an impaired glucose tolerance relative to control (Fig 1C). At this concentration, DT animals no longer gained weight and hence no further increase was attempted. As vanadium has been reported to inhibit the oral absorption of glucose,[26] an IVGTT was performed at 9 weeks. Relative to control, the D group showed significantly elevated fasting and peak plasma glucose and a markedly reduced glucose disappearance rate (Kg) (Fig 2A and C). In contrast, the glucose tolerance of the DT and DR groups were very similar, with both groups showing normal fasting and peak plasma glucose levels, although glucose disappearance rate was still reduced compared with control. Insulin secretory response was lower than control in CT rats and in all diabetic groups (Fig 2B).

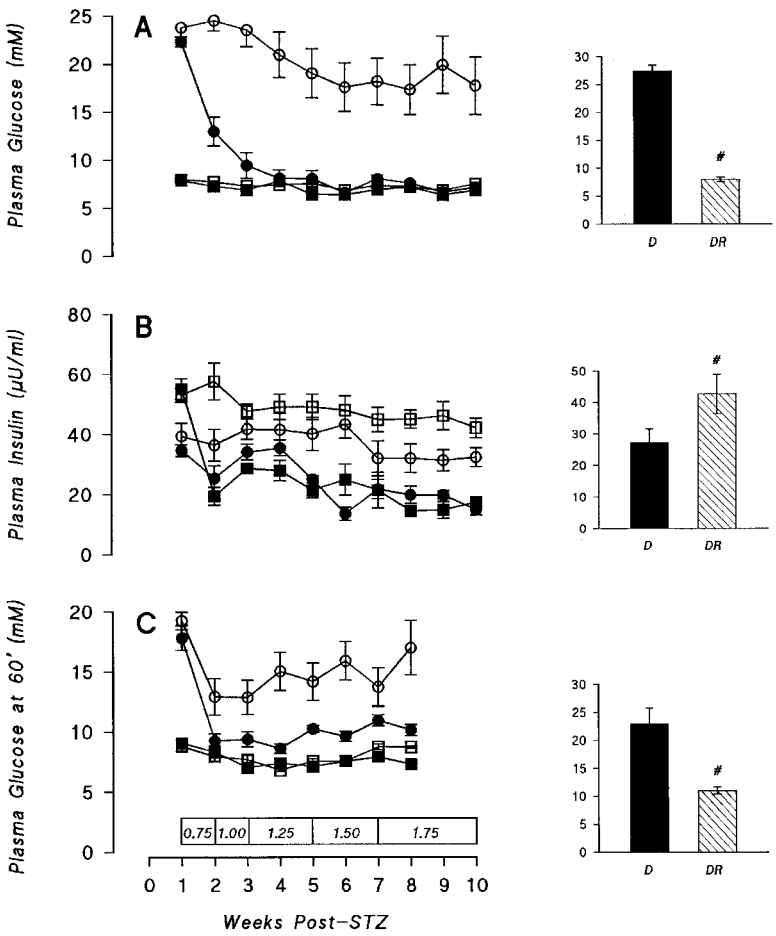


**Fig 1. Plasma glucose (A), insulin (B), and glucose tolerance as measured by glycemia at 60 minutes after a 1 g/kg oral glucose dose after overnight fasting (C) in untreated (□, n = 8) and vanadium-treated (■, n = 8) controls and untreated (○, n = 12) and vanadium-treated (●, n = 12) diabetic groups. Vanadyl concentrations administered in drinking water (mg/mL) are indicated above the X-axis. Insets show values in hyperglycemic (D, n = 6) and spontaneously reverted (DR, n = 6) diabetic groups at final time points. (#*P* < .05 *v* D).**

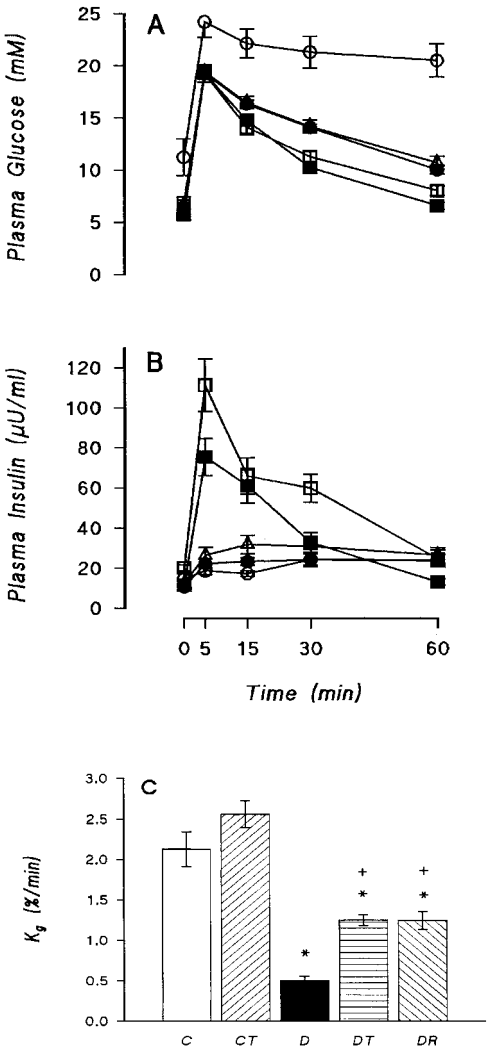


**Fig 2. Plasma glucose (A) and insulin (B) levels in response to an IV glucose load (0.5 g/kg) in overnight-fasted animals at 9 weeks post-STZ, in the untreated (C, □, n = 8) and vanadium-treated (CT, ■, n = 8) controls and hyperglycemic (D, ○, n = 6), vanadium-treated (DT, ●, n = 12) and spontaneously reverted (DR, ◁, n = 6) diabetic rats. (C) The glucose disappearance rate constant (Kg) was calculated from 5 to 30 minutes after the glucose bolus during the IVGTT. (*$P <$ .05 *v* C, +$P <$ .05 *v* D).**

*Pancreatic Insulin Content*

Among the diabetic animals, residual pancreatic insulin content was significantly higher in the DT (≈ 4-fold) and DR (6-fold) groups relative to D (Fig 3A). However, these levels remained considerably reduced as a percentage of control (1.7 ± 0.1 U/g): 18% (DR), 11% (DT), and 3% (D). As we have previously reported,[20] the presence of normoglycemia among the untreated diabetic rats was associated with pancreatic insulin content ≥ 12% of control and plasma insulin levels that were not different from control (Fig 3B).

*Effect of Vanadium Treatment on GLUT4 Translocation*

Total adipose tissue GLUT4 content (total sum of GLUT4 from 3 fractions, PM, HDM, and LDM), was reduced by 22% in the D group ($P <$ .01), and restored in vanadium-treated or spontaneously reverted diabetic animals (data not shown). In a series of experiments with control animals, translocation of GLUT4 from intracellular LDM to the PM pool in adipose tissue was optimally detected at 5 to 10 minutes after an IV glucose load. At 10 weeks of diabetes, glucose was administered by IV injection, and 7.5 minutes later adipose tissue was removed for preparation of subcellular membrane fractions. PM GLUT4 content from adipose tissue obtained from control animals before glucose injection (basal PM GLUT4) was used as a reference (set at 1.0 relative U/mg protein), and this value increased significantly by 50% after the glucose challenge (Fig 4A). Correspondingly, a significant decrease in GLUT4 content in the LDM was detected (Fig 4B), whereas no change was detected in the HDM fraction (Fig 4C). PM GLUT4 content in the basal state was also measured in a small number of hyperglycemic animals (n = 3) and was found to be similar to control (data not shown).

We chose to measure the subcellular distribution of GLUT4 at the time of maximal translocation to address the question whether vanadium treatment could result in increased cell

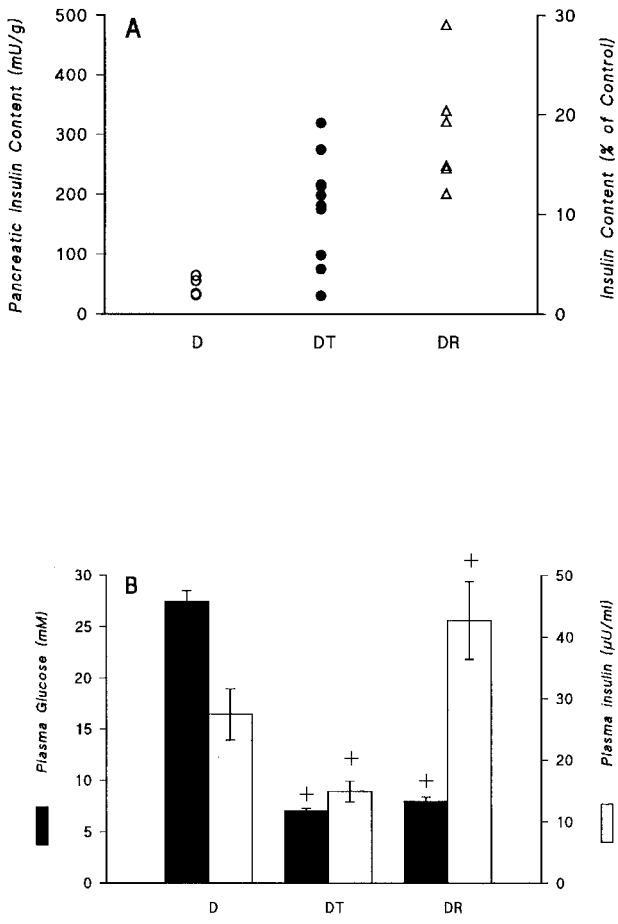


**Fig 3. Pancreatic insulin store (A) and fed plasma glucose and insulin (B) in hyperglycemic (D, ○, n = 6), vanadium-treated (DT, ●, n = 12), and spontaneously reverted (DR, ◁, n = 6) diabetic animals (+$P <$ .05 *v* D).**

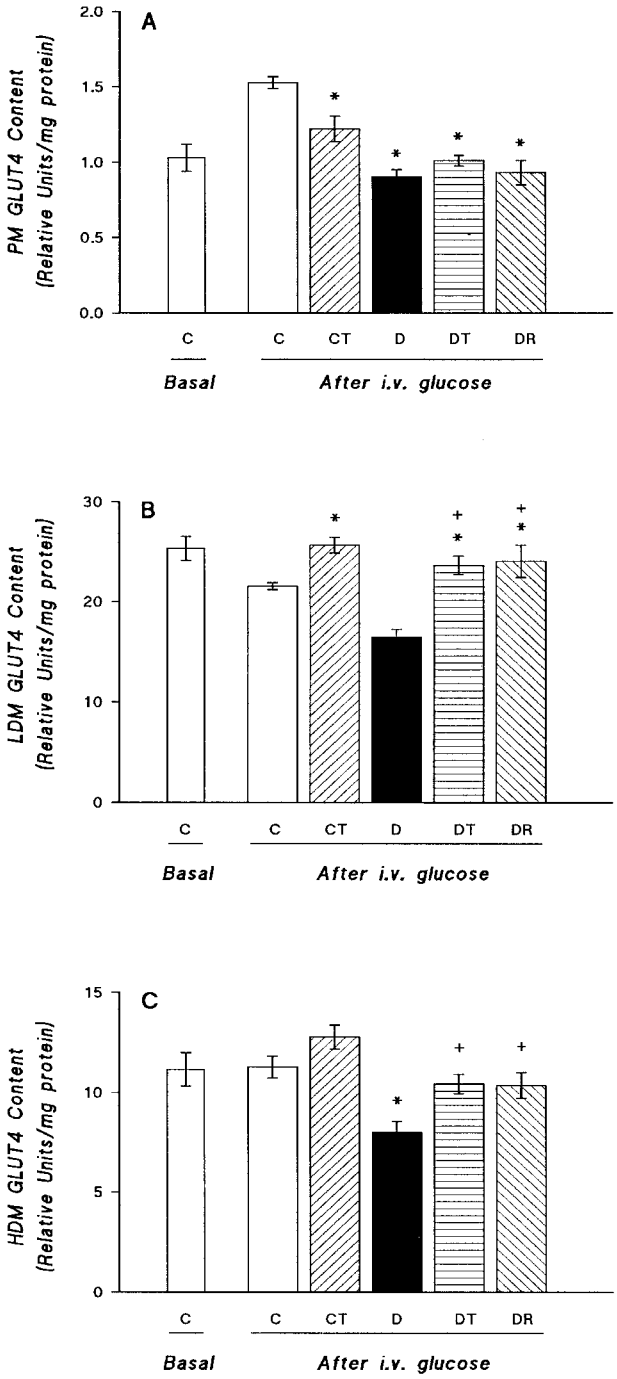


**Fig 4. GLUT4 content in adipose tissue PM (A), LDM (B), and HDM (C) fractions before (basal) and at 7.5 minutes after a 0.5 mg/kg IV glucose load in overnight-fasted animals at 10 weeks post-STZ (*$P <$ .05 *v* C; +$P <$ .05 *v* D). Adipose tissue was obtained from untreated (C, n = 8) and vanadium-treated (CT, n = 8) controls and hyperglycemic (D, n = 6), vanadium-treated (DT, n = 12), and spontaneously reverted (DR, n = 6) diabetic rats.**

surface GLUT4 after a physiologic glucose load during both control and diabetic conditions. In the hyperglycemic D group, peak PM GLUT4 content after a glucose load was 40% lower than in control and was not significantly different from control levels in the basal state. Indeed, peak cell surface GLUT4 content in vanadium-treated diabetic animals was not even increased over levels seen in the untreated diabetic group. Very similar results were observed in spontaneously reverted diabetic rats. Interestingly, vanadium treatment of control rats also led to a reduced appearance of GLUT4 in the PM fraction (by 20%) in response to a glucose load and a corresponding increased residence within the LDM fraction. Intracellular GLUT4 content in either vanadium-treated or spontaneously reverted diabetic animals was not significantly different from control in the basal state, suggesting that despite the restoration of GLUT4 expression, translocation remained impaired in these animals. Further analysis showed that PM GLUT4 content of all animals was highly correlated ($r = .85$, $P < .0001$) with ambient plasma insulin at 5 minutes after the glucose load (Fig 5A), however, there was no correlation with pancreatic insulin content (data not shown). Further, LDM GLUT4 content was highly correlated ($r = .87$, $P < .0001$) with residual pancreatic insulin stores in the diabetic groups (Fig 5B). In addition, among the diabetic animals, there was an inverse hyperbolic relationship between $AUC_g$ and pancreatic insulin content (Fig 5C), as we have shown previously.[20]

## DISCUSSION

The antihyperglycemic effects of vanadium in vivo remain incompletely understood, although the range of in vitro effects of vanadium on cell glucose transport and metabolism suggest several possible mechanisms. The main goal of the studies described here was to determine if the in vitro effects of vanadium on adipose tissue GLUT4 also occur at very low therapeutic vanadium concentrations generated in vivo in STZ-diabetic rats. Adipose tissue was chosen for study because it provides a convenient and relatively homogeneous tissue in which GLUT4 is rapidly and dramatically regulated in vitro in response to vanadium and insulin. In the present study of adipose tissue, as in previous studies with skeletal muscle and heart,[13,14] GLUT4 expression was decreased in STZ-diabetic rats, and normal expression was restored after vanadium treatment. Although GLUT4 expression was restored in vanadium-treated diabetic animals, subcellular localization of GLUT4 in adipose tissue during peak translocation was not altered in response to a glucose load. The failure of vanadium treatment to restore normal GLUT4 translocation in vivo is in contrast to actions observed in vitro. The plasma concentrations of vanadium achieved in vivo with the procedures described here are typically in the range of $10^{-6}$ to $10^{-5}$ mol/L.[20] Such in vivo concentrations are well below the range ($10^{-3}$ to $10^{-2}$ mol/L) required to stimulate GLUT4 translocation in vitro.[16,17] Indeed, lower concentrations of sodium orthovanadate (20 to 100 $\mu$mol/L) were completely ineffective in either directly activating glucose transport or enhancing the effects of insulin in isolated adipocytes.[27] We conclude that in vivo effects of vanadium on adipose tissue GLUT4 are selective and do not reflect all the actions observed (often at much higher concentrations) in vitro.

Additional quantitative features of GLUT4 control are also worth noting. The time-course for acute GLUT4 translocation observed in nondiabetic animals in response to a glucose load

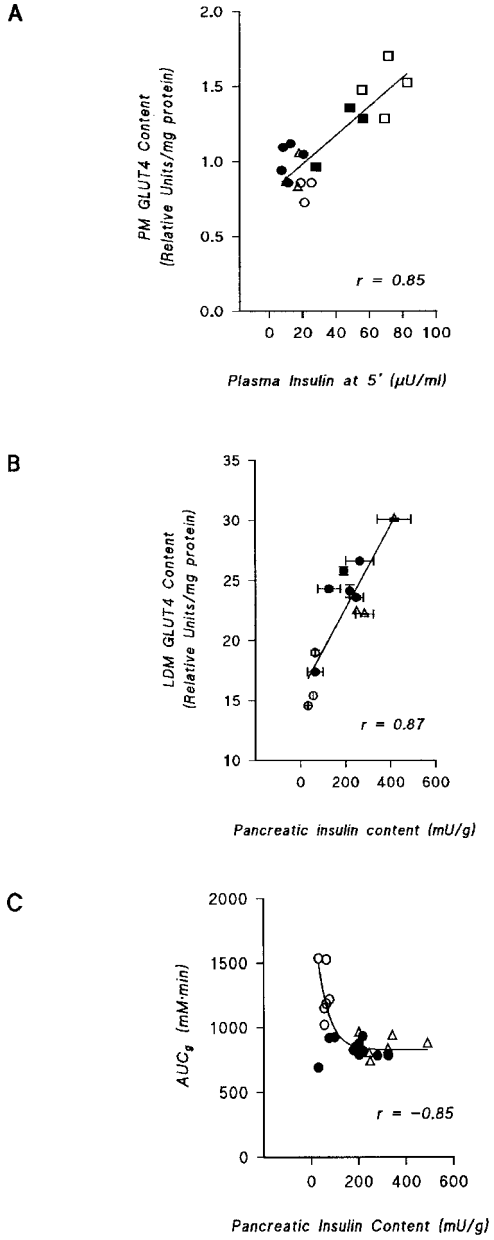


**Fig 5. Correlations between plasma insulin levels at 5 minutes after the glucose load and PM GLUT4 content (A) and between pancreatic insulin content and LDM GLUT4 content (B) and $AUC_g$ (C). Untreated (C, □) and vanadium-treated (CT, ■) controls, hyperglycemic (D, ○), vanadium-treated (DT, ●), and spontaneously reverted (DR, ◁) diabetic animals.**

in these in vivo studies is rapid (reaching a peak within 10 minutes after a glucose load). This time-course is comparable to that observed in studies of isolated adipocytes in vitro.[15,21,28] In contrast, the extent of GLUT4 translocation was quite modest (≈ 50%) by comparison to values observed in vitro, typically 2- to 5-fold.[28] In fact, the values obtained in the present in vivo studies are very similar to those previously reported in skeletal muscle in rats during an OGTT[29] and in the heart after administration of IV insulin.[30] These results indicate that the degree of GLUT4 translocation in vivo is considerably smaller than that observed in typical studies in vitro, perhaps because of the absence of physiologic "tonic" activation in the basal state in vitro, as well as the use of a physiologic stimulus in this and in other studies.[29,30]

Because of the apparent lack of effect of vanadium on PM GLUT4 in vivo, we explored the relationship between endogenous insulin levels and GLUT4 localization. This analysis showed a very close correlation between circulating insulin concentrations and PM GLUT4 content among all control and diabetic animals (Fig 5A). From these observations, it appears that GLUT4 translocation may be strongly influenced by circulating insulin concentrations and is not significantly influenced by vanadium in vivo.

The failure of vanadium to influence the cellular localization of GLUT4 in adipose tissue raises important questions about GLUT4 control in skeletal muscle, probably the dominant site for acute glucose disposal. It is possible that vanadium may influence GLUT4 traffic and glucose uptake in skeletal muscle, although it did not do so in adipose tissue. In this regard, the measure of whole-body glucose disposal (Kg, Fig 2) is important because it probably reflects the function of skeletal muscle in vivo. Because the marked suppression of acute glucose disposal seen in STZ-diabetic rats was only partially restored by treatment with vanadium, the defects in skeletal muscle glucose metabolism in STZ-diabetes are not completely corrected by vanadium treatment. If skeletal muscle GLUT4 control is also insensitive to vanadium, then improved glucose homeostasis might be dependent on effects of vanadium on other aspects of glucose transport and/or metabolism. For example, vanadium treatment has been shown to increase basal glucose uptake in liver and brain in vivo,[17,31] suggesting that insulin-independent transport (via GLUT1 and/or GLUT3) might be important targets for vanadium action. In this respect, glucose clearance by vanadium-treated animals was identical to that achieved by the spontaneous reversal of the diabetic state alone, suggesting that maintenance of basal normoglycemia might improve glucose-mediated glucose disposal during an IVGTT.[32,33]

It could be argued that the effect of vanadium might completely reflect on an improvement in pancreatic insulin content. Spontaneous reversal of diabetes occurs when pancreatic insulin content exceeds approximately 150 mU/g or ≥ 12% of control[20,31] and when circulating insulin is maintained in the normal range. Indeed, in the present study, animals that spontaneously reverted to normoglycemia had plasma insulin levels that were not different from control throughout the entire study. In contrast, vanadium-treated animals were able to maintain normoglycemia even at extremely low pancreatic insulin stores (<100 mU/g, <6% of control) and with markedly decreased plasma insulin levels. Overall, the results of the present and previous studies suggest that improved pancreatic insulin stores likely do not contribute significantly to the glucoregulatory effects of vanadium. These observations show the need for further studies of the effects of vanadium on glucose transport and metabolism in skeletal muscle and other tissues in vivo, and importantly, during basal, fed conditions. It should be noted however, that similar to the in vitro findings in rat adipocytes, GLUT4 translocation in cultured L6 myotubes and

cardiac muscle cells has been shown only at high vanadium concentrations ($10^{-3}$ mol/L).[34]

In conclusion, vanadium treatment of STZ-diabetic rats was accompanied by restoration of the total GLUT4 pool in adipose tissue, but did not influence acute regulation of GLUT4 as reflected by the extent of translocation to the PM after a physiologic glucose load. These results, despite the presence of normoglycemia and near-normal glucose tolerance, show the possible selectivity of effects of vanadium in vivo, relative to those shown at high concentrations in vitro. To fully understand the remarkable antihyperglycemic effects of vanadium, it is clearly crucial to establish the effects of vanadium that are still apparent at the low concentrations likely to be achieved in vivo.

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