Vanadate restores glucose 6-phosphate in diabetic rats: a mechanism to enhance glucose metabolism

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Vanadate restores glucose 6-phosphate in diabetic rats: a mechanism to enhance glucose metabolism. Am J Physiol Endocrinol Metab 279: E403–E410, 2000.—Vanadate mimics the metabolic actions of insulin. In diabetic rodents, vanadate also sensitizes peripheral tissues to insulin. We have analyzed whether this latter effect is brought about by a mechanism other than the known insulinomimetic actions of vanadium in vitro. We report that the levels of glucose 6-phosphate (G-6-P) in adipose, liver, and muscle of streptozotocin-treated (STZ)-hyperglycemic rats are 77, 50, and 58% of those in healthy control rats, respectively. Normoglycemia was induced by vanadate or insulin therapy or by phlorizin. Vanadate fully restored G-6-P in all three insulin-responsive peripheral tissues. Insulin did not restore G-6-P in muscle, and phlorizin was ineffective in adipose and muscle. Incubation of diabetic adipose explants with glucose and vanadate in vitro increased lipogenic capacity three- to fourfold (half-maximally effective dose = 11 ± 1 µM vanadate). Lipogenic capacity was elevated when a threshold level of ~7.5 ± 0.3 nmol G-6-P/g tissue was reached. In summary, 1) chronic hyperglycemia largely reduces intracellular G-6-P in all three insulin-responsive tissues; 2) vanadate therapy restores this deficiency, but insulin therapy does not restore G-6-P in muscle tissue; 3) induction of normoglycemia per se (i.e., by phlorizin) restores G-6-P in liver only; and 4) glucose and vanadate together elevate G-6-P in adipose explants in vitro and significantly restore lipogenic capacity above the threshold of G-6-P level. We propose that hyperglycemia-associated decrease in peripheral G-6-P is a major factor responsible for peripheral resistance to insulin. The mechanism by which vanadate increases peripheral tissue capacity to metabolize glucose and to respond to the hormone involves elevation of this hexose phosphate metabolite and the cellular consequences of this elevated level of G-6-P.

Vanadate mimics the actions of insulin in vitro (9, 11, 12, 22, 28, 45, 47, and reviewed in Refs. 3, 44, and 46). In vivo, vanadate therapy normalizes blood glucose levels in type 1 diabetes and in highly insulin-resistant type 2 diabetic rodents (2, 5, 19, 25, 26). It was observed that, after vanadate therapy, diabetic peripheral tissues restored significantly their capacity to metabolize glucose and to respond to insulin (2, 5). We have therefore hypothesized here that vanadate acts through an additional mechanism that functions in diabetic rodents in vivo and differs from (or complements) the known insulin-like actions of vanadium in vitro. Vanadate therapy did not raise the number or the efficiency of the insulin receptors, did not increase tyrosine phosphorylation of the receptor or of its substrates (3), and did not ameliorate the reduced level of glucose transporters (GLUT-4) (2, 3, 5, 25, 26). We have therefore hypothesized further that this effect is located downstream to endogenous tyrosyl phosphorylation and upstream to glucose transport activity. A recently developed in vivo/in vitro adipose-related experimental system (43) assisted us in elucidating the mechanism involved in this restoration.

EXPERIMENTAL PROCEDURES

Materials
d-[6-14C]glucose was purchased from New England Nuclear (Boston, MA). Collagenase type I (134 U/mg) was obtained from Worthington Biochemicals (Freehold, NJ). Porcine insulin was obtained from Eli Lilly (Indianapolis, IN). Krebs-Ringer bicarbonate (KRB) buffer, pH 7.4, contained (in mM) 110 NaCl, 25 NaHCO3, 5 KCl, 1.2 KH2PO4, 1.3 CaCl2 and 1.3 MgSO4. Sodium metavanadate (NaVO3) was from BDH Chemicals. All chemical reagents used in this study were of analytical grade.

Methods

Male Wistar rats (180–200 g) were supplied by the Departmental colony. Diabetes was induced by a single intravenous injection of freshly prepared solution of streptozotocin (STZ; 55 mg/kg body wt) in 0.1 M citrate buffer (pH 4.5). Rats were maintained at 24°C under conditions of control lighting and were fed ad libitum. Blood samples for analyses were taken from the tail veins and measured with a glucose analyzer (Beckman Instruments) by the glucose oxidase method (25). Normoglycemia in male Wistar rats is defined as blood glucose level in the range of 90–110 mg/dl. Blood glucose levels...
were measured 8–10 days after injection of STZ. To manifest equivalent degree in the severity of the disease, STZ-treated diabetic rats with circulating glucose levels in the range of 380–460 mg/100 ml glucose, measured after 5 h of food deprivation, were used further.

Diabetic adipose-tissue-related in vivo/in vitro experimental system. In a typical experiment, seven STZ rats were killed by decapitation 12–14 days after treatment with STZ. Epididymal fat pads were cut into small pieces with scissors and distributed into several petri dishes. About 1 g of tissue was incubated for 5 h at 37°C in a 100-mm plastic petri dish containing 10 ml of KRB buffer, pH 7.4, and 1% BSA. The medium was supplemented with glucose (2 mM) or NaVO₃ (1–100 μM) in various combinations according to the experimental conditions. The adipose tissue from each dish was then washed five times with KRB buffer-1% BSA and digested with collagenase for 30 min at 37°C to obtain intact adipocytes (36). The cells were then resuspended in the same buffer to obtain a 4% (vol/vol) suspension, put into plastic vials (0.5 ml/vial in quadruplicates) supplemented with [3-6,14C]glucose [final concentration 0.2 mM, 2,500 counts · min⁻¹ (cpm) · nmol⁻¹], and incubated for 60 min at 37°C under an atmosphere of 95% O₂-5% CO₂. The assay was terminated by adding toluene-based scintillation fluid (1.0 ml/vial), and radioactivity incorporated into the extracted lipids was determined (29). This procedure accurately reflects the lipogenic status of adipose cells.

Induction of normoglycemia in STZ rats before determining glucose 6-phosphate levels in peripheral tissues. Three agents were used in this study to induce normoglycemia in STZ rats. Treatment was started 10 days after administration of STZ. Groups of STZ rats (having free access to food and water) received for a period of 5 days daily administration of insulin (NPH insulin, 15 U and water) and vanadate ip (0.1 mmol/kg body wt) or NaVO₃ (1–100 μM) in various combinations according to the experimental conditions. The adipose tissue from each dish was then washed five times with KRB buffer-1% BSA and digested with collagenase for 30 min at 37°C to obtain intact adipocytes (36). The cells were then resuspended in the same buffer to obtain a 4% (vol/vol) suspension, put into plastic vials (0.5 ml/vial in quadruplicates) supplemented with [3-6,14C]glucose [final concentration 0.2 mM, 2,500 counts · min⁻¹ (cpm) · nmol⁻¹], and incubated for 60 min at 37°C under an atmosphere of 95% O₂-5% CO₂. The assay was terminated by adding toluene-based scintillation fluid (1.0 ml/vial), and radioactivity incorporated into the extracted lipids was determined (29). This procedure accurately reflects the lipogenic status of adipose cells.

RESULTS

Insulin-Responsive Tissues of STZ Rats Have Low Levels of G-6-P

Peripheral diabetic tissues (in particular, adipose and liver) were documented to have a lower level of key enzymes of glucose and fat metabolism (13, 33, 48, 54). We initially considered the possibility that this is the parameter restored by vanadium. Suckling rats also have low expression of glycolytic and lipogenic enzymes, and it was suggested that G-6-P is the glucose metabolite responsible to elevate mRNA (and activities) of these enzymes after rats have been weaned to a high-carbohydrate low-fat diet (10, 50). Although G-6-P is not expected to be rate limiting, we wished to determine whether this was indeed the case in peripheral tissues exposed to chronic hyperglycemia (14, 16, 30, 42). G-6-P levels in adipose tissue, liver, and muscle of healthy rats were 8.9 ± 0.4, 360 ± 20, and 930 ± 30 nmol/g tissue, respectively (Table 1). In STZ rats, levels dropped to 6.85 ± 0.3, 180 ± 10, and 540 ± 50 nmol/g, respectively. Thus hyperglycemia largely reduces G-6-P levels: in adipose tissue to 77%, in liver to 50%, and in muscle to 58% of control levels (calculated from Table 1).

Vanadate Therapy of STZ Rats Restores G-6-P Levels in All Three Insulin-Responsive Tissues

Table 1 summarizes a set of experiments in which STZ rats were treated with vanadate. G-6-P levels in peripheral tissues were determined when the rats had been normoglycemic for 2–3 days (experimental details in Table 1). G-6-P levels were 10.3 ± 1.0, 384 ± 17, and 960 ± 40 nmol/g wet tissue for adipose, liver, and muscle, respectively (Table 1). Comparison with the levels of healthy rats showed that vanadate therapy restored G-6-P levels to 116, 107, and 103% of normal values (Table 1).

Table 1. Glucose and G-6-P levels after vanadium therapy of STZ-treated diabetic rats

<table>
<thead>
<tr>
<th></th>
<th>STZ Rats</th>
<th>Control Healthy Rats</th>
<th>STZ Rats After 5 days of Vanadium Therapy</th>
<th>G-6-P % of Control Healthy Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose level, mg/100 ml</td>
<td>430 ± 30</td>
<td>100 ± 7</td>
<td>110 ± 15</td>
<td></td>
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<tr>
<td>G-6-P, nmol/g wet tissue</td>
<td></td>
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<tr>
<td>Adipose</td>
<td>6.85 ± 0.3*</td>
<td>8.9 ± 0.4</td>
<td>10.3 ± 1.0</td>
<td>116</td>
</tr>
<tr>
<td>Liver</td>
<td>180 ± 10†</td>
<td>360 ± 20</td>
<td>384 ± 17</td>
<td>107</td>
</tr>
<tr>
<td>Muscle</td>
<td>540 ± 50†</td>
<td>930 ± 40</td>
<td>960 ± 40</td>
<td>103</td>
</tr>
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</table>

Diabetes was induced by a single iv injection of streptozocin (STZ, 55 mg/kg body wt). Male Wistar rats (180–200 g) were used. Experiment was performed 10–12 days after treatment with STZ. STZ rats received daily administrations of vanadate ip (0.1 mmol/kg body wt at 10 AM). Rats were killed by decapitation. Adipose liver and muscle tissues were taken, frozen in liquid nitrogen, homogenized, and extracted with 10% HClO₄. Glucose 6-phosphate (G-6-P) level was determined by the methods of Lang and Michal (Ref. 21). *P < 0.05, †P < 0.01.
Induction of Normoglycemia by Phlorizin and Insulin; Effect on G-6-P Level

Phlorizin, an inhibitor of renal tubular reabsorption of glucose, is capable of correcting hyperglycemia in diabetic rats (38). It is devoid, however, of any known insulin (or vanadium) effects, either of a rapid metabolic or a prolonged nature (37). Correction of hyperglycemia by phlorizin can therefore suggest whether a certain abnormality originates solely from chronic hyperglycemia or if normoglycemia per se is sufficient to ameliorate this deficiency. Induction of normoglycemia with phlorizin over a period of 5 days partially restored hepatic G-6-P levels (32 ± 3%) but did not decrease or restore G-6-P levels of adipose and muscle tissue in STZ rats (Fig. 1). Induction of normoglycemia with insulin restored G-6-P levels in liver and in adipose (93% of normal value, Fig. 1). Like phlorizin and unlike vanadate, insulin failed to restore muscle G-6-P levels (Fig. 1). With respect to these findings, Rossetti and Laughlin (37) found that vanadate (but not phlorizin) normalizes glycogen repletion in diabetic skeletal muscle in vivo.

Partial Restoration of the Lipogenic Capacity of Diabetic Adipose Tissue by Glucose and Vanadate

We have recently established an adipose-related in vivo/in vitro experimental system in which the adipose lipogenic capacity was decreased by prolonged fasting in vivo, and its restoration was studied in vitro by incubating the fasted adipose explants for 5 h at 37°C under various experimental conditions (43). After intensive studies, the optimal parameters for this in vivo/in vitro experimental system were established as follows. 1) An incubation period of 5 h at 37°C in vitro was optimal for restoring low mRNA levels (and enzymatic activities) of lipogenic as well as glycolytic enzyme systems (16, 20, 30, 33). 2) Adipose explants were preferable to intact adipocytes, because they preserve protein-synthesizing capacity in vitro and hormonal activities up to a period of 24 h (27, 52). 3) D-[6-14C]glucose was the preferable glucose analog, because carbon 6 is incorporated into fat as fatty acids via acetyl-CoA and therefore reflects the activity of acetyl-CoA carboxylase (ACC) (24). Elevation of adipose lipogenic capacity was a particularly suitable parameter

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Fig. 1. Glucose 6-phosphate (G-6-P) levels in insulin-responsive tissues of streptozotocin-treated (STZ) rats after induction of normoglycemia with phlorizin and insulin. STZ rats (10 days after treatment with STZ) received daily administration over a period of 5 days of NPH insulin (15 U/rat sc), phlorizin (450 mg · day⁻¹ · kg body wt⁻¹, dissolved in 20% propylene glycol, ip), or none. Rats, maintained in a normoglycemic state for a period of ≥2 days, were killed by decapitation (on day 6). Adipose, liver, and muscle tissues were taken, and G-6-P levels were determined (see Methods). Values are means ± SD from 5 rats. *P < 0.05; **P < 0.01 vs. controls.
because it reflects the net (and desirable) result of the activity of several enzymes that, individually, might be altered in different directions in diabetes.

In this set of experiments, we utilized the same adipose experimental system except that adipose explants were taken from STZ rats maintained in hyperglycemic states for 10–12 days instead of from rats that had fasted (see Methods and Figs. 1–4). Incubation of diabetic adipose explants in KRB buffer, pH 7.4, for 5 h at 37°C with insulin (17 nM) or vanadate (100 μM) in the absence of glucose had no effect on adipose lipogenic capacity (Fig. 2A). The inclusion of 2 mM glucose slightly increased lipogenic capacity (1.3-fold, Fig. 2B), whereas the presence of vanadate (100 μM) markedly elevated it (~3.5-fold). When insulin (17 nM) was added instead of vanadate with the glucose, there was no change in lipogenicity, although this specific finding has to be taken with some caution, because the penetration capacity of insulin into adipose explants might be limited (a hypothesis under investigation).

Figure 2C shows the lipogenic capacity of diabetic adipose explants in the presence of 2 mM glucose and various concentrations of vanadate. In lipogenic capacity no increase is seen at 1 μM vanadate, whereas at 10 and 50 μM, lipogenic capacity was elevated 45 ± 3 and 95% of the maximum, respectively (Fig. 2C). The 50% effective dose (ED₅₀) was calculated to be 11 ± 3 μM (an average of three different experiments). Actinomycin D (50 μM) nearly fully suppressed (~95%) the effect of glucose (2 mM) and vanadate (100 μM, Fig. 2D), indicating a protein synthesis link between the glucose plus vanadate combination and lipogenicity. Actinomycin D alone in the presence of only 2 mM glucose did not decrease basal lipogenic capacity (Fig. 2D).

As we previously found with the fasted rat adipose model, vanadate also elevated lipogenicity upon replacing glucose with 2-deoxyglucose but not upon replacing glucose with 3-O-methylglucose (not shown). 2-Deoxyglucose undergoes a vanadate (or insulin)-evoked influx into the cell and is phosphorylated to 2-deoxyglucose 6-phosphate with no further metabolism.

Vanadate and Glucose Elevate G-6-P Levels in Diabetic Explants

In the experiments summarized in Fig. 3, diabetic adipose explants were incubated for 5 h at 37°C under various experimental conditions. G-6-P levels were then determined. In the absence and presence of 2 mM glucose (Fig. 3, columns 1 and 2), they were 2.9 ± 0.3 and 4.2 ± 0.3 nmol/g tissue. Vanadate at concentrations of 1, 10, and 100 μM with glucose elevated G-6-P.

![Figure 2](http://ajpendo.physiology.org/)

**Fig. 2.** Effect of glucose, insulin, and vanadate in vitro on lipogenic capacity of adipose explants from diabetic rats. Epididymal adipose tissue from STZ rats (12–14 days after treatment with STZ) was prepared for treatment in vitro and incubated for 5 h at 37°C with the indicated concentrations of glucose, insulin, or vanadate and actinomycin D as described in Methods. Adipocytes were then prepared, and a lipogenic assay was performed. Results are expressed as the amount of radioactivity (in counts/min) incorporated from D-[6-14C]glucose into the fat content of 3 x 10⁶ cells/h. Values are means ± SD from 5 rats. c.p.m., Counts/min. *P < 0.05; **P < 0.01 vs. no glucose treatment (for A, B, and C). **P < 0.01 vs. glucose treatment (D).
levels to 6.5 ± 0.3, 8.0 ± 0.4, and 9.7 ± 0.6 nmol/g, respectively (Fig. 3, columns 3, 4, and 5). Thus, in the presence of glucose, vanadate elevated G-6-P levels of diabetic adipose explants in vitro.

A Threshold Concentration of G-6-P Is Required For Elevating Lipogenicity of Diabetic Adipose Tissue In Vitro

Figure 4 aligns the data of G-6-P levels and the lipogenic status of diabetic adipose tissue after 5 h of incubation in vitro under various experimental conditions. A threshold level of −7.5 nmol G-6-P/g adipose tissue was noted above as the point at which lipogenic capacity was elevated. In the presence of glucose or glucose and 1 µM vanadate, G-6-P levels rose to 4.2 and 6.5 nmol/g tissue, whereas lipogenicity was quite unaffected. At the higher concentrations of vanadate with glucose, when G-6-P levels had reached 8.0 ± 0.4 and 9.7 ± 0.8 nmol/g, lipogenic capacity rose steeply (Fig. 4). These results are on par with the values found in both diabetic and healthy adipose tissue in vivo (Table 1, and dashed lines in Fig. 4). A G-6-P level of 6.85 nmol/g tissue (in STZ rats) reflects the poor lipogenic capacity of adipose tissue from the diabetic rat, whereas 8.9–10.3 nmol/g accounts for undisturbed or largely restored lipogenic capacity in vivo.

DISCUSSION

We have long suspected that the therapeutic actions of vanadium in diabetic rats in vivo are manifested through a mechanism different from that by which vanadium facilitates the rapid metabolic actions of insulin in vitro (reviewed in 44, 46). This notion gained credence when diverse laboratories observed that, after withdrawal of vanadium therapy, diabetic rats remained normoglycemic and anabolic for days or even weeks (26, 34, 40). Because neither endogenous tyrosyl phosphorylation nor the ability of the diabetic muscle or adipose tissue to encourage glucose influx was affected by this therapy (2, and 26 reviewed in 3), we further hypothesized that the basis for the therapeutic action of vanadium might be an increase in the synthesis of key enzymes in carbohydrate and fat metabolism. These are known to be suppressed in diabetes (13, 48, 54). There are also two physiological conditions characterized by low levels of glycolytic and lipogenic enzymes in peripheral tissues: immaturity (10, 16, 50) and starvation (20, 31–33). We therefore searched for a missing link common to these three conditions.

Incubation in vitro of adipose explants from suckling rats for 5 h at 37°C with 20 mM glucose elevated fatty acid synthase and ACC mRNA levels five- to sevenfold (16). The augmented lipogenic enzyme gene expression was thought to be associated with the glucose metabolite G-6-P. These same enzymatic activities are rapidly decreased in starvation, and refeeding with a carbohydrate diet restores their level and activity in liver and in adipose tissue (20, 31, 32). In our in vivo/in vitro experimental system, adipose lipogenic capacity was decreased by 20 h of fasting and was restored in vitro by glucose together with vanadate. Actinomycin D suppressed this elevation. We also concluded that, in this particular experimental system, G-6-P is the metabolite of glucose involved in the transcriptionally regulated elevation of lipogenic capacity (43).
In view of these findings, we wondered whether G-6-P were a rate-limiting metabolite in diabetes as well. Theoretically, chronic hyperglycemia would at least be expected to elevate (rather than to reduce) G-6-P levels. Prolonged incubation of adipose explants (14), rat skeletal muscle (41, 42), or liver cells (30) with high concentrations of glucose indeed elevates G-6-P levels. This was not the case, however, in STZ rats in vivo, where G-6-P actually fell to 77, 50, and 58% of control values in adipose, liver, and muscle (Table 1). The decrease in hepatic G-6-P was not surprising, because glucokinase activity in STZ rats is essentially lacking (18, 49). Also, the level of liver Glc-6-Pase protein in partially pancreatectomized diabetic rats is elevated three- to fourfold (23). The decrease in adipose G-6-P level and to a greater extent in muscle tissue, however, was not expected. Despite intense investigation, the decrease of hexokinase II activity in the muscle and adipose tissue of STZ rats was minimal, and in some cases the level of hexokinase I even increased (i.e., Ref. 7).

Vanadate Therapy of STZ Rats Restores G-6-P Levels

Vanadate therapy normalized blood glucose levels and restored adipose, liver, and muscle G-6-P to normal levels (Table 1). Vanadate, however, does not elevate the low levels of circulating insulin in this diabetic model (19, 34). We can therefore suggest that the hyperglycemia is a major factor suppressing G-6-P in the three insulin-responsive tissues of STZ rats. Our findings with phlorizin and insulin, however, appear to indicate that the subsequent induction of normoglycemia per se is capable of partially restoring hepatic G-6-P levels but is an insufficient condition to do so in adipose and muscle (Fig. 1). Additional actions manifested by vanadate and insulin (in adipose) or by vanadate but not insulin (in muscle) are required for restoring G-6-P (Fig. 1). From a diabetological standpoint, these findings appear to have an important clinical message: if the decrease in G-6-P is a major deteriorating factor in diabetes (as we suggest here), this deficiency cannot be corrected by insulin in muscle (Fig. 1).

Vanadate Elevates G-6-P in Diabetic Adipose Explants In Vitro

Our earlier study with fasted rats showed that vanadate elevated G-6-P levels significantly in adipose explants (43). We interpreted this as a dual effect of vanadate in facilitating glucose uptake and in inhibiting adipose Glc-6-Pase. As with the fasted adipose explants, vanadate also elevates G-6-P in the diabetic adipose explants (Fig. 3). Adipose Glc-6-Pase activity was not reduced in the diabetic adipose tissue and was similarly inhibited by vanadate (IC50 = 7.0 ± 0.3 μM, results are not shown).

Restoration of the Lipogenic Capacity of Diabetic Adipose Explants by Vanadate

In the presence of glucose, vanadate elevated the lipogenic capacity of diabetic adipose explants, an action that could be suppressed by actinomycin D (Fig. 2). Maximal elevation (in the presence of 2 mM glucose and 100 μM vanadate) was ~3.5-fold, with one-half the maximal effect at 11.0 ± 0.2 μM vanadate (Fig. 2). We therefore suggest that elevating G-6-P levels over 85% of normal in the diabetic adipose explants in vitro or to 95–116% of control levels in the diabetic rat model in vivo significantly renews the glycolytic and lipogenic capacities of insulin-responsive tissues. This is a major ameliorating function of vanadium in diabetic rodents. These results are parallel to those in the fasted adipose explants (43). For the sake of objectivity, however, it should be stressed that starvation is a “healthier” condition than diabetes (i.e., physiological rather than pathological). In the fasted adipose explant model, lipogenicity (under maximal activating conditions) increased 8- to 10-fold, as opposed to the more modest elevation (3.5-fold) in the diabetic adipose explants (Fig. 2). Obviously, vanadium therapy cannot overcome several additional defects known to be induced by chronic hyperglycemia. For example, the content of muscle GLUT-4 glucose transporters is not increased by this therapy. Nevertheless, the overall glucose disposal capacity of the muscle tissue is largely restored (2, 51).

It should be mentioned at this point that Brichard et al. (4) found lipogenic enzyme gene expression elevated in liver but not in adipose after oral vanadium therapy. The difference with our study can be due simply to the higher-dose vanadium therapy we have applied here and the lower capacity of adipose, as opposed to liver, to accumulate vanadium (~4-fold lower, Ref. 17). Under the conditions of vanadium therapy we applied here (daily sc administration of 0.1 mmol/kg body wt over a period of 5 days), adipose tissue is largely enriched with vanadium (unpublished observations).

The therapeutic actions of vanadium, especially in the diabetic muscle, obviously require further investigation. As mentioned earlier, vanadium therapy does not elevate the reduced content of GLUT-4 glucose transporters, although glucose disposal by muscle is restored (2, 51). Glycogen synthase activity of the diabetic muscle as measured in the cell-free system is not reduced (6, 8, 15), yet glycogen reserves are depleted, and this parameter is corrected by vanadate (37, 51). Perhaps our study represents the first report of a gross alteration, in muscle, of a signal metabolite capable of elevating enzyme gene expression in principle. G-6-P is an allosteric activator of glycogen synthase (53). The substantial reduction in muscle G-6-P found here (42% decrease, Table 1) is sufficient to account for a significant decrease in glycogen synthesis despite the fact that glycogen synthase levels and activity have not been reduced by chronic hyperglycemia (manuscript in preparation). By use of 31P NMR spectroscopy, lower levels of G-6-P compared with control subjects were also observed in human diabetic patients who were exposed to hyperglycemic and hyperinsulinemic conditions (39).

In summary, we find here that G-6-P levels are reduced in insulin-responsive tissues exposed to prolonged hyperglycemia to a much greater extent than
we ever previously assumed. We provide a strong argument to show that vanadate operates in diabetic rats through an additional G-6-P-restoring pathway, and that diabetes has much in common with immaturity and starvation.

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