Synergistic Interaction of Magnesium and Vanadate on Glucose Metabolism in Diabetic Rats

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The effect of vanadate (V) alone, magnesium (Mg) alone, and the combination of Mg plus V (MgV) on insulin-mediated glucose disposal and glucose tolerance was investigated in normal and streptozotocin-induced diabetic rats. MgV, magnesium sulfate ($MgSO_4$) and sodium metavanadate (NaV) were added to the drinking water of normal or diabetic rats (~300 g) for 3 weeks. After 3 weeks of V treatment (both MgV and NaV), diabetic rats demonstrated a normal meal tolerance test without any increase in the plasma insulin response. Rats also received a euglycemic insulin clamp (12 mU/kg · min for 120 minutes) with 3-^{3}H-glucose infusion to quantify total body glucose disposal, glycolysis (^3H_2O production), and glycogen synthesis (total body glucose disposal minus glycolysis). Total glucose disposal was decreased in diabetic versus control rats (29 ± 2 *v* 35 ± 2 mg/kg · min, $P < .01$) and returned to levels greater than the nondiabetic control values after MgV (41 ± 2, $P < .01$). Supersensitivity to insulin was not observed in diabetic rats treated with NaV (34 ± 1). Glycogen synthesis was increased by both MgV and NaV treatment (23 ± 21, $P < .01$ and 18 ± 1, $P < .05$ *v* 14 ± 2 mg/kg · min) in diabetic rats. A small increase in glycolysis was observed in $MgSO_4$ and MgV rats (18 ± 1 and 18 ± 1 *v* 16 ± 1, $P < .05$). NaV alone had no effect on glycolysis. Thus, Mg has a synergistic effect with V to increase muscle glycogen synthesis in diabetic rats. In normal rats, neither $MgSO_4$ nor NaV had any effect on glucose utilization. However, MgV increased glucose disposal to rates that were significantly higher than the rate in untreated control rats ($P < .05$). Based on these results, MgV is superior to either V alone or Mg alone in improving insulin sensitivity and glycogen synthesis in diabetic rats.

INSULIN RESISTANCE is a characteristic feature in humans with diabetes mellitus,[1,2] as well as animal models of diabetes.[3,4] Based on this important pathogenic feature, there has been great interest in the development of medications that enhance insulin sensitivity. Vanadium is a trace element that has been shown to possess insulinomimetic properties both in vitro[5-7] and in vivo.[7-10] Its mechanism of action is believed to result from an inhibition of protein tyrosine phosphatases, leading to an activation of cytosolic tyrosine kinase[11] or insulin receptor tyrosine kinase activity.[12]

Consistent with its insulinomimetic properties, several recent studies have demonstrated that vanadate (V) can improve insulin sensitivity and glucose tolerance in human type 2 diabetic subjects.[13-15] Magnesium (Mg) ion is a cofactor for many enzymes and also has been shown to increase tyrosine kinase activity.[16] Rossetti et al[8] examined the effect of treatment with a cocktail (V, lithium, zinc, and Mg) of trace elements and demonstrated an improvement in glucose tolerance and insulin sensitivity when Mg/zinc was added to V/lithium-treated diabetic rats. In man, Paolisso et al[17] have shown that in thiazide-treated, Mg-deficient, insulin-resistant, nondiabetic hypertensive individuals, Mg treatment for 8 weeks improved insulin sensitivity by enhancing flux through the glucose oxidative pathway. The same group,[18] using the euglycemic insulin clamp technique, also demonstrated a beneficial effect of Mg supplementation on insulin sensitivity in type 2 diabetic patients. However, no previous study has specifically evaluated the efficacy of combined MgV treatment in an insulin-resistant animal model of type 2 diabetes mellitus.

MATERIALS AND METHODS

Animals

Eight groups of male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) weighing 180 to 200 g were studied. All rats were given free access to food and water, housed in individual cages in an air-controlled room, and subjected to a standard light (6 AM to 6 PM)/dark (6 PM to 6 AM) cycle. On the day after arrival, animals were injected with vehicle (groups I to IV) or streptozotocin 35 mg/kg intravenously via the tail vein (groups V to VIII). One week after vehicle or streptozotocin injection, MgV 0.3 mg/mL (Baker Norton Pharmaceuticals, Miami, FL; groups II and VI), $MgSO_4$ 0.3 mg/mL (Sigma, St. Louis, MO; groups III and VII), and sodium metavanadate (NaV) 0.3 mg/mL (Sigma; groups IV and VIII) were added to the drinking water (Table 1). No test compounds were added to the drinking water of animals in groups I and V, and these groups served as the control for nondiabetic (groups II to IV) and diabetic (groups VI to VIII) animals, respectively. In diabetic rats treated with V, there was a progressive reduction in water intake, which paralleled the decline in blood glucose. Therefore, the V concentration in the drinking solution was increased every 3 days with the goal of maintaining postprandial plasma glucose between 100 and 150 mg/dL. The variation in the daily intake of V was 10% ± 1%. The concentration of $MgSO_4$ in the drinking water was not changed during the study period.

Experimental Design

Every 3 to 4 days after the start of Mg and/or V, food was removed from the cage at 6 AM and tail vein blood was obtained at 8 AM for plasma glucose determination. At 3.5 weeks after streptozotocin or vehicle injection (ie, 2.5 weeks after the start of Mg and/or V), rats were fasted overnight and subjected to a meal tolerance test at 8 AM with 2 g rat chow (Teklad LM-485; Harlan Teklad, Madison, WI). Rats spontaneously consumed all of the rat chow within 5 to 10 minutes. Blood for plasma glucose and insulin determinations was collected from the tail vein before and 60 and 120 minutes after the chow was eaten. On the day following the meal tolerance test, rats were anesthetized with sodium pentobarbital (50 mg/kg body weight intraperitoneally) and indwelling catheters were inserted into the right internal jugular vein and the left carotid artery. Both catheters were exteriorized through the skin at the back of the neck. Four to 5 days postsurgery, rats received a euglycemic insulin clamp. Only rats who reached their preoperative

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Table 1. Body Weight, Food and Water Intake, and Daily Mg and V Intake in Nondiabetic and Diabetic Groups

Group	No. of Rats	Intake of Compound (mg/d)	Mg (mg/d)	V (mg/d)	Body Weight (g)	Incremental Body Weight (g/d)	Food (g/d)	Water (mL/d)
I. Nondiabetic	12	—	—	—	319 ± 9	7.4 ± 0.4	29 ± 1	39 ± 1
II. Nondiabetic + MgV	10	6.7 ± 0.3	0.7 ± 0.03	3.1 ± 0.1	258 ± 7*	4.7 ± 0.3*	31 ± 2	23 ± 1
III. Nondiabetic + $MgSO_4$	7	11.5 ± 0.7	2.3 ± 0.1	—	352 ± 20	7.0 ± 0.5	30 ± 1	38 ± 1
IV. Nondiabetic + NaV	6	7.1 ± 0.6	—	2.9 ± 0.2	284 ± 12	6.5 ± 0.4	24 ± 2	26 ± 2
V. Diabetic	11	—	—	—	252 ± 17*	3.2 ± 0.7*	43 ± 3*	158 ± 21*
VI. Diabetic + MgV	11	11.5 ± 1.3	1.3 ± 0.1	5.3 ± 0.6	260 ± 13*	2.4 ± 0.6*	25 ± 2	31 ± 2
VII. Diabetic + $MgSO_4$	10	24.2 ± 5.0	4.9 ± 1.0	—	300 ± 20	3.5 ± 1.0	36 ± 2*	110 ± 20*
VIII. Diabetic + NaV	9	10.5 ± 1.3	—	4.4 ± 0.5	290 ± 15	4.1 ± 0.8	29 ± 1	31 ± 2

NOTE. Results are the mean ± SEM.

*$P < .05$—.01 *v* group I (nondiabetic control).

weight within 4 to 5 days after surgery were studied. On the basis of this criterium, one animal was excluded.

Euglycemic Insulin Clamp

Before the start of the insulin clamp, a blood sample was obtained for determination of the plasma V concentration in six diabetic rats in the MgV group and six diabetic rats in the NaV group. At 8 AM, following a 24-hour fast, porcine insulin (Sigma) was infused at 12 mU/kg · min for 120 minutes and euglycemia was maintained by adjusting the glucose infusion rate based on the negative-feedback principle.[19,20] At the time the insulin infusion was started, rats also received a primed (2 μCi)-continuous (0.15 μCi/min) infusion of 3-^{3}H-glucose (DuPont-NEN, Boston, MA). Blood samples for determination of plasma tritiated glucose specific activity and ^3H_2O were obtained every 10 minutes after the start of tritiated glucose. Blood for plasma insulin assay was drawn at −10, 0, 90, and 120 minutes. The total amount of blood drawn during any study was less than 4 mL. To prevent intravascular volume depletion and anemia, a solution (1:1 vol/vol) of an equivalent amount of fresh whole blood (obtained by heart puncture from littermates of the experimental animal) and heparinized saline (10 U/mL) was infused at a constant rate throughout the tritiated glucose infusion period. At the end of the 120-minute insulin clamp study, rats were injected with pentobarbital (60 mg/kg body weight) and the hindlimb muscle was removed and freeze-clamped with aluminum tongs precooled in liquid nitrogen. All tissue samples were stored at −80°C.

Chemical Determinations

The plasma glucose level was measured by the glucose oxidase method (Beckman Glucose Analyzer; Beckman Instruments, Fullerton, CA) and plasma insulin by radioimmunoassay using rat (fasting insulin) or human (during the insulin clamp) standards (Rat Insulin Kit; Linco, St Louis, MO). The plasma vanadium concentration was determined by atomic absorption spectrophotometry (Elemental Research, North Vancouver, Canada). Methods for the determination of plasma 3-^{3}H-glucose specific activity have been previously described.[19-21] Briefly, plasma proteins were precipitated by adding 100 μL $Ba(OH)_2$ and 100 μL $ZnSO_4$ to 50 μL plasma (Somogyi procedure). After centrifugation, half of the supernatant was evaporated to dryness at 55°C to eliminate ^3H_2O, reconstituted in 0.1 mL water, mixed with 3 mL Scintiverse II (Fisher, Pittsburgh, PA), and counted in a Beckman LS 6000 IC beta scintillation counter. The other half of the supernatant (containing ^3H_2O) was counted directly without evaporation. All samples were tested in duplicate. Plasma ^3H_2O radioactivity was calculated by subtracting the dpm in an aliquot of the Somogyi supernatant that was evaporated to dryness from an unevaporated aliquot.

Calculations

Whole-body glucose uptake and endogenous glucose production. A steady-state plateau of plasma 3-^{3}H-glucose specific activity was achieved within 30 minutes after the start of [3-^{3}H]-glucose infusion in each study. During this steady-state period, the rate of endogenous glucose appearance (Ra) equals the rate of glucose disappearance (Rd), and the glucose turnover rate was calculated by dividing the [3-^{3}H]-glucose infusion rate (dpm per minute) by the steady-state plasma 3-^{3}H-glucose specific activity (dpm per milligram).[20,21] In the basal state, Ra equals the rate of endogenous (primarily hepatic) glucose production (EGP). In the insulin-stimulated state, Ra equals the rate of EGP plus the rate of exogenous glucose infusion. Therefore, EGP = Ra − exogenous glucose infusion. In the insulin-stimulated state, Rd equals the rate of whole-body glucose uptake.

Whole-body glycolysis and whole-body glucose storage. ^{3}H in the C-3 position of glucose is lost selectively to water during glycolysis. Therefore, plasma tritiated counts are present either as ^3H_2O or as [3-^{3}H]-glucose. Rates of whole-body glycolysis were determined from the increment per unit time in ^3H_2O (dpm per liter per minute) multiplied by the total body water mass and divided by the [3-^{3}H]-glucose specific activity (dpm per milligram).[20,21] Plasma H_2O is assumed to be 93% of the total plasma volume, and total body H_2O mass is assumed to be 65% of the body weight.[22] The rate of whole-body glycolysis was determined during the last 20 minutes of [3-^{3}H]-glucose infusion. The appearance of ^3H_2O in plasma over this period was linear in all studies, as judged by linear regression analysis. This is in agreement with previous publications.[22] The rate of whole-body glucose storage was calculated by subtracting the rate of whole-body glycolysis from the rate of whole-body glucose disposal (Rd). The calculated rate of whole-body glucose storage primarily reflects glycogen synthesis[22] plus a small amount of glucose that is converted to lipid.[21]

Muscle glycogen synthesis. The muscle glycogen synthetic rate was determined as previously described.[22] Briefly, aliquots of the tissue homogenate of the hindlimb muscle (200 μL) were used to determine the amount of tritium label in glycogen. Glycogen was precipitated by washing in 10 vol absolute ethanol and incubating for 1 hour at −20°C. The procedure was repeated three times, and then the precipitate was collected, dried, and dissolved in water before scintillation counting. The muscle glycogen synthetic rate was calculated by dividing the number of tritium counts in glycogen per gram of tissue by the time-weighted mean plasma 3-^{3}H-glucose specific activity, as previously described.[22]

Statistics

Multiple comparisons were performed using two-way ANOVA on the eight groups of rats. When ANOVA showed a significant difference,

both Fisher's least-significant difference test and the unpaired two-tailed Student's *t* test were used to define differences between individual rat groups. The criterion for significance was set at P less than .05. All data are presented as the mean ± SEM.

RESULTS

Body Weight, Food and Water Consumption, Mg and V Intake, and Plasma Vanadium Concentration

In general, diabetic rats tended to gain slightly less weight than their nondiabetic counterparts. Both nondiabetic and diabetic rats treated with MgV gained less weight than their counterparts treated with NaV. No diarrhea was observed in any group, specifically the groups treated with MgV. Both food and water intake were markedly increased in untreated diabetic rats (group V) and in diabetic rats receiving $MgSO_4$ (group VII). The amount of vanadium consumed by diabetic rats in groups VI and VIII and nondiabetic rats in groups II and IV was similar. Both the normal (group III) and diabetic (group VII) rats ingesting $MgSO_4$ consumed more elemental Mg per day than their nondiabetic (group II) and diabetic (group VI) counterparts who were receiving MgV in the drinking water. The plasma vanadium concentration was similar in diabetic rats treated with NaV and MgV (609 ± 71 *v* 623 ± 112 ng/mL, respectively).

Morning (8 AM) Blood Glucose Concentration

The morning blood glucose concentration (2 hours after removal of food from the cage) in diabetic rats (group V) was 330 to 430 mg/dL versus 120 to 125 mg/dL in nondiabetic rats (group I). Treatment with MgV and NaV reduced the 8 AM morning blood glucose from 413 ± 32 to 124 ± 8 mg/dL and from 286 ± 39 to 137 ± 11 mg/dL, respectively (both $P < .001$). The decrement in morning glucose with MgV ($\Delta = 289 \pm 33$ mg/dL) was significantly greater ($P < .001$) than the decrement observed with NaV ($\Delta = 149 \pm 16$). $MgSO_4$ also decreased the 8 AM morning glucose in diabetic rats from 303 ± 32 to 263 ± 37 mg/dL ($\Delta = 40 \pm 18$ mg/dL, $P < .05$). In nondiabetic control rats, NaV and MgV both caused a slight reduction (P = NS) in the 8 AM morning blood glucose concentration, but this decline was not different from the decline observed in the nondiabetic control group that received no treatment (Fig 1).

Meal Tolerance Test

In untreated diabetic rats (group V), fasting plasma glucose increased from 240 ± 56 mg/dL to 390 ± 69 at 1 hour and 361 ± 71 at 2 hours following ingestion of the meal. $MgSO_4$ treatment in diabetic rats improved the meal tolerance test (glucose, 107 ± 11 mg/dL at 0 hours and 211 ± 40 at 2 hours, both $P < .01$ *v* untreated diabetic rats) but did not return the glucose level to normal ($P < .01$ *v* nondiabetic control rats). Treatment with both MgV and NaV completely normalized the meal tolerance test. There was no difference in meal tolerance in any of the four nondiabetic groups (Fig 2).

Basal plasma insulin concentrations were slightly reduced in diabetic rats (0.46 ± 0.12 ng/mL) versus control rats (0.64 ± 0.12 ng/mL, P = NS); 1- and 2-hour plasma insulin concentrations were decreased 30% to 40% in diabetic (group V) versus nondiabetic (group I) rats (Fig 2). $MgSO_4$ significantly increased the plasma insulin response ($P < .01$ *v* group V) but did not completely return it to normal ($P < 0.05$ *v* group I). MgV and NaV had no effect on insulin secretion in diabetic rats. In nondiabetic rats, Mg tended to increase insulin secretion, while both MgV and NaV tended to decrease insulin secretion. However, none of these changes reached statistical significance.

Insulin Clamp: Plasma Glucose and Insulin Concentrations

In nondiabetic rats, the plasma glucose concentration during the insulin clamp was maintained at 98 ± 2, 102 ± 2, 99 ± 2, and 100 ± 2 mg/dL in groups I to IV. In diabetic rats, plasma glucose was clamped at 102 ± 1, 97 ± 2, 100 ± 4, and 104 ± 2 mg/dL. The fasting plasma insulin concentration was similar in all four nondiabetic groups and averaged 0.77 ± 0.06 ng/mL. The fasting insulin concentration was reduced 40% to 60% in all diabetic groups: 0.44 ± 0.12, 0.28 ± 0.06, 0.49 ± 0.08, and 0.41 ± 0.07 ng/mL in groups V to VIII, respectively. During the insulin clamp, steady-state plasma insulin concentrations were similar in all eight groups and averaged 258 ± 27 and 249 ± 21 μU/mL in nondiabetic and diabetic rats, respectively.

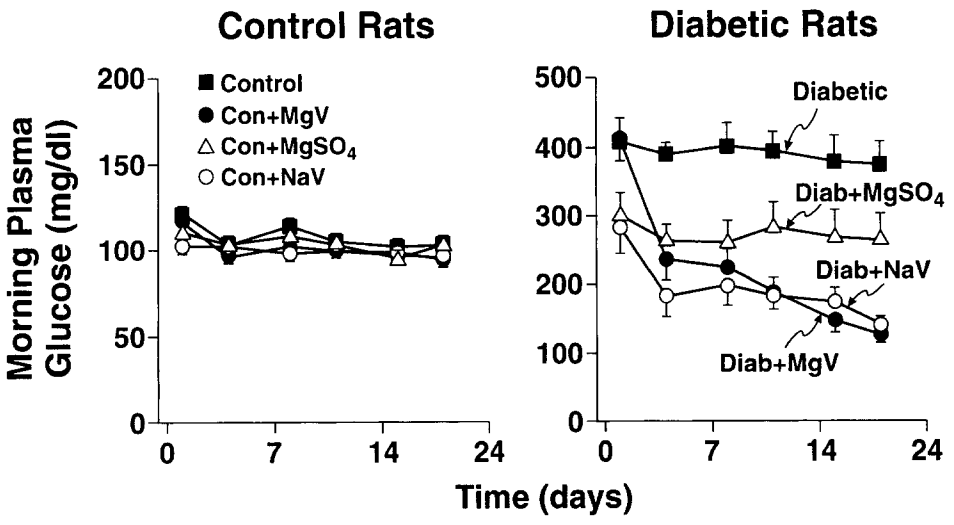

Fig 1. Time course of the effect of Mg, V, and MgV treatment on the morning (8 AM) plasma glucose concentration in diabetic and control rats.

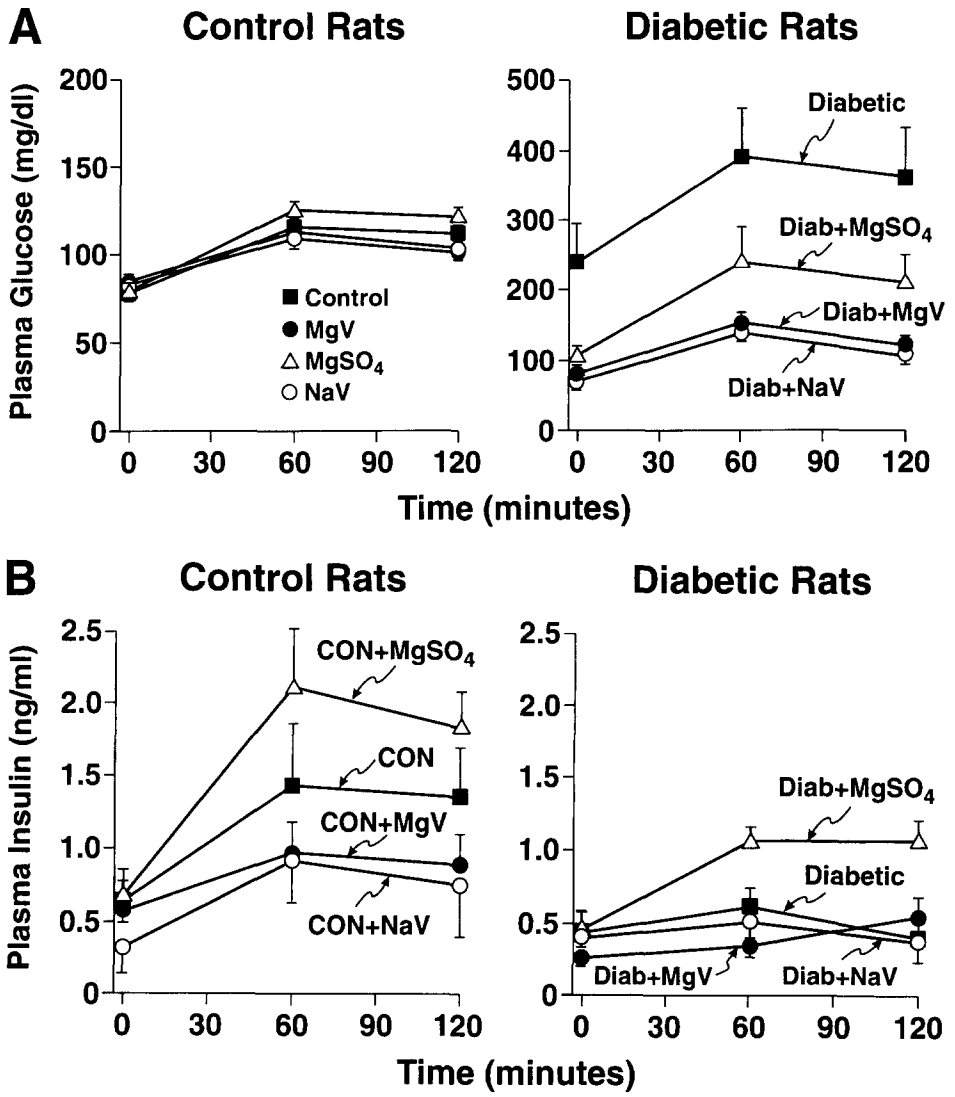

Fig 2. Effect of Mg, V, and MgV treatment on the meal tolerance test in diabetic and control rats.

Insulin Clamp: Whole-Body Glucose Uptake and EGP

Whole-body insulin-mediated glucose disposal was significantly reduced in diabetic versus nondiabetic control rats (29.4 ± 1.5 *v* 35.2 ± 1.5 mg/kg · min). Treatment with NaV increased the rate of whole-body insulin-mediated glucose disposal (34.0 ± 1.0 mg/kg · min, $P < .01$ *v* diabetic rats) to values similar to those in nondiabetic control rats. $MgSO_4$ had a modest effect to improve whole-body glucose disposal in response to insulin (33.7 ± 1.4, $P < .05$ *v* diabetic rats). When diabetic rats received combination therapy with MgV (group VI), the rate of insulin-mediated glucose disposal (40.6 ± 2.0) increased to values significantly greater than those observed in nondiabetic control animals ($P < .05$) and in diabetic rats treated with NaV ($P < .05$). In nondiabetic rats, neither NaV nor $MgSO_4$ had any effect on insulin-mediated whole-body glucose disposal, while MgV increased the rate to a higher level than observed in control (group I) rats (40.4 ± 1.6 *v* 35.2 ± 1.5 mg/kg · min, $P < .05$) (Fig 3).

Because the tracer was started at the beginning of insulin infusion, EGP was not measured in the fasting state. In response to insulin, EGP was suppressed to 2.0 ± 0.4 mg/kg · min in

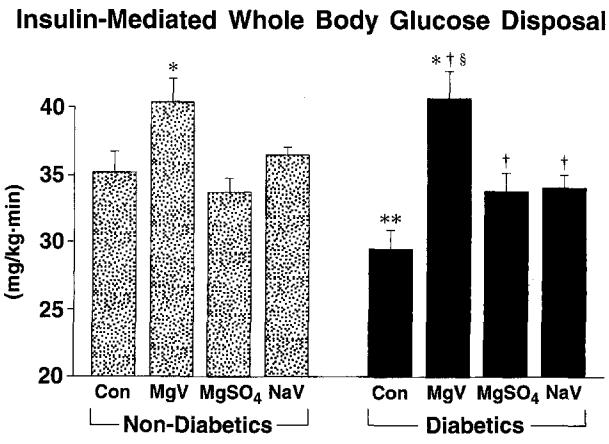

Fig 3. Effect of Mg, V, and MgV treatment on whole-body insulin-mediated glucose disposal. *$P < .05$ *v* controls and controls treated with NaV; **$P < .01$ *v* controls; †$P < .05$ *v* diabetic rats; §$P < .05$ *v* diabetic rats treated with NaV.

nondiabetic control rats, compared with 3.8 ± 0.6 in diabetic rats ($P < .05$). In diabetic rats treated with MgV (0.3 ± 0.3) and NaV (1.2 ± 0.2), suppression of EGP was significantly greater than in controls ($P < .05$). The decline in EGP in diabetic rats treated with MgV was significantly greater versus diabetic rats treated with NaV ($P < .05$). In diabetic rats, $MgSO_4$ enhanced the insulin-mediated suppression of EGP (1.5 ± 0.5) to levels observed in nondiabetic controls.

Insulin Clamp: Glycolysis and Glycogen Synthesis

The insulin-mediated whole-body glycolytic rate was similar in nondiabetic control (group I) rats (15.0 ± 0.9 mg/kg · min) and streptozotocin diabetic (group V) rats (15.8 ± 0.9 mg/kg · min). In nondiabetic (17.6 ± 0.6) and diabetic (18.1 ± 0.8) rats treated with MgV, the insulin-mediated glycolytic rate increased ($P < .05$) versus the respective control groups. $MgSO_4$ also increased the insulin-mediated glycolytic rate (19.5 ± 0.9) above that observed in diabetic (group V) rats ($P < .05$). NaV had no effect on the insulin-stimulated glycolytic rate (Fig 4).

Insulin-stimulated whole-body glycogen formation, measured as the difference between the rates of whole-body glucose disposal and glycolysis, was reduced 33% in diabetic versus nondiabetic control rats (13.6 ± 1.6 *v* 20.2 ± 0.9 mg/kg · min, $P < .001$). In diabetic rats, MgV increased insulin-stimulated whole-body glycogen synthesis to 22.5 ± 1.0 mg/kg · min ($P < .001$ *v* diabetics and $P < .10$ *v* controls). NaV increased whole-body glycogen formation modestly to 17.6 ± 0.9 mg/kg · min ($P < .05$ *v* diabetic and $P < .01$ *v* diabetic plus MgV). $MgSO_4$ had no significant effect on glycogen synthesis in diabetic rats. In nondiabetic rats, MgV had a modest stimulatory effect on insulin-mediated glycogen formation (22.7 ± 0.9 mg/kg · min, $P < .10$ *v* untreated nondiabetic rats). Neither $MgSO_4$ nor NaV or MgV had any effect on whole-body glycogen formation in nondiabetic rats.

The muscle glycogen synthetic rate, measured by tracer accumulation in muscle glycogen during the insulin clamp study, was significantly decreased in diabetic versus control rats (770 ± 64 *v* 1,107 ± 125 μg/g wet weight per 2 hours, $P < .05$). Treatment of diabetic rats with MgV (1,362 ± 70) increased the muscle glycogen synthetic rate to values significantly greater than obtained with NaV (1,049 ± 96, $P < .05$) or $MgSO_4$ (821 ± 94, $P < .05$).

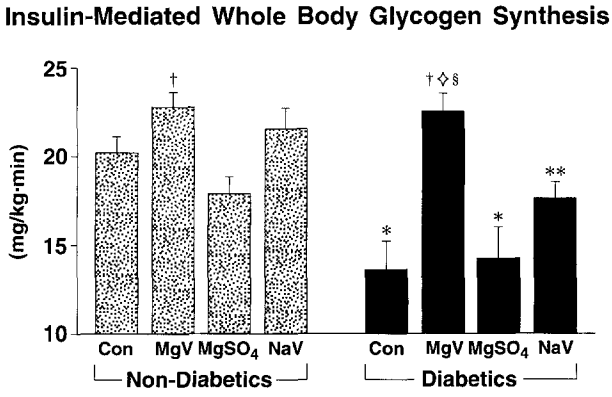

Fig 4. Effect of Mg, V, and MgV treatment on whole-body insulin-mediated glycogen synthesis. *$P < .01$ *v* controls; ◇$P < .001$ *v* diabetic rats; †$P < .10$ *v* control rats; §$P < .01$ *v* diabetic rats treated with NaV; **$P < .05$ *v* diabetic rats.

DISCUSSION

A number of studies both in vitro[5-7,12,23] and in vivo[7-15,23] have demonstrated that V has insulinomimetic properties in a variety of tissues in both animals and humans with type 2 diabetes mellitus. As reviewed by Goldfine et al,[12] the most consistently observed effect of V is the phosphorylation of tyrosine residues of the insulin receptor. Although some evidence suggests that V directly phosphorylates the insulin receptor,[23] most studies indicate that vanadium compounds inhibit phosphotyrosyl protein phosphatases, which indirectly enhances the phosphorylation state of the insulin receptor.[12,24] In vivo V augments insulin sensitivity,[7-15] and this is associated with increased muscle glucose transport[25,26] and glycogen synthesis.[6,7,9,12,27,28] The present findings are entirely consistent with these previous publications (Fig 3 and 4). It is noteworthy that V had no stimulatory effect on glycolysis (Fig 4) and all of the improvement in whole-body insulin sensitivity resulted from enhanced glycogen synthesis. During the euglycemic insulin clamp, the great majority of glucose disposal occurs in muscle. The stimulatory effect of V on muscle glycogen formation observed in the present study has been documented previously.[6,7,9,27,28] Conflicting reports have appeared concerning the effect of V on basal EGP and its suppression by insulin.[13,14,29] Our results indicate that V reduces the elevated basal rate of EGP and corrects the defect in the insulin-mediated suppression of HGP, as demonstrated by Halberstam et al[29] and Cohen et al.[14]

Despite the intensive investigation of vanadium compounds as insulinomimetic agents, relatively few studies have examined the effect of other trace elements on insulin action in vivo.[8] This is surprising, considering the important role of these trace elements as cofactors for many enzymes involved in carbohydrate metabolism. In the present study, we have examined the effect of one such trace element, Mg, alone and in combination with V. Mg was chosen because it is involved in the pyruvate dehydrogenase complex[30] and insulin receptor tyrosine kinase activity.[16,31] Moreover, insulin augments Mg entry into the cell by activating Ca^{++}-Mg^{++}-ATPase activity,[32] and the ability of insulin to enhance Mg entry has been shown to be impaired in type 2 diabetes mellitus.[33,34]

When given to diabetic rats, Mg reduced the fasting glucose concentration, improved the meal tolerance test, and enhanced insulin sensitivity. However, unlike V, all of the improvement in whole-body insulin sensitivity was due to increased glycolysis, without any change in glycogen synthesis (Figs 3 and 4). This observation is consistent with the important role of Mg in the regulation of pyruvate dehydrogenase activity[30] and several recent studies indicating that Mg supplementation can enhance insulin sensitivity in nondiabetic hypertensive[17] and type 2 diabetic[18] subjects by augmenting the flux through the glucose oxidative pathway without altering glycogen synthesis. It also is possible that Mg may affect cytosolic tyrosine kinase or the oxidation state of vanadium.[35] In both control and diabetic rats, Mg also was associated with an increase in insulin secretion. Therefore, it is not possible to dissect the individual contributions of enhanced insulin secretion versus enhanced insulin sensitivity to the improvement in glucose homeostasis. Previous

studies in man also have demonstrated a stimulatory effect of Mg on insulin secretion.[36]

Especially dramatic was the effect of Mg in combination with V. The decrement in fasting glucose was significantly greater with MgV compared with NaV, and similar fasting glucose levels were reached in both groups (Fig 1). However, because the starting fasting glucose concentration was significantly different between MgV and NaV groups, it is difficult to draw definitive conclusions about the biologic potency of the two treatment regimens. More meaningful insight into the biologic potency of MgV versus NaV can be gained from examining the effect of these two agents on whole-body insulin sensitivity and glycogen synthesis. Whole-body insulin sensitivity in both diabetic and nondiabetic rats treated with MgV was increased to values that were significantly greater versus the respective control groups and significantly greater versus diabetic and control rats treated with NaV (Fig 3). In both diabetic and nondiabetic rats, MgV increased whole-body and skeletal muscle glycogen formation to supernormal levels ($P < .01$) and to values that were significantly greater than observed in both diabetic and nondiabetic rats treated with NaV (Fig 3). These results indicate that Mg and V have a synergistic effect to increase whole-body insulin sensitivity and glycogen synthesis. Unlike NaV, which had no effect on whole-body glycolysis, MgV significantly enhanced flux through the glycolytic pathway in both diabetic and nondiabetic rats. Insulin-mediated suppression of EGP also was greater with MgV compared with NaV.

In summary, the present results indicate that in diabetic rats, both Mg and V have important effects to improve insulin sensitivity via increasing flux through the glycolytic and glycogenic pathways, respectively. However, when used in combination, Mg and V have a synergistic action to augment whole-body insulin sensitivity and glycogen synthesis. To the best of our knowledge, these findings represent the first demonstration of a synergistic interaction between Mg and V to improve insulin sensitivity, and suggest a potential role for combination therapy with trace elements in type 2 diabetes mellitus and other insulin-resistant states.

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