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COMMENT

Vanadyl Sulfate Improves Hepatic and Muscle Insulin Sensitivity in Type 2 Diabetes*

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ABSTRACT

Vanadyl sulfate ($VOSO_4$) is an oxidative form of vanadium that *in vitro* and in animal models of diabetes has been shown to reduce hyperglycemia and insulin resistance. Small clinical studies of 2- to 4-week duration in type 2 diabetes (T2DM) have led to inconsistent results. To define its efficacy and mechanism of action, 11 type 2 diabetic patients were treated with $VOSO_4$ at a higher dose (150 mg/day) and for a longer period of time (6 weeks) than in previous studies. Before and after treatment we measured insulin secretion during an oral glucose tolerance test, and endogenous glucose production (EGP) and whole body insulin-mediated glucose disposal using the euglycemic insulin clamp technique combined [3-^{3}H]glucose infusion. Treatment significantly improved glycemic control: fasting plasma glucose (FPG) decreased from 194 ± 16 to 155 ± 15 mg/dL, hemoglobin A_{1c} decreased from 8.1 ± 0.4 to 7.6 ± 0.4%, and fructosamine decreased from 348 ± 26 to 293 ± 12 μmol/L (all $P < 0.01$) without any change in body weight. Diabetics had an increased rate of EGP compared with nondiabetic controls (4.1 ± 0.2 *vs.* 2.7 ± 0.2 mg/kg lean body mass·min; $P < 0.001$), which was closely correlated with FPG ($r = 0.56$; $P < 0.006$). Vanadyl sulfate reduced EGP by about 20% ($P < 0.01$), and the decline in EGP was correlated with the reduction in FPG ($r = 0.60$; $P < 0.05$). Vanadyl sulfate also caused a modest increase in insulin-mediated glucose disposal (from 4.3 ± 0.4 to 5.1 ± 0.6 mg/kg lean body mass·min; $P < 0.03$), although the improvement in insulin sensitivity did not correlate with the decline in FPG after treatment ($r = -0.16$; P = NS). Vanadyl sulfate treatment lowered the plasma total cholesterol (223 ± 14 *vs.* 202 ± 16 mg/dL; $P < 0.01$) and low density lipoprotein cholesterol (141 ± 14 *vs.* 129 ± 14 mg/dL; $P < 0.05$), whereas 24-h ambulatory blood pressure was unaltered. We conclude that $VOSO_4$ at maximal tolerated doses for 6 weeks improves hepatic and muscle insulin sensitivity in T2DM. The glucose-lowering effect of $VOSO_4$ correlated well with the reduction in EGP, but not with insulin-mediated glucose disposal, suggesting that liver, rather than muscle, is the primary target of $VOSO_4$ action at therapeutic doses in T2DM. (*J Clin Endocrinol Metab* **86:** 1410–1417, 2001)

VANADIUM IS A trace element believed to be important for normal cell function and development (1–4). It is present in all tissues, but its exact role in glucose homeostasis in man has yet to be established. Numerous *in vitro* and *in vivo* studies have shown that vanadium has insulin-like effects in liver, skeletal muscle, and adipose tissue (1–4). Vanadium at relatively high concentrations *in vitro* and *in vivo* inhibits phosphotyrosine phosphatases (PTPs), thus enhancing insulin receptor phosphorylation and tyrosine kinase (IRTK) activity (2–4). However, several studies have demonstrated that vanadium can stimulate glucose uptake independently of any change in IRTK activity (5–11), suggesting that there are additional mechanisms for its insulin-mimetic effects.

In rat models of obesity and type 2 diabetes mellitus (T2DM), vanadium improves glucose metabolism by restoring normal hepatic and skeletal muscle insulin sensitivity (6, 7, 12–23). In liver, vanadium compounds have been reported to inhibit lipogenesis (6, 16, 17, 19–21) and gluconeogenesis (6, 19, 22, 24) and to stimulate glycolysis (12, 19, 24) and glycogen synthesis (12, 15, 16, 21). In skeletal muscle, vanadium augments glucose uptake (13, 14, 16, 18, 23, 25), primarily by stimulating glycogen formation (13, 23). In contrast to the large number of *in vitro* and *in vivo* animal studies, there is limited information about the metabolic effects of vanadium in humans. Two oxidized forms of vanadium, vanadyl (tetravanadate) and vanadate (pentavalent state), have been examined in T2DM with conflicting results. Goldfine *et al.* (26) treated five patients with sodium metavanadate for 2 weeks and failed to observe any reduction in fasting plasma glucose (FPG) or hepatic glucose production (HGP). More encouraging results have been reported with vanadyl sulfate ($VOSO_4$), possibly because vanadyl appears to be the active intracellular form of vanadium (1, 4, 27). Treatment with small oral doses of $VOSO_4$ (~1.0 mg/kg per day) for short periods of time (2–4 weeks) led to a modest 15–20% decrease in FPG (~30 mg/dL) (28, 29). In these studies, discordant results were reported concerning the effect of $VOSO_4$ on liver and muscle. Boden *et al.* (29) found only a mild improvement in insulin-mediated suppression of HGP, but no effect on basal HGP or insulin-stimulated whole body glucose uptake, glycogen synthesis, or glycolysis (29). In

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contrast, Cohen *et al.* (28) reported that $VOSO_4$ enhanced muscle and liver insulin sensitivity. However, the patients had received an overnight insulin infusion to normalize FPG (28), thus preventing any assessment of the effect of $VOSO_4$ on basal HGP, which is the primary determinant of FPG (30).

The metabolic effects of vanadium are known to be dose dependent and to require more than 4 weeks for a complete response (7, 16, 31–33). Therefore, we hypothesized that the modest glucose-lowering effect of vanadium and the discrepant results concerning its mechanism of action in these previous trials (26, 28, 29) could be related at least in part to suboptimal vanadium dosing and the short duration of treatment. The purpose of the present investigation was to reexamine the effect of $VOSO_4$ on glycemic control, insulin secretion, basal endogenous glucose production, and insulin-mediated whole body glucose disposal (which primarily represents muscle) in T2DM, when the trace element is administered for a longer period and at a higher dose than employed in previous studies.

Subjects and Methods

Subjects

Eleven type 2 diabetic patients [Caucasians; four women and seven men; age, 59 ± 2 yr; duration of diabetes, 4 ± 1 yr; body mass index, 28.9 ± 1.1 kg/m^2; hemoglobin A1c (HbA_{1c}), 8.4 ± 0.4%; fructosamine, 347 ± 19 mmol/L; FPG, 188 ± 15 mg/dL] were studied. Five subjects were being treated with diet alone, and six with a sulfonylurea. The sulfonylurea dose was stable for at least 6 months before study entry, and the dose was maintained constant throughout the study. Sulfonylureas were discontinued only on the morning of the day on which the oral glucose tolerance test (OGTT) and insulin clamp were performed (see below). Body weight was stable for at least 3 months before participation. No subject was excessively sedentary or participated in any unusual or strenuous exercise. Other than diabetes, no subject had any clinical or laboratory evidence of cardiac, pulmonary, renal, hepatic, or any other organ system disease, as determined by a complete medical history, physical examination, electrocardiogram, routine blood work, and urinalysis. Six subjects were taking a stable dose (at least 3 months) of an angiotensin-converting enzyme inhibitor, which remained unchanged during the study. Participants were not receiving any other medications known to affect carbohydrate metabolism or insulin secretion. Five healthy Caucasian nondiabetic subjects (two women and three men; age, 50 ± 3 yr; body mass index, 28.0 ± 1.4 kg/m^2; HbA_{1c}, 4.9 ± 0.3%; fructosamine, 248 ± 16 μmol/L; FPG, 98 ± 6 mg/dL) received a euglycemic insulin clamp and served as the control group. All nondiabetic volunteers had a negative family history of T2DM and a normal oral glucose tolerance test. Each subject gave written, informed, voluntary consent before participation.

Study design

After the initial screening, eligible subjects were instructed by a dietician to ingest a weight-maintaining American Diabetes Association diet and were seen weekly for 4 weeks to ensure dietary compliance. During this period they were encouraged to maintain their dietary intake and physical activity constant. If after the 4-week run-in period participants were considered to be compliant with the diet and if their weight had remained stable, they were admitted to the Clinical Research Center for two baseline metabolic studies, which were carried out at 0730 h after a 12-h overnight fast: 1) a 75-g OGTT, and 2) a euglycemic insulin clamp, as described below. The studies were performed with an interval of 3–7 days. Subjects consumed at least 200 g carbohydrates for 3 days before each study. Because vanadium compounds have been reported to reduce blood pressure (2, 3), we performed 24-h ambulatory blood pressure monitoring before and after treatment.

After completing the OGTT and insulin clamp patients were started on 25 mg $VOSO_4$ (8 mg elemental vanadium; Vanulin, Diabetic Nutriceuticals, Concord, CA) twice daily with breakfast and dinner. The dose was increased every 2–3 days. At 2 weeks all participants were receiving a total daily dose of 150 mg $VOSO_4$, divided in 50-mg doses given with breakfast, lunch, and dinner. Most patients achieved this dose after a week with good tolerance. Subjects were requested to maintain their usual dietary habits and level of daily activity throughout the study. Patients were followed weekly during the titration period and every 2 weeks thereafter. On each visit to the Clinical Research Center subjects were questioned about their dietary intake and physical activity pattern, overall well-being, compliance with the medication (confirmed by pill count), and side-effects using a standard questionnaire. Vital signs, physical exam, and home glucose-monitoring results were recorded. Blood was drawn for plasma glucose, fructosamine, and HbA_{1c} determinations on each visit. The total duration of treatment (titration plus stable dose) was 6 weeks. At the end of the 6 weeks, the OGTT, insulin clamp, and 24-h ambulatory blood pressure monitoring were repeated. Plasma vanadium levels were determined on the euglycemic insulin clamp days before and after the 6-week treatment period, and 6 weeks after discontinuation of $VOSO_4$ treatment.

Description of metabolic studies

OGTT. After an overnight fast, two blood samples were drawn through an iv catheter placed in an antecubital vein (−15 and 0 min), and subjects then received a 75-g glucose drink. Blood was drawn every 30 min for the next 2 h to measure plasma glucose, insulin, and free fatty acid (FFA) levels.

Euglycemic insulin clamp. Insulin sensitivity was measured before and after treatment in all participants with a 40 mU/m^2·min euglycemic insulin clamp (34). In brief, a 20-gauge Teflon catheter was inserted into an antecubital vein for the infusion of test substances ([3-^{3}H]glucose, insulin, and 20% dextrose). After this, a vein of the dorsum of the hand was retrogradely cannulated with a second catheter, and the hand was placed in a thermoregulated box at 65 C for arterialization of the venous blood. Both iv lines were kept patent with a slow infusion of normal saline. At 0730 h, a primed-continuous infusion of [3-^{3}H]glucose (NEN Life Science Products, Boston, MA) was started and continued until the end of the study. The [3-^{3}H]glucose constant infusion rate was of 0.2 μCi/min, and the tritiated glucose prime was calculated as follows: 20 μCi × FPG/100. After allowing 180 min for isotopic equilibration, four baseline blood samples were taken at 10-min intervals (−30, −20, −10, and 0 min) for the determination of plasma tritiated glucose specific activity, plasma glucose, FFA, and insulin concentration. After the determination of basal glucose turnover, a 2-h euglycemic insulin (40 mU/m^2·min) clamp was performed using a primed-continuous insulin infusion as previously described (34). In the diabetic subjects, after the start of insulin no glucose was infused until the plasma glucose concentration had declined to 100 mg/dL, at which level it was maintained by adjustment of a variable glucose infusion based upon the negative feedback principle (34). After 80 min, five blood samples were drawn at 10-min intervals (80–120 min) for measurement of plasma glucose, FFA, insulin levels, and plasma glucose radioactivity.

Analytical procedures

Plasma glucose was measured in duplicate using the glucose oxidase method with a Glucose Analyzer II (Beckman Coulter, Inc., Fullerton, CA). HbA_{1c} was measured using a DCA 2000 (Bayer Corp.; normal range, 4.0–6.0%). Fructosamine was measured by the nitroglutetrazolium reduction method (Roche Molecular Biochemicals, Indianapolis, IN; normal, <285 μmol/L). Total cholesterol, high density lipoprotein cholesterol, and triglycerides were measured by standardized enzymatic procedures using a Hitachi 917 autoanalyzer (Roche Molecular Biochemicals). Plasma insulin concentrations were determined by RIA (Enzymun-Test, Roche Molecular Biochemicals). Plasma FFA concentrations were measured by standard colorimetric methods (Wako Chemicals USA, Inc., Richmond, VA). Intra- and interassay coefficients of variation were 4.2% and 4.5% for insulin and 1.1% and 3.3% for FFA, respectively. Plasma vanadate concentrations were measured according to the method of Mongol *et al.* (35), by Elemental Research, Inc. (North Vancouver, Canada). Test samples (200-μL aliquots) had 2% HNO_3 added and were placed in a hot water bath at 95 F for 2 h. After cooling,

100 μL of 1 parts/million Y were added as an internal standard, and samples and standards were then analyzed by atomic absorption spectrometry. For determination of plasma glucose specific activity, plasma was deproteinized (36) and centrifuged for 30 min at 3500 × *g*, and the clear supernatant was evaporated to dryness at 55 C in a Speed-Vac evaporator (Savant Instruments, Farmingdale, NY). The pellet was resuspended in 1 mL distilled water, mixed with 5 mL Scintiverse II (Fischer Scientific, Pittsburgh, PA), and counted in an LS 6000IC scintillation counter (Beckman Coulter, Inc.). Twenty-four-hour blood pressure measurements were performed using an ambulatory blood pressure recording device (Takeda TM2420, A&D Engineering, Inc., CA) with readings every 15 min between 0700–2300 h and every 30 min between 2300–0700 h.

Calculations

During the basal period, the rate of endogenous plasma glucose appearance [which primarily represents HGP after an overnight fast (37)] equaled the rate of plasma glucose disappearance and was calculated by dividing the [3-^{3}H]glucose infusion rate (disintegrations per min/min) by the steady state plasma tritiated glucose specific activity (disintegrations per min/μmol) during the last 40 min of tracer equilibration. As the infusion of insulin results in nonsteady state conditions, the rate of plasma glucose appearance was calculated using Steele's nonsteady state equation (38), as modified by DeBodo *et al.* (39), using a pool fraction of 0.65 (40) and a volume of distribution of 200 mL/kg (39). A steady state plateau of plasma [3-^{3}H]glucose specific activity was achieved in all subjects during the last 40 min of the basal and euglycemic insulin clamp periods. During the euglycemic insulin infusion period, endogenous glucose production (EGP) was computed as the difference between the exogenous glucose infusion rate and the isotopically measured rate of plasma glucose appearance. Negative numbers for EGP were not observed in any study. The rate of total body insulin-mediated glucose disposal was calculated by adding the residual rate of EGP to the rate of exogenous glucose infusion.

Turnover rates are expressed as milligrams per kg lean body mass (LBM). Determinations of LBM and bone mineral density were performed using x-ray fan beam scanning of total body with a QDR4500SL bone densitometer (Hologic, Inc., Bedford, MA).

Statistical analysis

All data are presented as the mean ± SEM. Statistical significance was determined by ANOVA for repeated measures over time (FPG, fructosamine, and HbA_{1c}). Paired two-tailed Student's *t* test was applied where appropriate, such as for metabolic variables measured before and after $VOSO_4$ treatment (OGTT, EGP, and whole body insulin-mediated glucose disposal). Statistically significant differences between the diabetic and control groups were determined by unpaired *t* test. Where appropriate, regressions were calculated by least squares linear regression analysis. All data were analyzed using the statistical package from StatView (SAS Institute, Inc., Cary, NC). Comparisons were considered statistically significant at $P < 0.05$.

Results

Vanadyl sulfate tolerance and adverse events

Food intake and physical activity remained stable during the study. Weight remained within ±2% in all patients (at study entry, 78.3 ± 4.3 kg; after 4-week run-in, 78.1 ± 4.2 kg; after 6 weeks of treatment, 77.7 ± 4.2 kg). Tolerance to $VOSO_4$ was good, as evaluated by history, physical exam, and routine laboratories. Diarrhea (n = 4) and abdominal discomfort (n = 2) were the only significant side-effects. Symptoms occurred only during the titration period. In three of the four patients symptoms subsided (~2 weeks) with continuation of treatment. In one patient diarrhea was persistent, requiring a reduction in the dose of $VOSO_4$ treatment to 75 mg/day after 3 weeks. This patient also had a history of gastrointestinal intolerance to metformin and acarbose monotherapy. No abnormalities in blood chemistries, complete blood count, or urinalysis were observed in any subject. Three subjects had the lumbar spine and hip bone mineral density measurements repeated before and after treatment with no detectable change.

Metabolic response to treatment: FPG, HbA_{1c}, fructosamine, and lipids (Fig. 1)

FPG, fructosamine, and HbA_{1c} did not change significantly during the 4-week run-in period (FPG, 188 ± 15 *vs.* 194 ± 16 mg/dL; HbA_{1c}, 8.4 ± 0.4% *vs.* 8.1 ± 0.4%; fructosamine, 347 ± 19 *vs.* 348 ± 26 μmol/L; *vs.* study entry, respectively, all P = NS). FPG began to decrease by the second week of treatment (from 194 ± 16 to 175 ± 15 mg/dL; $P < 0.03$) and dropped progressively during the 6-week treatment period, reaching a nadir of 155 ± 15 mg/dL by 6 weeks ($P < 0.01$ *vs.* pretreatment; Fig. 1). The decline in the plasma fructosamine level closely paralleled the decrease in FPG and had decreased significantly by the fourth week of treatment (348 ± 26 *vs.* 316 ± 19 μmol/L; $P < 0.05$), reaching a value of 293 ± 12 μmol/L ($P < 0.01$) at 6 weeks (Fig. 1). Consistent with its longer half-life, the decline in the HbA_{1c} lagged behind that of fructosamine, but was significantly reduced after 6 weeks of $VOSO_4$ treatment (8.1 ± 0.4% *vs.* 7.6 ± 0.4%; $P < 0.01$).

Treatment with $VOSO_4$ significantly reduced plasma total

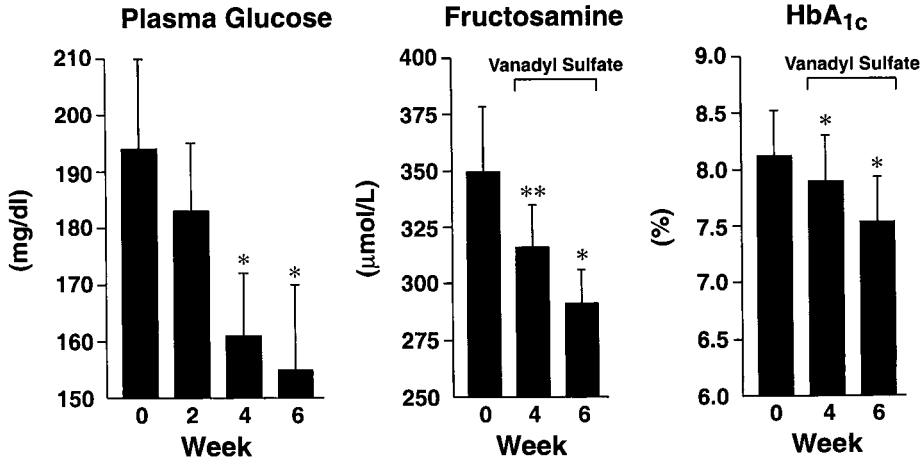

FIG. 1. Effect of 6 weeks of treatment with vanadyl sulfate on FPG, serum fructosamine, and HbA_{1C} in poorly controlled T2DM patients. Results represent the mean ± SE. *, $P < 0.01$; **, $P < 0.05$ (*vs.* pretreatment).

cholesterol [at study entry, 223 ± 14; pretreatment (after a 4-week run-in), 227 ± 14; $VOSO_4$, 205 ± 15 mg/dL; both $P <$ 0.01) and low density lipoprotein cholesterol (at entry, 137 ± 14; pretreatment, 141 ± 14 mg/dL; $VOSO_4$, 129 ± 14 mg/dL; both $P < 0.05$). There was no change in plasma high density lipoprotein cholesterol (at entry, 42 ± 5; pretreatment, 45 ± 4; $VOSO_4$, 39 ± 3 mg/dL; P = NS) or triglyceride levels (at entry, 194 ± 28; pretreatment, 205 ± 41; $VOSO_4$, 196 ± 40 mg/dL; P = NS) after $VOSO_4$ treatment.

OGTT (Fig. 2)

During the OGTT, FPG was significantly lower after $VOSO_4$ treatment (199 ± 15 *vs.* 172 ± 15 mg/dL; $P < 0.02$; Fig. 2, *left panel*). The plasma glucose concentration was reduced about 30 mg/dL during the entire OGTT, and this decrement was almost entirely accounted for by the decrease in FPG. Treatment with $VOSO_4$ did not reduce the fasting plasma insulin levels (15 ± 2 *vs.* 16 ± 3 μU/mL; P = NS). There was a trend for plasma insulin to be lower during the OGTT (Fig. 3, *right panel*), consistent with improved insulin sensitivity, as shown in the insulin clamp studies (see below). The fasting plasma FFA concentration (830 ± 67 *vs.* 719 ± 43 μmol/L; P = NS) and the mean plasma FFA concentration after glucose ingestion (514 ± 76 *vs.* 439 ± 31 μmol/L; P = NS) were unchanged after $VOSO_4$ treatment.

Plasma glucose, insulin, and FFA concentrations during the euglycemic insulin clamp

The steady state plasma glucose concentration during the euglycemic insulin clamps were similar before and after treatment (102 ± 5 *vs.* 99 ± 3 mg/dL; P = NS), with a coefficient of variation of less than 4% in all studies. The steady state plasma insulin concentrations were well matched during the insulin clamp studies performed before and after treatment (pre- *vs.* posttreatment, 63 ± 7 *vs.* 64 ± 5 μU/mL). The fasting plasma FFA concentration (816 ± 40 *vs.* 779 ± 48 μmol/L; P = NS) and the decrease in plasma FFA concentration during insulin infusion (262 ± 24 *vs.* 221 ± 21 μmol/L; P = NS) were not significantly different before *vs.* after treatment.

Endogenous glucose production (EGP) (Figs. 3 and 4)

Basal EGP, which primarily reflects glucose release by the liver (37), decreased by about 20% after $VOSO_4$ treatment (4.1 ± 0.2 to 3.4 ± 0.2 mg/kg LBM·min; $P < 0.005$; Fig. 3). There was a close correlation between the FPG and basal EGP ($r = 0.56$, $P < 0.006$; Fig. 4A) as well as between the reduction in basal EGP with treatment and the improvement in FPG ($r = 0.60$; $P < 0.05$; Fig. 4B). EGP was nearly completely suppressed during the insulin infusion period of the euglycemic insulin clamp before and after $VOSO_4$ treatment (0.2 ± 0.02 *vs.* 0.2 ± 0.01 mg/kg LBM·min, respectively; P = NS) and equally suppressed in the nondiabetics (0.2 ± 0.01 mg/kg LBM·min). Despite the reduction in basal EGP after $VOSO_4$ therapy, the rate of EGP was still 26% higher than that in nondiabetic control subjects (3.4 ± 0.2 *vs.* 2.7 ± 0.2 mg/kg LBM·min; $P < 0.01$).

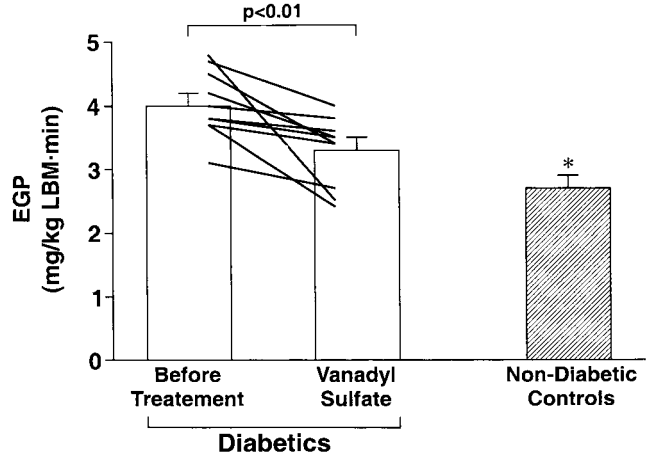

FIG. 3. Basal EGP before (*left column*) and after (*center column*) 6 weeks of vanadyl sulfate treatment in poorly controlled T2DM patients and in matched nondiabetic controls (*right column*). EGP was significantly reduced by treatment ($P < 0.01$), but remained significantly higher compared with that in nondiabetic controls (*, $P < 0.01$, posttreatment vanadyl sulfate *vs.* nondiabetic controls). Results represent the mean ± SE.

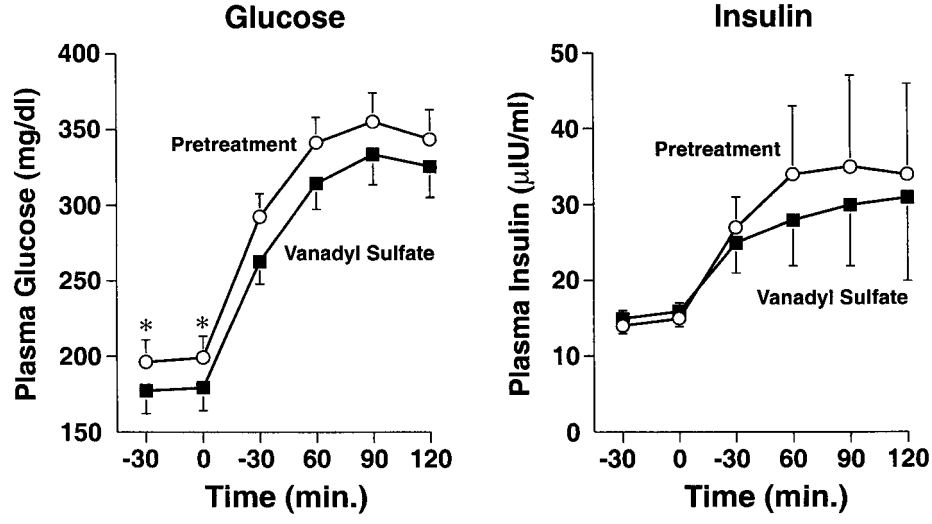

FIG. 2. Effect of 6 weeks of treatment with vanadyl sulfate on plasma glucose (*left panel*) and plasma insulin (*right panel*) concentrations during an OGTT in poorly controlled T2DM patients. Results represent the mean ± SE. *, $P <$ 0.02 *vs.* pretreatment.

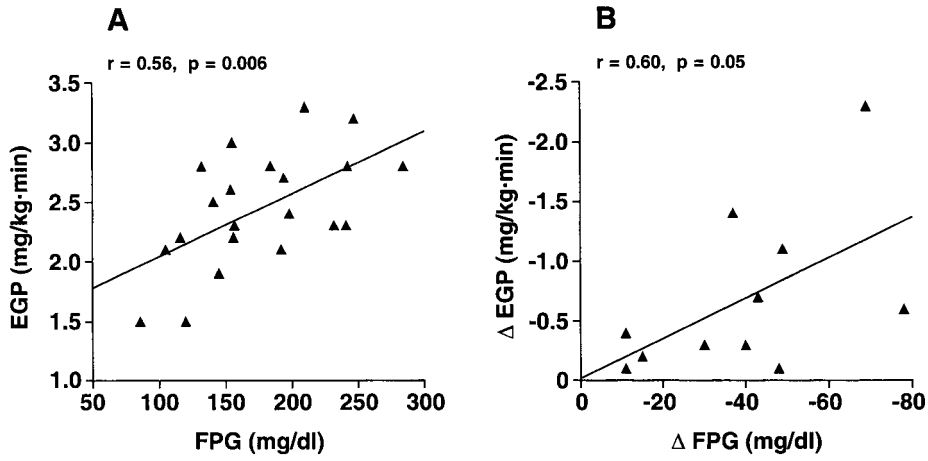

FIG. 4. A, Correlation between the rate of basal EGP and the FPG concentration in type 2 diabetic patients (data from before and after treatment combined). B, Correlation between the decrement in the rate of basal EGP and the decrement in the FPG concentration after 6 weeks of vanadyl sulfate treatment.

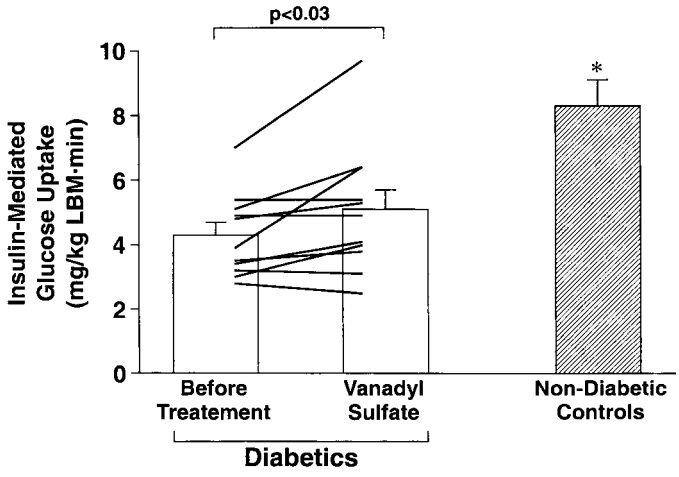

FIG. 5. Whole body insulin-mediated glucose disposal during the 40 mU/m^2·min euglycemic insulin clamp, performed before (*left column*) and after (*right column*) treatment with vanadyl sulfate. Whole body insulin-mediated glucose disposal was significantly improved by vanadyl sulfate treatment ($P < 0.03$), but remained significantly lower compared with that in matched nondiabetic controls (*, $P < 0.01$, posttreatment vanadyl sulfate *vs.* nondiabetic controls). Results represent the mean ± SE.

Whole body insulin-mediated glucose disposal (Fig. 5)

In the basal state, steady state conditions prevail, and the rate of glucose disappearance (Rd) is identical to the rate of glucose appearance. During the euglycemic insulin clamp (Fig. 5), whole body insulin-mediated glucose disposal improved significantly with $VOSO_4$ treatment from 4.3 ± 0.4 to 5.1 ± 0.6 mg/kg LBM·min ($P < 0.03$). However, it remained approximately 40% lower than that in nondiabetic controls (8.3 ± 0.8 mg/kg·min; $P < 0.001$). There was no significant correlation between insulin-mediated glucose disposal and the reduction in basal EGP (r = −0.47), FPG (r = −0.16), fructosamine (r = 0.22), or HbA_{1C} (r = −0.59; all P = NS).

Blood pressure

Blood pressure was unchanged when measured on each out-patient visit by the same investigator (K.C.) using a standardized procedure (data not shown). To obtain more detailed information, we measured blood pressure during 24 h with an ambulatory blood pressure-recording device. The mean 24-h blood pressure was unchanged by $VOSO_4$ treatment (before, 139 ± 4/92 ± 3; after, 147 ± 6/91 ± 4 mm Hg; P = NS). When diurnal and nocturnal blood pressures were analyzed separately we also failed to observe any differences before and after treatment. Mean heart rate was unchanged during the 24-h ambulatory blood pressure measurements (before, 77 ± 2; after, 78 ± 2 beats/min).

Plasma vanadium concentration

Fasting plasma vanadium levels were undetectable (<10 μg/L) before treatment and increased to 104 ± 18 μg/L after 6 weeks of $VOSO_4$ administration. Six weeks after discontinuation of $VOSO_4$ treatment, levels returned to the undetectable range (<10 μg/L).

Discussion

Over the last decade there has accumulated a large body of data that demonstrates that vanadium compounds improve glucose homeostasis and enhance insulin sensitivity in animal models of diabetes (1–4). However, only three studies have examined the effect of vanadium in type 2 diabetic subjects with rather conflicting results (26, 28, 29). We hypothesized that the inconsistent results could be explained by the short duration of these clinical trials (2–4 weeks), the small number of subjects who were studied (5–8 patients), and/or the relatively low doses of $VOSO_4$ that were employed (28, 29). It should be noted that the effect of vanadium is dose dependent (31–33, 41, 42) and requires at least 4 weeks for a full response (7, 16, 32, 43). Therefore, in the present study we treated poorly controlled type 2 diabetic subjects with 150 mg/day $VOSO_4$ for 6 weeks and quantitated glucose tolerance and hepatic and peripheral (muscle) tissue sensitivity to better define the mechanism of action of vanadium. Our results demonstrate, for the first time in subjects with type 2 diabetes, that an important mechanism of action of $VOSO_4$ is to reduce basal EGP, which primarily reflects glucose produced by the liver (37). The reduction in HGP closely correlates with the decline in FPG. Consistent with previous results (28, 29), $VOSO_4$ also improved insulin-mediated glucose disposal without any change in body weight.

During the oral glucose tolerance test, plasma insulin lev-

els did not change, confirming results from animal (7, 14, 16, 23, 43) and human (28) studies that $VOSO_4$ does not stimulate insulin secretion. Indeed, all of the reduction in the glucose AUC during the OGTT was secondary to a reduction in FPG (Fig. 2). In the present study basal EGP was about 50% higher in diabetics *vs.* healthy controls and correlated closely with the elevation in FPG ($r = 0.56$; $P < 0.006$). Treatment decreased the rate of basal EGP by about 20% ($P < 0.005$), and the reduction in FPG was closely correlated with the reduction in basal EGP ($r = 0.60$; $P < 0.05$). The ability of $VOSO_4$ to ameliorate hepatic insulin resistance and reduce the rate of basal EGP is in agreement with studies performed in animals (6, 16, 17), but such a response has not previously been reported in type 2 diabetic patients (26, 28, 29). A modest improvement in the suppression of EGP during euglycemic hyperinsulinemia has been reported in patients with type 2 diabetes (28, 29), but neither of these studies observed a decrease in basal EGP with vanadium treatment. As in the present study EGP was nearly completely suppressed during the insulin clamp performed before $VOSO_4$ treatment, we were unable to examine whether vanadium enhanced hepatic sensitivity to a physiological increment in the plasma insulin concentration. Our ability to detect a suppressive effect of $VOSO_4$ on basal EGP compared with previous studies (28, 29) may have resulted from the higher doses of $VOSO_4$ used and/or the longer duration of therapy. Alternatively, the reduction in basal EGP may be an intrinsic effect of $VOSO_4$ that is not shared by sodium metavanadate (26).

The mechanism(s) by which $VOSO_4$ reduces hepatic glucose output remains to be determined. In obese hyperinsulinemic insulin-resistant Zucker (*fa/fa*) rats, vanadate salts have been shown to restore to normal insulin receptor binding and to decrease protein tyrosine phosphatase (PTPase) activity (44). Recently, it has been proposed that in the liver vanadate inhibits Src homology 2 domains that contain PTPase activity (45). However, other investigators have failed to see an increase in liver insulin receptor autophosphorylation (6, 7) despite a glucose-lowering effect of vanadate, which suggests a site of action that is distal to the insulin receptor tyrosine kinase. Consistent with this later scenario, vanadium has been reported to restore in the liver the activity of key glycolytic (12, 19, 24) and gluconeogenic enzymes, such as phosphoenolpyruvate carboxykinase (6, 19, 22) and glucose-6-phosphatase (22, 46, 47). The effect on glucose-6-phosphatase is of particular interest, because chronic hyperglycemia has been shown to stimulate EGP by up-regulating the glucose-6-phosphatase complex, the final step involved in the release of glucose by the liver (48).

Vanadyl sulfate also may reduce HGP by indirect mechanisms, ameliorating gluconeogenesis by reducing plasma FFA levels and/or other gluconeogenic substrates. However, the plasma lactate concentration is not affected by $VOSO_4$ treatment (28), and plasma FFA levels and FFA turnover are either unchanged (29) or only mildly improved (49). We found no significant change in plasma FFA levels when measured in the fasting state, during the OGTT, or in response to insulin-mediated FFA suppression during the insulin clamps. Therefore, at the doses used in the present study, modulation of FFA metabolism is unlikely to play a major role in the reduction of HGP during $VOSO_4$ treatment.

Vanadyl sulfate increased insulin-stimulated glucose disposal in patients with T2DM. This observation is consistent with the results reported by Cohen *et al.* (28), in which enhanced glycogen synthesis accounted for more than 80% of increased glucose disposal. However, an improvement in insulin sensitivity was not found by other investigators (26, 29). This inconsistency may be related to the small number of subjects included in previous studies and/or differences in patient population, duration of treatment, vanadium dose, or vanadium preparation. Our results are in agreement with a number of *in vitro* and *in vivo* studies that have demonstrated that vanadium salts improve skeletal muscle glucose disposal (13, 14, 18, 23, 25, 50–54) while stimulating glucose transport (25, 51–55) and glycogen synthesis (13, 23, 56). Although vanadium salts are regarded as PTPase inhibitors capable of enhancing IRTK activity (1–4), vanadyl (the intracellular form of vanadium) is a relatively weak PTPase inhibitor (2), and stimulation of glucose uptake in muscle seems dissociated from an effect on insulin receptor autophosphorylation (5). Recent studies suggest that vanadium may activate signaling steps downstream of the insulin receptor in muscle tissue, at the level of IRS-1 and phosphatidylinositol 3-kinase (55) and/or protein kinase B (51, 57), sites of action also reported in adipocytes (58, 59). Stimulation of muscle glucose uptake by vanadium may also involve pathways in addition to those stimulated by insulin (51, 54).

In the present study we chose to administer $VOSO_4$, because it has no significant toxicity when given to rats for 1 yr at doses approximately 50- to 100-fold higher than those used in this study (60, 61). Although more work is needed in humans to assess its long-term clinical safety (*i.e.* potential for body accumulation), administration of $VOSO_4$ to weight-training athletes for up to 12 weeks has not been associated with any toxicity (62). Despite the higher doses of $VOSO_4$ used in the present study, the incidence of gastrointestinal side-effects (mild diarrhea and abdominal discomfort) was lower than that in previous studies (26, 28, 29). The low incidence of side-effects in the present study could have resulted from the gradual titration to maximum doses over a 2-week period, based upon gastrointestinal tolerance, rather than initiating treatment with the full dose (26, 28, 29). None of our patients complained of anorexia or lost weight, consistent with previous clinical studies (26, 28, 29, 62).

This is the first time that the effect of vanadium on blood pressure is examined by monitoring 24-h ambulatory blood pressure. Vanadyl sulfate has recently been reported to reduce blood pressure in rat models of hypertension and diabetes (63), but we failed to see a clinically significant effect on systolic or diastolic blood pressure. Nevertheless, a cardiovascular benefit of $VOSO_4$ was to reduce total and low density lipoprotein cholesterol. Such an effect has been reported in healthy subjects (64) and more recently in T2DM (26, 28, 29).

In summary, 6 weeks of $VOSO_4$ treatment in T2DM patients improves glycemic control by reducing basal EGP and enhancing skeletal muscle insulin sensitivity. Although clinical use awaits further assessment of its long-term safety, $VOSO_4$ was well tolerated, and side-effects

were uncommon, and primarily related to the gastrointestinal tract. Newer and more potent vanadium compounds, with fewer gastrointestinal side-effects, are currently under development (2, 4, 65) and may offer a new option for the treatment of T2DM.

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