Oral Vanadyl Sulfate Improves Insulin Sensitivity in NIDDM but Not in Obese Nondiabetic Subjects

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We compared the effects of oral vanadyl sulfate (100 mg/day) in moderately obese NIDDM and nondiabetic subjects. Three-hour euglycemic-hyperinsulinemic (insulin infusion 30 mU · m^{-2} · min^{-1}) clamps were performed after 2 weeks of placebo and 3 weeks of vanadyl sulfate treatment in six nondiabetic control subjects (age 37 ± 3 years; BMI 29.5 ± 2.4 kg/m^2) and seven NIDDM subjects (age 53 ± 2 years; BMI 28.7 ± 1.8 kg/m^2). Glucose turnover ([3-^{3}H]glucose), glycolysis from plasma glucose, glycogen synthesis, and whole-body carbohydrate and lipid oxidation were evaluated. Decreases in fasting plasma glucose (by ~1.7 mmol/l) and HbA_{1c} (both $P < 0.05$) were observed in NIDDM subjects during treatment; plasma glucose was unchanged in control subjects. In the latter, the glucose infusion rate (GIR) required to maintain euglycemia (40.1 ± 5.7 and 38.1 ± 4.8 μmol · kg fat-free mass [FFM]$^{-1}$ · min^{-1}) and glucose disposal (R_d) (41.7 ± 5.7 and 38.9 ± 4.7 μmol · kg FFM^{-1} · min^{-1}) were similar during placebo and vanadyl sulfate administration, respectively. Hepatic glucose output (HGO) was completely suppressed in both studies. In contrast, in NIDDM subjects, vanadyl sulfate increased GIR ~82% (17.3 ± 4.7 to 30.9 ± 2.7 μmol · kg FFM^{-1} · min^{-1}, $P < 0.05$); this improvement in insulin sensitivity was due to both augmented stimulation of R_d (26.0 ± 4.0 vs. 33.6 ± 2.22 μmol · kg FFM^{-1} · min^{-1}, $P < 0.05$) and enhanced suppression of HGO (7.7 ± 3.1 vs. 1.3 ± 0.9 μmol · kg FFM^{-1} · min^{-1}, $P < 0.05$). Increased insulin-stimulated glycogen synthesis accounted for >80% of the increased R_d with vanadyl sulfate ($P < 0.005$), but plasma glucose flux via glycolysis was unchanged. In NIDDM subjects, vanadyl sulfate was also associated with greater suppression of plasma free fatty acids (FFAs) ($P < 0.01$) and lipid oxidation ($P < 0.05$) during clamps. The reduction in HGO and increase in R_d were both highly correlated with the decline in plasma FFA concentrations during the clamp period ($P < 0.001$). In conclusion, small oral doses of vanadyl sulfate do not alter insulin sensitivity in nondiabetic subjects, but it does improve both hepatic and skeletal muscle insulin sensitivity in NIDDM subjects in part by enhancing insulin's inhibitory effect on lipolysis. These data suggest that vanadyl sulfate may improve a defect in insulin signaling specific to NIDDM. *Diabetes* 45:659–666, 1996

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FFA, free fatty acid; FFM, fat-free mass; GIR, glucose infusion rate; HGO, hepatic glucose output; MAP, mitogen-activating protein; OGTT, oral glucose tolerance test; R_d, glucose disposal rate; STZ, streptozocin.

Vanadium, a trace element widely distributed in nature and normally present in human tissues, has been shown to have insulinomimetic properties, including stimulation of glucose uptake, glycogen synthesis, and glucose oxidation in adipocytes, hepatocytes, and skeletal muscle in vitro (1–3). More recently, in vivo effects of vanadate on carbohydrate metabolism have been characterized, including normalization of insulin-mediated glucose disposal in 90% partially pancreatectomized diabetic rats (4) and improvement of glucose tolerance in streptozocin (STZ)-induced diabetic rats (5). Finally, in humans with NIDDM, we have demonstrated an increase in insulin-mediated glucose uptake and glycogen synthesis as well as inhibition of hepatic glucose production with vanadate treatment (6).

The mechanism of vanadium-associated enhancement of insulin action is not known. Correction of chronic hyperglycemia in partially pancreatectomized rats with vanadate has been associated with increased activation of tyrosine kinase in hepatic insulin receptors (7), and vanadate has been shown to inhibit insulin receptor kinase-associated phosphotyrosine phosphatase activity (8,9). Glucokinase and phosphoenolpyruvate carboxykinase gene activation have been attributed to vanadium (10), and postreceptor effects such as enhanced activity of mitogen-activating protein (MAP) kinase have been postulated (11). Additionally, induction of insulin-independent pathways mediated by changes in intracellular pH or Ca^{2+} has been suggested (12). We reasoned that if the effect of vanadate were related to reversing and/or improving specific defects in insulin signaling, then its efficacy may be limited to insulin-resistant states characterized by such defects, such as NIDDM. Conversely, insulin resistance due to other causes, such as those associated with obesity, aging, or physical inactivity, may not be ameliorated with vanadate treatment.

We therefore compared the effects of a vanadium compound on whole-body glucose metabolism during hyperinsulinemic clamps in NIDDM subjects and moderately insulin-resistant obese nondiabetic subjects of similar BMI. Vanadyl sulfate was the compound selected because its use in previous short-term studies was not associated with overt toxicity (13,14).

RESEARCH DESIGN AND METHODS

Subjects. We studied seven NIDDM and six nondiabetic individuals using a modified single-blind placebo-controlled experimental design. Some of the data from five of the seven diabetic subjects have been previously reported (6). The NIDDM subjects (age 53 ± 2 years, diabetes duration 6.3 ± 0.8 years [means ± SE]) were maintained on their current

TABLE 1
Subject characteristics

	Sex (M/F)	Age (years)	FFM (kg)	BMI (kg/m^2)	Diabetes	
					Duration (years)	Treatment
NIDDM subject						
1	M	58	48.2	24.4	5	Glipizide 7.5 mg q.d.
2	M	55	75.6	30.8	5	Glipizide 10 mg b.i.d.
3	M	61	47.6	28.0	4	Glyburide 5 mg q.d.
4	M	46	64.4	38.0	5	Glyburide 2.5 mg q.d.
5	F	47	31.9	24.4	9	Glyburide 25 mg q.d.
6	M	48	43.5	25.1	7	Glyburide 5 mg q.d.
7	M	57	63.3	28.7	10	Diet alone
Mean ± SE		53 ± 2	53.5 ± 5.6	28.7 ± 1.8	6.3 ± 0.8	
Control subjects (n = 6)		37 ± 3	62.4 ± 3.6	29.5 ± 2.4		

treatments (diet alone in one subject, oral hypoglycemic agents in the other six) throughout the study (Table 1). None were ever treated with insulin, and all were in good health. Compared with the control subjects, the NIDDM subjects had similar BMI (28.7 ± 1.8 and 29.5 ± 2.4 kg/m^2, respectively, NS) and fat-free mass (FFM) (53.5 ± 5.6 and 62.4 ± 3.6 kg, respectively, NS), but NIDDM subjects were older than control subjects (53 ± 2 and 37 ± 3 years, $P < 0.002$). Fitness for study was determined by history, physical examination, hematological and biochemical tests, and electrocardiography. Subjects with anemia, bleeding disorders, diarrhea, or recent weight changes were excluded. All subjects were instructed to keep their diet, physical activity, and lifestyle as constant as possible throughout the study period. Informed written consent was obtained in accordance with the policy of the Committee on Clinical Investigations of the Albert Einstein College of Medicine.

Study design. After an initial run-in period of 6 weeks, paired 3-h euglycemic-hyperinsulinemic clamps were performed after administration of placebo capsules twice daily for 2 weeks and after treatment with vanadyl sulfate (50 mg twice daily) for 3 weeks in capsules identical to placebo (vanadyl [IV] sulfate hydrate obtained from Spectrum Chemical, Gardenia, CA, and prepared by the hospital pharmacy). Before study initiation and at the end of each study period, clinical history, blood pressure, weight measurements, urinalysis, and hematological and biochemical profiles, including serum lipids, were obtained. Subject compliance, ascertained by pill count, exceeded 95%.

Procedures. On the morning of the clamp, which was performed after an overnight fast, subjects were given their study capsule. Each clamp was preceded by a 2-h equilibration period during which [3-^{3}H]glucose (high-performance liquid chromatography–purified; Du Pont-NEN, Boston, MA) was infused (continuous infusion rate 0.15 μCi/min) via an indwelling catheter. In NIDDM subjects, a variable insulin infusion (0.5–3.0 U/h) was used to establish and maintain euglycemia before the clamp. To obtain arterialized venous blood samples, a contralateral hand vein was cannulated in retrograde fashion and the hand was maintained in a warming chamber (65°C). After equilibration, a primed continuous insulin infusion was begun and maintained at 30 mU · m^{-2} · min^{-1} for 180 min. Simultaneously, a 20% glucose solution was infused at variable rates to maintain euglycemia; [3-^{3}H]glucose was added to the exogenous glucose infusate to maintain plasma glucose specific activity (15). Blood was sampled at 5- to 30-min intervals for measurement of plasma glucose, glucose specific activity, plasma insulin, free fatty acids (FFAs), cortisol, growth hormone, and lactate. Continuous indirect calorimetry was performed for two 20-min periods before and at 140 min after clamp initiation. Protein oxidation was estimated from urinary urea production measured over the duration of the procedure (16). On completion of the study, euglycemia was achieved by infusing glucose, terminating the insulin infusion, and providing a meal.

To estimate FFM, body composition was assessed by either body impedance analysis (RJL Systems, Detroit, MI) or body water determination assayed by bolus administration of tritiated water (40 μCi; Du Pont-NEN) and measurement of plasma radioactivity after equilibration (coefficients of determination between body impedance analysis and tritiated water methodologies for determination of total body water average ~0.9) (18).

Analytical procedures. Plasma glucose was measured with a Beckman glucose analyzer (Fullerton, CA) with the glucose oxidase method. Plasma [3-^{3}H]glucose radioactivity was measured in duplicate on the supernatants of barium hydroxide–zinc sulfate precipitates (Somogyi procedure) of plasma samples after evaporation to dryness to eliminate tritiated water. Plasma tritiated water specific activity was determined by liquid scintillation counting of the protein-free supernatant (Somogyi filtrate) before and after evaporation to dryness. Because tritium on the C-3 position of glucose is lost to water during glycolysis, it can be assumed that plasma tritium is present in either tritiated water or [3-^{3}H]glucose (19,20). This method to estimate whole-body glycolysis has been validated under hyperinsulinemic conditions in humans (21). Plasma insulin, growth hormone, and cortisol were determined by radioimmunoassay (22). Plasma FFA was measured by a colorimetric method (23), and plasma lactate was measured using an enzymatic spectrophotometric assay (24). For indirect calorimetry, air flow, and O_2 and CO_2 concentrations in the expired and inspired air were measured by a computerized open-circuit system (Delta Trak, Sensormedics, Yorba Linda, CA). Urinary nitrogen was measured by the Kjeldahl procedure (16). HbA_{1c} was measured by ion-exchange chromatography with an upper limit of normal of 6.2%.

Calculations. Glucose turnover during clamp experiments was calculated using Steele's equation (25). Rates of glycolysis from plasma glucose were estimated from the increment per unit time in tritiated water (dpm · ml^{-1} · min^{-1}) times body water mass (ml) per [3-^{3}H]glucose specific activity (dpm/mg), as previously validated (21). Glycogen synthetic rates were estimated as the difference between glucose disposal rate (R_d) and glycolysis. Carbohydrate oxidation was calculated from O_2 consumption and CO_2 production (corrected for protein oxidation) with the equations of Lusk (26). Data for glucose turnover, carbohydrate and lipid oxidation, and plasma hormone and substrate concentrations represent the mean values during the last 60 min of the basal period and the final 60 min of hyperinsulinemia. Results of the glucose turnover measurements are expressed in terms of fat-free body mass.

Statistical analysis. Repeated measurement analyses of the variables were performed using PROC MIXED (SAS/STAT software system version 6.07). The random effect considered in this mixed model was the error measurement of the individual subject, and the within-individual fixed effects were stage of study (placebo 1, vanadyl sulfate, placebo 2) and the specific time points during physiological (clamp and oral glucose tolerance test [OGTT]) studies in each stage. For laboratory data with one observation per study period per subject, only the stage effect was considered as a fixed effect. Compound symmetry variance-covariance structure was assumed in the analysis. The likelihood ratio test was used in fitting the best model for those variables with both stage and time effects. Variables with multiple time points in each of the three study stages were hierarchically fitted using quadratic polynomials, lines of different slope, lines of equal slope, and lines of zero slope. To compare the quadratic and linear polynomial models, we calculated the difference of twice the log-likelihood function and compared the result with the critical value with 3 df in a χ^2 distribution. Using the best fitted model for each variable, comparisons among the different stages were performed by the approximate F test and t test.

RESULTS

Fasting plasma glucose concentrations and HbA_{1c} values measured at the initial evaluation before placebo treatment were compared with those after the 2-week placebo period to ascertain stability of these parameters at baseline. In NIDDM subjects, fasting plasma glucose averaged 12.8 ± 2.4

TABLE 2
Clinical and laboratory data

	NIDDM subjects		Control subjects	
	Placebo	Vanadyl	Placebo	Vanadyl
Body weight (kg)	87.4 ± 8.9	86.5 ± 8.6	90.2 ± 5.8	90.7 ± 5.9
Systolic blood pressure (mmHg)	127 ± 4	134 ± 7	121 ± 2	120 ± 4
Diastolic blood pressure (mmHg)	83 ± 3	83 ± 4	81 ± 4	78 ± 3
Fasting glucose (mmol/l)	12.3 ± 1.3	10.6 ± 0.9*	5.4 ± 0.1	5.6 ± .02
HbA_{1c} (%)	9.4 ± 0.5	8.8 ± 0.5†	nl < 6.2	
Triglycerides (mmol/l)	7.07 ± 1.37	5.62 ± 1.31	3.76 ± 0.64	4.63 ± 0.82
Cholesterol (mmol/l)	5.25 ± 0.44	4.94 ± 0.39*	4.94 ± 0.31	5.07 ± 0.36
HDL (mmol/l)	0.91 ± 0.08	0.91 ± 0.08	1.01 ± 0.03	0.93 ± 0.03
LDL (mmol/l)	2.87 ± 0.23	2.87 ± 0.21	3.52 ± 0.28	3.49 ± 0.21
White blood cells/ml (10^3)	6.1 ± 0.9	6.0 ± 0.8	5.4 ± 0.4	6.0 ± 0.8
Hematocrit (%)	42.8 ± 0.8	41.5 ± 1.1*	41.7 ± 1.8	40.7 ± 2.0
Platelet count (10^3)	211 ± 26	231 ± 24	213 ± 26	220 ± 28

Data are means ± SE. *$P < 0.05$ compared with placebo; †$P < 0.01$ compared with placebo.

and 12.3 ± 1.3 mmol/l, respectively (NS), and the corresponding HbA_{1c} values were 9.4 ± 0.5 and 9.4 ± 0.5%. After the 3-week treatment with vanadyl sulfate, both fasting plasma glucose (10.6 ± 0.9 mmol/l) and HbA_{1c} (8.8 ± 0.5%) were significantly lower ($P < 0.01$ for both). In nondiabetic subjects, plasma glucose was unchanged over the course of the study (5.4 ± 0.1 and 5.5 ± 0.2 mmol/l, after placebo and vanadyl sulfate, respectively; NS). Likewise, fasting plasma insulin concentrations were unaffected by vanadyl sulfate (100 ± 29 and 122 ± 36 pmol/l, respectively; NS).

Other clinical and laboratory data for the NIDDM subjects are shown in Table 2. Body weight remained stable in all subjects throughout the study duration. Appetite was unchanged, and no subjects complained of symptoms of anorexia during therapy. Blood pressure, liver function tests, renal function, and urinalysis were similar during all study periods. A small but significant decrease in serum cholesterol from 5.25 ± 0.44 to 4.94 ± 0.39 mmol/l was noted in the NIDDM subjects during vanadyl sulfate treatment ($P < 0.05$), in association with unchanged serum HDL and LDL cholesterol levels and a small decline in serum triglyceride concentrations from 7.07 ± 1.37 to 5.62 ± 1.31 mmol/l (NS). Hematological indexes indicated a very small decline in hematocrit (~1%) in only the NIDDM subjects.

Transient gastrointestinal side effects were experienced by all subjects during vanadyl sulfate administration, but no subject required termination from the study. These included nausea, mild diarrhea, and abdominal cramps, which subsided after 1 week of treatment. In addition, some subjects noted dark discoloration of their stool during vanadium therapy; however, stool examinations were negative for occult blood.

We investigated the mechanism of action of vanadyl sulfate on various insulin-mediated processes during euglycemic-hyperinsulinemic clamp. Plasma glucose concentrations remained constant throughout the clamp and for the period of analysis averaged 5.1 ± 0.5 and 5.1 ± 0.04 mmol/l in NIDDM and 4.9 ± 0.02 and 5.0 ± 0.05 mmol/l in nondiabetic subjects after placebo or vanadyl sulfate treatment, respectively (Fig. 1). Specific activities of plasma glucose remained constant and tritiated water specific activities were comparable among groups (data not shown).

In NIDDM subjects, before initiation of the clamp but after the insulinization period, plasma insulin concentrations (Fig. 1) averaged 277 ± 26 pmol/l during placebo and 263 ± 8 pmol/l during vanadyl sulfate administration (NS). In control subjects, plasma insulin concentrations averaged 93 ± 3 and 110 ± 4 pmol/l before placebo and vanadyl sulfate clamps, respectively (NS). During the clamp, plasma insulin levels were unaltered from 30 min onward and identical in NIDDM (456 ± 13 and 455 ± 8 pmol/l) and nondiabetic (454 ± 3 and 450 ± 3 pmol/l) subjects during placebo and vanadium treatment, respectively (NS).

The glucose infusion rates (GIRs) required to maintain euglycemia (Fig. 2) were virtually identical in nondiabetic subjects (40.1 ± 5.7 and 38.1 ± 4.8 μmol · kg FFM^{-1} · min^{-1}) after placebo and vanadyl sulfate treatment periods, respectively (NS). In NIDDM subjects, the GIR rose significantly from 17.3 ± 4.7 μmol · kg FFM^{-1} · min^{-1} during placebo to 30.9 ± 2.7 μmol · kg FFM^{-1} · min^{-1} ($P < 0.05$) during vana-

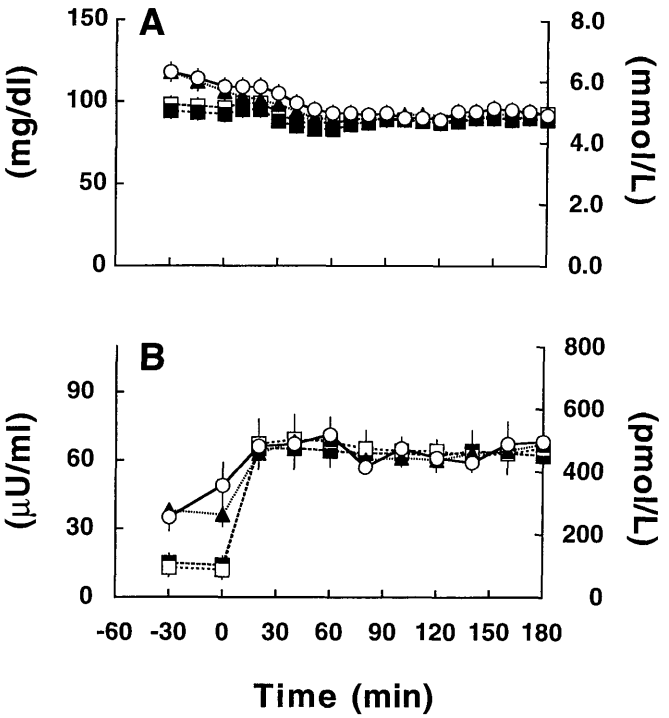

FIG. 1. Plasma glucose (*A*) and plasma insulin (*B*) during clamp studies after placebo and after vanadyl sulfate treatment. Results in nondiabetic subjects (□, placebo; ■, vanadyl sulfate) are compared with studies in NIDDM subjects (○, placebo; ▲, vanadyl sulfate).

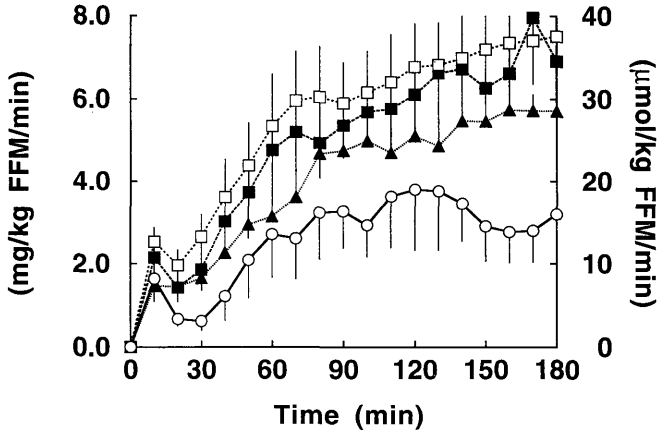

FIG. 2. GIR required to maintain euglycemia during clamp studies after placebo and after vanadyl sulfate treatment. In NIDDM subjects, the GIR during the final 60 min of the clamp was greater after vanadyl sulfate treatment compared with after placebo ($P < 0.05$) and approached the GIR obtained during experiments in nondiabetic control subjects, which was unaltered by drug treatment. See Fig. 1 for symbols.

dium treatment and approached the rates in the nondiabetic control subjects.

Before initiation of the clamps, glucose output averaged 7.52 ± 1.07 and 7.74 ± 1.37 μmol · kg FFM^{-1} · min^{-1} on placebo and vanadyl sulfate in nondiabetic subjects and 16.02 ± 2.29 and 12.99 ± 1.21 μmol · kg FFM^{-1} · min^{-1} in NIDDM subjects. Basal glucose output was greater in NIDDM subjects on both occasions compared with the corresponding values in control subjects ($P < 0.05$ for both). Glucose output during the final 60 min of the clamp is shown in Fig. 3. In nondiabetic subjects, glucose output was completely suppressed in response to euglycemic hyperinsulinemia after both study periods. In contrast, glucose output, minimally suppressed during hyperinsulinemia in NIDDM subjects after the placebo period (7.7 ± 3.1 μmol · kg FFM^{-1} · min^{-1}), was almost completely suppressed after vanadyl sulfate treatment (1.3 ± 0.9 μmol · kg FFM^{-1} · min^{-1}, $P < 0.005$).

Similar findings were observed for glucose uptake (Fig. 4). In NIDDM subjects, glucose uptake averaged 19.06 ± 1.71 and 18.08 ± 1.48 μmol · kg FFM^{-1} · min^{-1} before hyperinsulinemic clamps after placebo and vanadyl sulfate treatment, respectively (NS). During the final 60 min of the clamp,

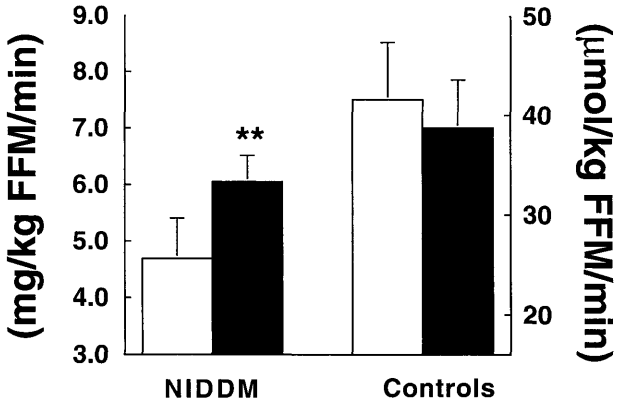

FIG. 3. Rates of glucose uptake during the final 60 min of hyperinsulinemia during placebo (□) and vanadyl sulfate (■) treatment. In NIDDM subjects, insulin-mediated glucose uptake was significantly greater during vanadyl sulfate treatment compared with placebo ($P < 0.05$). In control subjects, glucose uptake was similar during placebo and vanadyl sulfate treatment.

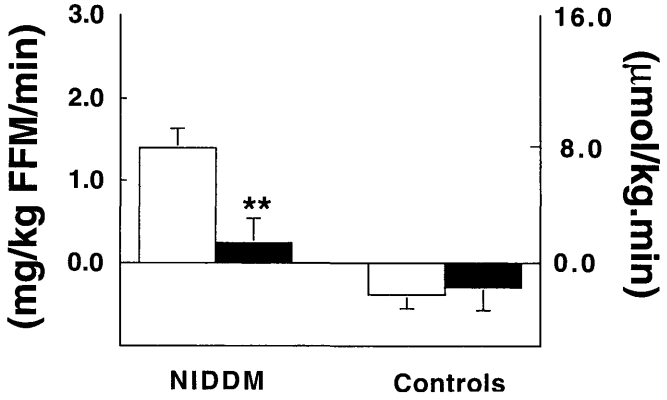

FIG. 4. Rates of glucose output during the final 60 min of hyperinsulinemia during placebo (□) and vanadyl sulfate (■) treatment. In NIDDM subjects, glucose production was significantly lower during vanadyl sulfate treatment compared with placebo ($P < 0.05$). In control subjects, glucose production was completely suppressed during placebo and vanadyl sulfate treatment.

however, glucose uptake increased to only 26.0 ± 4.0 μmol · kg FFM^{-1} · min^{-1} during placebo but to 33.6 ± 2.22 μmol · kg FFM^{-1} · min^{-1} after vanadyl sulfate treatment ($P < 0.05$). Quantitatively, this improvement in insulin-mediated glucose disposal in NIDDM subjects was similar to the enhanced insulin-induced suppression of glucose output observed with vanadyl sulfate. In nondiabetic subjects, glucose disposal was unchanged by treatment and averaged 41.7 ± 5.7 and 38.9 ± 4.7 μmol · kg FFM^{-1} · min^{-1} during placebo and vanadyl sulfate treatment, respectively (NS). The rates of glucose uptake in control subjects were not significantly different from the rates achieved in NIDDM subjects after 3 weeks' treatment with vanadyl sulfate.

The augmented glucose disposal observed with vanadyl

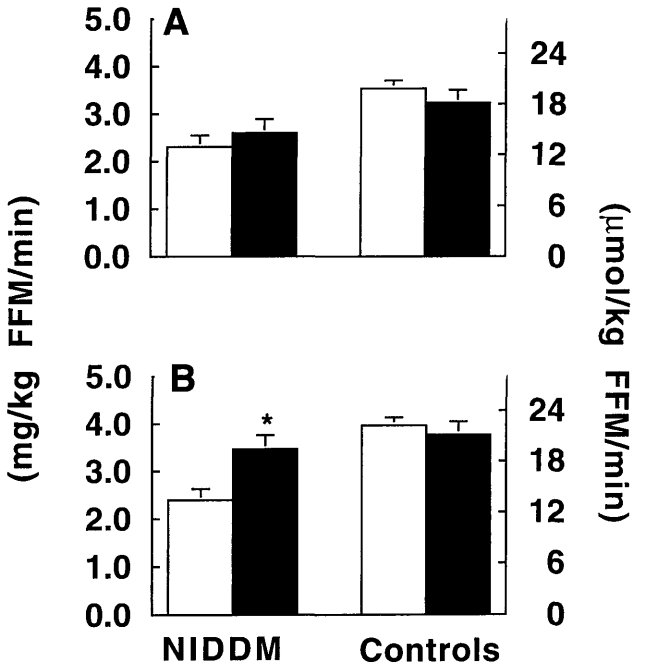

FIG. 5. Rates of plasma glucose flux through glycolysis (*A*) and glycogen synthesis (*B*) during the final 60 min of hyperinsulinemia during placebo (□) and vanadyl sulfate (■) treatment. In NIDDM subjects, with vanadyl sulfate treatment, there was an increase in insulin-stimulated glycogen synthesis, which accounted for ~80% of the increase in glucose uptake with vanadyl sulfate treatment. In control subjects, these rates were unchanged with drug treatment.

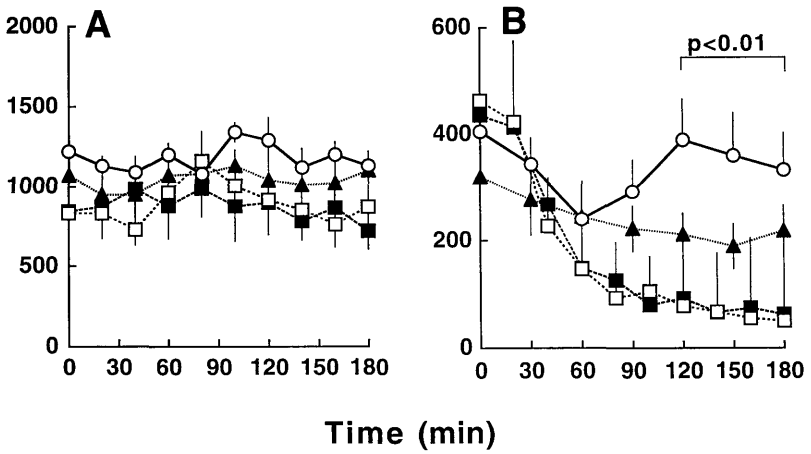

FIG. 6. Plasma lactate (*A*) and FFA (*B*) concentrations (μmol/l) during placebo and vanadyl sulfate treatement in NIDDM and control subjects. See Fig. 1 for symbols.

sulfate therapy in NIDDM subjects was associated primarily with an increase in glycogen synthesis (Fig. 5), which rose from 13.3 ± 4.0 μmol · kg FFM^{-1} · min^{-1} after placebo to 19.2 ± 2.1 μmol · kg FFM^{-1} · min^{-1} after vanadyl sulfate ($P < 0.005$). Plasma-derived glucose flux through glycolysis (Fig. 5) increased minimally (12.8 ± 1.3 to 14.4 ± 1.7 μmol · kg FFM^{-1} · min^{-1}, NS) with vanadium treatment. In nondiabetic subjects, both glycogen synthesis and glycolysis were unaltered with vanadyl sulfate administration (Fig. 5).

Carbohydrate and lipid oxidation rates estimated from indirect calorimetry were also examined. Carbohydrate oxidation increased similarly during hyperinsulinemia with placebo and vanadyl sulfate treatment (approximately twofold) in control subjects, averaging 20.3 ± 3.3 and 16.0 ± 1.4 μmol · kg FFM^{-1} · min^{-1}, respectively (NS). In NIDDM subjects, carbohydrate oxidation was unchanged during hyperinsulinemia with placebo (11.8 ± 2.5 μmol · kg FFM^{-1} · min^{-1}) but increased ~33% to 15.2 ± 2.2 μmol · kg FFM^{-1} · min^{-1} ($P < 0.05$) with vanadyl sulfate treatment. Conversely, lipid oxidation, unchanged with placebo, decreased ~15% ($P < 0.05$) with vanadium treatment in NIDDM subjects only.

Plasma concentrations of cortisol and growth hormone were unchanged during the placebo versus vanadyl treatment in both groups. In nondiabetic subjects, for placebo and vanadyl sulfate periods, respectively, plasma cortisol averaged 204 ± 44 and 221 ± 61 nmol/l; for NIDDM subjects, the corresponding values were 303 ± 28 and 303 ± 28 nmol/l ($P < 0.05$ vs. nondiabetic subjects). The corresponding values for plasma growth hormone were 0.2 ± 0.1, 0.3 ± 0.1, 2.0 ± 0.8, and 1.1 ± 0.7 μg/l (NS within and between groups).

Plasma FFA and lactate concentrations are depicted in Fig. 6. Plasma lactate was unaltered during hyperinsulinemia with placebo and with vanadyl sulfate treatment in both NIDDM and control subjects. During the final 60 min of hyperinsulinemia, plasma lactate averaged 1,170 ± 90 and 1,050 ± 100 μmol/l in NIDDM subjects and 850 ± 118 and 816 ± 81 μmol/l in nondiabetic subjects after placebo and vanadyl sulfate, respectively (NS). In the NIDDM subjects, before clamp initiation, plasma FFA averaged 390 ± 69 and 317 ± 125 μmol/l during placebo and vanadyl sulfate treatment, respectively (NS). During euglycemic hyperinsulinemia, plasma FFA remained unchanged with placebo (361 ± 68 μmol/l) but declined by 35% after vanadium administration to 206 ± 40 μmol/l ($P < 0.01$). In nondiabetic subjects, plasma FFA levels declined similarly during the final 60 min of hyperinsulinemia (62 ± 19 and 73 ± 15 μmol/l, placebo and vanadyl sulfate treatment, respectively [NS], declining from corresponding basal values of 467 ± 12 and 461 ± 131 μmol/l).

The relationship between the changes in plasma FFA concentrations during hyperinsulinemia and the improvement in insulin sensitivity is shown in Fig. 7. The magnitude of the decrease in plasma FFA (calculated as the arithmetic difference between the effects of euglycemic hyperinsulinemia during placebo and vanadyl sulfate treatment) was highly correlated with both the suppression of glucose output ($r^2 = 0.364$, $P < 0.001$) and the stimulation of glucose uptake ($r^2 = 0.562$, $P < 0.001$).

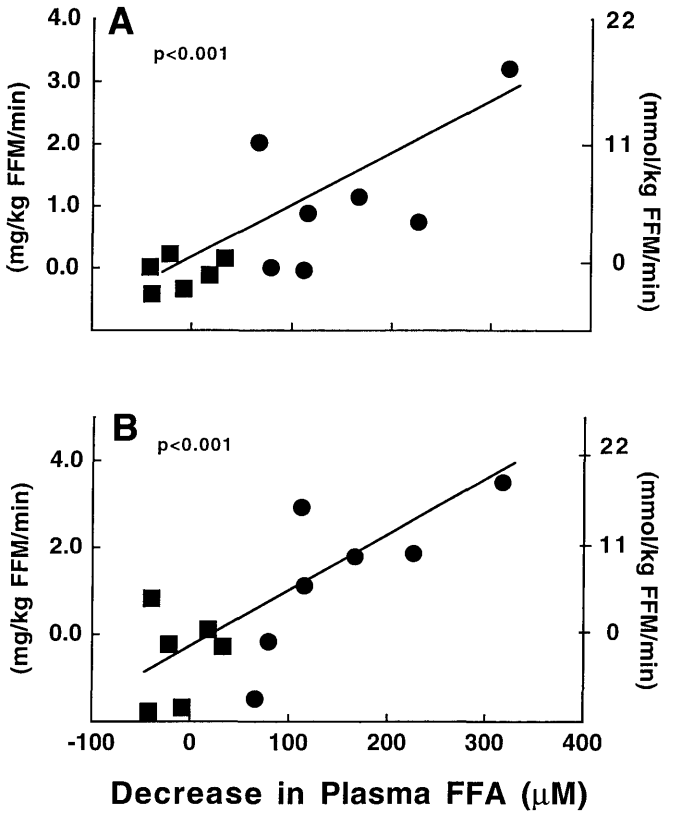

FIG. 7. The insulin-induced decrement in plasma FFA (differences between placebo and vanadyl sulfate averaged over the final 60 min of each clamp) were correlated with improvement in suppression of glucose output (*A*) and glucose uptake (*B*) among all study subjects. The decrease in plasma FFA correlated positively with both enhanced suppression of glucose production ($r^2 = 0.364$, $P < 0.001$) and enhanced glucose uptake ($r^2 = 0.562$, $P < 0.001$).

DISCUSSION

The purpose of this study was to compare the effect of vanadyl sulfate on glucose homeostasis in a group of moderately obese insulin-resistant NIDDM subjects and obese nondiabetic subjects with mild insulin resistance. We demonstrated that in NIDDM subjects vanadyl sulfate treatment was associated with significant reductions in fasting plasma glucose and HbA_{1c} levels, whereas no effect on fasting plasma glucose was noted in obese control subjects. Moreover, vanadyl sulfate treatment was associated in only the NIDDM subjects with an increase in insulin-mediated peripheral glucose disposal and greater suppression of hepatic glucose output. The peripheral effect was largely mediated by enhanced stimulation of glycogen synthesis, with little change in plasma glucose flux through glycolysis. Furthermore, the improvements in peripheral and hepatic insulin sensitivity with vanadyl sulfate in NIDDM subjects were associated with reduced lipid oxidation rates and plasma FFA concentrations during hyperinsulinemia. In contrast, there was no evidence in the nondiabetic control subjects of significant changes in hepatic glucose production or increased peripheral glucose uptake or inhibition of lipolysis after vanadyl sulfate treatment. These data suggest that vanadyl sulfate ameliorates defects in insulin action specific for NIDDM subjects and that its effects on glucose metabolism may be caused, in part, by enhanced insulin-mediated inhibition of lipolysis.

Vanadium and its associated compounds are found in all living organisms in varying concentrations (27). Early studies focused on its role as a potent inhibitor of Na^+-K^+-ATPase activity (28). Recently, the insulinomimetic properties of vanadium compounds have generated great interest. Indeed, as early as 1899, Lyonnet et al. (29) reported a decrease in glycosuria in two patients with diabetes treated with sodium vanadate. In 1985, Heyliger et al. (30) reported the first in vivo effects of vanadium on carbohydrate metabolism in experimental diabetes. We have previously reported that vanadyl sulfate treatment resulted in improved insulin resistance and hyperglycemia in humans with NIDDM treated for 3 weeks with 100 mg daily of vanadyl sulfate and that these effects persisted for at least 3 weeks after the vanadium was withdrawn (6). Recently, Goldfine et al. (11) reported that basal MAP kinase activity was stimulated in circulating monocytes taken from NIDDM subjects treated with sodium metavanadate for 2 weeks. These varying results suggest that vanadium compounds might possess inherently different activities with respect to insulin-like actions or that heterogeneity among study populations may account for the differences in effects of vanadium salts. It should be emphasized, however, that our previous study, as well as the present report, examined the effects of vanadyl sulfate in a placebo-controlled experiment to account for potential study effects that could independently affect hyperglycemia.

Most studies examining the in vivo mechanisms of vanadium action have been conducted in rats. Vanadium has been shown to reduce hyperglycemia via suppression of hepatic gluconeogenesis (2,10), as well as increased skeletal glycogen synthase activity (2,4,6). These effects have been attributed to inhibition of phosphotyrosine phosphatase activity as well as improved basal insulin receptor tyrosine kinase activity (4,8,9). Other in vitro studies have suggested that vanadate may exert effects through activation of MAP kinase activity independent of insulin receptor autophosphorylation (31).

In the present study, the potent effect of vanadyl sulfate to enhance tissue responsiveness to insulin in NIDDM subjects was in marked contrast to its lack of an effect in nondiabetic obese subjects. We must first acknowledge that this observation could be secondary to the improvement in hyperglycemia-induced defects in insulin action and/or secretion produced by vanadyl sulfate in NIDDM subjects. This explanation is unlikely, however, since *1*) the extent and duration of improved hyperglycemia were quite small and brief; *2*) we have not previously observed improvement in defective insulin secretion during OGTTs in NIDDM subjects with similar improvement in hyperglycemia resulting from vanadyl sulfate (6); and *3*) we have no evidence that hyperglycemia was improved by non–insulin-mediated effects on appetite, body weight, or glycosuria. In addition, it is clear that our obese control subjects were resistant to insulin action. When compared with lean control subjects (28 ± 2 years, BMI 24.2 ± 0.7 kg/m^2) who had glucose disposal rates of 9.59 ± 1.28 $mg \cdot kg\ FFM^{-1} \cdot min^{-1}$ during euglycemic hyperinsulinemia (unpublished observations), the obese control subjects in the present study had values that were ~30% lower ($P < 0.001$). Moreover, a number of studies have suggested that vanadium may indeed exert increased effects in insulin-resistant states. A recent study reported that vanadate-stimulated 2-deoxyglucose transport rates in vitro were significantly higher in insulin-resistant muscle than were insulin-stimulated rates in muscle from human skeletal muscle biopsies (32). This was coupled with the fact that vanadate, in conjunction with insulin, stimulated glucose transport in muscle from lean individuals at a higher rate than insulin alone. Furthermore, sodium orthovanadate administered for 5 weeks to nondiabetic rats showed no significant effects on glucose homeostasis (33).

We acknowledge that our study may have failed to detect an effect of vanadium on forms of insulin resistance other than that associated with obesity. For example, aging-related insulin resistance was not examined, and our NIDDM subjects were indeed older than the control subjects. In addition, as noted above, our obese subjects were only moderately insulin resistant; this also may explain a lack of effect. Despite these caveats, the present data are consistent with the assumption that vanadium may function on a pathway or pathways other than normally regulated insulin-signaling pathways and would thus have a preferential effect in diabetes.

Data in the literature regarding the reduction of glucose toxicity are not entirely consistent with our results. In NIDDM, the correction of hyperglycemia has a less consistent or pronounced effect on insulin action than in IDDM, perhaps because of the concomitant presence of underlying inherited insulin resistance in NIDDM (34). When phlorizin was used to lower plasma glucose without using insulin in a pancreatectomized diabetic rat model, normalization of plasma glucose did not correct the severe impairment in skeletal muscle glycogen synthesis (4,35). On the other hand, vanadate treatment in these animals restored both muscle glycogen synthase activity and plasma glucose concentrations to normal (35). Our results in humans are similar in that vanadyl sulfate improved hyperglycemia modestly but had a major impact on the defects in basal skeletal muscle glycogen synthase activity (6) and in vivo glycogen synthesis in

response to hyperinsulinemia. Nevertheless, some of the effects of vanadyl sulfate we report herein might be secondary to improvement of hyperglycemia. For example, a recent study demonstrated that use of phlorizin in STZ-induced diabetic rats for only 1 week significantly improved pathways regulating hepatic gluconeogenesis, though the authors attributed most of these effects to reduction of hyperglucagonemia (36).

On the other hand, there are data that are contrary to the present findings. Oral vanadium has been shown to augment insulin sensitivity in the skeletal muscle of normal rats (37) as well as in insulin-resistant obese Zucker rats (38) and insulin-resistant senescent rats (39). These results, however, reflect effects of either high doses and/or the differences in vanadium compounds used and thus may not be directly pertinent to our results. For example, Leighton et al. (40) showed that peroxovanadate significantly increased glycogen synthesis and glucose oxidation in rat soleus muscle from both lean and obese nondiabetic Zucker rats. Carey et al. (32) also reported that human muscle incubated in the presence of 30 mmol/l sodium vanadate displayed direct vanadate-stimulated glucose transport in the presence of insulin. Indeed, this latter report suggested that some components of obesity-related insulin resistance may not be reversible by sodium vanadate, though their study subjects were morbidly obese, with BMI values averaging 48 kg/m^2, whereas BMI in our subjects averaged ~30 kg/m^2.

Enhanced inhibition of lipolysis during hyperinsulinemia was observed with vanadyl sulfate treatment in NIDDM subjects in our study. Recently, other in vivo studies have focused on vanadium's effects on lipid metabolism. Brichard et al. (41) reported that 3 weeks of vanadate treatment in STZ-induced diabetic rats completely restored acetyl-CoA carboxylase mRNA and partially restored fatty-acid synthase mRNA levels. Taken together with our findings, these data suggest that enhancement of insulin-mediated actions on carbohydrate metabolism may be due, in part, to augmented insulin sensitivity in the regulation of fat metabolism. The reduction of plasma FFA concentrations and lipid oxidation could then conceivably reduce the substrate competition in the periphery (Randle effect) and the gluconeogenic substrate delivery to the liver. We also observed small decreases in serum cholesterol and triglycerides in the NIDDM subjects. Similar findings have been reported in STZ-induced diabetic rats, in which normalization of cholesterol, phospholipids, and triglycerides in plasma lipoprotein fractions occurred with vanadate treatment (41).

The dose of vanadyl sulfate we used was well tolerated in both groups of subjects. Except for some minor gastrointestinal discomfort and stool discoloration, subjects were relatively asymptomatic, and no one withdrew from the study. The anorexic effect of vanadium compounds reported in previous studies (42) was not observed in our treatment group. No subject reported a change in dietary intake, and weight remained stable throughout the course of therapy. Because the dose of vanadyl sulfate used in the present study (~1–2 $mg \cdot kg^{-1} \cdot day^{-1}$) was markedly lower than the effective dose used in previous rat studies (~125 $mg \cdot kg^{-1} \cdot day^{-1}$) associated with weight loss, anorectic effects of vanadium may be dose dependent. Finally, studies of at least 1 year of treatment with vanadium in the rat failed to show significant toxic effects (43).

Many other systemic effects have been reported in studies using vanadium compounds, including hemolysis and impaired erythropoiesis (44). Because vanadium is excreted mainly by the kidney (45), its effect on renal blood flow has been well studied and its effects on mesangial cell proliferation have been documented (46). Dose-dependent decreases in renal function have been reported in some studies (47), but other long-term studies have reported a renoprotective effect of vanadate in diabetic rats (48). Though hematological and renal indexes remained unchanged throughout the course of the present study, the relatively short duration of treatment precludes any definitive conclusions regarding vanadyl sulfate's long-term effects. However, absence of hematological and renal toxicity has been reported after 1 year of treatment with vanadium (49). The potential long-term benefits or side effects of vanadium treatment in humans remain to be explored.

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