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In vivo and *in vitro* studies of vanadate in human and rodent diabetes mellitus

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Abstract

In vivo vanadate and vanadyl have been shown to mimic the action of insulin and to be effective treatment for animal models of both Type I and Type II diabetes. The molecular mechanism of action of the vanadium salts on insulin sensitivity remains uncertain, and several potential sites proposed for the insulin-like effects are reviewed. In human trials, insulin sensitivity improved in patients with NIDDM, as well as in some patients with IDDM after two weeks of treatment with sodium metavanadate. This increase in insulin sensitivity was primarily due to an increase in non-oxidative glucose disposal, whereas oxidative glucose disposal and both basal and insulin stimulated suppression of hepatic glucose output (HGP) were unchanged. Clinically, oral vanadate was associated with a small decrease in insulin requirements in IDDM subjects. Of additional benefit, there was a decrease in total cholesterol levels in both IDDM and NIDDM subjects. Furthermore, there was an increase in the basal activities of MAP and S6 kinases to levels similar to the insulin-stimulated levels in controls, but there was little or no further stimulation with insulin was seen. Further understanding of the mechanism of vanadium action may ultimately be useful in the design of drugs that improve glucose tolerance. (Mol Cell Biochem **153:** 217–231, 1995)

Key words: vanadate, vanadium, insulin dependent diabetes mellitus, noninsulin dependent diabetes mellitus, mechanism of action, phophatases

Introduction

In the late 1800's vanadium was proposed to have medicinal value and to be of benefit in nutrition, diabetes, atherosclerosis, anemia, metabolism of lipids, prevention of dental caries, and treatment of infection, especially tuberculosis and syphilis [1, 2]. Over the past 15 years, considerable evidence has accumulated to show that vanadium salts, specifically tetravalent vanadyl, usually found as the divalent cation VO^{2+}, and pentavalent vanadate, VO_3^-, will mimic insulin action in a number of isolated cell systems and produce dramatic glucose lowering effects when given orally to animal models of both Types I and II diabetes mellitus [1, 3]. *In vitro*, vanadate was first found to mimic insulin action on hexose uptake and glucose metabolism in rat adipocytes in about 1980 and has since been demonstrated to have insulin-like actions in many cell types [3, 4]. The insulinomimetic properties include stimulation of hexose transport in rat adipocytes [3, 4] and rat skeletal muscle [5], stimulation of lipogenesis [6], inhibition of lipolysis [7], stimulation of glucose oxidation [4], and stimulation of glycogen synthase in rat adipocytes [8]. In addition to the effects on glucose metabolism, these compounds appear to enhance K^+ uptake in cardiac muscle cells [9] and stimulate DNA synthesis in cultured cells [10–12] similar to insulin (reviewed in Shechter [13]).

Animal studies

In vivo vanadate and vanadyl have been shown to be effective treatment for animal models of both Type I and Type II diabetes [13–18]. In rodent models of Type I diabetes, oral vanadate has been found to improve blood glucoses in STZ-

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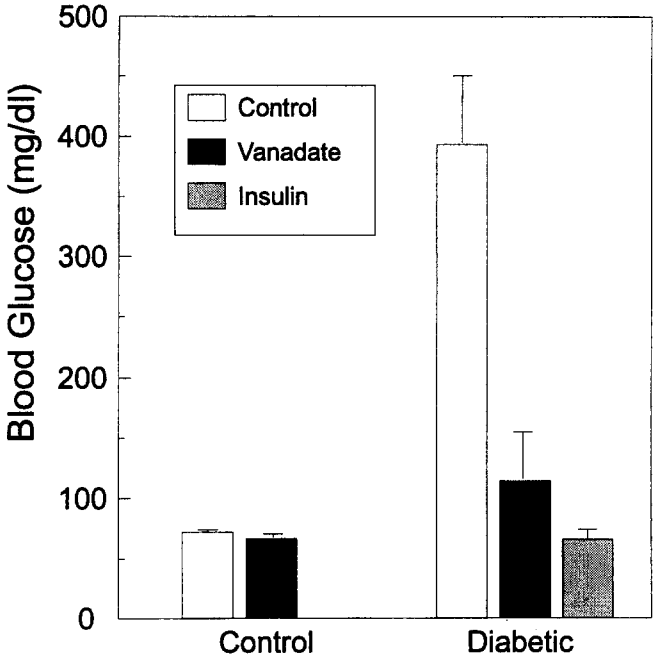

Fig. 1. The effect of vanadate on blood glucose in the STZ-diabetic rat.

diabetic rat. Treatment for 5 days with either insulin (14 U/rat/day) or with vanadate (0.6 mg/ml in the drinking water) produced a near normalization of blood glucose in the diabetic rats within 3–4 days, without any exogenously administered insulin (Fig. 1) [19]. Similar improvements in glucose were seen in the BB rat, in the presence of decreased, although continued insulin [20]. The catabolic state induced by the diabetes was reversed in both models by vanadate treatment, and the animals became anabolic. In addition to the improvement in glucose levels, 2 weeks of vanadate therapy restored to normal the decreased hepatic glycogen, fructose-2,6-bisphosphate concentrations and 6-phosphofructo-2-kinase activity present in the STZ-diabetic rat [21]. These beneficial effects were reversible following the removal of vanadate from the drinking water. Furthermore, abnormalities in isolated working heart function and glycerol output from adipose tissue are corrected by vanadyl treatment, and there is an apparent persistence of a therapeutic effect demonstrated up to 13 weeks following completion of a three week treatment period [22].

Vanadate administered in the drinking water has also been shown to lower blood glucose to near normal levels in three models of NIDDM, including ob/ob and db/db mice, and fa/fa rats [14–16]. Ob/ob and db/db mice are two commonly studied rodent models of type II diabetes mellitus, characterized by obesity, hyperglycemia, hyperinsulinemia and a blunted response to insulin at the receptor and postreceptor levels. To investigate the effects of vanadate on blood glucose levels rodent models of NIDDM, vanadate (0.25 mg/ml drinking water) was administered to ob/ob and db/db mice

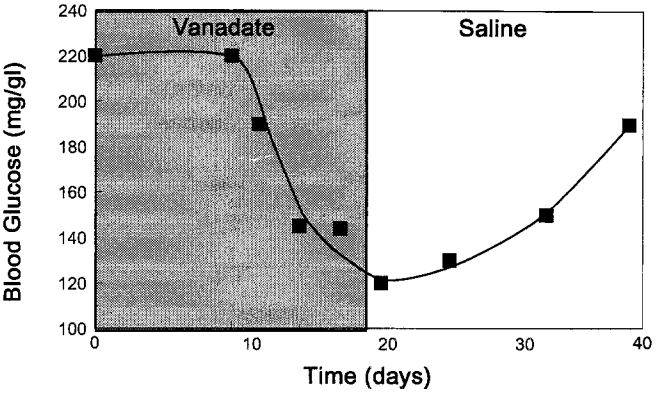

Fig. 2. The effect of vanadate on blood glucose in ob/ob mice.

for three weeks [14]. Fasting blood glucose levels were reduced from 236–143 mg/dl in ob/ob mice and from 170–114 mg/dl in ob/+ mice (Fig. 2), and from 228–141 mg/dl in db/db mice and from 126–81 mg/dl in db/+ mice. Vanadate also lowered glucose levels in the fed state, improved oral glucose tolerance and restored early insulin secretion [16]. In both mouse models of Type II diabetes, some effect of vanadium was seen after 2–10 days of treatment, but the effect was not maximal until 25–50 days. This is in contrast to the rapid effects seen after 3–4 days in the STZ-diabetic rats noted above. There was no effect on body weight. The effect of vanadate of blood glucose levels was reversible and after withdrawal the blood glucose levels gradually returned to the original hyperglycemic level. There was no evidence of hepatotoxicity by electron microscopic examination after 47 days of treatment. Furthermore, disappearance rates for intravenous glucose was doubled in treated animals as compared to the controls, the insulin response to the glucose challenge reappeared, hepatic glycogen content doubled, and pancreatic insulin stores were increased. There was also an increase in basal glucose oxidation rates by muscle (hemidiaphragm) in the vanadate-treated animals, although insulin stimulation was similar to that of the control [16].

When given to obese, hyperinsulinemic fa/fa rats, vanadate also improved oral glucose tolerance without decreasing body weight or changing counter-regulatory hormones [15]. In euglycemic, hyperinsulinemic clamp studies, the glucose infusion rate required for stable glycemia was higher in the vanadate treated rats due to higher rates of peripheral glucose disposal. There was no effect of vanadate on inhibition of hepatic glucose production by insulin [23]. In obese mice or rats treated with vanadate, there was no change in the percent lean body mass, again suggesting that the effects of vanadate do not require a major decrease in food intake.

Mechanisms of vanadium's insulin-like actions

The exact mechanism of the vanadium salts effects remains uncertain. In both STZ-diabetic rats and db/db mice oral administration of vanadate improves blood glucoses without increasing serum insulin levels [18, 24], thus indicating that the primary site of action is on insulin target tissues. Insulin action at the cellular level is complex (reviewed in refs [25, 26] (Fig. 3). Insulin initiates its actions by binding to its tetrameric membrane receptor. This receptor is a member of the family of receptor tyrosine kinases and following insulin binding undergoes autophosphorylation on multiple tyrosine residues which in turn activates the receptor kinase toward other substrates. In most cells, the major substrate is a high molecular weight cytosolic protein termed IRS-1 (insulin receptor substrate-1) [27, 28]. IRS-1 possesses 22 potential sites of tyrosine phosphorylation, many in repetitive sequence motifs (YMXM and YXXM). Following phosphorylation IRS-1 serves as a 'docking protein' for other intracellular proteins, including enzymes like phosphatidylinositol 3-kinase (PI 3-kinase) and SHPTP2, and adaptor proteins such as Grb2 [29]. The latter links IRS-1 to a series of closely linked serine/threonine kinases and phosphatases such as the MAP kinases, S6 kinases and protein phosphatase-1A via the ras-GTPase system. These serine kinase act on enzymes like glycogen synthase, transcription factors, and other proteins to produce many of the final biological effects of the hormone. In adipose tissue and muscle, insulin stimulation also increases glucose uptake by promoting translocation of an intracellular pool of glucose transporters to the plasma membrane [30]. Exactly how this action is linked to the phosphorylation cascade is unknown, but several recent studies suggest that this is down-stream of PI 3-kinase and may involve a member of the Rab family of GTPases [31]. In the absence of continued insulin secretion, the cellular actions of the hormone are 'turned off' in two ways: first insulin ei-

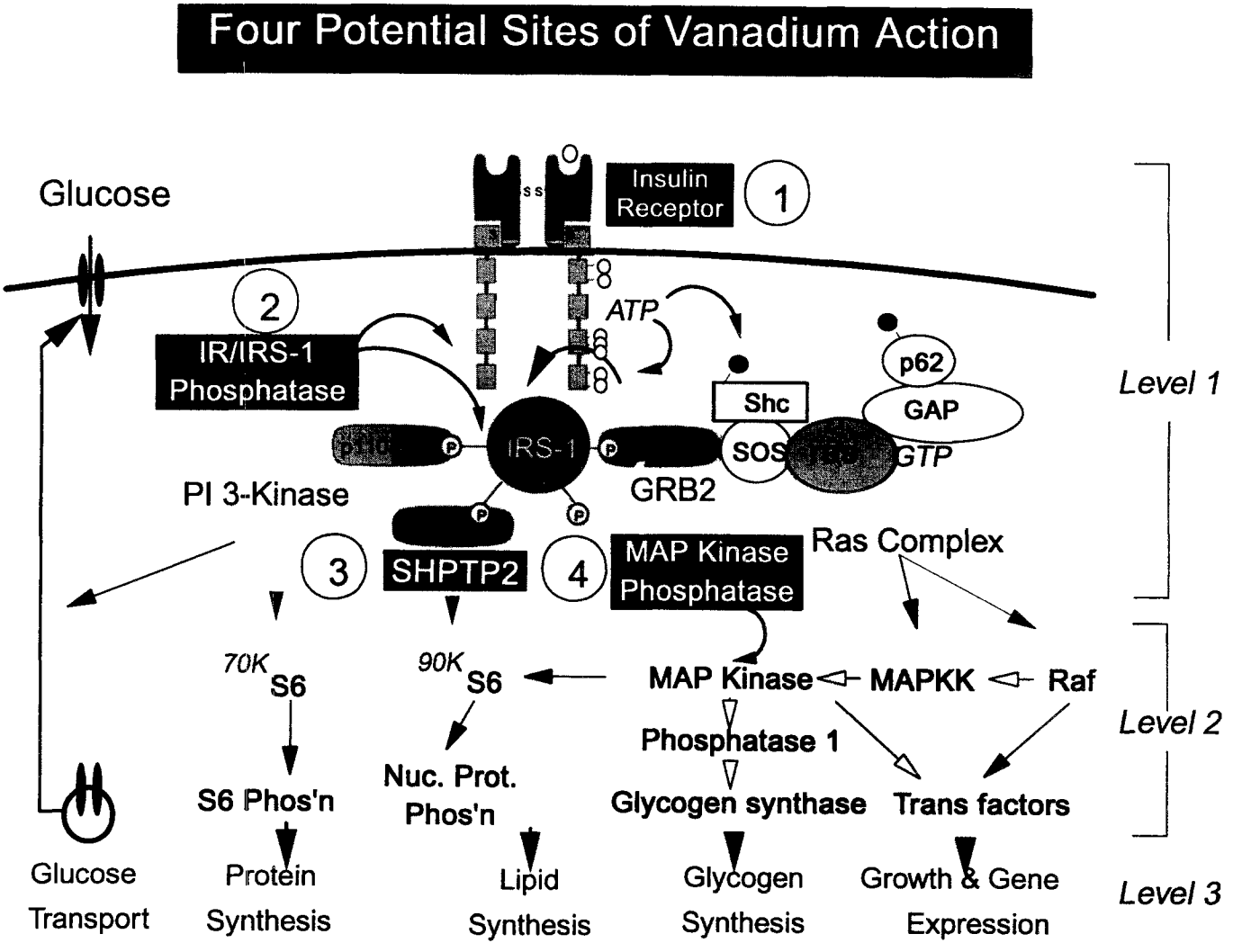

Fig. 3. The cellular mechanism of insulin action and possible sites of vanadium effects.

ther dissociates from its receptor or is internalized and degraded [32], and secondly the activated receptor kinase and IRS-1 molecule are dephosphorylated by the action on a group of enzymes called phosphotyrosine phosphatases [33].

Several potential sites have been proposed for the insulin-like effect of vanadium (Fig. 3). To determine possible mechanisms of vanadate action, serum insulin levels, insulin receptor function and some postreceptor sites of insulin action were analyzed. Plasma insulin levels in both ob/ob and db/db mice showed only small changes following vanadate therapy which could not account for the normalization of glucose. As previously reported [34], a decrease in insulin binding to partially purified insulin receptor is present in ob/ob mice as compared to control ob/+ mice. Vanadate treatment tended to down-regulate the level of insulin binding in the ob/+ treated animals, but had no effect on the already reduced level of insulin binding in ob/ob mice, consistent with a post-receptor mechanism of vanadate action [19].

Most early studies focused on the possibility that vanadate stimulates phosphorylation of the insulin receptor either directly or through its inhibitory effect on phosphotyrosyl protein phosphatase (PTPases). Tamura *et al.* [35] have presented evidence that vanadate might directly stimulate insulin receptor β-subunit tyrosine autophosphorylation. However this action has not been observed in several other studies [19, 36, 37], such that when tissues were taken from vanadate-treated diabetic animals, no increase wass observed in either receptor or substrate phosphorylation [19]. Furthermore, the effect of vanadate on hexose transport is not inhibited by quercitin, a compound which inhibits both insulin receptor tyrosine kinase and insulin-stimulated hexose uptake [38]. Shisheva and Shechter [39] have postulated that vanadate stimulates a soluble cytosolic tyrosine kinase, thus bypassing the need for activation of the insulin receptor itself. By contrast to vanadate itself, peroxides of vanadium, also called pervanadates, do appear to produce an activation of the insulin receptor tyrosine kinase as measured by ^{32}P incorporation into a synthetic substrate, and in some studies are even more potent than insulin in this regard [40].

The best studied site of action for vanadium compounds is at the level of the PTPases. As phosphorylation of tyrosine residues on the β-subunit of the insulin receptor and on the insulin receptor substrate IRS-1 form the first two steps in transduction of the insulin signal at the intracellular level, in normal tissues, the level of tyrosine phosphorylation of these and other proteins is a delicate balance between the action of tyrosine kinases such as the insulin receptor and the dephosphorylation of tyrosine residues by specific PTPases. There are two major classes of PTPases: one class is represented by large, transmembrane molecules which resemble receptors (although no ligands have been found to date) and the other are smaller cytoplasmic enzymes [33]. Genes for over 20 of these PTPases have been cloned, however, the role of many these enzymes in normal physiology has yet to be defined [41, 42]. Vanadate is a potent inhibitor of cellular PTPases, especially the cytosolic PTPases [41, 43]. Thus, one could imagine that vanadate would enhance insulin receptor and/or substrate phosphorylation indirectly by inhibiting the dephosphorylation of these proteins. In isolated cell systems, this effect of vanadate is easily demonstrable, albeit at somewhat higher concentrations than those achieved *in vivo* during treatment of diabetic animals, or humans (Fig. 4).

The effects of insulin and vanadate on *in vitro* PTPase activity in the well-differentiated Fao rat hepatoma cell line was studied using a ^{32}P-labeled peptide corresponding to the major site of insulin receptor autophosphorylation [44]. Of the PTPase activity in Fao cells, 14% was in the cytosolic fraction, whereas 86% was in the particulate fraction; this latter fraction also had a 4-fold higher specific activity against the insulin receptor phosphopeptide. PTPase activity in both fractions was inhibited by zinc at concentrations of 0.1–1.0 mM. Vanadate was a much more potent inhibitor than zinc, and was also differentially active in the two cell fractions producing complete inhibition of the cytosolic PTPase at 20 μM, whereas inhibition of the particulate enzymes required 200 μM for maximal effect (Fig. 4). The effect on cytosolic PTPases is in the range observed during oral administration to rodents and humans (see Section 4). The cytosolic and particulate enzymes could also be distinguished by their ability to be inhibited by the phosphorylated insulin receptor-related peptide. Insulin treatment of cells produced a very slight inhibition of cytosolic PTPase activity and a modest stimulation of membrane PTPase activity, but did not reproduce the dramatic effects of vanadate.

We have also studied PTPase activity in *in vivo* models of IDDM and NIDDM. We and others had previously shown that in spite of increased insulin receptor concentrations in animal models of type I diabetes, such as the streptozotocin (STZ) rat and the BB rat, insulin receptor autophosphorylation and kinase activity is decreased [45]. PTPase activity was measured in cytosolic and particulate fractions of liver

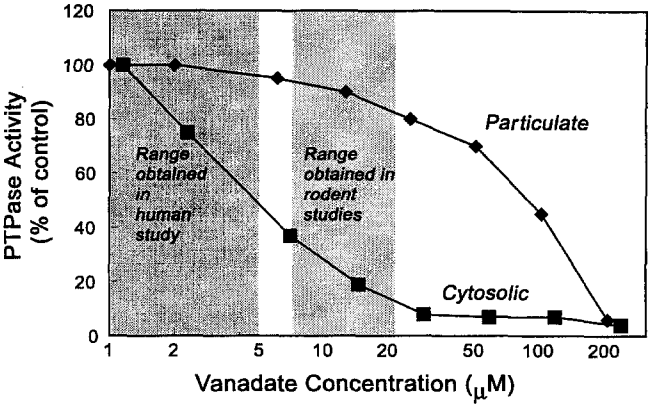

Fig. 4. The effect of vanadate on PTPase activity in Fao cells.

in STZ and BB diabetic rats before and after vanadate therapy using a phosphopeptide substrate with a sequence identical to the major insulin receptor autophosphorylation site [19]. In STZ- and BB-diabetic rats, cytosolic PTPase activity increased to 180% of the control value. In the particulate fraction, PTPase activity was also increased up to 80% at eight days of diabetes in the STZ rat, however this increase was not sustained at 30 days. Thus insulin deficient diabetes is associated with marked alterations in hepatic PTPase activity.

To investigate the effects vanadate on *in vivo* PTPase activity STZ-diabetic rats and BB rats were treated for 5 days with either insulin (14 U/rat/day) or vanadate (0.6 mg/ml in the drinking water) which produced a near normalization of blood glucose. Three days of treatment with either insulin or vanadate resulted in a reduction of PTPase activity in the particulate fraction (Fig. 5). In contrast, no change in cytosolic PTPase activity was seen with either insulin or vanadate. Thus, in two animal models of Type I diabetes there is an early increase in cytosolic and membrane PTPase activity, and treatment with either insulin or vanadate improves glucose homeostasis and reduces membrane PTPase activity.

Similar to changes in insulin receptor tyrosine kinase activity seen in IDDM, insulin receptor kinase activity is also reduced in hepatocytes [46], adiposcytes [47] and skeletal muscle tissue [48] isolated from obese NIDDM subjects. In animal models of NIDDM, cytosolic and membrane associated PTPase activity in ob/ob mice was 55% of that seen in ob/+ mice as assayed using a phosphorylated peptide substrate *in vitro*, whereas in the db/db mice, only the cytosolic PTPase was reduced [19]. Vanadate treatment did not alter the cytosolic or particulate PTPase activity in either ob/ob or db/db mice, in contrast to the findings in insulin deficient models of diabetes mellitus described above. Thus, under the conditions of these experiments, vanadate<apos>s therapeutic effect was observed without any measurable change in hepatic PTPase activity. Furthermore, vanadate treatment was not associated with increases in the low levels of insulin receptor β-subunit or insulin receptor substrate (IRS-1) phosphorylation. Also, no new phosphotyrosyl proteins were detected in the vanadate treated animals. These studies demonstrate that vanadate is an effective hypoglycemic agent in these two rodent models of NIDDM and suggest that vanadate acts distally to the insulin receptor and the insulin receptor substrate IRS-1 and do not involve the PTPase activity in NIDDM models.

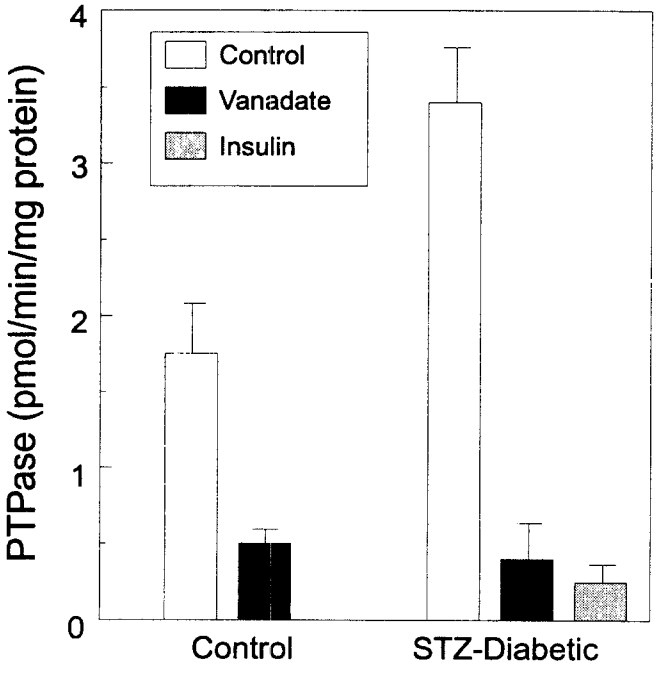

Fig. 5. Effect of vanadate on particulate PTPase activity in STZ-diabetic rats.

Three other post-receptor sites of vanadate action have been explored in a limited manner. In animals that have received vanadate in the drinking water, the uptake of 3-O-methyl glucose, a non-metabolized analogue of glucose, is doubled in muscle and liver tissues of both control and STZ- rats suggesting that the observed lowering of blood glucoses is due to enhanced tissue glucose uptake and metabolism [24]. It is worth noting, however, that insulin itself does not increase hepatic glucose uptake, hence the increased transport of 3-O-methylglucose observed in liver is not simply another insulin-like action. In isolated rat fat pads, vanadate increases the activity of the low K_m (Type IV) cAMP phosphodiesterase (PDE) in the microsomal fraction [49]. Preincubation of the fat pads with inhibitors of protein kinase C results in a partial inhibition of the vanadate effect, but has no effect on insulin-stimulated PDE. Vanadate/glutathione complexes also inhibit the membrane-bound PDE IV in eosinophils [50].

In an attempt to further characterize the possible mechanism by which vanadate improves blood glucose levels in ob/ob mice, several key enzymes and transport proteins involved in carbohydrate metabolism were analyzed at the mRNA level, including PEPCK and the Glut2 glucose transporter [37, 51–53]. Despite the increased gluconeogenesis present in these diabetic animals, ob/ob mice exhibited 90% reductions in hepatic levels of PEPCK mRNA, and 2–5 fold increases in glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and β-cell/liver glucose transporter (GLUT2) mRNA levels, as compared to their lean ob/+ controls. Vanadate treatment resulted in an increase in PEPCK mRNA levels similar to that seen with 24–48 h of starvation [54]. Vanadate also stimulates PEPCK mRNA in hepatoma cells in culture. However, vanadate had no effect on PEPCK mRNA levels in ob/+ controls and did not alter mRNA levels of any of the other proteins analyzed. While vanadate does have effects to increase the expression of several of these

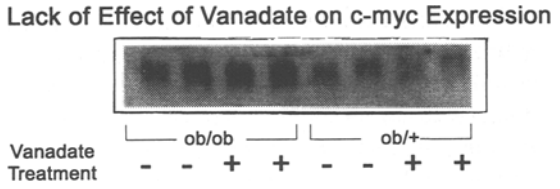

Fig. 6. mRNA levels of c-myc in ob/ob mouse hepatocytes.

proteins at the mRNA level, it is not at all clear if these account for any of the insulin-like effects. Indeed, the effect on PEPCK is exactly opposite that of insulin itself.

Since chronic vanadate treatment has been noted to produce a somewhat altered appearance of cultured fibroblasts suggestive of transformation, mRNA levels of the proto-oncogene c-myc were analyzed. Although 3–4 fold higher levels of c-myc mRNA were seen in the liver of ob/ob mice as compared to their lean controls, six weeks of vanadate administration did not alter the steady-state mRNA levels of this proto-oncogene (Fig. 6).

Human studies of the use of vanadium salts in diabetes mellitus

To investigate the efficacy and mechanism of action of vanadate as oral hypoglycemic agent, 5 subjects with IDDM (age 45 ± 6 yrs, BMI 24.0 ± 0.8 kg/m^2, HbA1c 11.1 ± 1.0%)and 5 with NIDDM (age 52 ± 3 yrs, BMI 28.7 ± 1.7 kg/m^2, HbA1c 10.9 ± 2.0%) were studied before and after oral sodium metavanadate ($NaVO_3$) 125 mg/day. Subjects were studied for 4 weeks including a 1 week baseline period, 2 weeks of treatment with vanadate, and a 1 week post-treatment period. Baseline laboratory testing included glycosylated hemoglobin and fructosamine levels, chest radiograph, electrocardiogram, CBC, chemistry profile, coagulation times, thyroid function tests, urine analysis, and urine microalbumin. Subjects were instructed to monitor blood glucoses four times a day (Lifescan, One Touch Meter) and keep records of insulin dosage and dietary intake for the first, baseline, week. At the end of the first study week, subjects were admitted to the General Clinical Research Center and a two step euglycemic, hyperinsulinemic clamp study was performed to quantitate insulin sensitivity. Deuterated glucose was used to measure endogenous hepatic glucose production and continuous indirect calorimetry was used to measure substrate oxidation and energy expenditure. In addition, subjects with NIDDM on oral hypoglycemic therapy and/or diet alone were evaluated with a 2 h hyperglycemic clamp to assess insulin secretion on the morning preceding the euglycemic clamp.

Subjects were then given sodium metavanadate 125 mg orally each day (50 mg with breakfast, 50 mg with lunch, and 25 mg with supper) for two weeks. Blood glucose monitoring, as well as insulin and dietary logs were continued. Physical examination, blood tests and urine profiles were repeated weekly throughout the study to evaluate for biochemical evidence of toxicity. At the completion of the third study week (the second week of therapy with vanadate), the hyperglycemic clamp (in NIDDM) and the euglycemic insulin clamp studies (in all subjects) were repeated, and the sodium metavanadate was discontinued. The subjects were then followed for an additional week to monitor for delayed adverse effects.

Pharmacology in humans

Although a few subjects experienced mild nausea when vanadate therapy was initiated, this rapidly dissipated. Only one study subject could not tolerate the full dose of sodium metavanadate due to nausea and vomiting. He became asymptomatic when the dose was reduced to 25 mg po tid, and this dose was continued throughout the study. One subject noted increased salivation. Serum vanadium levels were measured by electrothermal atomization atomic absorption spectroscopy at the Metals Laboratory of the Mayo Clinic. The assay has a limit of sensitivity of 1 ng/ml (< 20 nM). Human serum vanadium concentrations are consistently less than this in normal subjects by this assay (T. P. Moyer, personal communication). In addition, 24 h collections of urine were obtained for analysis of vanadium content at the end of the two week treatment period. Serum V levels varied from 19–261 ng/ml (Table 1). Mean vanadium levels were 187

Table 1. Serum and urine vanadium levels measured by atomic absorption spectroscopy at the conclusion of 2 weeks of $NaVO_3$ therapy.

Vanadium levels:		
IDDM		
Patient	Serum	Urine
		(ng/ml) (μg/l)
MV[1]	114	145
KG	139	982
BB	283	683
SH	238	2293
RR	162	792
Mean ±	187.2 ± 28.3	979.0 ± 319.0
NIDDM		
Patient	Serum	Urine
	(ng/ml)	(μg/l)
VS	136	372
RM	19	155
BF	224	418
MB	70	305
WT	261	1638
Mean ± SE	142.0 ± 40.6	577.6 ± 240.4

Source: Serum and urine vanadium levels were measured by graphite furnace atomic absorption spectroscopy. (Special thanks to Dr. Thomas P. Moyer, Ph. D. Mayo Clinic, Rochester, Minnosota)

Note: [1]Dose of vanadate reduced to 75 mg. po qd, divided.

and 142 ng/ml (~3 mM) for the IDDM and NIDDM groups, respectively. There was a 15 fold-range of serum vanadium levels in the NIDDM group which did not correlate with either apparent bioactivity or side effects. Urinary vanadium excretion was about 1–2 mg/day or slightly less than 1% of the administered dose.

Effect of vanadate on insulin sensitivity and insulin action

As a primary end-point of short term therapy, insulin sensitivity was measured using two-step euglycemic, hyperinsulinemic clamps at 0.5 mU insulin/kg/min and 1.0 mU insulin/kg/min [55]. Patients were studied in the postabsorptive state after blood glucose was normalized by administration of a low-dose insulin infusion overnight prior to the study. The clamp studies were performed with 6,6-[$^2H_2$]-glucose to quantitate endogenous glucose production [56]. The clamp studies were performed with continuous indirect calorimetry to assess oxidative versus non-oxidative glucose disposal [57, 58] at baseline and during the last 60 min of each step of the clamp.

Although two of the five subjects with IDDM showed an improved rate of glucose utilization after treatment with sodium metavanadate, on average there was no change in the rate of glucose utilization with either the low (3.6 ± 0.6 *vs* 3.2 ± 0.5 mg/kg/min) or high (8.0 ± 0.8 *vs* 9.0 ± 1.1 mg/kg/min) insulin dose. In contrast, insulin sensitivity improved in all subjects with NIDDM during therapy. Glucose disposal increased by 29% at the 0.5 mU insulin dose (1.7 ± 0.4 *vs* 2.2 ± 0.3 mg/kg/min) and by 39% at the 1.0 mU insulin dose (4.1 ± 1.0 *vs* 5.7 ± 1.3 mg/kg/min) (combined p=0.03) (Figs 7A and 7B). In this small group, there was no apparent correlation between the serum vanadium level achieved and the change in insulin sensitivity.

Oxidative and non-oxidative glucose disposal were calculated from indirect calorimetry data (Fig. 8). Basal oxidative and non-oxidative glucose disposal were similar in subjects with IDDM and NIDDM before and after treatment. The improved insulin sensitivity in the patients with NIDDM during vanadate therapy appeared to be accounted for entirely by increased non-oxidative glucose disposal (1.6 ± 1.0 *vs* 2.9 ± 0.8 mg/kg/min, at the higher insulin dose; $p < 0.03$), with no change in oxidative glucose utilization. Subjects with IDDM demonstrated no significant change in the proportion of oxidative and non-oxidative glucose disposal at either insulin dose.

In contrast to its effect on peripheral glucose utilization, vanadate therapy had no measurable effect on hepatic glucose production (HGP). Basal HGP was unchanged before and after vanadate in both IDDM and NIDDM (Fig. 9). Furthermore, suppression of HGP was similar pre- and post-vanadate in both study groups during both lower and higher dose insulin clamps. These data suggest that the improvement in glucose utilization in NIDDM patients was due to enhanced insulin sensitivity in peripheral tissues (i.e., muscle) without a change in hepatic insulin sensitivity.

Insulin secretion

To assess the effects of vanadate on insulin secretion in subjects with NIDDM not requiring insulin, hyperglycemic clamps [55] were performed before and at the end of treatment. Subjects were studied in the fasted state on the day prior to the euglycemic clamp with blood glucose normalized by administration of a low-dose insulin infusion during the night prior to the study. After the collection of baseline samples for hormones and substrates, the plasma glucose concentration was raised by 100 mg/dl above fasting, and maintained at this level for 2 h. First phase insulin and C-peptide secretion was calculated as the increment in mean plasma insulin or C-peptide level during the first 10 minutes of the hyperglycemic clamp, second phase secretion was calculated as the increment in mean levels during the 10–120 min time period (Fig. 10). C-peptide levels in the basal state (0.69 ± 0.07 *vs* 0.70 ± 0.02 pM/ml) and during the first 10 min of hyperglycemia (0.76 ± 0.07 *vs* 0.81 ± 0.04) and the last 110 min of hyperglycemia (1.16 ± 0.17 *vs* 1.14 ± 0.17) were unchanged after vanadate therapy. Similarly, there was no significant change in basal, first phase or second phase insulin secretion.

Diabetic control

After 2 weeks on vanadate individuals with IDDM showed a small (14%), but significant, decrease in mean daily insulin requirements (39.1 ± 6.6 to 33.8 ± 4.7 units insulin/day, $p < 0.05$), but no change in mean blood glucose (MBG) (157 ± 15 *vs* 152 ± 8 mg/dl). The decrease in insulin requirements did not correlate with the improvement in insulin sensitivity or with any change in caloric intake. There was no change in the dose of the oral hypoglycemic agent in any NIDDM subject. In view of the short duration of the study, it was not surprising that there was no significant change in glycohemoglobin, although values in both groups decreased by ~10% (11.1 ± 1.0 *vs* 10.2 ± 0.8, 10.9 ± 2.0 *vs* 10.2 ± 1.1% in IDDM and NIDDM, respectively). Fructosamine decreased slightly during the baseline week , but no further drop was seen after two weeks on vanadate. No significant changes in average blood glucose or weight were demonstrated in either cohort (Fig. 11).

Effect of vanadate treatment on cholesterol

Vanadate has previously been studied in humans for its potential in treatment of hypercholesterolemia [59]. Serum cholesterol decreased over the course of two week treatment with vanadate in both IDDM and NIDDM subjects (175.2 ± 6.3 *vs* 165.0 ± 8.7 mg/dl, $p = 0.06$, 267.6 ± 29.0 *vs* 204.2 ± 17.9, $p < 0.05$, respectively) (Fig. 12). This is similar in magnitude to the decrease seen in non-diabetic individuals [59]. Apolipoprotein A-1 and apolipoprotein B levels did not

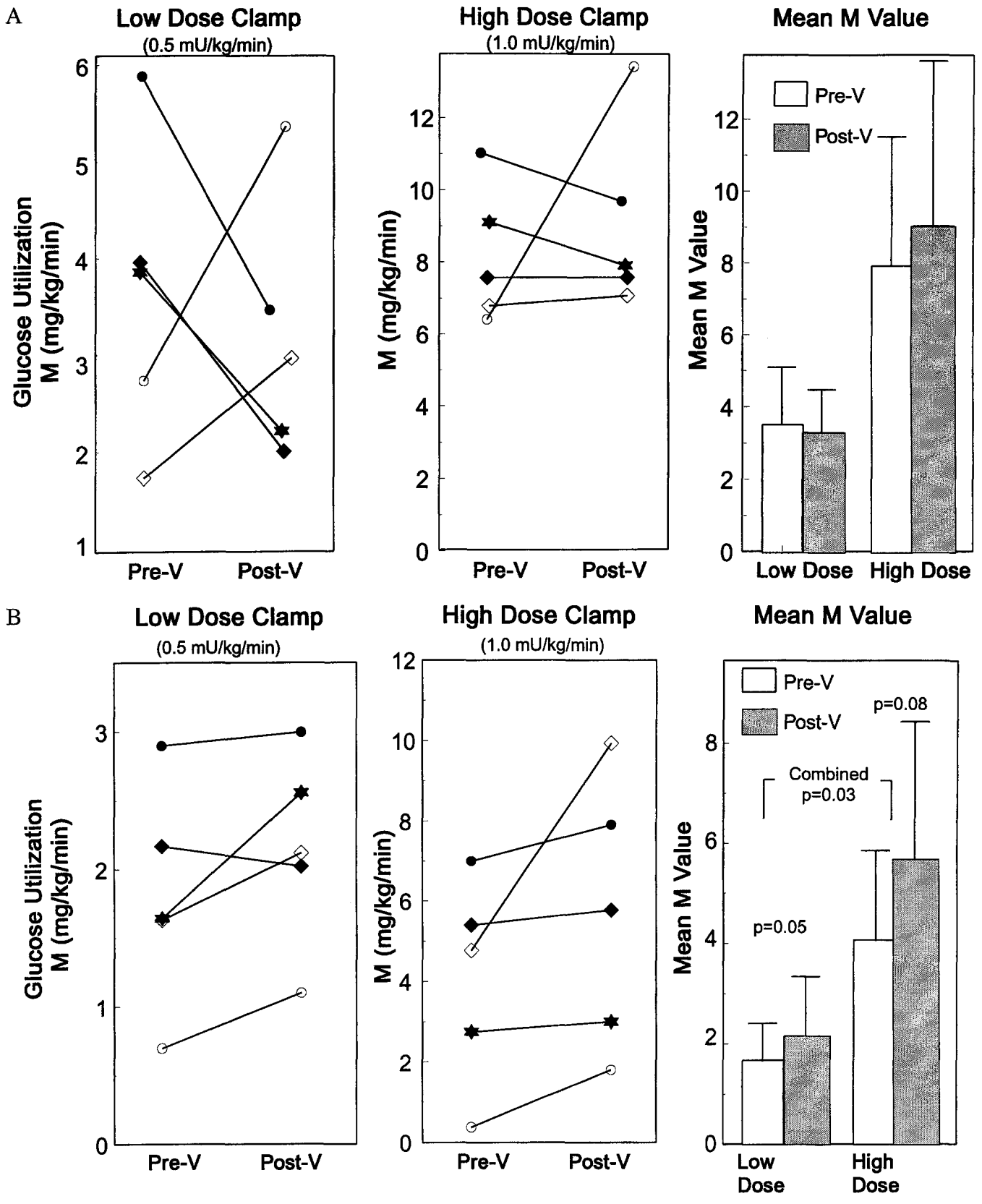

Fig. 7. Effect of vanadate on insulin sensitivity in patients with IDDM (A) and NIDDM (B). Figures on the left represent individual responses, and the figure on the right demonstrates the mean ± SE for the group. Statistical analysis was performed using paired, one tailed, *t*-tests.

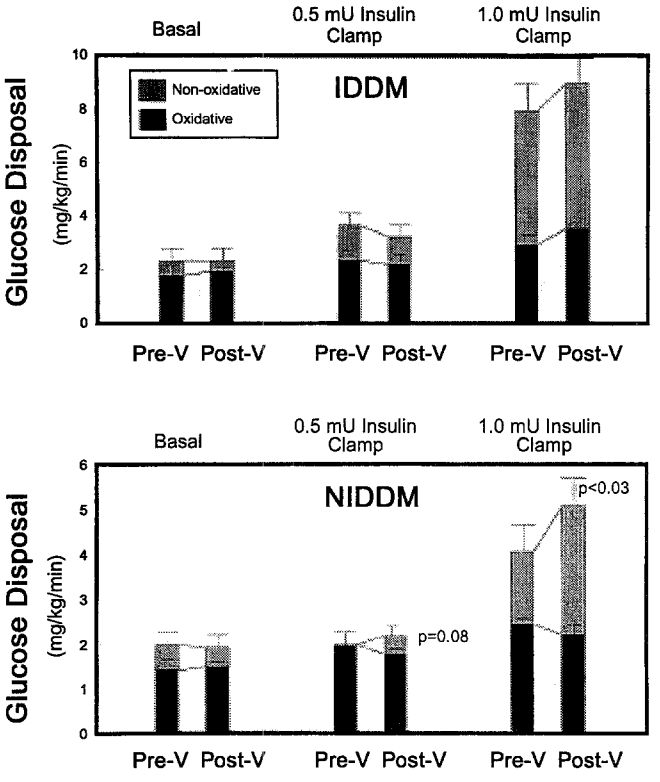

Fig. 8. Oxidative and non-oxidative glucose disposal in IDDM (top panel) and NIDDM (bottom panel) before and after two weeks on sodium metavanadate.

change significantly in either IDDM or NIDDM subjects or in the combined cohort.

Effects of vanadate treatment on map and s6 kinases

MAP and S6 kinases are insulin-stimulated enzymes which have been shown to play important roles in insulin signalling downstream of the insulin receptor [60–62]. To assess possible mechanisms of vanadate action on this post-receptor pathway, MAP and S6 kinase activity was measured in circulating mononuclear cells isolated from subjects pre- and post-vanadate therapy and stimulated with insulin *in vitro*. Each subject's sample was assayed in parallel with that of an age-matched healthy control who did not take vanadate.

In non-diabetic subjects, MAP and S6 kinase activity increased about 2-fold after *in vitro* stimulation with 100 nM insulin. In contrast, there was no significant insulin stimulation of MAP or S6 activity in leukocytes taken from untreated patients with IDDM or NIDDM. Following two weeks of oral therapy in diabetic subjects with sodium metavanadate, there was a 1.7–3.9-fold increase in *basal* MAP and S6 kinase activity in both IDDM and NIDDM to levels similar to those produced by *in vitro* stimulation with insulin in healthy controls. However, following vanadate, there was little further stimulation with insulin *in vitro* in cells from both IDDM and NIDDM subjects (Fig. 13).

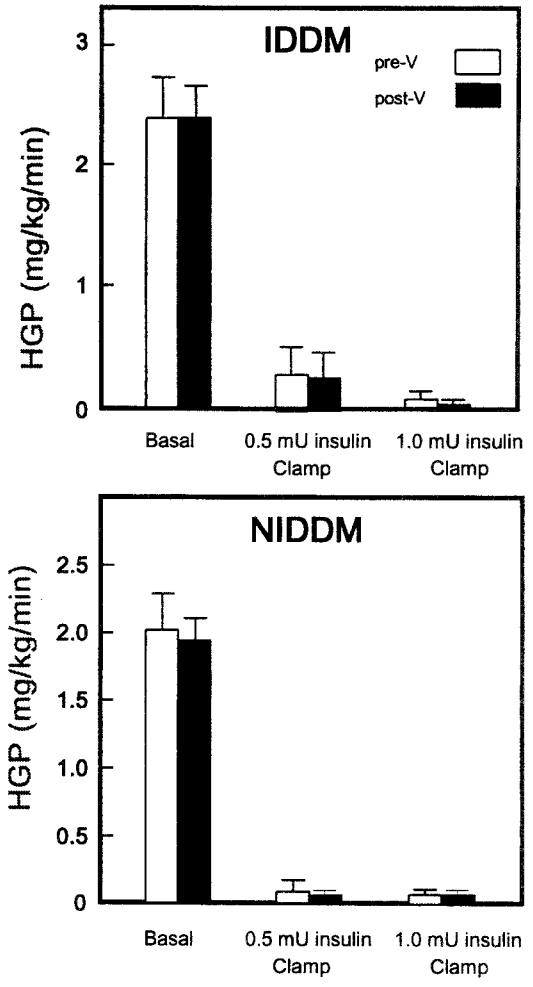

Fig. 9. Hepatic glucose production (HGP) in subjects with IDDM (top panel) and NIDDM (bottom panel) in the basal state and at the steady state of each step of the euglycemic clamp.

Na^+/Li^+ countertransport and na^+/H^+ exchange

Increased Na^+/Li^+ countertransport activity is seen in a subset of patients with essential hypertension and has been attributed to insulin resistance and hypertension [63]. Vanadyl sulfate has been shown to lower plasma insulin and systolic blood pressure without affecting plasma glucose in animal models of hypertension, including the SH rat [64] and the fructose-fed rat [65]. Insulin has been shown to increase the maximal transport rate [V_{max}] and substrate concentration for half maximal velocity [K_m] of Na^+/Li^+ exchange, as well as the V_{max} and K_m for Na^+, but not the K_m for intracellular H^+ [66]. These effects are mimicked by okadaic acid, an inhibitor of protein phosphatases. Thus, we measured the effect of vanadate and vanadyl on Na^+/Li^+ and Na^+/H^+ transport activity in a limited number of patients. Although the V_{max} of Na^+/Li^+ countertransport activity was not affected by 2 weeks of sodium metavanadate (0.36 ± 0.09 *vs* 0.38 ± 0.14 mmol/L cell/h), the K_m was significantly increased (40.8 ± 18.9 *vs* 70.8

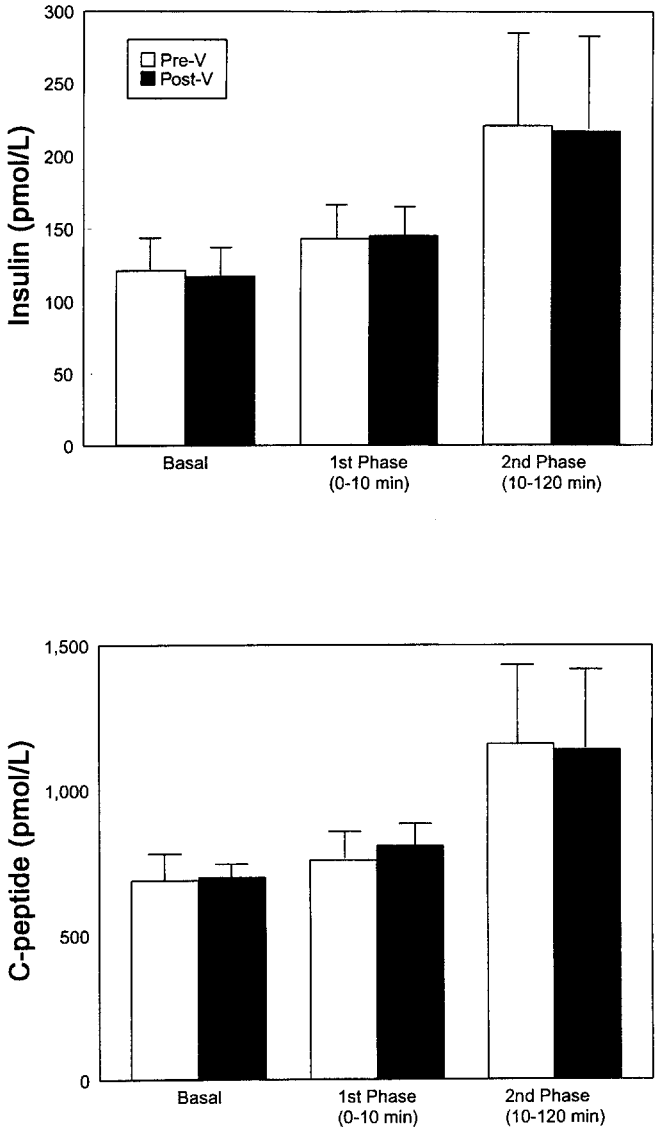

Fig. 10. Insulin and C-peptide levels during a + 5.5 mmol/L hyperglycemic clamp in individuals with NIDDM on oral agent and/or diet treatment.

± 21.2 mM, $p < 0.05$). (Fig. 14) Furthermore, the V_{max} of Na^+/H^+ exchange tended to increase (12.3 ± 6.3 *vs* 18.8 ± 5.5 mmol/L cell/h, $p = 0.08$). This is in contrast to the results of Simonson and Wolpert [67] who found that Na^+/Li^+ transporter activity did not change in obese individuals despite dietary intervention which improved insulin sensitivity. Since hypertensive subjects have an elevated V_{max} Na^+/Li^+ and Na^+/H^+ transport activity, and there is an association between hyperinsulinemia, insulin resistance and hypertension, one might expect vanadium treatment to improve insulin sensitivity and to lower the V_{max} of Na^+/H^+ exchange. The area of interaction of insulin, sodium-ion exchange and vanadium requires further investigation.

Side effects and toxicity

No biochemical evidence of toxicity after short term treatment with sodium metavanadate was detected on the screening laboratory profiles which included electrolytes, BUN, creatinine, liver function studies, thyroid functions, urine analysis and a complete blood count. The major side effect was gastrointestinal intolerance, including vomiting in one patient, which limited the total daily dose to 75 mg daily. Four subjects also reported initial mild diarrhea; this required no therapy and was self limiting in all cases. One subject noticed increased salivation. There was also a single episode of hypoglycemia requiring assistance in a subject with IDDM with a history of hypoglycemia unawareness. All other episodes of hypoglycemia were detected on routine blood glucose monitoring, were asymptomatic or not associated with neuroglycopenia and required no external assistance.

Implications and future directions

Over the past 15 years, considerable evidence has accumulated to show that vanadium salts will mimic insulin action in a number of isolated cell systems and produce glucose lowering effects when given orally to animal models of both IDDM and NIDDM [19]. The present investigation shows that two weeks of treatment with sodium metavanadate at a dose of 125 mg po daily appears to be relatively well tolerated, and improves insulin sensitivity in patients with NIDDM, as well as in some patients with IDDM. The increase in insulin sensitivity is primarily due to an increase in non-oxidative glucose disposal, whereas oxidative glucose disposal and both basal and insulin stimulated suppression of hepatic glucose output (HGP) were unchanged. Thus the improvement in insulin action in patients receiving vanadate is due primarily to increased peripheral (i.e., skeletal muscle) insulin sensitivity, although it is difficult to rule out an effect on hepatic insulin sensitivity, since HGP was maximally suppressed even by low dose insulin. Clinically, this is associated with a small decrease in insulin requirements and glycohemoglobin. Of additional benefit, there is a decrease in total cholesterol levels in both IDDM and NIDDM subjects. Furthermore, there is an increase in the basal activities of MAP and S6 kinases to levels similar to the insulin-stimulated levels in controls, but there is little or no further stimulation with insulin. Although this study focused on sodium metavanadate, in preliminary studies, we and others [68] have seen similar effects with oral vanadyl sulfate treatment.

The present data in humans are encouraging and consistent with previous studies in animal models of both human IDDM and NIDDM [4, 14–18, 21, 24, 69, 70]. The smaller effect observed in humans as compared to rodents may reflect differences in dose, attained blood levels, and time course of

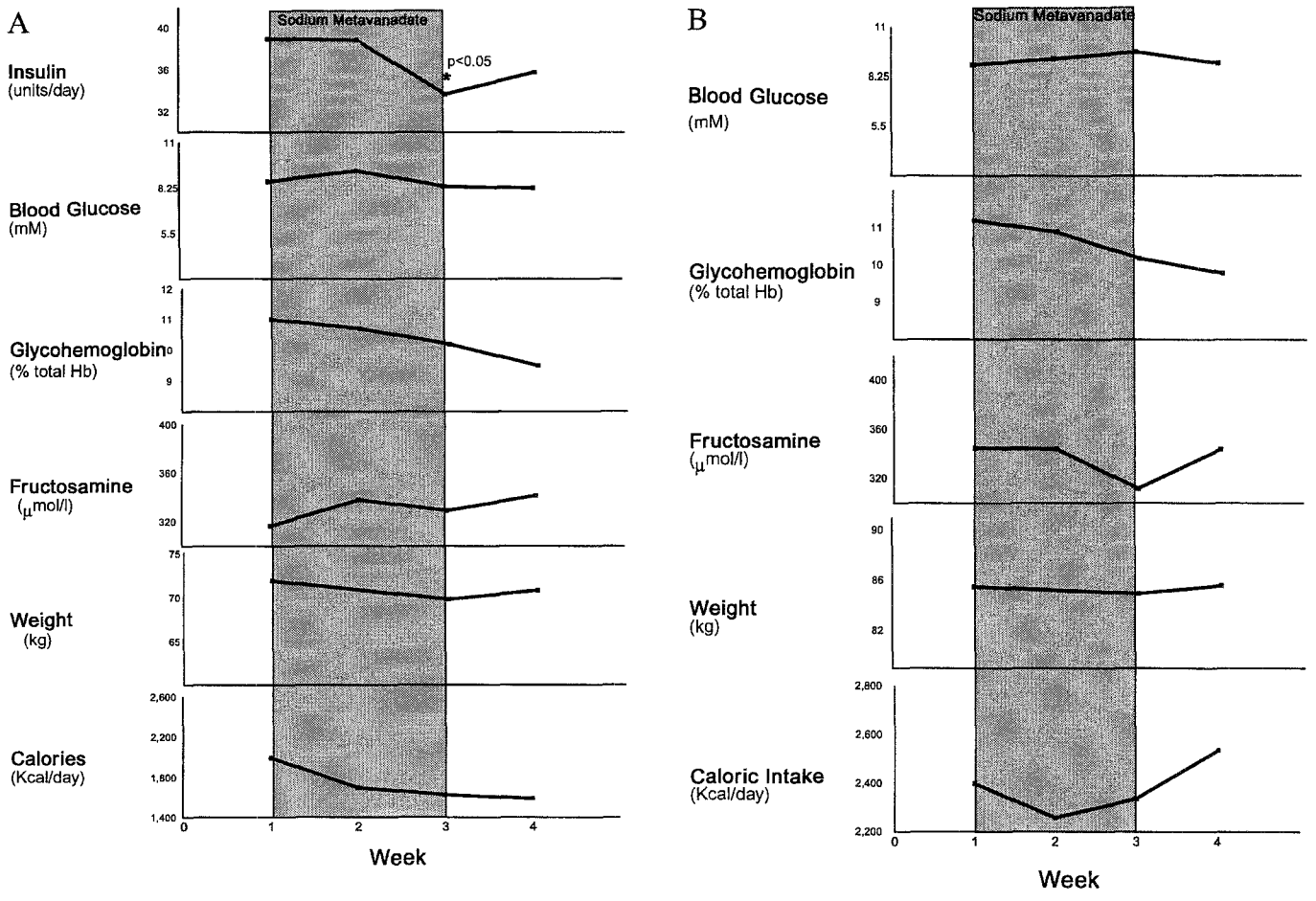

*Fig. 11.*Clinical parameters in IDDM (A) and NIDDM (B) patients receiving sodium metavanadate. Each panel shows the mean value for each parameter at the indicated time in the study.

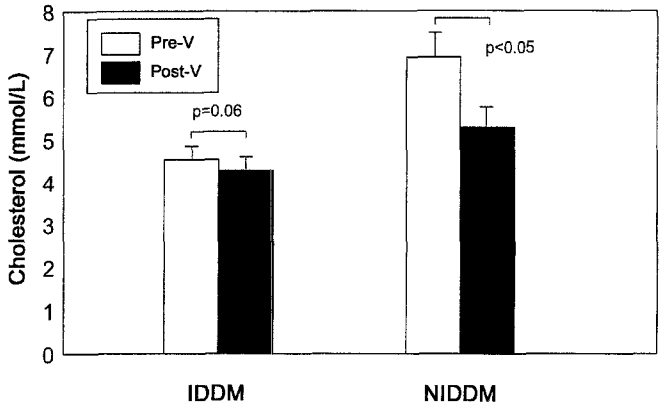

Fig. 12. Serum cholesterol levels before and after treatment with sodium metavanadate.

action. For example, in rodents, the dose of oral vanadate which has been found to improve blood glucoses is about 100 mg/kg/day, while the dose used in the current study was about 1.5 mg/kg/day. Likewise the blood level of vanadium achieved in rodent studies is between 10 and 20 μM, while the blood level in the present human study was between 1 and 5 μM. Finally, in humans the entire treatment period was only two weeks. In STZ-diabetic and BB rats (in the presence of continued, reduced insulin doses) vanadate added to the drinking water lowers glucose to near normal values within 3–4 days [18, 24, 69], however, the effect of vanadate to lower blood glucose to near normal levels in ob/ob and db/db mice takes 10–20 days [14, 16]. Thus, studies of longer duration in humans are warranted in order to fully evaluate the potential of this compound on glucose metabolism in humans.

It has recently been suggested that one factor which may contribute to the hypoglycemic action of vanadate is a decrease in food intake [71]. While there are some mild, transient gastrointestinal symptoms in humans receiving vanadate, we were unable to detect a significant difference in food intake as assessed by dietary history and food records or body weight during the vanadate treatment period in either NIDDM or IDDM, thus decreased intake does not appear

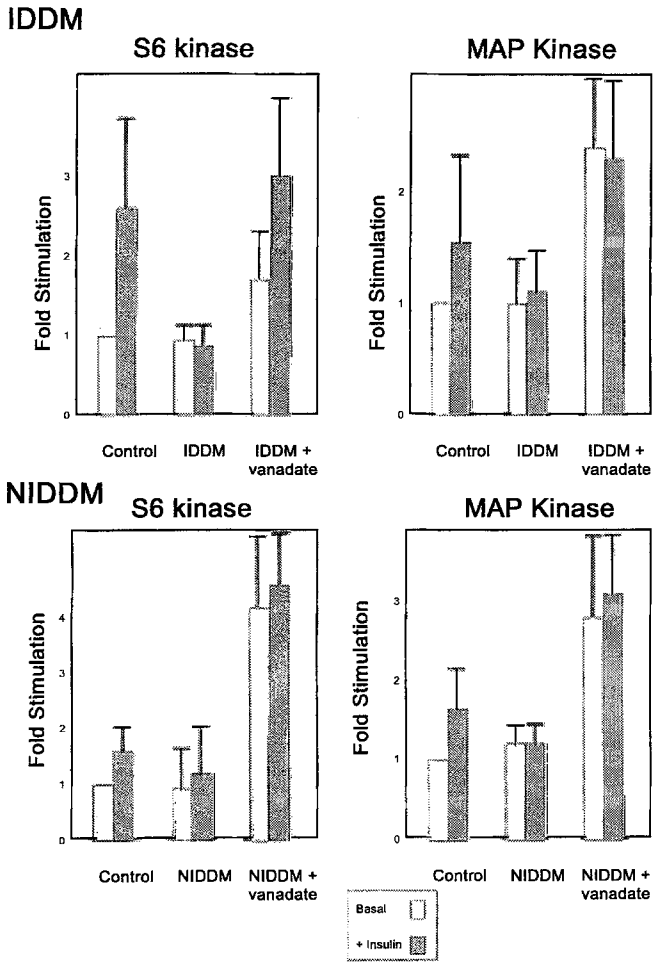

Fig. 13. MAP and S6 kinase activity in monocytes of control subjects, IDDM and NIDDM patients in the basal state and after two weeks on vanadate. (control individuals were not treated with $NaVO_3$).

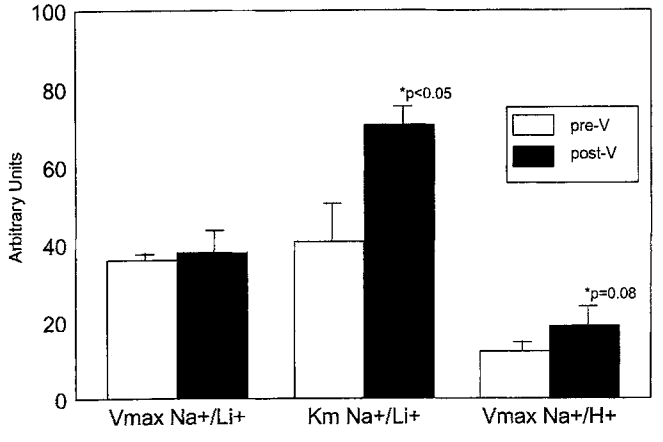

Fig. 14. Na^+/Li^+ and Na^+/H^+ transport activity in human erythrocytes before and after sodium metavanadate.

to be the mechanism of action in humans. These data are consistent with most previous animal data. In obese mice or rats treated with vanadate, there is no change in the percent lean body mass suggesting that the effects of vanadate do not require a major decrease in food intake [14, 16, 24]. When given to obese, hyperinsulinemic fa/fa rats, vanadate also improves oral glucose tolerance without decreasing body weight or changing counter-regulatory hormones [53, 70]. The differences in reported findings could be due to different responses in different animal models studied, different vanadium salts administered, different dosages employed, or the nutritional status (fed vs. fasted) of the rats at the time of study.

The exact tissue site of action of vanadium salts is remains unclear. In ob/ob mice, the disappearance rate for intravenous glucose is doubled in treated animals as compared to controls, and the insulin response to the glucose challenge reappears [14, 16]. In addition, hepatic glycogen content doubles, and pancreatic insulin stores are increased. There is also an increase in basal glucose oxidation by the hemidiaphragm in vanadate-treated animals, although insulin stimulation is similar to that of the control [70]. In vanadate treated STZ diabetic rats, euglycemic, hyperinsulinemic clamp studies indicate higher rates of peripheral glucose disposal, but no effect of vanadate on inhibition of hepatic glucose production by insulin [69]. In humans, we found no change in basal rates of oxidative or non-oxidative glucose disposal, and no change in oxidative glucose disposal at either low or high insulin levels, but a modest increase in non-oxidative glucose disposal in the NIDDM subjects. This improvement in glucose utilization in humans appears to be due to enhanced insulin sensitivity in muscle, since there was no change in basal or stimulated insulin secretion during the hyperglycemic clamp and no effect of vanadate on hepatic glucose production.

Improved insulin sensitivity, however, may be related to actions which increase basal activities of S6 and MAP kinases. Like insulin, sodium orthovanadate administration has been shown to increase both S6 phosphorylation and S6 activation in rat liver [72, 73]. In the present study we found that untreated IDDM and NIDDM subjects had a decreased insulin stimulation of activity of both MAP and S6 kinases. Sodium metavanadate increased the basal activities of MAP and S6 kinases to levels similar to the insulin-stimulated levels in controls, but there was little or no further stimulation with insulin. These data suggest that vanadate enhances the intrinsic activity of these enzymes, perhaps by inhibition of a phosphatase that would inhibit MAP kinase.

A potential additional clinical benefit of vanadate treatment was the reduction in serum cholesterol levels in the NIDDM patients. These results are consistent with the observations of Curran *et al.* who found that non-diabetics given oxytartratovanadate for eight weeks had significant reductions in total and free cholesterol levels [59]. The mechanism of this effect is most likely on cholesterol synthesis. Vanadium salts inhibit cholesterol synthesis by interference with formation and utilization of mevalonic acid [74]. Vanadium compounds

may inhibit coenzyme A and thus interfere with the conversion of HMG to β-methyl crotonate [75]. Furthermore, early studies indicate that deposition of cholesterol is reduced and mobilization of predeposited cholesterol is increased in rabbits fed vanadium [76, 77].

Additional *in vivo* and *in vitro* studies with the vanadium compounds are warranted to better understand the mechanism of action of these interesting compounds.

Acknowledgements

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