Available online at www.sciencedirect.com

Metabolism

www.metabolismjournal.com

A tea/vanadate decoction delivered orally over 14 months to diabetic rats induces long-term glycemic stability without organ toxicity

Tod A. Clark[a], Clayton E. Heyliger[b], Melanie Kopilas[c], Andrea L. Edel[c], Asad Junaid[d], Floribeth Aguilar[c], Donald D. Smyth[e], James A. Thliveris[f], Michele Merchant[c], Hae K. Kim[c], Grant N. Pierce[c,*]

[a] Department of Surgery, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada R3E OW3
[b] Department of Physiology, Ross University School of Veterinary Medicine, St. Kitts
[c] Department of Physiology, University of Manitoba, and The Canadian Centre for Agri-food Research in Health and Medicine, St. Boniface Hospital, Winnipeg, Manitoba, Canada R2H 2A6
[d] Department of Internal Medicine, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada R3E OW3
[e] Department of Pharmacology, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada R3E OW3
[f] Departments of Human Anatomy and Cell Sciences, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada R3E OW3

ARTICLE INFO

Article history:
Received 3 March 2011
Accepted 13 October 2011

ABSTRACT

Vanadium can induce potent hypoglycemic effects in type 1 and type 2 diabetes mellitus animals, but toxic adverse effects have inhibited the translation of these findings. Administration of vanadate in a black tea decoction has shown impressive hypoglycemic effects without evidence of toxicity in short-term studies. The purpose of this study was to investigate the hypoglycemic action and the toxic adverse effects of a tea/vanadate (T/V) decoction in diabetic rats over a 14-month treatment period. Streptozotocin-induced type 1 diabetes mellitus rats were orally gavaged with 40 mg sodium vanadate in a black tea decoction only when blood glucose levels were greater than 10 mmol/L. Glycemic status and liver and kidney function were monitored over 14 months. All of the diabetic rats in this treatment group (n = 25) required treatment with the T/V decoction at the start of the study to reduce blood glucose levels to less than 10 mmol/L. Diarrhea was uncommon among the T/V-treated animals during the first week of T/V treatment and was absent thereafter. There was no evidence of liver or kidney dysfunction or injury. From 2 to 6 months, fewer animals required the T/V treatment to maintain their blood glucose levels. After 9 months of treatment, none of the diabetic animals required any T/V to maintain their blood glucose levels at less than 10 mmol/L. Oral administration of a T/V decoction provides safe, long-acting hypoglycemic effects in type 1 diabetes mellitus rats. The typical glycemic signs of diabetes were absent for the last 5 months of the study.

Author contributions: Tod Clark was responsible for the organization of the paper, writing the manuscript, and some experimental data collection. Clayton Heliger was responsible for preparation of the extract and was involved in designing the study. Melanie Kopilas, Andrea Edel, Asad Junaid, Floribeth Aguilar, Don Smyth, Michele Merchant, and Hae Kim were responsible for data collection. Jim Thliveris was responsible for the electron micrograph work. Grant Pierce was responsible for coordinating the overall project and for supervision and was involved in the writing of the manuscript.

* *Corresponding author*. Tel.: +1 204 235 3206; fax: +1 204 235 0793.
E-mail address: gpierce@sbrc.ca (G.N. Pierce).

0026-0495/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2011.10.007

1. Introduction

Vanadium compounds suspended in water and administered orally can effectively control hyperglycemia in animal models of diabetes [1]. However, this has been associated with liver and kidney toxicity, and severe diarrhea that can ultimately lead to high mortality rates [2-6]. Many laboratories have since attempted to chemically modify the vanadium to increase its tolerability in the subjects, and this has met with mixed success [7-11]. Alternatively, we have suspended sodium orthovanadate in a tea extract known to have antidiarrheal and beneficial gastrointestinal properties [12]. This tea/vanadate (T/V) decoction demonstrated long-lasting hypoglycemic action in streptozotocin (STZ)-induced diabetic animals with no observable adverse effects over an 8-week period [13]. However, it is not known if this hypoglycemic action continues for longer periods (more than 1 year) and if evidence of organ toxicity would appear if the compounds were administered for these longer periods. The primary purpose of this study, therefore, was to determine if this hypoglycemic action continued for long periods and if these extended periods would reveal any toxic long-term adverse effects.

2. Methods

2.1. Animals

Male Sprague-Dawley (SD) rats (175-200 g) were obtained from the University of Manitoba Central Animal Care Services, housed on wood-chip bedding in polycarbonate cages, and offered free access to both food (LabDiet 5P00, Prolab, Richmond, IN, USA) and water. The animals were maintained at 20°C with 50% humidity throughout the study. The experimental period was 64 weeks. The Animal Care Review Committee of the University of Manitoba approved this study. Animals were euthanized with a single intraperitoneal injection of a 9-mg/mL ketamine:0.9-mg/mL xylazine cocktail. Plasma was collected and stored at –20°C for subsequent analysis.

2.2. Insulin-dependent diabetic model

Rats were lightly anesthetized with 2% isoflurane and 2% O_2 gas, and diabetes was induced by a single tail vein injection of STZ at a dosage of 55 mg/kg body weight as described previously [14,15]. Control animals received an injection of buffered vehicle alone. Animals were allowed to adjust to their diabetic state for 4 days before any treatment. The Animal Care Review Committee would not allow the STZ-induced diabetic rats to be maintained in an untreated condition for longer than 10 weeks. It was also no longer considered to be ethically responsible to include a diabetic group treated with vanadate in a water solution as has been done previously [2-6] in view of the nonspecific organ toxicity reported. Thus, we could not include this control group in the present study and have instead relied on previously published accounts of vanadate toxicity when delivered to diabetic rats in a water solution.

2.3. Blood glucose analysis

Blood glucose levels were assessed in all animals using a Bayer Glucometer Elite testing system (Bayer, Toronto, Canada). A distal tail snip generated the 5-μL quantity of blood necessary for analysis. Daily glucose levels were done at 9:00 AM regularly by removing the scab formed on the tail; or if the tail was too irritated for this procedure, a glucometer lancet device was used to puncture the tail for a sample.

2.4. Treatment of diabetes

A decoction of sodium orthovanadate (Sigma, St Louis, MO) was made by suspending 20 mg vanadate per milliliter tea extract. The tea extract was prepared by bringing 1.0 L of ddH_2O to a boil and then adding 60 g of Chinese Lychee Black Tea leaves (Golden Sail brand, China), as described elsewhere [13,16,17]. The sodium orthovanadate was suspended into the tea with stirring, and then the solution was stored in the dark at room temperature until used within approximately 5 hours. Fresh solution was made daily.

Any animal with a blood glucose level greater than 10 mmol/L was orally gavaged at 4:00 PM daily with the T/V treatment solution. Rats with daily blood glucose levels less than 10 mmol/L were considered to have glycemic levels that were only slightly greater than control levels (~7 mmol/L). These were considered manageable, and the animal was not treated with the T/V decoction that day. Animal treatment groups included 64-week T/V-treated diabetic rats (T/V) (n = 27), 10-week diabetic rats (D) gavaged with water (n = 12), 64-week nondiabetic animals gavaged with water (ND) (n = 13), and 10-week nondiabetic animals gavaged with water (n = 12). Each animal received 2 mL of treatment solution, corresponding to a total vanadium dose of 40 mg.

2.5. Plasma/urine analysis

Biochemical diagnostic kits (Sigma) were used for the assessment of alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglycerides (TGs), and cholesterol levels within plasma. A Vet test 8008 spectrophotometer (IDEXX Laboratories, Westbrook, MN, USA) was used for albumin, alkaline phosphatase (ALKP), amylase, blood urea nitrogen (BUN), creatinine, globulin, total bilirubin, total protein, and uric acid analysis. Urine specific gravity was assessed with a hand refractometer (Atago, Bellevue, WA, USA), and plasma insulin levels were measured with an enzyme-linked immunosorbent assay.

2.6. Morphology of pancreatic islet cells

Pancreata were collected from all groups and processed for electron microscopy using standard techniques [18]. Thick sections were cut, stained with toluidine blue, and viewed under the light microscope for routine orientation. Thin sections were cut, stained with uranyl acetate and lead citrate, viewed, and photographed in a Philips EM201 microscope (Phillips, Einhoven, Germany). To eliminate observer

bias, tissues were examined using coded grids without foreknowledge of their source.

2.7. *Statistical analysis*

The data were analyzed statistically using an analysis of variance test followed by a Student-Newman-Keuls post hoc test. Results are reported as the mean ± SE. Statistical significance was determined at a *P* level of <.05.

3. Results

This study was carried out for 64 weeks (448 days). The exact time points that we used to carry out the analyses of the selected parameters varied (6, 8, or 10 weeks, etc). These measurement time points varied to avoid the influence that conducting one measurement (eg, a glucose tolerance test) may have on another measurement (eg, sampling blood for creatinine analyses).

The beneficial effects of vanadate on the glycemic status of STZ-induced diabetic animals have been suggested to be due to a repression of appetite [19]. Therefore, water and food intake was measured. Water intake was significantly higher in the untreated diabetic animals, and food intake was significantly lower at 6 weeks in comparison to the nondiabetic controls and the T/V-treated diabetic animals at 15 weeks (Fig. 1A, B). The T/V-treated diabetic rats also had a significantly higher water intake than the nondiabetic controls. Water and food intake was relatively constant over the time course of the experiment and did not differ at 34 weeks or 48 to 54 weeks between the nondiabetic controls and the T/V-treated diabetic animals.

The metabolic status and the integrity of selected organ systems were monitored with plasma markers over the course of the study. As shown in Fig. 2A, serum amylase levels were lower in the untreated diabetic animals in comparison to the nondiabetic control animals at 10 or 64 weeks and to the T/V-treated diabetic animals at 64 weeks.

Nondiabetic control animals exhibited blood glucose levels of approximately 6 to 8 mmol/L at the beginning of this study (Fig. 2B). Streptozotocin administration to the animals resulted in blood glucose levels of approximately 22 mmol/L in both the untreated diabetic and the T/V animal groups. Fifteen animals remained untreated over a 10-week period. Blood glucose levels actually rose to approximately 28 mmol/L and remained at that level. However, diabetic rats treated with T/V exhibited a rapid and significant decrease in blood glucose levels over the course of the study. This was maintained at approximately 8 mmol/L until 200 days into the study, after which it gradually declined to levels indiscernible from the nondiabetic controls over this period (Fig. 2B). Glycated hemoglobin levels were measured over the course of the study (Fig. 2C). Whereas the untreated diabetic rats exhibited elevated levels of glycated hemoglobin at 10 weeks, the nondiabetic controls at 10 and 64 weeks had significantly lower glycated hemoglobin levels. Glycated hemoglobin levels in the T/V-treated diabetic animals at 64 weeks were not statistically different from the control animals. An oral glucose tolerance test was also undertaken. A bolus of glucose given by gavage to untreated diabetic animals resulted in a rapid increase in blood glucose

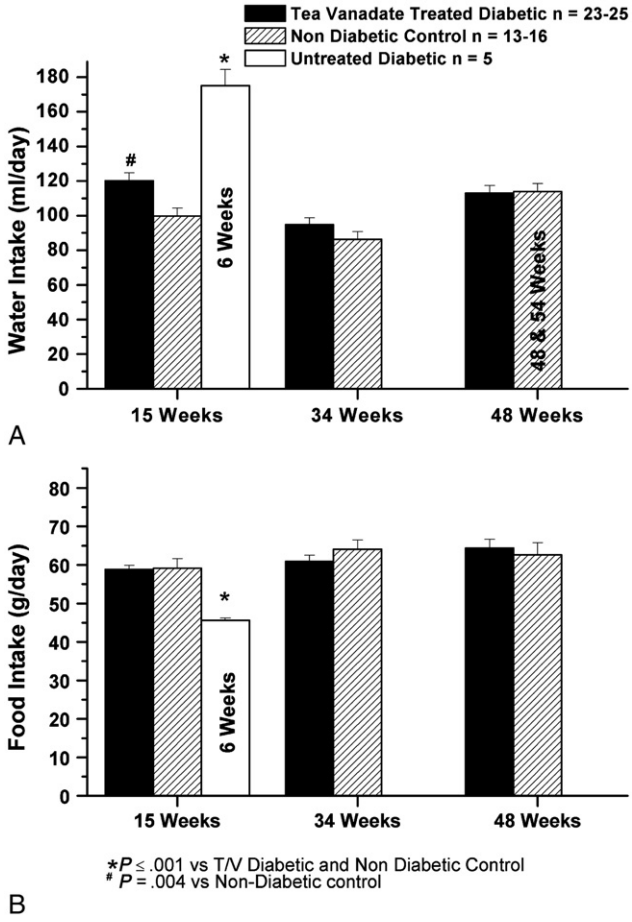

Fig. 1 – The effects of a T/V treatment on (A) water or (B) food intake in diabetic SD rats treated for 1 year. *P ≤ .001 vs T/V-treated diabetic and nondiabetic control rats; #P = .004 vs nondiabetic control rats. Data represent mean ± SE for T/V-treated diabetic, nondiabetic control and untreated diabetic rats. n = 25, 16, and 5, respectively.

levels that was maintained over the 6-hour testing period (Fig. 2D). In contrast, the nondiabetic controls at the same time point (8 weeks into the study) exhibited a significant increase in blood glucose in response to the glucose load; but this was short lived and returned to basal levels within 2 hours. There was a significant blunting in the response in comparison to the untreated diabetic animals at the same time point. At 52 weeks into the study, age had clearly blunted the response to the administration of the glucose load. The rise in blood glucose levels in response to the glucose load was significantly less than that in the untreated diabetic rats. The nondiabetic controls at 52 weeks and the T/V-treated diabetic animals exhibited similar responses in blood glucose during the glucose tolerance test (Fig. 2D).

The T/V treatment was only administered when the rats exhibited blood glucose levels greater than 10 mmol/L. As shown in Fig. 3A, all of the rats in the T/V group (n = 25) required a gavage treatment with the T/V decoction at the start of the study to maintain blood glucose levels at less than 10 mmol/L. From months 2 to 6 of the study, although fewer animals required the T/V treatment to maintain their blood glucose levels, most of the diabetic animals still required the

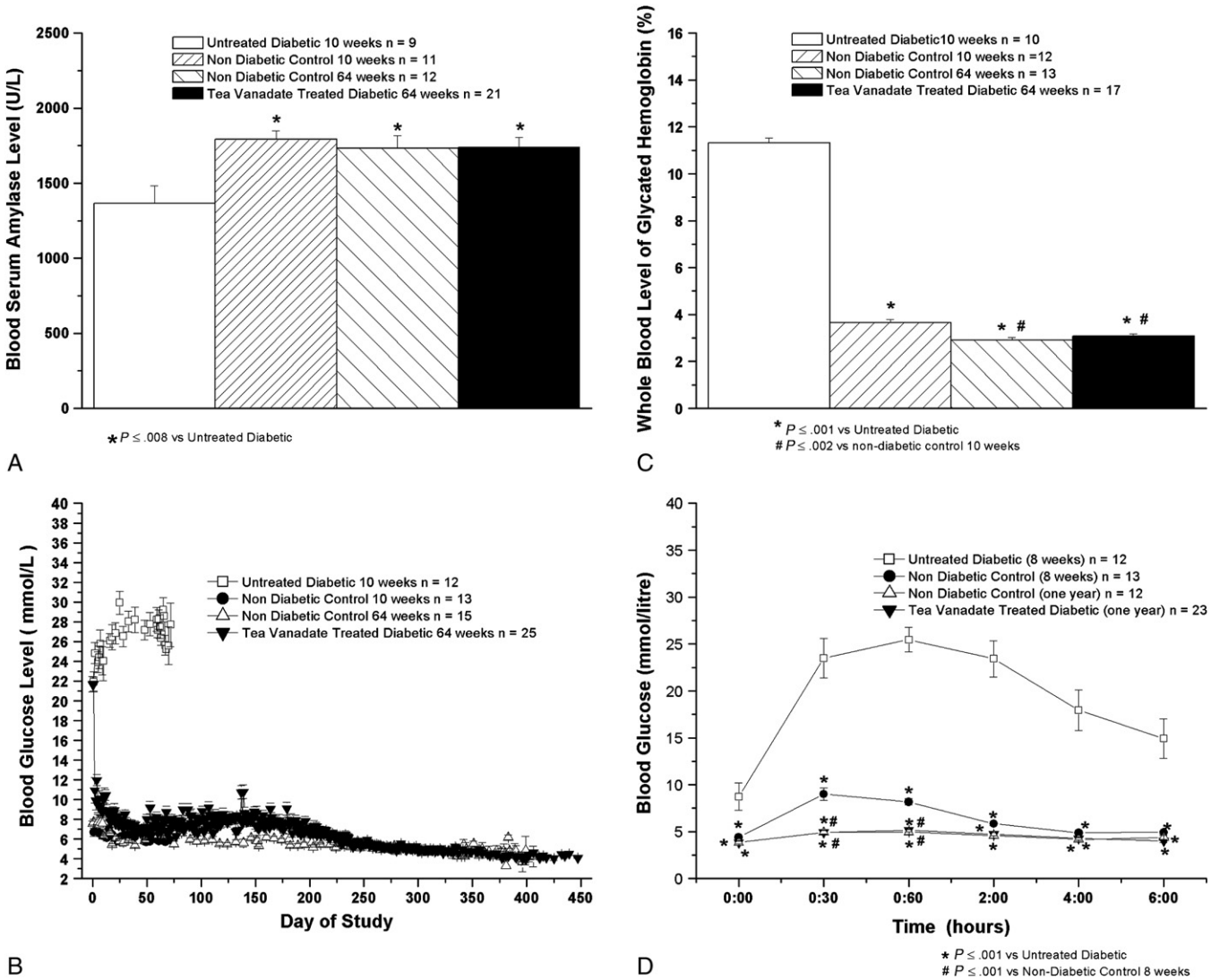

Fig. 2 – The effects of T/V treatment on blood serum constituents. A, Amylase. *P ≤ .008 vs untreated diabetic rats. B, Glucose. C, Glycated hemoglobin. *P ≤ .001 vs untreated diabetic rats, #P ≤ .002 vs 10-week nondiabetic controls. Data represent mean ± SE for 10-week untreated diabetic, 10-week nondiabetic control, 64-week non-diabetic control, and 64-week T/V-treated diabetic rats; and n = 10 to 12, 11 to 13, 12 to 15, and 17 to 25 in graphs A to C, respectively. D, The effects of T/V treatment on glucose tolerance in response to a load of glucose in diabetic SD rats treated for 1 year. Data represent mean ± SE for 10-week untreated diabetic, 10-week nondiabetic control, 64-week nondiabetic control, and 64-week T/V-treated diabetic rats. n = 12, 13, 12, and 23, respectively. *P ≤ .001 vs untreated diabetic rats; #P ≤ .001 vs 8-week nondiabetic control rats.

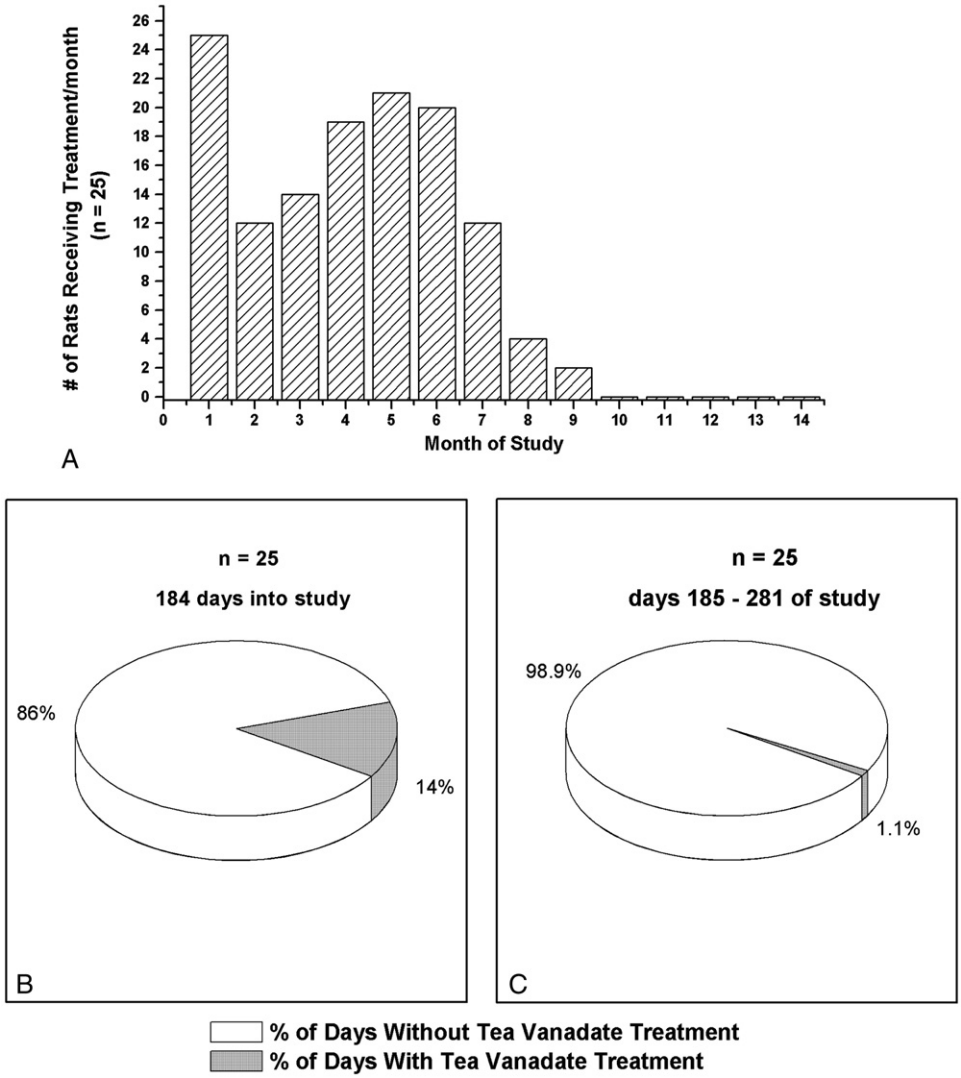

Fig. 3 – A, Total number of rats receiving T/V treatments each month of the study. Percentage of days requiring treatment for diabetic SD rats treated with T/V. B, Days 1 to 183 of the study. C, Days 184 to 365 of the study. n = 25 animals for each half of the study.

T/V treatment to reduce their blood glucose levels to less than 10 mmol/L. However, after 9 months of treatment, none of the animals required any T/V to maintain their blood glucose levels at less than 10 mmol/L. Using another perspective of these data, the diabetic animals required T/V treatment for 14% of the days for the first 184 days of the study (Fig. 3B). Most (86%) of the days did not require any T/V treatment at all. By days 185 to 281 of the study, the animals required T/V treatment only 1.1% of these days. Almost 99% of the time, the 25 animals did not require any treatment whatsoever to maintain their blood glucose levels at less than 10 mmol/L (Fig. 3C). After 281 days, none of the diabetic animals required any further treatment with T/V because they all maintained their blood glucose levels at less than 10 mmol/L every day.

The effects of the treatment interventions on serum lipid levels were also examined (Fig. 4). The induction of diabetes in the rats did not result in a significant increase in total cholesterol levels at 10 weeks in comparison to nondiabetic controls. The nondiabetic controls exhibited higher circulating cholesterol levels at 64 weeks than the nondiabetic controls at 10 weeks. Cholesterol levels were significantly higher in the T/V-treated diabetic animals than in the nondiabetic controls at 64 weeks (Fig. 4A). Induction of diabetes in the untreated animals resulted in a significant rise in circulating TG levels in comparison to nondiabetic control animals at 10 weeks (Fig. 4B). The nondiabetic controls exhibited an increase in circulating TG levels at 64 weeks in comparison to their values at 10 weeks. The T/V treatment of the diabetic animals did not change this age-related response. The TG levels were significantly higher at 64 weeks in both groups in comparison to both groups at 10 weeks.

Circulating insulin levels were measured in untreated diabetic animals at 8 weeks after STZ injection, and in control and T/V-treated animals 1 year into the study (Fig. 5). As expected, there was a significant decrease in plasma insulin concentration in diabetic animals in comparison to control. Tea/vanadate treatment of the diabetic animals resulted in a partial but not complete restoration of circulating insulin levels.

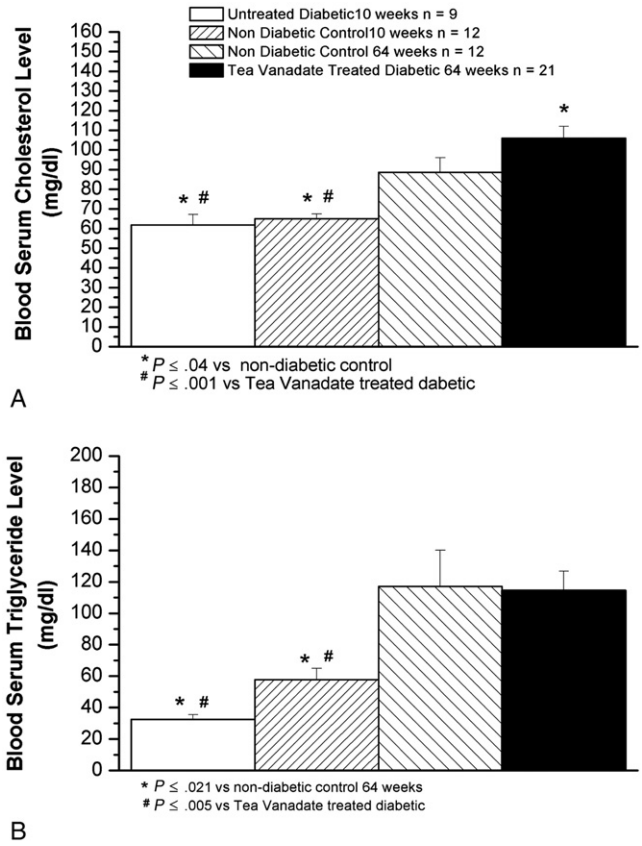

Fig. 4 – Biochemical analysis of serum lipids. A, Serum cholesterol levels. *P = .04 vs 64-week nondiabetic control rats; #P ≤ .001 vs T/V-treated diabetic rats. B, Serum TG levels. Data represent mean ± SE for 10-week untreated diabetic, 10-week nondiabetic control, 64-week nondiabetic control, and 64-week T/V-treated diabetic rats. *P = .021 vs 64-week nondiabetic control rats; #P ≤ .005 vs T/V-treated diabetic rats. n = 9, 12, 12, and 21, respectively.

The morphology of pancreatic tissue from control and diabetic animals was evaluated by electron micrography, and representative results are shown in Fig. 6. Pancreatic α- (A) and β- (B) cells, including their secretory granules (Sg), were readily distinguishable in control tissues (Fig. 6A). Untreated diabetic animals exhibited β-cells with loss of cytoplasmic integrity, but α-cells were normal (Fig. 6B). Tea/vanadate-treated diabetic animals exhibited β-cells with near-normal cellular integrity, but they contained fewer Sg (Fig. 6C). Data were obtained from a number of animals as well. Five randomly selected blocks of pancreatic tissue were examined from 5 animals per experimental group. Two to 4 islets were identified in each block. β-Cells from control animals typically contained numerous Sg and an abundance of flattened or partially dilated profiles of rough endoplasmic reticulum (RER). The majority of β-cells from nontreated diabetic animals showed loss of cytoplasmic integrity. Occasionally, normal-appearing cells were seen as well as cells with dilated profiles of RER and sparse Sg. There were notably more β-cells from diabetic animals treated with the T/V decoction. They also showed more marked dilation of the RER and Sg than was observed in nontreated diabetic rats. In addition, there were fewer cells with loss of cytoplasmic integrity than in untreated diabetic rats. Normal-appearing β-cells were also observed.

Long-term vanadate treatment has been reported to induce organ damage [2-6]. We examined markers of organ damage in blood samples from the animals in this study. Several markers of liver damage were studied. As shown in Fig. 7A, serum ALKP levels were significantly higher in the untreated diabetic rats at 10 weeks than in the nondiabetic control samples at the same time point. The T/V treatment of diabetic animals over the 64-week period resulted in ALKP levels that were similar to those of the nondiabetic controls at 10 and 64 weeks. Similar qualitative results were found in AST (Fig. 7B) and ALT (Fig. 7C).

Several markers were examined as indicators of liver and/or kidney dysfunction (Fig. 8). Serum bilirubin levels were unchanged in any of the 4 groups over the course of this study (Fig. 8A). Untreated diabetic animals had a significantly lower level of albumin in the serum than nondiabetic controls at 10 weeks (Fig. 8B). Tea/vanadate treatment of the diabetic animals over 64 weeks resulted in normalized albumin levels in comparison to control levels at 10 and 64 weeks. Serum protein exhibited a similar qualitative response as serum albumin among the groups (Fig. 8C). A similar qualitative response was also exhibited by the 4 groups in the serum globulin levels (Fig. 8D).

Other specific markers for kidney damage were investigated (Fig. 9). No changes in serum creatinine levels were found in any of the groups over the course of the study (Fig. 9A). Serum BUN levels were significantly decreased in the control animals at 64 weeks in comparison to the same animals at 10 weeks (Fig. 9B). There was no significant difference between the T/V-treated diabetic animals at 64 weeks and the nondiabetic controls during the same time points. A similar qualitative trend was exhibited in serum uric acid levels across the 4 groups (Fig. 9C), but this did not achieve statistical

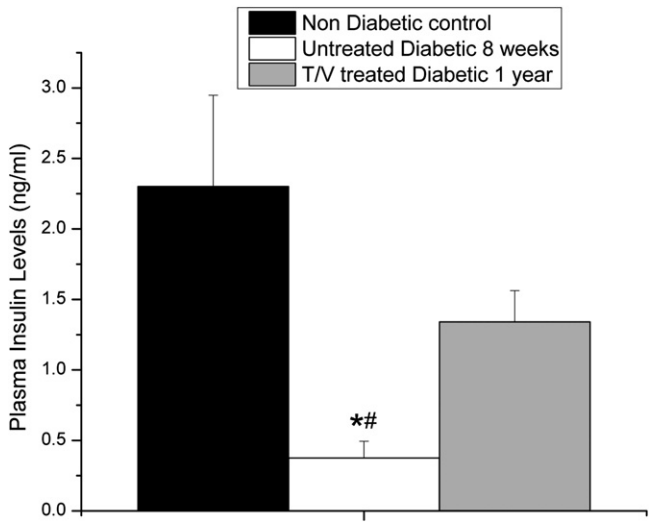

Fig. 5 – Effects of T/V treatment on plasma insulin levels in control and STZ-induced diabetic rats. Values represent mean ± SE of n = 12, 5, and 20 in ND, D, and T/V-treated diabetic animals, respectively. The ND and T/V data were obtained at the 1-year time point, whereas the D data were obtained after 8 weeks. *P < .05 vs ND; #P < .05 vs T/V-treated D.

significance. Urine specific gravity was also significantly lower in nondiabetic controls at 10 weeks in comparison to the untreated diabetic controls at the same time point (Fig. 9D). The urine specific gravity values at 64 weeks were also lower than the untreated diabetic values at 10 weeks, and the T/V-treated diabetic animals at 64 weeks had similar specific gravity levels in their urine as the 2 control groups (Fig. 9D).

Additional measurements of kidney integrity were conducted. Kidney tubular volume did not change in any of the groups over the course of this study (Fig. 10A). The glomerular filtration rate (GFR) as indicated by inulin clearance was significantly higher in the untreated diabetic animals at 10 weeks in comparison to the nondiabetic control animals (Fig. 10B). The nondiabetic controls at 13

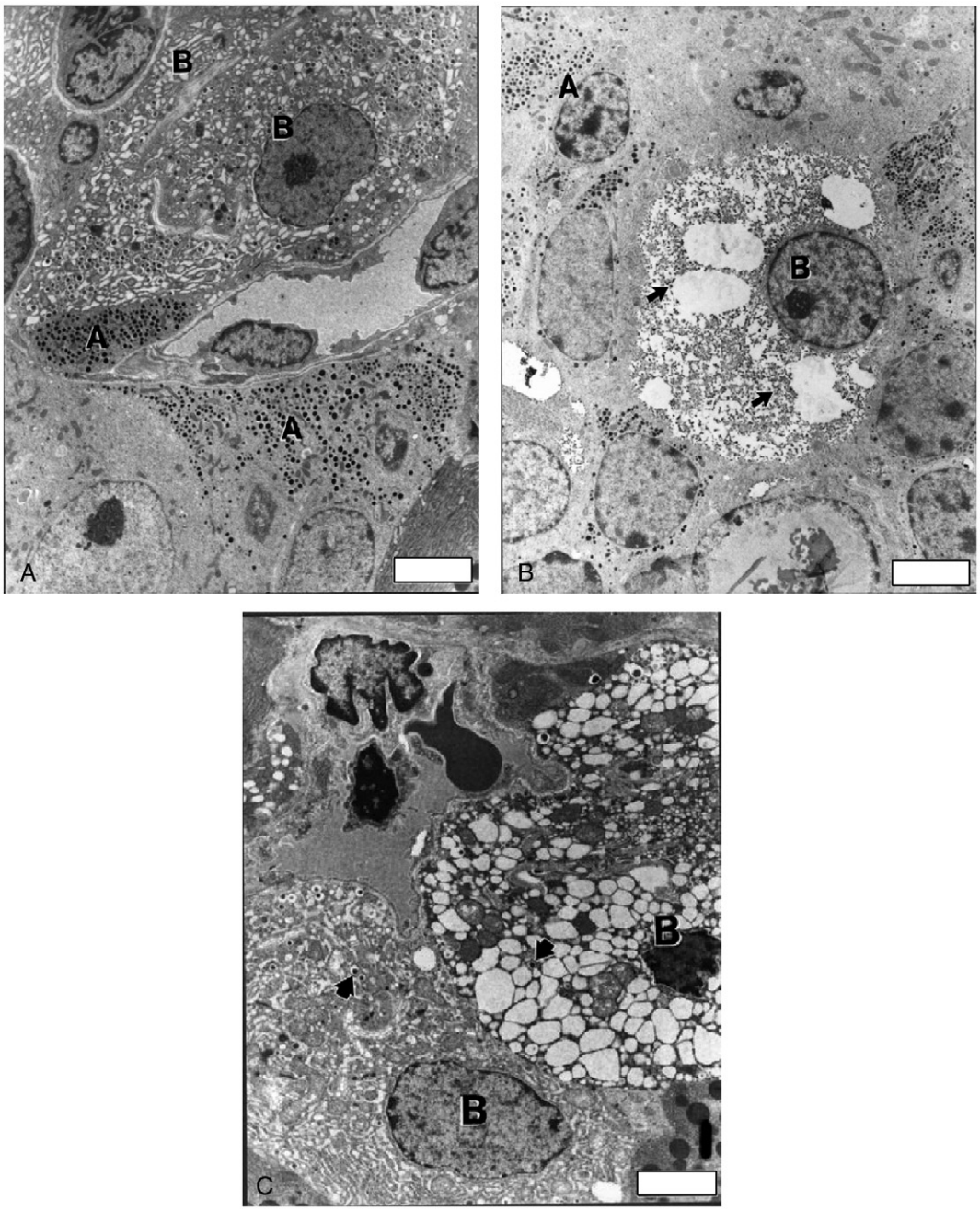

Fig. 6 – Effects of T/V treatment on the insulin-producing pancreatic ß-cells in STZ-induced diabetic rats. A, An electron microscopic image of pancreatic α-cells (A) and ß-cells (B) from a control nondiabetic rat 14 months into the study. Secretory granules can be readily distinguished between the 2 cell types. B, An untreated diabetic rat 8 weeks after STZ injection. ß-Cells exhibit a marked loss of cytoplasmic integrity (arrows), whereas α-cells remain normal. C, An electron microscopic image of pancreatic ß-cells from an STZ-induced diabetic T/V-treated rat 14 months into this study. The cellular integrity is near normal but with sparse numbers of Sg (arrows). Original magnification ×4000.

months into the study exhibited significantly lower GFR values than the 10-week untreated diabetic animals. At 13 months, the T/V-treated diabetic animals exhibited a GFR that was not significantly different than the 2 control groups; nor was it significantly different than the untreated diabetic animals at 10 weeks.

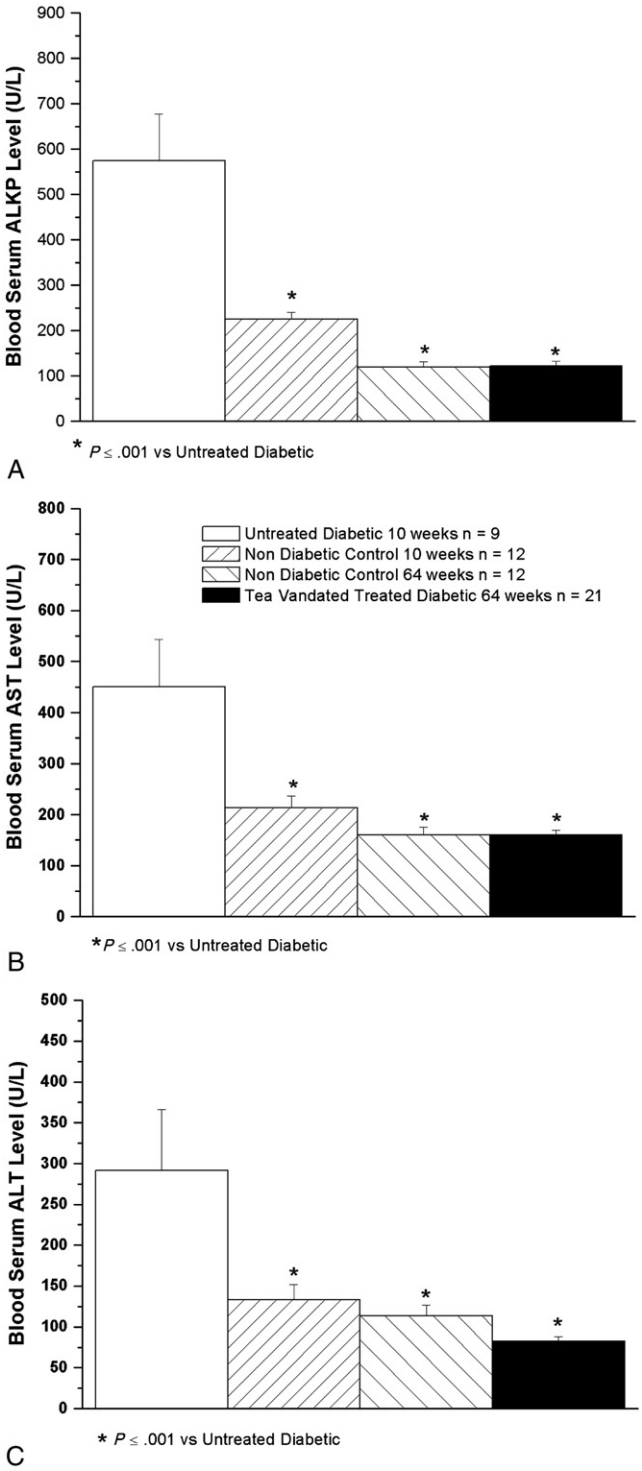

Fig. 7 – Blood serum enzymes as indicators of liver disease. A, Alkaline phosphatase. B, Aspartate aminotransferase. C, Alanine aminotransferase. Data represent mean ± SE for 10-week untreated diabetic, 10-week nondiabetic control, 64-week nondiabetic control, and 64-week T/V-treated diabetic rats. n = 9, 12, 12, and 21, respectively. *P ≤ .001 vs untreated diabetic rats.

Several general characteristics of the animals' health were observed during the course of the study. Approximately 75% of the untreated diabetic animals exhibited diarrhea over the 10-week observational period, whereas none of the nondiabetic controls exhibited diarrhea at any point in the study. Only 2 of the 25 T/V-treated diabetic animals exhibited diarrhea over the 1-year course of this study. Untreated diabetic animals had an incidence rate for cataracts of nearly 60% at 10 weeks. Control animals did not exhibit any cataracts, nor did the T/V-treated diabetic animals over the year of observation. No animals died over the 10 weeks in the control or untreated diabetic groups. However, approximately 10% of the control and T/V-treated diabetic animals died over the course of 1 year. This was not significantly different between the 2 groups. Animals were examined for abnormal growths on their bodies over the course of the experimental study. None were detected in young animals at the 10-week intervention. However, approximately 50% of the animals exhibited growths of some sort on their bodies at 1 year. This was not significantly different between the nondiabetic control animals and the T/V-treated diabetic animals.

4. Discussion

Vanadium use as a therapeutic clinical agent has been limited to date because of its adverse effects. The elimination of both diarrhea and mortality in the T/V-treated animals at 3 months suggests that the antidiarrhetic properties of the decoction work synergistically with the hypoglycemic action of sodium orthovanadate. Over a full year, T/V treatment was associated with an extremely low incidence of diarrhea and a mortality rate equivalent to that in nondiabetic animals. The mechanism by which the T/V decoction reduces vanadium toxicity is undetermined. However, 2 of the possibilities include reducing vanadium uptake or altering the direct effects by which vanadate causes diarrhea. The former option is less likely in view of previously published data showing that vanadium levels in plasma and in the organ tissues were not significantly different from those associated with vanadium administered in water [17]. It has also been observed throughout our studies that most cases of diarrhea occur in the hours immediately following the first dose of vanadium, and this is supported by other investigations [1,13,16]. As such, the importance of the T/V in inhibiting vanadate absorption acutely across the gut wall appears minimal. A more likely postulate concerning the ability of T/V to protect animals from both diarrhea and mortality is that the tea will react with the vanadate to reduce direct vanadium toxicity.

Diarrhea is not the only adverse effect induced by vanadium ingestion. High quantities of vanadium are also reported to alter liver and kidney function [2-6]. Indeed, these effects are so well appreciated in the literature and by those investigating vanadate effects in diabetes that it was not possible to gain ethical approval in our study for a group of animals treated with vanadate alone in water. However, we did not observe any of these effects when vanadate was

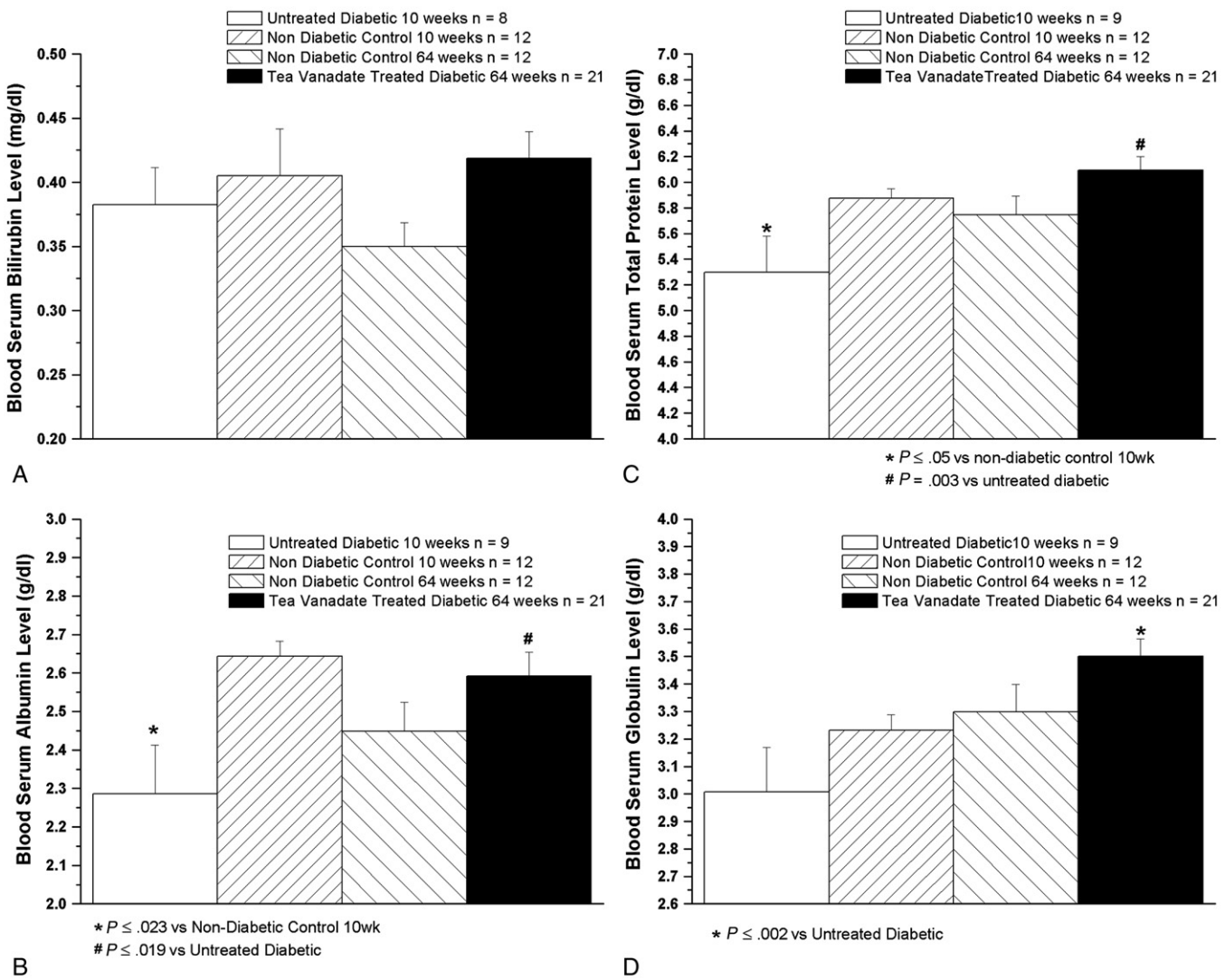

Fig. 8 – Blood serum proteins as indicators of liver or kidney disease. A, Bilirubin. B, Albumin. *P = .023 vs 10-week nondiabetic control rats; #P = .019 vs untreated diabetic rats. C, Total protein. *P ≤ .05 vs 64-week nondiabetic control rats; #P = .003 vs T/V-treated diabetic rats. D, Globulin. *P ≤ .002 vs untreated diabetic rats. Data represent mean ± SE for 10-week untreated diabetic, 10-week nondiabetic control, 64-week nondiabetic control, and 64-week T/V-treated diabetic rats. n = 9, 12, 12, and 21, respectively.

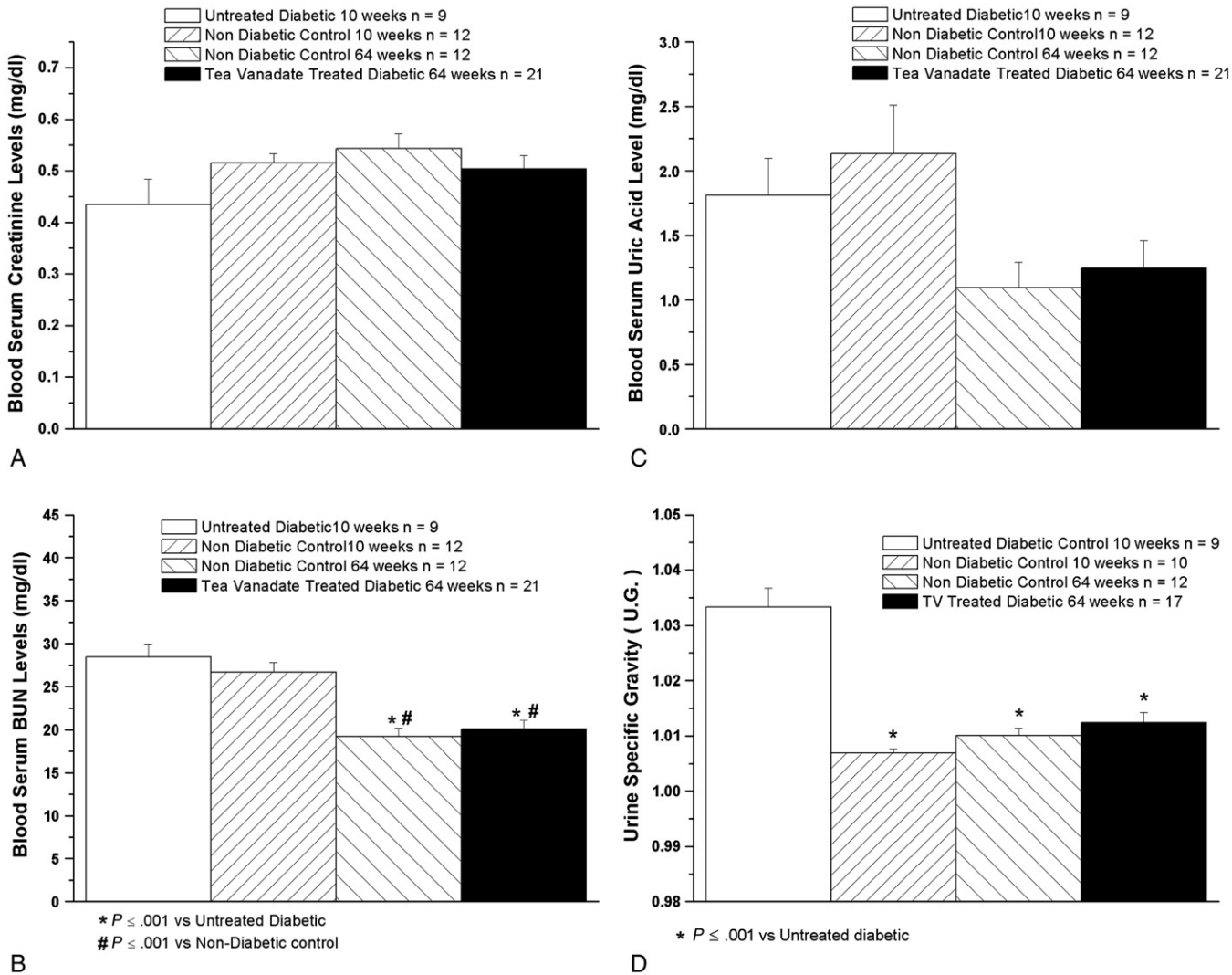

Fig. 9 – Specific markers of kidney damage. A, Serum creatinine levels. B, Serum/BUN levels *$P \leq .001$ vs untreated diabetic rats; #$P \leq .001$ vs 10-week nondiabetic control rats. C, Serum uric acid levels. D, Urine specific gravity. *$P \leq .001$ vs untreated diabetic rats. Data represent mean ± SE for 10-week untreated diabetic, 10-week nondiabetic control, 64-week nondiabetic control, and 64-week T/V-treated diabetic rats. n = 9, 12, 12, and 21, respectively, for graphs A to C; n = 9, 10, 12, and 17, respectively, for graph D.

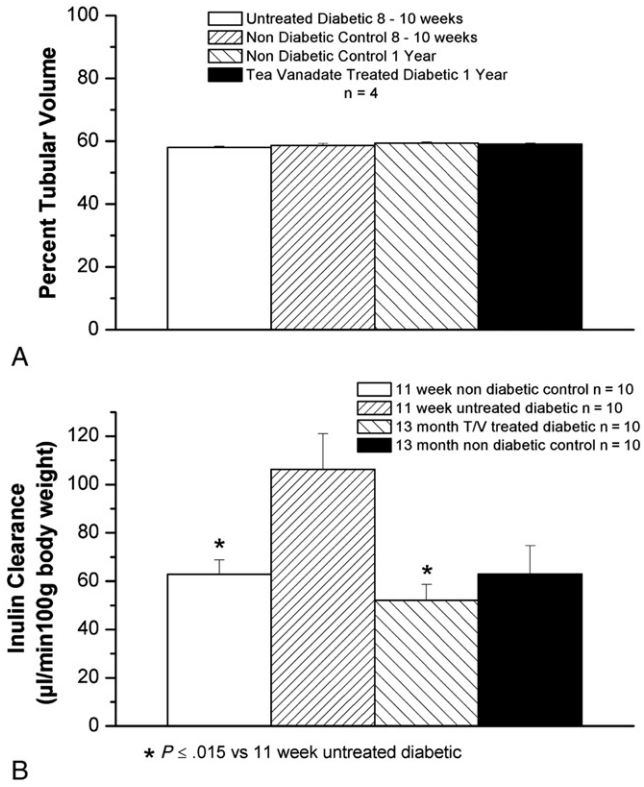

Fig. 10 – Measurement of kidney integrity. A, Tubular volume. Data represent mean ± SE for 8- to 10-week untreated diabetic, 8- to 10-week nondiabetic control, 1-year nondiabetic control, and 1-year T/V-treated diabetic rats. n = 4 for each. B, Glomerular filtration rate. Data represent mean ± SE for 11-week untreated diabetic, 11-week nondiabetic control, 13-month nondiabetic control, and 13-month T/V-treated diabetic rats. n = 10 for each. *P ≤ .015 vs 11-week untreated diabetic rats.

administered with the tea decoction. The efficacy of the T/V decoction to reduce tissue toxicity was substantiated by a large panel of biochemical analyses. Significant improvements in plasma creatinine, urine specific gravity, and normal ALT levels in the T/V-treated diabetic animals suggest that kidney function was normal. After extended periods of treatment with T/V, ALKP activity, amylase, AST, BUN, and uric acid were also restored to normal levels. Our data, therefore, would suggest that animals treated with the T/V decoction have completely normal liver and kidney function. Thus, the animals appear to have no observable toxicity.

The central beneficial feature that the diabetic animals displayed was a normalized blood glycemic control. The mechanism by which the vanadate lowers the blood glucose levels is still not completely understood. Some have suggested that appetite suppression alone produces hypoglycemia [16]. However, others have contested this postulate [9]. Our results are consistent with the latter study [9] and present strong evidence of a return to a normal glycemic profile using a T/V decoction. After a period of more than 1 year, diabetic animals treated with T/V regained normal body mass. Both water and food intake was normalized.

A more plausible possibility for the hypoglycemic action of vanadate may involve its effects on plasma insulin concentration. The T/V-treated diabetic animals exhibited significantly increased plasma insulin levels in comparison to the untreated diabetic animals. This provides evidence for either regeneration of β-cells within the pancreatic islets or functional stimulation of those β-cells not destroyed by STZ. The latter may be more plausible because we observed significantly higher plasma insulin levels in Zucker diabetic fatty rats treated with T/V as well [16]. Furthermore, electron microscopic analysis of pancreatic islets confirmed necrosis in insulin secretary β-cells in both T/V-treated diabetic animals and in untreated diabetic rats. However, more hyperactive β-cells were clearly evident in the T/V-treated diabetic animals than in the untreated diabetic rats (Fig. 6). These data argue against β-cell regeneration as a potential mechanism and argue that, in the T/V-treated diabetic rats, the remaining functional β -cells are more active to control blood glucose.

Two other possibilities exist. Tea has known antioxidant properties that may also protect from any free radical generation induced by vanadate alone. It is also possible that vanadate may have an intracellular site of action that is independent of its potential effects on β-cell function. An intracellular phosphorylation process is critical for glucose transporter (GLUT4) movement to the cell surface to induce glucose transport. This movement would be stimulated by phosphatase inhibition. Vanadate is a potent phosphatase inhibitor [7,20]. A clear understanding of the specific phosphatase that vanadate may target to alter glucose homeostasis would represent critical targeting information.

Regardless of the mechanism of action of the T/V decoction, it is clear that the administration of the T/V decoction has impressive long lasting effects. *The T/V decoction allows the animals to exist in a normal glycemic state for extended periods of time without any treatment.* This is likely due to its accumulation within storage sites in the body [17] and a slow release from these sites into the circulation. It is worth emphasizing that the diabetic animals receiving the T/V decoction did not require any treatment to maintain a normal glycemic status for the final 5 months of this study. This effect is noteworthy in itself. Although one may question our use of less than 10 mmol/L glucose levels as the definition of a glycemic condition that did not require T/V treatment, one cannot question the normal glycemic values exhibited by the T/V animals during these final 5 months of the study. The blood glucose levels at this time were indistinguishable from the control rats during this time (Fig. 2). Furthermore, the benefits offered by an oral route of administration cannot be underestimated considering the millions of insulin-dependent diabetic patients that currently require intramuscular injections on a daily basis. The long-term control of glycemic status by T/V has been shown in both types 1 and 2 diabetes mellitus animals [13,16], although it is much more prolonged in the former model of diabetes.

An untreated diabetic group was not permitted to remain in our study for the full 14 months. One may argue that the STZ-induced diabetes may have spontaneously reversed during this time and, therefore, the long-lasting normoglycemic status in the T/V group was actually due to a spontaneous reversal of the diabetic state and had nothing to do with the T/V treatment. However, spontaneous recovery of STZ diabetes is first

observed 8 to 12 months following STZ administration [21], whereas we observed long-term glycemic control within the first weeks after T/V administration. Furthermore, it takes 16 months for complete spontaneous recovery of STZ diabetes to occur [21]; but it was clearly exhibited in the majority of T/V-treated diabetic rats by 3 months. Long-term control of glycemic status occurred more than half a year before spontaneous recovery of the diabetic state would be expected and, therefore, could not possibly explain our findings. Furthermore, others have reported that glucose homeostasis is irreversibly damaged by STZ administration and/or a long-term diabetic state [22]. Tea/vanadate-treated diabetic rats exhibited none of these defects. Finally, we should have observed death in a large percentage of the STZ-diabetic rats; and we observed no deaths in our STZ-diabetic T/V-treated rats.

A systematic review of oral vanadium therapy in type 2 diabetes mellitus subjects recommended the need for larger-scale randomized controlled trials to be conducted [23]. Others [24] have also found beneficial actions using ligands for vanadyl ions; but again, there was a clear need for larger, more conclusive trials. In view of the superior effects we have observed using T/V in type 1 vs type 2 diabetes mellitus animals [13,16], its translation to type 1 diabetes mellitus clinical trials may be justified.

Funding

This study was supported by an operating grant provided by the Canadian Institutes for Health Research. The indirect costs of the research were supported by the St Boniface Hospital and Research Foundation.

Conflicts of Interest

None of the authors have any conflicts of interest to declare.

REFERENCES

[1] Heyliger CE, Tahiliani AG, McNeill JH. Effect of vanadate on elevated blood glucose and depressed cardiac performance of diabetic rats. Science 1985;227:1474-7.
[2] Domingo JL. Vanadium and diabetes. What about vanadium toxicity? Mol Cell Biochem 2000;203:185-7.
[3] Domingo JL, Gomez M, Llobet JM, Corbella J, Keen CL. Oral vanadium administration to streptozotocin-diabetic rats has marked negative side-effects which are independent of the form of vanadium used. Toxicology 1991;66:279-87.
[4] Boden G, Chen X, Ruiz J, van Rossum GD, Turco S. Effects of vanadyl sulfate on carbohydrate and lipid metabolism in patients with non–insulin-dependent diabetes mellitus. Metabolism 1996;45:1130-5.
[5] Srivastava AK. Anti-diabetic and toxic effects of vanadium compounds. Mol Cell Biochem 2000;206:177-82.
[6] Goldfine AB, Patti ME, Zuberi L, et al. Metabolic effects of vanadyl sulfate in humans with non–insulin-dependent diabetes mellitus: in vivo and in vitro studies. Metabolism 2000;49:400-10.
[7] Shisheva A, Ikonomov O, Shechter Y. The protein tyrosine phosphatase inhibitor, pervanadate, is a powerful antidiabetic agent in streptozotocin-treated diabetic rats. Endocrinology 1994;134:507-10.
[8] Yuen VG, Orvig C, Thompson KH, McNeill JH. Improvement in cardiac dysfunction in streptozotocin-induced diabetic rats following chronic oral administration of bis(maltolato) oxovanadium(IV). Can J Physiol Pharmacol 1993;71:270-6.
[9] Yuen VG, Orvig C, McNeill JH. Effects of bis(maltolato) oxovanadium(IV) are distinct from food restriction in STZ-diabetic rats. Am J Physiol 1997;272(1 Pt 1):E30-5.
[10] Pandey SK, Anand-Srivastava MB, Srivastava AK. Vanadyl sulfate-stimulated glycogen synthesis is associated with activation of phosphatidylinositol 3-kinase and is independent of insulin receptor tyrosine phosphorylation. Biochemistry 1998;37:7006-14.
[11] Orvig C, Thompson KH, Battell M, McNeill JH. Vanadium compounds as insulin mimics. Met Ions Biol Syst 1995;31: 575-94.
[12] Peirce A. The American Pharmaceutical Association practical guide to natural medicines. First Edition. New York: Stonesong Press; 1999.
[13] Clark TA, Heyliger CE, Edel AL, Goel DP, Pierce GN. Codelivery of a tea extract prevents morbidity and mortality associated with oral vanadate therapy in streptozotocin-induced diabetic rats. Metabolism 2004;53:1145-51.
[14] Pierce GN, Dhalla NS. Myofibrillar ATPase activities in diabetic myocardium. J Mol Cell Cardiol 1981;13:1063-9.
[15] Pierce GN, Kutryk MJB, Dhalla NS. Alterations in calcium binding by and composition of the cardiac sarcolemmal membrane in chronic diabetes. Proc Natl Acad Sci U S A 1983;80:5412-6.
[16] Clark TA, Edel AL, Heyliger CE, Pierce GN. Effective control of glycemic status and toxicity in Zucker diabetic fatty rats with an orally administered vanadate compound. Can J Physiol Pharmacol 2004;82:888-94.
[17] Edel AL, Kopilas M, Clark TA, et al. Short-term bioaccumulation of vanadium when ingested with a tea decoction in streptozotocin-induced diabetic rats. Metabolism 2006;55:263-70.
[18] Luft J. Improvements in epoxy resin embedding methods. J Biophys Biochem Cytol 1961;9:409-14.
[19] Malabu UH, Dryden S, McCarthy HD, Kilpatrick A, Williams G. Effects of chronic vanadate administration in the STZ-induced diabetic rat. The antihyperglycemic action of vanadate is attributable entirely to its suppression of feeding. Diabetes 1994;43:9-15.
[20] Swarup G, Cohen S, Garbers D. Inhibition of membrane phosphotyrosyl protein phosphatase activity by vanadate. Biochem Biophys Res Commun 1982;107:1104-9.
[21] Rakieten N, Gordon BS, Beaty A, Cooney DA, Schein PS, Dixon RL. Modification of renal tumorigenic effect of streptozotocin by nicotinamide: spontaneous reversibility of streptozotocin diabetes. Proc Soc Exp Biol Med 1976;151:356-61.
[22] Inoue K, Cetkovic-Cvrlje M, Eizirik DL, Grill V. Irreversible loss of normal beta-cell regulation by glucose in neonatally streptozotocin diabetic rats. Diabetologia 1994;37:351-7.
[23] Smith DM, Pickering RM, Lewith GT. A systematic review of vanadium oral supplements for glycaemic control in type 2 diabetes mellitus. Quarterly J Med 2008;101:351-8.
[24] Thompson KH, Lichter J, LeBel C, Scaife MC, McNeill JH, Orvig C. Vanadium treatment of type 2 diabetes: a view to the future. J Inorg Biochem 2009;103:554-8.